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RESEARCH ARTICLE

Virulence Factors of Uropathogenic *Escherichia coli* in Diabetic Patients of Al-Muthanna Province

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Abstract

Background and aims: There are evidences that patients with diabetes have an increased risk of asymptomatic bacteriuria and urinary tract infections (UTIs). UTIs are the most common bacterial infection in diabetic patients. The present study aimed to describe the characteristics of *E. coli* of Al-Muthanna province patients with urinary tract infection by identifying the virulence genes (*FimH*, *Pap* and *HlyA*). Material and methods: The study population included 240 diabetic and non-diabetic patients with UTIs as well the healthy control, Urine and blood sample was collected at Al Hussein Educational Hospital, between October 2017 to July 2018. Serum sample were collected and stored for immunological tests. Standard protocol was followed to isolate and identify organism and the data were analyzed using Microsoft Excel 2013. Results: Of the total 240, The results of the study were 41 (33.3%) isolates of Diabetes patients (DM group), 42(34.1%) isolates of Non-Diabetes patients (ND group) with urinary pathogenic *E. coli* (UPEC) and selected 40 (32.5%) of control group. The age duration of 46-61 years were most prevalence of UPEC infections either DM group (56.1%) or ND group (35.7%) while the gender factor was shown significantly in DM and ND females (78.1% and 97.6% respectively) compare to males. Twenty two (53.7%) of diabetes urban community were found to have UPEC infections while the rural infected population were 19 (46.3%), Non-diabetes group were recoded 17 (40.5%) , 25 (59.5%) positive cases of UPEC in urban and rural communities respectively. Fimbriae severity virulence genes of DNA (*fimH* , *pap*) and alpha-hemolysis gene (*HlyA*) of were extracted of 83 UPEC isolates, it was then identified using the PCR amplification technique. The results of molecular diagnosis shown that *fimH* was detected in 78% in DM patients had higher than of ND patients (57%) of UPEC strains while the percent of *Pap* and *HlyA* were (31.7%, 12.2%) in DM patients compare to ND patients (*Pap* /38.1%) and (*HlyA* /7.1%) of UPEC strains respectively. Conclusion: UTIs are frequent in diabetic patients. For the reason of the great proportion of asymptomatic forms among diabetic patients, the urine culture should be performed in all hospitalized patients with diabetes.

Introduction

Uropathogenic *Escherichia coli* (UPEC) is the major causative agent of urinary tract Infections (UTI) including cystitis and pyelonephritis. In ascending infections, colonization of fecal organisms in the urethra leads to the upward spread of bacteria to the bladder (causing cystitis) and kidneys (causing pyelonephritis). UPEC contains several virulence factors that facilitate its colonization and invasion of host cells.

Surface virulence factors (adhesins) of UPEC are among the most important virulence factors. As the main attachment factor, P fimbriae is particularly associated with pyelonephritis and is encoded by pap genes. Another adhesion that acts as a virulence factor is S fimbrial adhesion, which is coded by *sfa* genes [1, 2, and 3]. UPEC is the cause of community-acquired UTIs and a large portion of nosocomial UTIs, accounting for substantial medical costs and morbidity and mortality worldwide. The ability of UPEC to cause symptomatic UTIs is associated with expression of a broad spectrum of virulence factors, with adhesive molecules being arguably the most important determinants of pathogenicity [4, 5].

Fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys (causing pyelonephritis), or the prostate in males. Because women have a shorter urethra than men, they are 14 times more likely to suffer from an ascending UTI [6]. Uropathogenic *E. coli* use P fimbriae (pyelonephritis-associated pili) to bind urinary tract urothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood-group antigen of erythrocytes and uroepithelial cells. Approximately 1% of the human population lacks this receptor, and its presence or absence dictates an individual's susceptibility or non-susceptibility, respectively, to *E. coli* urinary tract infections and uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells [7, 8, and 9].

The microbial virulence of UPEC has been linked to many factors. The most prominent is type I fimbriae, which are filamentous bacterial appendages that are capped by *FimH*. Type 1 fimbriae promote tight bacterial binding to the matrix of uroplakin complexes on the surface of superficial bladder epithelial cells [10, 11]. Similarly, the presence of flagella enhances virulence by permitting the organism to migrate toward the urothelium against the flow of the urinary stream.

After binding, UPEC invades the uroepithelium where it may either establish a state of commensalism or cause a severe, symptomatic infection characterized by a rapid innate host response with cytokine secretion, recruitment of immune cells to the site of infection and successful elimination of bacteria, or progressive disease with acute tissue destruction [12].

Materials and Methods

Setting

All urine and blood sample collected from cases of clinical diabetic and non- diabetic patients who had urinary tract infections were admitted to diabetic and endocrine center of Al-Samawa city. A detailed clinical history, location and age. Two hundred patients and fourty control (urine sample) were collected from infected with UTIs patients that divided into two groups diabetic and non-diabetic group , age around (14-77) years older during the period extended from October 2017 to July 2018.

Culture

Urine samples collected from the two groups were grown in brain heart infusion broth for 24 hours to make the bacteria regenerated inside the broth then after incubation period the cultured bacteria have been sub cultured to (urinary tract infection chromogenic agar) for the presumptive detection and differentiation of organisms causing UTI. At the same time subcultured the growing bacteria on (*E.coli* Enterobacteria chromogenic agar) for the differentiation of *E.coli* from another bacteria that causing UTI, and for more identification of *E.coli* from another bacteria we sub culture into MacConkey agar where *E.coli* lactose fermenting, after that Strains biochemically confirmed as *E.coli* were kept in brain heart infusion agar. The biochemical tests were then applied according to the product instructions.

DNA Isolation

Bacterial genomic DNA of UPEC isolates were extracted by using (Quick Bacteria Genomic DNA Extraction Kit, Dongsheng biotech.China), and done according to manufacturer's instruction.

Detection of uro-virulence genes in *E. coli*

In this present study, multiplex PCR reactions were used for detection of some virulence factors of *E. coli* isolate from patients with urinary tract infection in Al-Muthanna province. Table 1 showed the primers used for detection of UPEC virulence genes and the PCR programs including cycle, time, temperature and volumes are shown in Table 2. The amplified products were visualized by ethidium bromide staining after gel electrophoresis of 6 µL of the final reaction mixture in 1 % agarose. The UPEC strains were confirmed by the gene including by detecting virulence genes by PCR method.

Table 1: Oligonucleotide Primers Sequence used for detection of virulence genes in UPEC strains

Gene	Primer name	Primer sequence (5'-3')	Size of product (bp)	References
HlyA	hly1 (F)	AACAAGGATAAGCACTGTTCTGGCT	1177	**(Yamamoto *et al.*, 1995) [13]**
	hly2 (R)	ACCATATAAGCGGTCATTCCCGTCA		
FimH	fim1 (F)	GAGAAGAGGTTTGATTTAACTTATTG	559	**(Struve and Krogfelt, 1999) [14]**
	fim2 (R)	AGAGCCGCTGTAGAACTGAGG		
Pap	pap3 (F)	GCAACAGCAACGCTGGTTGCATCAT	336	**(Yamamoto *et al.*, 1995) [13]**
	pap4 (R)	AGAGAGAGCCACTCTTATACGGACA		

Table 2: Cycling conditions for standard PCR

Step	Time	Temperature	Comment
Initial heat activation:	15 min	95°C	HotStarTaq DNA Polymerase activation.
3-step cycling:			
Denaturation	30 s	94°C	
Annealing	90 s	60°C	Used 60°C as the starting temperature. If the lowest T_m of your primer mixture is below 60°C, used 57°C as the starting
Extension	90 s	72°C	Optimal for targets up to 1.5 kb in length.
Number of cycles	30–45		
Final extension:	10 min	72°C	

Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2013 version1.0 for significant relationship between UPEC infection and patients' age, community type, in diabetes and non-diabetes patients.

Results

UPEC were isolated from eighty three patients (41.5 %) while the rest of the patients were negative (117, 58.5%). The present study showed positive cases in 41 (33.3%) of Diabetes patients group (DM), 42 (34.1%) of Non- Diabetes patients group (ND) and 40 (32.5%) of cure persons (control group). Duration of diabetes with UPEC was 14 to 29 years in 2 (4.9 %) patients, 30 to 45 years in 6 (14.6 %) patients, and 45 to 61 years in 23 (56.1%) and greater than 64 to 77 years in 10 (24.4 %) patients while the negative.

Results were shown in 59 (59%) patients. Group 2 (ND) was shown 12 (28.6%), 13 (31%), 15 (35.7%) and 2 (4.7%) positive cases of UPEC in different age group in this study (58, 58%) with negative findings of control group, Table (3).

Table 3: Distribution of UPEC isolates according to age groups

Age (year)	Diabetes			Non- Diabetes			*P value*	Control		
	Positive	Negative	%	Positive	Negative	%		Positive	Negative	%
14-29	2	6	4.9	12	23	28.6	0.003	0	20	0
30-45	6	15	12.2	13	25	25.5	0.094	0	11	0
46-61	23	31	30.3	15	7	19.7	0.132	0	5	0
62-77	10	7	45.4	2	3	9.1	0.007	0	4	0
Total	41	59	20.5	42	58	21	0.902	0	40	0

There were 21.9 % of diabetes male population infected with UPEC in this study higher than the females population that was 78.1 %.

On the other hand, the infected females in group 2 (Non- diabetes) have also a higher incidences of UPEC (97.6 %) compared to the male population (2.4%), Table (4).

Table 4: Distribution of UPEC isolates according to gender groups

Gender	Diabetes			Non-Diabetes			Control		
	Positive	Negative	%	Positive	Negative	%	Positive	Negative	%
Male	9	27	21.9	1	22	2.4	0	14	0
Female	32	32	78.1	41	36	97.6	0	26	0
Total	41	59	41	42	58	42	0	40	0

After PCR technique on positive urine culture for presence of UPEC, it was detected that *FimB* gene of fimbrial antigen with 78% in diabetes patients had higher results than of Non-diabetes patients (57%) with significant different ($p < 0.05$), in contrast to each *Pap* and *Hly A* genes detection of both diabetes and non-diabetes patients. The occurrence of virulence factors of diabetic UPEC in the present study for P pili *Pap* and alpha-hemolysis *Hly A* were (31.7%) and (12.2%) while those genes had been given (38.1%) and (7.1%) for non-diabetes patients respectively. The detection of virulence factors genes were variety according of gender and age parameters as shown in Table (5). Figures (1) showed positive molecular bands of virulence factors genes in the study.

Figure 1: Positive molecular bands of virulence factors genes (*FimH*/559bp, Pap/336bp and *Hly A*/1177pb)

Table 5: Prevalence of virulence genes in UPEC strains of present study

Virulence gene	Diabetes (%)	Non-Diabetes (%)	Gender (%) of MD & ND		Age (year) of MD & ND				
			Male	Female	14-29	30-45	46-61	62-77	%
FimB +	32 (78)	24 (57)	7 (8.4)	49 (59.1)	11	11	25	9	67.5
FimB -	9	18	3 (3.6)	24 (28.9)	3	8	13	3	32.5
Pap +	13 (31.7)	16 (38.1)	4 (4.8)	25 (30.1)	5	5	11	8	34.9
Pap -	28	26	6 (7.3)	48 (57.8)	9	14	27	4	65.1
Hly A +	5 (12.2)	3 (7.1)	1 (1.2)	7 (8.4)	1	2	3	2	9.6
Hly A -	36	39	9 (10.9)	66 (79.5)	13	17	35	10	90.4

Discussion

UPEC could be one of the main reasons of urinary tract infections and contribute to the development of complications in patients with diabetes [15]. In the present study the rate of *UPEC* isolation in the diabetic and non-diabetic females (78.1 and 97.6 % respectively) was higher than diabetic and non-diabetic male (21.9 and 2.4 % respectively).It has shown in several reports that women are at increased risk to infect UTI then men [16], and it is shown that UTI is mostly female disease due to short urethra [17, 18].

Observed that *E.coli* isolation rate in both males and females of diabetic patients were lower than of non-diabetic group [19]. Results of the present investigation showed the infection incidence rate of *UPEC* in diabetes (20.5 %) was not significant value than that found in non-diabetes (21%). This difference between those groups might be explained by the oldest age average of diabetes (46-61 year) is most likely to infect with *UPEC* than other age groups. Many studies worldwide have also reported an increase *UPEC* in the oldest age of diabetes patients [20]. Higher prevalence of virulent strains of *E. coli* in diabetic patients was concerned finding of our study especially that *fimB* genes of virulent strains with significant different ($p< 0.05$) compare with non-diabetes as well as the difference was obvious in comparison to the age and gender factors (table 3). Schwan, W. R. explained that type 1 pilus of the most important virulence factors involved in attachment mediating by a set of *fim* genes in an operon and this pili type controlled by phase variation phenomenon [21].

Otherwise, the present study shown that there was no significant difference between virulent strains of *UPEC* in detection of *Pap* or *Hly A* genes, in a study which was conducted in order to investigate of *UPEC* virulence factors in diabetes and non-diabetes patients [22], study explained that there was not much significant difference in distribution of virulence factors of UPEC causing UTI from diabetes and non-diabetes patients when *Pap* genes was most prevalent in both groups of patients, followed by *Hly A* gene[23]. In conclusion, our results provide evidence that older ages as well as females gender of diabetes more susceptible to infect with UPEC in urinary tracts which could associated with adhesion factors.

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