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Bacteriuria with Unusual Uropathogens in Diabetics in Thi-Qar Province

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***Abstract*— This study was designed to investigate the causative agents of recurrent urinary tract infection in diabetic patients in Thi-Qar Province . The study included 87 Iraqi participants of whom 46 diabetics and 41 non diabetics healthy control . Urine samples were collected from each participant and cultured on blood and MacConkey agars ,followed by macro and microscopic identification and biochemical tests. API 20E and Vitek 2 compact systems were used as a confirmatory tests . The findings showed ,of the total 87 participants 60(69%) gave a positive growth for urine culture. The results also showed thatT2DM patients were more susceptible to UTI with positive urine growth of 23/26 (88%) compared to T1DM patients who showed 14/20 (70%), and the majority of cases were female. Escherichia coli is the most common isolates accounting for the majority of cases 29 (48%), followed by Klebsiellapneumoniae9(15%) and *Acinetobacter radioresistens* 8(13.3%).It can be concluded that diabetic patients are more susceptible to UTI, especially T2DM. According to the present results, the immune disturbance due to diabetic patients is taking tall on T2DM patients even with asymptomatic bacteriuria.**

***Keywords*— Diabetes , Inflammation , Escherichia coli**

I. INTROUDUCTION

Urinary tract infection [UTI] is one of the most common infections in diabetic patients, predisposing these patients to significant morbidity and being one of the most common routes for sepsis (Hamdan *et al.*, 2015). UTIs are more common and severe in patients with diabetes. Immune system dysfunction, deprived metabolic control, and lacking bladder emptying due to autonomic neuropathy may all contribute to these patients' increased risk of urinary tract infections. The majority of UTI cases in diabetes are caused by Gram-negative bacteria from the patient's gut microbiota (Taher *et al.*, 2021). In these patients, the spectrum of UTI ranges from asymptomatic bacteriuria (ASB) to lower UTI (cystitis), pyelonephritis, and severe urosepsis (Ornan *et al.*, 2015). Serious UTI complications, such as emphysematous cystitis and pyelonephritis, renal abscesses, and renal papillary necrosis, are more common in type 2 diabetes patients than in the general population (Walsh and Collyns, 2017). Type 2 diabetes is a risk factor not only for community-acquired UTI but also for health-care-associated UTI, catheter-associated UTI (Öztürk and Murt, 2020) ,and post-renal transplant-recurrent UTI (Shams *et* a*l*., 2017).Furthermore, these patients are more likely to have UTI caused by resistant pathogens, such as extended-spectrum β-lactamase-positive Enterobacteriaceae, fluoroquinolone-resistant uropathogens, carbapenem-resistant Enterobacteriaceae, and vancomycin-resistant Enterococci(Aswani *et al*., 2014). The study aims to identification of the causative bacteria associated with UTI in diabetes and comparing them with non diabetics healthy groups.

II. METERALS AND METHODS

A . Design of Study

The study included 87 participants of whom 46 were diabetics of both types (20 and 26) for Type I and Type II respectively , along with non diabetics control group of both genders. The samples were collected between the period of October 21st to December 7th of 2021 from patients who attended the diabetes section in Al Nasseriya teaching hospital. The age range of participants were (8-85). Inclusion criteria; diabetics of either types .Exclusion criteria patients with underlying endocrine disease other than diabetes. In addition to 41non diabetics control group.

B. Methods

Urine samples were collected from each participants in sterile containers then transported to the laboratory for further analysis via ice box. The urine were processed routinely then cultured on blood and MacConkey agars. The positive growth samples were further analyzed by Macroscopic characteristics of the colonies and Microscopic examination by Gram's stain. Then the colonies were examined for their biochemical profile using routine biochemical tests (Oxidase , catalase , IMViC) and confirmatory tests (API 20E and Vitek2 compact system) for some isolates .

The statistical analysis of this study was performed with the statistical package for social sciences (SPSS) 20.0 and Microsoft Excel 2013. Numerical data with normal

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distribution were described as mean and standard deviation, independent sample t-test used for comparison between two groups. Categorical data were described as count and percentage. Statistically significant differences were determined at $p<0.05$.

C. Statistical Aanalysis

The statistical analysis of this study was performed with the statistical package for social sciences (SPSS) 20.0 and Microsoft Excel 2013. Numerical data with normal distribution were described as mean and standard deviation, chi square was used for categorical and data were described as count and percentage. Statistically significant differences were determined at $p<0.05$.

III. Result and Discussion

A total of 87 subjects were enrolled in this study, 46 (52.87 %) of them were patients diagnosed with the diabetes mellitus and 41(47.13%) were a healthy control group Figure (1). In diabetics patients the T2D patients were enrolled in the current study being the most prevalence with (56.52%) and(43.48 %) for T1D.This finding is consistent with the fact that the minority of cases of T1DM diabetes are presented by previous prevalence studies conducted worldwide (IDF, 2013).

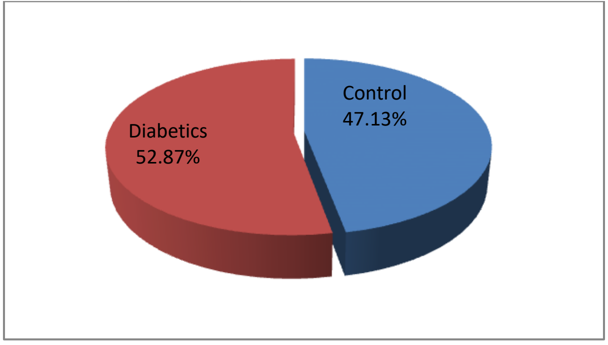


Figure (1): shows the percentage of diabetics and control subjects .

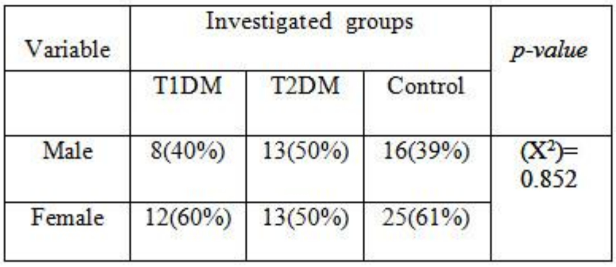
Age wise the study population was between the age of 8 and 85 Elderly individuals appear to be more susceptible to T2D. The maximum number of T2D patients was found within age group 41-60 years 17 (65.4%), T2DM patients age mean (54.4±10.1) , which in line with a local study by Mansour *et al.* (2020), that reported the mean age of type II diabetics being (51.4±3 years).The results seem to disagree with a study by Ajayi and Radhakrishnan (2016) in, the most common age group inflected with UTI is above 60 years of age rather than meddle aged population which the current study presents. While the maximum number of T1D patients was found within age group ≤20years 10 (50%).And, the age mean and standard deviation of T1DM (26.3±8.9 years), that in line with previous study by Goldberg *et al.* (2020) which concluded that the T1DM prevalence at a younger age (21.2±3 years). The Table(1) shows distributionof the diabetic patients and control cases within the age groups.

Table(1) : Age distribution of diabetic patients and healthy subjects**.**

Age groups	DM type I		DM type II		Healthy	
	No.	%	No.	%	No.	%
≤20	10	50	0	0	8	19.51
21-40	6	30	3	11.5	13	31.71
41-60	3	15	17	65.4	12	29.27
≥61	1	5	6	23.1	8	19.51
Total	20	100	26	100	41	100

Regarding to gender (male and female) distribution in both groups is listed in Table (2) . Forty percentage of the T1DM were males and 60% were females . However, for T2DM, the gender was distributed equally being thirteen (50%) samples each, Table (2). The results is compatible with Almahfoodh *et al.* (2017) .As they found the incidence of T1DM in females is higher than males, as it showed in the present study, in which the female to male ratio is 1.5:1. Also compatible with Mansour *et al.* (2014) in Iraq and apparently worldwide such as Kuwait (Mohammad *et al.*, 2021), Egypt (El-Ziny *et al.*, 2014) ,Thailand (Rittiphairoj et al., 2022) and Korea (Chae *et al.*, 2020).This might be due to the fact that female have more hormonal changes through their lives and a body fat than male and thus have tendency to develop an inflammatory and metabolic diseases as fat have major role in the production of inflammatory cytokine(Karastergiou *et al.*, 2012). Gender wise T2DM was equal accounting for 50% of each gender. Also Randomly selected control group was 47% of the total study population distributed into 39% male and 61% female.

Table (2): Gender distribution of patients and healthy



Variable	Investigated groups			*p-value*
	T1DM	T2DM	Control	
Male	8(40%)	13(50%)	16(39%)	(X^2)= 0.852
Female	12(60%)	13(50%)	25(61%)	

The role of bacterial infection in the diabetic mellitus were investigated . The results of urine culture showed that of the total 87 participants 60 (69%) showed a positive growth for urine culture Table(3) . Positive urine culture for T1DM at 14 (70%) , T2DM 23(88.5%) and for control group 56% of them showed a positive growth. Female gender was mere susceptible to UTI in all investigated groups.

Table(3): Study population with positive bacteriuria

Subject	Male	%	Female	%	Total	%
T1DM	5	35.71	9	64.29	14	23.33
T2DM	11	47.83	12	52.17	23	38.33
Control	9	39.13	14	60.87	23	38.33
Total	25	41.67	35	58.33	60	100

The results are compatible with a previous study by Al-Tulaibawi (2019) in Misan province, Iraq, which showed that diabetic females are more susceptible to UTI. Seemingly the same results were concluded by (Alhamdany, 2018) at Babylon province .Also the same results were reported by (Taher *et al*., 2021) at Al-Kut province.

The uropathogen isolates were as follow; *Escherichia coli* was the leading pathogen, which was isolated from all test groups: T1DM,T2DM and control group being 8/14 (57.14%) ,12/23 (52.17%) and 9/23 (39.13%) respectively, accounting for 29/60(48.33%)of the total positive isolates from the test population, followed by *Klebseilla pneumoniae* isolates from both T2DM and control group being 5/23(21.74%) and 4/23(17.39%) respectively accounting for 15% of the total isolates. *Acinetobacter radioresistens*, which is unusual uropathogen came in third, isolated from all participant groups 2/14(14.29%), 3/23(13.04%) and 3/23(13.04%) from T1DM ,T2DM and control group respectively, and accounting for 13.33% of the total positive isolates, Table (3) listed the other uropathogens isolated from cultured urine

The leading isolated uropathogen was *E. coli* which in line with previous study by Al-Tulaibawi (2019) in Misan province , which showed the supremacy of *E.coli* over the other causative agents in regards to urine samples. Furthermore, agrees with the same terms as Tektook and Al-Lehibi, (2017)at the specialized center For endocrinology and diabetes of Baghdad city, Iraq. Globally, Hamdan *et al*., (2015) in Sudan and Aswani *et al.* (2014) India. Also the findings regarding *E. coli* being the common isolated bacteria are also in agreement with Aamir *et al.* (2021) in which the *E. coli* reign supreme to the most common isolate, not only to symptomatic but also to asymptomatic UTI in Pakistani diabetics.

Yet it seems to disagree with (Abdullah, 2019) which describe *Staphyloccocusaureus* to be the top one isolate from UTI patients, rather than *E. coli*.

The second highest causative agent was *Klebsiella pneumoniae* with 15% of the total UTI specimens 80% female comparing to 20% male , as it presented by previous study by (Abdullah, 2019). *Acinetobacter radioresistens* , a non-fermentative Gram-negative coccobacilli that are ubiquitous in the environment and a rare agent of human disease that is frequently misidentified(Wang *et al*., 2019). The results showed the infection with *A. radioresistens* more common in male rather than female regarding UTI being (67% and 33%) for male and female respectively , Which somehow breaks the notion that females being more susceptible to UTI than males. However , there had been a scarcity of data reported on the role of *A. radioresistens* as a uropahtogen other than a case report study, of a combined bacteriuria of *A. radioresistens* and coagulase negative Staphyloccoci in 70 years old woman presented with bacteremia by *Alloscardoviaomnicolens* secondary to UTI in Japan by (Ogawa *et al*., 2016) and a study done by (Chaudhury, 2018) in which the isolated Acinetobacter species from different bodily fluid ,the urine comprised about (5.88%) of those (4.7 %) are *A. radioresistens*.

Least isolated bacteria only one isolate each, were *Pseudomonas aeruginosa*, *Escherichiavulneris* and *Rahnellaaquatilis*, the later being unusual uropathogen in Iraqi diabetics.*Rahnella* spp. is a member of the *Enterobacteriaceae* family that is facultative anaerobic that fix nitrogen. This genus was described back in 1976 and was in the beginning classified as an *Enterobacteriaceae* Group H based on numerical taxonomy. Then , the new genus was renamed *Rahnella* as a result of the development of molecular techniques. It has been isolated from fresh water and soil (Al-Bayatti *et al*., 2012).

The *Rahnell*a genus comprise three species: *R. aquatilis*, *R. genomospecies 2*, and *R. genomospecies 3*, which can only be distinguished using molecular techniques (Martins *et al*., 2015). As it been described by (Al-Bayatti *et al*., 2012) ,that drinking water of Tigris river source may contaminated by *Rahnella aquaitilis* and possible transferred to human by using the water source. Interestingly , less commonly encountered pathogens were isolated such as *Pantoea* spp2. and *Pseudomonas oryzihabitans* from relatively young participants at (6.67%) and (1.67%) respectively of the total isolates.

Pantoea bacterium is a gram-negative rod that is widely distributed in nature and has been isolated from a variety of ecological niches, including plants, water, soil, insects, and animals, but it is considered a rare opportunistic pathogen to humans.

Following that, *Pantoea*spp. isolates from clinical sources have increased, causing bloodstream infections, urinary tract infections, osteomyelitis, eye infections, and meningitis (Cruz *et al*., 2007; Gajdács, 2019). The current study is in line with a recent study conducted by Fazaa and Darwish (2020) in AL-Qadisyiah province /Iraq , in which isolation of *Pantoea*spp. was about (6.9%). Also the results showed *Pantoea* spp. may target the younger ages and this finding agree with Büyükcam *et al.* (2018) .

Given the fact that the control population was chosen for being healthy (not diabetic ,no endocrine diseases and no symptoms of UTI), the presence of bacteriuria only emphasize the fact that the total number of UTI cases are hugely underreported for being asymptomatic in nature. Of the total study population about 38.33% were healthy control group with bacteriuria, more than a half of control participants (56%) showed asymptomatic UTI, with female accounting for the majority of cases . These result are in total agreement with (Ali and Neamah, 2021) who surveyed the prevalence of asymptomatic bacteriuria in patients on hemodialysis which reported the females have an higher percentage of asymptomatic bacteriuria 56.7% . Also the present study results agrees at the same terms with Raz (2003) and Taher *et al*. (2021) regarding the prevalence of asymptomatic UTI along with fact that female are more vulnerable to such infection due hormonal changes and the proximity of the urethra to the anus (Abou Heidar *et al*., 2019).

Table(4): The results of cultured urine in investigated groups

Culture results	Isolated numbers			Total	%
	T1DM	T2DM	Healthy		
E. coli	8	12	9	29	33.3
K.pneumoniae	0	5	4	9	10.35
A.radioresistens	2	3	3	8	9.2
Pantoea spp	0	0	4	4	4.6
Enterobacteraerogenes	0	1	2	3	3.5
P. mirabilis	2	0	0	2	2.3
R. aquatilis	1	0	0	1	1.15
P.aeruginosa	1	0	0	1	1.15
E. vulneris	0	1	0	1	1.15
A. shydrophilia	0	1	0	1	1.15
P. oryzihabtans	0	0	1	1	1.15
*NG**	6	3	18	27	31
Total	20	26	41	87	100

IV. CONCLUTION

In accordance with these findings, the study concluded that diabetic patients are more likely to develop UTIs with a variety of pathogens including rare and uncommon opportunistic pathogens. This conclusion supports of the fact that diabetes alters the normal host system which may be potential causes of urinary tract development such as increased adhesion of microorganisms to urothelial cells and granulosa cell dysfunction, or as a result of abnormal intracellular calcium metabolism

V. ACKNOWLEDGMENT

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VI. ETHICAL CONSIDERATION

An official permission was granted form the Hospital officials and verbal and written consent were received by the participants enrolled in this study

VII. CONFLICT OF INTEREST

The authors declare no conflicts of interest

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