THE FREQUENCY OF *CORYNEBACTERIUM UREALYTICUM* IN PATIENTS WITH RENAL STONES

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Abstract- Urinary stones are prevalent and *Corynebacterium urealyticum*, a urease-positive bacteria, is a frequent urinary tract pathogen. However, the association of this organism with urinary stones is not sufficiently studied in Iran. This study was carried out to determine the prevalence of C. *urealyticum* in patients with urinary stones referred to the urology sections of Tehran University of Medical Sciences. This study was performed using case-control method. A total of 150 urine samples from patients with urinary stones and the same number from the control group without urinary stones or infection were collected. The two sets of samples were cultured and biochemically identified. The pH of urine samples also measured with pH paper. *C. urealyticum* was detected in 11.34% of cases which was significantly higher than that in control group ($P < 0.01$). The frequency of alkaline urine was four times more in the patients than those in control group which was statistically significant ($P< 0.01$). According to the results, *C. urealyticum*, which is known to cause alkaline urine, might enhance formation of stones. Further studies using cohort or experimental methods are recommended.

Acta Medica Iranica, 42(4): 285-288; 2004

Key words: Urinary infectious stones, urease positive bacteria, *Corynebacterium urealyticum.*

INTRODUCTION

One of the most important and common problems of urinary system is the formation of renal stones. Identification of renal stones has a history of 7000 years. Factors like nutrition, environmental conditions, sex, genetics and presence of urease positive bacteria has been reported to play a role in formation of renal stones.

Corynebacterium urealyticum, a urease positive bacteria (previously named group D2), through decomposition of urea and production of ammonium hydroxide can result in an increase in urine pH (1-4). This increase not only has a toxic effect on the renal epithelium but also predispose to supersaturation of ammonium-magnesium phosphate and apatite carbonate (1). It, therefore, leads to crystallization and deposition of crystals and ultimately to production of renal stones (1). Ammonium magnesium phosphate (struvite) stones work as nuclei for colonization of bacteria (5), causing more serious infections, increased incidence of pyelonephritis, alkaline incrusted cystitis and recurrent urinary tract infections (6,7). Taking into consideration the resistance of C. *urealyticum* to many antibiotics, elevation of urine pH will produce unfavorable conditions that may reduce the effect of antibiotics (8).

Several studies have been done concerning the pathogenicity of urease-positive bacteria, including

Received: 30 Dec. 2001, Revised: 19 Jan. 2004, Accepted: 16 May 2004

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C. *urealyticum*, and their role in production of urinary tract infections and formation of renal stones. Among them is a study of 82 patients infected with C. *urealyticum* which showed that 72% of them had alkaline urine (9). The presence of a calculus along with C. *urealyticum* infection has been reported in a transplanted kidney (10). The infection was treated successfully with vancomycine after the removal of the stone. Another study in which urease-positive bacteria were inoculated into the urinary bladder of a mouse demonstrated an increase in urine pH. A renal stone was also noticed in the sacrified animal (11). In another study, the stone removed from a renal allograft was of the struvite type, and the isolated bacteria were urease-positive (12). The patient had recurrent infection with *Proteus mirabilis* before the formation of stone. In a prospective study for investigation of the relationship between UTI and stone formation, 270 children were followed for 27 years (13). The study showed that infection caused by urease-positive bacteria provide an environment predisposing to stone formation.

In the present case-control study we attempted to determine the frequency of C. *urealyticum* in patients with renal stones.

MATERIALS AND METHODS

In this study, urine samples were obtained from two groups of individuals: first group included 150 patients with renal stones documented by clinical and radiological signs, attending the urology departments of two general hospital in Tehran. The second group comprised of 150 subjects free of urinary tract infection or any signs of renal stone or renal abnormality. They were selected to be relevant to the patient's group in sex and age. Informed consent was obtained from all patients.

Mid stream clean-catch urine samples were collected. For this purpose, the patients were first instructed how to collect the specimen correctly. The urine samples were transferred to the laboratory in sterile screw-capped glass bottles for culture. One milliliter of each sample was inoculated on blood agar plate using a sterile syringe, and incubated for 48 hours at 37ºC. The isolated colonies were subcultured on blood agar plate. The original plates were kept in the incubator for an additional 24 hours for more growth. *C. urealyticum* cannot grow on ordinary media and needs more than 24 hours for growth. Detectable growth is usually noticed after 48 hours and occasionally after 72 h. C. *urealyticum* is a gram positive bacillus, produces pinpoint, gray smooth convex non-hemolytic colonies. The isolated colonies in pure cultures were identified by gram staining and biochemical testing, *i.e.* catalase test, urea hydrolysis and sugar fermentation (14). The pH of urine was measured by the use of urine filter-paper strips.

RESULTS

Of the 150 patients in group one, 105 (70%) were males and 45 (30%) were females. The lowest and highest ages were 23 and 72, respectively. Overall, *C. urealyticum* was isolated from 20 subjects: 17 (11.4%) from patients and 3 (2%) from the control group. Of the 17 strains of C. *urealyticum* isolated from the patient group, 13 strains were isolated from alkaline urine and 4 strains from acidic or neutral urine samples. Only 3 strains were isolated from the control group: 2 strains from alkaline urine and 1 strain from acidic urine. The frequency of C. *urealyticum* in both patient and control groups according to urine pH is demonstrated in table 1.

The results also showed that 34 patients and 8 controls had alkaline urine. The Frequency of urine samples with different pH in case and control groups is shown in table 2. Alkaline urine was significantly more frequent in patients with renal stones ($P< 0.01$).

Table1. Frequency of *C. urealyticum* in both case and control groups according to urine pH*

Urine pH	Patients with CU	Controls with CU	Total
5-6	1	1	2
6.1-7	3	0	3
7.1-8	5	1	6
8.1-9	8	1	9
Total	17	3	20

Abbreviation: CU, *Corynebacterium urealyticum.*

*Data are given as number.

Table 2. Frequency of urine samples with different pH in case and control groups *

Group	Urine pH				Total
	5-6	6.1-7	7.1-8	8.1-9	
Case	84(56)	32(21.3)	21(14)	13(8.7)	150(100)
Control	117(78)	25(16.6)	5(34.3)	3(2)	150(100)

*Data are given as number (percent).

DISCUSSION

Approximately 15% of urinary stones are infection stones (15). They form rapidly and have frequent recurrences (16). Chemical analysis of 100 calculi from 100 patients performed by Sohshang *et al.* showed that about 47% of the cases had positive urine culture. The stones were composed mainly of calcium oxalate and/or phosphate followed by struvite, and mixed stones (17). The urease positive urinary tract infection is found to be a precondition for the formation of infected stones (15), although urease-negative bacteria may also be involved in renal stone formation (18). However, a study showed that a urease-negative mutant of *P. mirabilis* is unable to initiate stone formation (19).

The increase in urine pH, which occurs in the presence of some bacteria including *C. urealyticum* is among factors predisposing infectious stone formation (2, 3). It is shown that urease is necessary to split urea to ammonia and CO_2, leading to formation of ammonia ions and development of alkaline urine both of which are preconditions for the formation of struvite and carbonate apatite crystals (15). In literature, there is evidence of association between *C. urealyticum* infection of urine and stone formation. A study on 82 patients infected with *C. urealyticum* showed that 72% of them had alkaline urine (9). In another study, Diamond *et al.* followed up 270 children for 27 years and found that infection caused by urease-positive bacteria provided an environment predisposing to stone formation.

Since 1% of healthy individuals harbor *C. urealyticum* in the distal portion of urethra, and due to the presence of pilli, which enable the bacteria to adhere firmly to uroepithelial cells, the urine may become contaminated when passed out. Therefore, the presence of these bacteria even in acidic or neutral urine is possible. This has been proved by the presence of some strains of *C.urealyticum* in both patient and control groups. In order to get rid of this biasing factor, it is recommended to collect the urine samples with bladder puncture. However, this method is not usually used for many reasons.

In the present study, the pH of some urine samples of both groups was alkaline in the absence of *C. urealyticum*. This may be due to the presence of other urease-positive bacteria, *i.e. Proteus mirabilis*, *Klebsiella pneumonia*, or other organisms, among which *Proteus* plays the most important role in increasing the urine pH.

In conclusion, the present study confirmed the important role of *C. urealyticum* in increasing the pH of urine, and its possible role in formation of infectious renal (struvite) stones. According to these results, we recommend that urine samples of patients with renal stones be routinely tested for the presence of *C. urealyticum*, with prolonged culture on selective medium (20). If the culture is negative, PCR method is recommended (21, 22). Vaccination against *Proteus mirabilis* infection to prevent colonization and urolithiasis has been recommended by Li and Mobley (19). More investigation is necessary to make recommendations about prevention of *C. urealyticum*.

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