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Association of *Oxalobacter Formigenes* with the Stools of Urinary Tract Stone Patients in Basrah Province

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ABSTRACT

One hundred stool samples were obtained, fifty (30 from male and 20 from female) from patients with urinary stone disease and the other fifty from healthy individuals (38 from male and 12 from female) as a control. The incidence of urolithiasis was more in males (60%), kidney (74%), ureter (24%) and bladder (2%). Calcium-oxalate stone was higher (74%) than non-oxalate stone. Specific *oxc* gene for *Oxalobacter formigenes* was detected in 96 % of healthy samples comparing with patients. Between stone types, Calcium- oxalate was 74% in patients which have only 24.3% *oxc* comparison with 75.7% negative, while non - calcium oxalate (26%) was in patients with 69.2% of *oxc*. The last results showing the positive role of *Oxalobacter formigenes* to reduce the calcium-oxalate stone formation in Basrah province.

Keywords: *Oxalobacter Formigenes*, Stone, Basrah, Urinary

I. INTRODUCTION

Urinary stones are important health problems in a wide parts of the world, globally 5–15% of the population affected by this disease (1). Urine microscopy is an indispensable part of urinalysis provides a valuable information about the urinary tract infections and urinary stones, sometimes, the type of crystalline provides primary information on the type of the stone (2).

The epidemiology of urolithiasis differs according to the geographical area, age, sex, anatomical location and stone components (3,4). Urinary stone is a hardened deposit of dissolved minerals and salts in urine that form within the urinary tract, thus when the normal crystallization conditions of urine and high concentrations of body excretions may not dissolve completely, the crystals may aggregate and precipitate to form urinary stones (5). Chemically, there are several types of urinary stones such as calcium, uric acid, magnesium-ammonium phosphate and cysteine stones (6). Several studies have reported that most urinary stones are primarily composed of calcium oxalate (2,7,8). The highest concentration of urinary oxalate is an important risk factor for calcium oxalate stone formation (9). Oxalate is a highly oxidized and harmful compound that is synthesized by a large number of plants and microorganisms (10). In the case of human lacking the enzymes needed to regulate the oxalate levels in the body, oxalate homeostasis is maintained by microflora in the gastrointestinal tract (11).

Oxalobacter formigenes is colonized the large intestine of many vertebrates including humans (12). This bacterium uses oxalate as an exclusive source for getting carbon and energy (13). Having two essential enzymes to oxalate degradation: The first is formyl–CoA transferase (*frc*) activating an oxalate to oxalyl–CoA (14, 15), and the second is oxalyl-CoA decarboxylase (*oxc*) which decarboxylates the oxalyl-CoA to formyl-CoA (16,17). Several studies reported that the incidence of calcium oxalate urinary stones has been shown to increase with the absence of intestinal oxalate degrading *Oxalobacter formigenes* (8,12,18, 19, 20). The absence of *O. formigenes* from intestine microflora permits more absorption of dietary oxalate in the colon leading to increase the oxalate excretion in the urine, a high concentration of urinary oxalate is called hyperoxaluria

leading to calcium oxalate urinary stone formation (8,20,21).

In the last decade, many individuals of different sex and ages suffered of urinary stone disease were reported as inpatients and outpatients of Basrah province. Thus, this study was to find the frequency of urinary stone according to some factors. Moreover, the role of *Oxalobacter formigenes* to reduce stone formation among this sample of a population.

II. METHODS AND MATERIAL

A. Sample Collection

One hundred stool samples were obtained from volunteers between 20-60 years old during October to December in 2014 from the Urological Lithotripsy Unit / Al-Basrah General Hospital in Al-Basrah province. Fifty samples (30 from male and 20 from female) from patients with urinary stone disease and fifty from healthy individuals (38 from male and 12 from female) as a control were collected. All stool samples were accumulated by sterilized containers (Dollphi) by wooden sticks, additionally, fifty urine samples were collected from the same patients of urinary stone disease to detect the type of crystals (22). Questionnaires had completed covering the information pertaining to sex, age and the anatomical location of the stone.

B. Microscopical Examination of Urine Samples

Urine sediment was performed by centrifuging to determine the type of crystals using a microscopical examination (23).

C. Preparation and Extraction of DNA from Stool Samples

Preparation of stool samples for bacterial DNA extraction was described by Staccy-Phipps *et al*., (24). Approximately 100 mg of stool was suspended in a plain tube containing 1.5 ml of PBS and centrifuged at 2000-2500 rpm for 3 minutes to remove debris. The supernatant was transported by a micropipette to 1.5 ml of eppendroff tube and centrifuged at 13000 rpm for 5 minutes to precipitate the bacterial as a pellet.

Extraction of genomic DNA from a bacterial pellet was performed by Exi prep ^{TM} plus Bacteria Genomic DNA Kit (BIONEER, USA) using Automated Nucleic Acid Extraction System (BIONEER, USA), then DNA was detected by gel of 1% agarose containing 1% ethidium bromide and electrophoresed at 60 volt for 1.5 hour.

D. PCR for *oxc*

PCR primers OxF-forward 5'-AATGTAGAGTTGACTGA-3' and OxF-reverse 5'-AATGTAGAGTTGACTGA-3' (15) was used to amplify *oxc* gene in eppendroff tube (20 µl) mixture (BIONEER, USA) consisting of 5 µl mastermix, 10 pmol primer (1.5 µl) for each, 1.5 µl DNA template and 10.5 µl nuclease free water. PCR program was 94Cº for 1min, 35 cycles of 94Cº denaturation for 1min, 53Cº annealing for 1min, 72Cº extension for 1min, and 72Cº for 5min.

The *oxc* gene bands of 416 bp were observed by adding 5µl of PCR product in 2% agarose gel with 1% ethidium bromide and electrophoresed after adding 5µl of 100bp DNA ladder (BIONEER, USA) then photographed by Samsung camera.

E. Statistical Analysis

Statistical analysis was accomplished by Chi-square test by Statistical Package for Social Sciences (SPSS).

III. RESULT AND DISCUSSION

Results

A. Frequency of some factors in patients with urinary stones

Table (1) showed the frequency of urinary stones was more in males (60%) with a significant difference at $P<0.05$. Anatomical location of urinary stone showing high in kidney (74%) comparing with ureter and bladder with high signification ($P<0.01$). Furthermore, calcium–oxalate stone was higher (74%) than other stone types at $P<0.01$. While the occurrence of urinary stones was 54% in the age group 40-60 year.

Table (1): Frequency of some factors in patients with urinary stone disease

Gender n (%)		Age (Year) n (%)		Site of stone n(%)			Type of stone n(%)	
*Male	Female	20-39	40-60	**Kidney	Ureter	Bladder	**Ca-oxalate	Non-oxalate
30(60)	20(40)	23(46)	27(54)	37(74)	12(24)	1(2)	37(74)	13(26)

*: P<0.05, **: P<0.01

B. *oxc* gene of *Oxalobacter formigenes*

Oxalobacter formigenes was denoted when the *oxc* gene band appeared on the agarose gel at the position 416bp (figures 1 to 3). Table (2) showed 48 (96%) of healthy samples were positive for *Oxalobacter formigenes* with high significant differences (P<0.01) comparing with only 18 (36%) from patients of urinary stones. Nevertheless, in patients, the *oxc* gene was absent in 32 (64%) cases with significant differences (P<0.05). Between the types of urinary stones, calcium-oxalate was 37 (74%) than that of non-calcium oxalate stones 13 (26%) with high significant differences (P<0.01). Only 9 (24.3%) of calcium-oxalate stone samples were positive for *Oxalobacter formigenes* than negative 28 (75.7%) with high significant differences (P<0.01) while 9 (69.2%) from 13 stool samples with non-calcium stones were positive versus 4 (30.8%) negative with high significant differences (P<0.01).

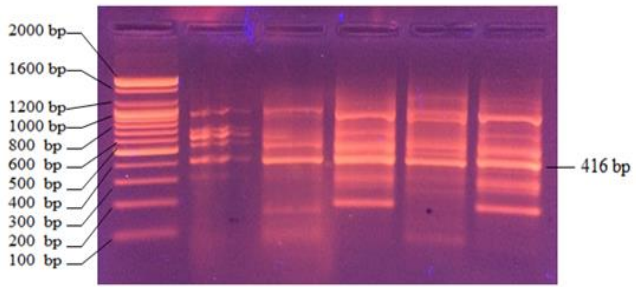

Figure 1: Gel electrophoresis of *oxc* gene of *O. formigenes* from stool samples of healthy volunteers. Lane M: 100bp DNA ladder, all lanes from 1-5 were positive for *O. formigenes*.

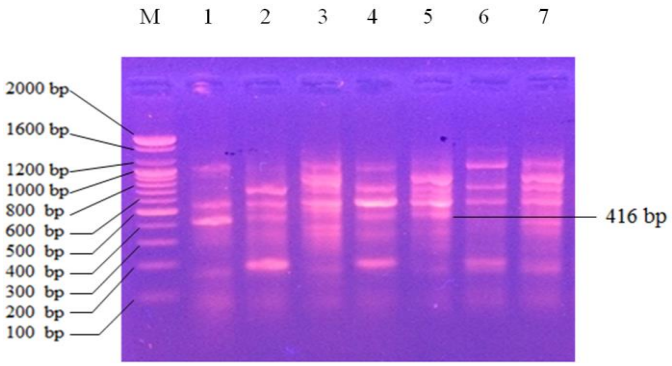

Figure 2: Gel electrophoresis of *oxc* gene of *O. formigenes* from stool samples of patients with non-calcium oxalate urinary stone. Lane M: 100 bp DNA ladder. Lane 1, 2, 4 and 5 were positive for *O. formigenes* while Lane 3, 6 and 7 were negative.

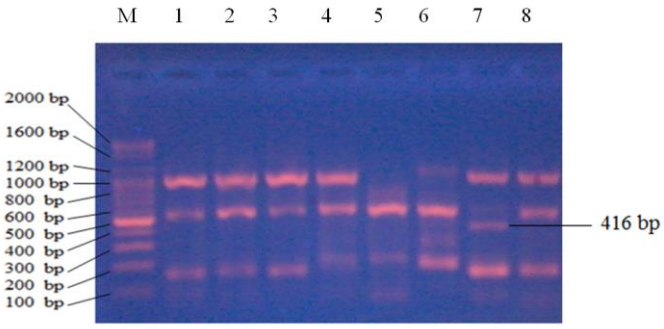

Figure 3: Gel electrophoresis of *oxc* gene of *O. formigenes* from stool samples of patients with calcium oxalate urinary stone. Lane M: 100bp DNA ladder. Lane 7 was positive for *O. formigenes* while Lane 1, 2, 3, 4, 5, 6 and 8 were negative.

Table 2: Frequency of *oxc* gene of *Oxalobacter formigenes* in stool samples among healthy volunteers and patients with urinary stones

*Frequency of *oxc* gene in healthy volunteers n (%)		Frequency of *oxc* gene in patients with urinary stones n (%)			
Yes * 48 (96)	No 2 (4)	Yes 18(36)		No ** 32 (64)	
		Calcium oxalate stone 37 (74)		Non-calcium oxalate stone 13 (26)	
		Yes 9 (24.3)	No * 28 (75.7)	Yes * 9 (69.2)	No 4 (30.8)

*: P<0.01, **: P<0.05

Discussion

The incidence of urinary stone was higher in males (60%) which agreement with several studies. Since, in male to female ratio was from 1.15:1 in Iran, 1.5:1 in USA, 1.6:1 in Thailand, 5:1 in Saudi Arabia and in Iraq was from 2:1 to 4:1 (25, 26,27,28,29) respectively. Urolithiasis is more occurrence in older people (40-60 years) than the young. However, the age effecting is not clearly understood (3,7), but it could be due to the type of multi-nutrition and digestion among these age groups. On the other hand, the majority of urinary stones were common in the kidneys and primirly composed of calcium oxalate, which may related to the low power of the urine stream in kidney inducing the precipitation of stones. However, these results are in agreement with those reported by others (4, 7,30). All these factors indicate the important role of nutrition education either by the amount of water drinking in the day or by the type of foods. Human lacks the enzymes to metabolize oxalate, so they depend on micro-intestinal flora to manipulate oxalate homeostasis (11). *O. formigenes* is very difficult for culturing because is a very fastidious, present in a very small amounts of stool and its required to special obligate anaerobic condition for culturing (12). Therefore, molecular identification considered as the best technique to detect this bacterium (15).

O. formigenes expresses a unique gene required for catabolising oxalate (oxc, encoding oxalyl coenzyme A decarboxylase), this gene has been cloned and sequenced (17). Sequencing of the 5′ ends of *oxc* of *O. formigenes* was identified with unique and highly conserved regions, allowing for synthesis a specific PCR primer pair (18). The amplification of whole stool DNA with this specific primer pair provides a rapid diagnostic tool to detect *O. formigenes*.

In the present study of Basrah province, *O. formigenes* was detected in healthy volunteers more than in patients with urinary stone, this result is in agreement with other studies (21,31). This finding showed that the detection rate of *O. formigenes* in patients with calcium-oxalate stone was lower than with non-oxalate stone. Furthermore, 64% of patients with non-oxalate stone were positive for *O. formigenes*. All the above findings have emphasized the role of *O. formigenes* to reduce oxalate in healthy volunteers and supported the presence of *O. formigenes* plays an important role in reducing the risk of calcium–oxalate stones. Nevertheless, the gel appeared the presence of many bands beside the specific *oxc* (416bp) band in the same specimen, this may be due to the stool samples containing a large amount of extracted DNAs for different bacterial species, leading to bind primers with different incorrect sites of DNA. This multi-bands view is similar with Kawak *et al*., (21), bands view (Figure 4) who showed variable amplification patterns in the same sample, but all samples showed a dominant PCR band to the specific gene size.

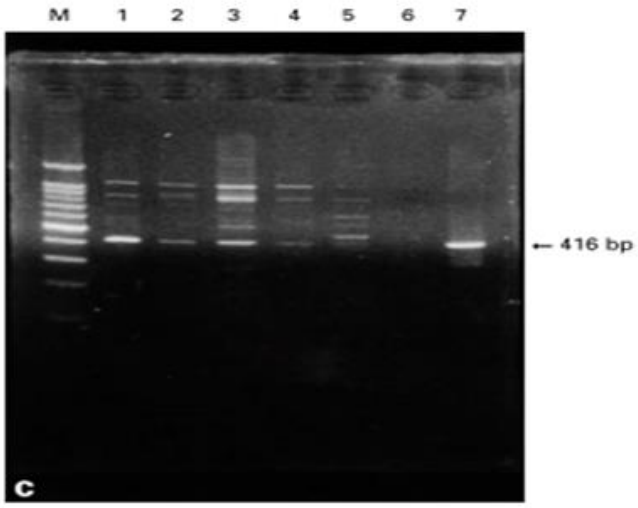

Figure 4: Specific *oxe* gene band beside variable amplification patterns in each sample (21)

In conclusion, the main frequency of the stone in Basrah province was appeared in male, kidney and calcium-oxalate was the predominant type of stone. *O. formigenes* was prevalent in healthy volunteers and in patients with non-oxalate urinary stones, which indicate the main role of *O. formigenes* to reduce the calcium-oxalate stone forming.

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