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Original Research Article

Bacteriological evaluation of bladder calculi: a study

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ABSTRACT

Background: Despite modern antibiotic therapy and technological advances in lithotripsy, the presence of infection in patients with urinary stones, as well as with infectious stones is still a significant cause of morbidity and mortality. Recent findings lend more theories as to how infection leads to stone formation.
Methods: Bacteriological study was conducted on pre-operative urine and operated bladder stones. Pre-operative urine samples were collected aseptically for macroscopic and microscopic examination. Both pre-operative urine and operated renal stones were processed for bacteriological culture. The isolated microorganisms were identified by standard techniques.
Results: Urinary tract infection was present in 27.88% cases. Majority of cases urine culture was positive (12.5%). *E. coli* was the commonest organism pseudomonas aerogenosa 3.84% *klebsiella aureogenosa* 1.92%, *staphylococcus aureus* and *proteus* 0.96% present in and mixed organism was found in 3.84% cases.
Conclusions: This study revealed the following aspect: The incidence of urinary stone was higher in patient having predisposing factor: recurrent UTI, Urinary Stasis, Inadequent water intake, anatomical abnormality in urinary tract. The prepoderent microorganism found in stone and urine is *E coli*, followed by *Pseudomonas aeruginosa*, *Staphylococcus aureus, Proteus*.

Keywords: Bladder calculus, *E-coli*, Stone bacteria, Stone core culture

INTRODUCTION

Renal stone disease has been recognized in many parts of the world since antiquity. It is one of the most painful and most common urological disorders.[1] Bladder calculi account for 5% of urinary calculi usually occur because of stricture, obstruction, recurrent UTI, stasis, inadequate intake of water, catheterization. Uncorrected anatomical abnormalities.[2]

Among all the site in human body urinary tract form the most frequent site for stone formation usually form when when concentrated urine reside long in bladder.[3] As urine stagnantes mineral in urine form various crystal that might be cause for vesical calculus. One in ten individual will experience a urinary stone, bacteria have long been recognized to contribute urinary stone, more common calcium oxalate and calcium phosphate. however high incidence of UTI is possible cause of culture positive urinary stones indicate its possible association. Bacteria can be culture from stone themselves.[4] *E-coli* and *pseudomonas spp*.

Were the most common bacteria isolated from stone culture followed by the urease-splitting bacteria typically involved in struvite stone formation. Therefore, the present study was undertaken to evaluate bacteriological spectrum of renal stones and culture of their pre-operative urines. Aim and objective was to study the bacteria in the bladder calculus.

METHODS

The present study was conducted on 50 patients of bladder stones admitted in the Urology and Surgical departments of RKDF Hospital during the time period

between September 2017 to March 2018 for management of bladder stones. Bacteriological study was conducted on pre-operative urine and operated bladder stones.

Screening

Pre-operative urine samples were collected aseptically for macroscopic and microscopic examination. UTI has been screened by semi quantitative method which could be the cause of bladder stones.[5] Both pre-operative urine and operated renal stones were processed for bacteriological culture. From all 50 patients diagnosed by the urologist as having urinary stones were included in this study. Informed consent was obtained from all participants. There were no refusals to participate. Patients were examined by a physician; those with lower urinary tract stone disease, renal stone disease with renal failure, renal tumors and previous history of renal stones were excluded.

Procedure

Processing of stones for bacteriological culture was done as described by Ohkawa et al.[6] The renal stones were thoroughly rinsed in sterile physiological saline and then crushed with a sterile hack-saw.[7] The crushed stone core was cultured in 5ml thioglycolate broth which was incubated at 37°C for 18-24 hours and then subcultures were made on blood agar and MacConkey's agar plate for isolation of etiological agents.[8] The isolated organisms then subculture on nutrient agar and plate were incubated at 37°C overnight. The typical colonies were identified as lacose fermenting and non-lactose fermenting then stains were characterized as *E coli*, *staphylococci aureus*, *Klebsiella aerogens*, *Pseudomonas* by standard conventional techniques and biochemical tests.[9]

RESULTS

Incidence of vesical calculus was 1.01% out of total surgical admissions and out of total genitourinary system disease cases. Males predominated the females with male to female ratio 9:1. Majority of cases were from low socio-economic income group. Majority of cases reaction of urine was acidic (79.80%) and in (16.34%) it was alkaline. Urinary tract infection was present in 50.50% cases.

Majority of cases urine culture was positive (22.36%). *E. coli* was the commonest organism. *pseudomonas aerogenosa* 2.36% *Klebsiella aureogenosa* 1.92%, *staphylococcus aureous* and *proteus* 5.26% present in and mixed organism was found in 3.84% cases. *E. coli* is the predominant organism found both in urine and core culture of stone. Incidence of vesical calculus is progressively decreasing in urban region because of improved diet, nutrition, infection control and modification of life style. It is evident that the majority of cases of vesical calculus were non vegetarian.

However, the reported results may under-represent the bacteria in urinary stones because the standard urine culture protocols used by clinical microbiologists are designed to identify clinical infections by known uropathogens. Bacteria and Urinary Stone Disease (USD) are clinically associated because they often occur in the same patients and USD patients often have positive urine and/or stone cultures. Whether they are mechanistically associated is an emerging research topic. Selective aggregation to some crystal types seen in USD patients, increased crystal clumping in the presence of bacteria, bacteria-induced lower urine citrate levels and increased CaOx (calcium oxalate) deposits and stone matrix protein expression when bacteria are present as opposed to CaOx deposits alone are initial findings that might provide the basis to begin to unravel the reasons for the clinical USD-bacteria associations. Important future directions will include: sequencing a larger number of stones to determine if the bacteria present are consistent between stone composition and other covariates such as sex and age a devising model systems to test mechanistic overlap between USD and the bacteria isolated from urinary stones.

DISCUSSION

It appears that the bacteriological testing of urine samples does not always reflect the bacteriology of urinary tract stones, which is in agreement with the results of previous studies.[10] This might be due to an intermittent release of a small number of microorganisms from the stone, which may or may not be isolated from urine.[11] The explanation for the presence of bacteria within the calculi may be due to insignificant intermittent bacteriemia, from where the bacteria are excreted in renal pelvis and may act as a nidus for deposition of crystals either by damaging the mucous coat or perhaps also by acting as a nidus for crystallization of salts.[12] Thus, a vicious cycle starts, the infection leading to stone formation and then the stone causing infection.[13] Most of the current literature on the subject focuses on pathogenesis of infectious urinary stones.[13] Griffith et al, showed that bacterial urease is a primary cause of infection stones.[14] The remaining literature highlights difficult cases, outcomes of treatments, and overall reviews of the subject.[15] Further investigation is critically needed to improve the outcomes of patients suffering from infections with urinary stones and infectious stones.

The bacteriological study of urine and stone samples revealed that commonest pathogens were *E.coli*, *Pseudomonas aeruginosa*, *Enterobacter spp*. and *Proteus spp. E.coli* is not a urease producing organism and is not considered to be a stone producing micro-organism. However, the present study revealed that *E. coli* was predominant microorganism recovered from mixed stones (calcium oxalate, triple phosphate and calcium phosphate). The present findings are consistent with the study of Dajani and Bratell et al.[16] For many patients, clues to the stone formation are obtained with an

extensive search for risk factors. Such an outcome most certainly reflects our incomplete understanding of the stone formation or the way we usually collect and analyze urine.[17] Despite the obvious shortcomings, it is important to reveal a correlation between the various risk factors by a careful medial history with a radiographic examination as well as an analysis of stone, blood and urine composition and an effective individualized treatment.

Table 1: Incidence of bladder calculus.

Author	Year	Place study	Incidence of vesica calculas %
Kabra SG	1972	Rajasthan	3.4%
Fazil YM	1977	Kerala	0.88%
Kumar R	1980	Aligarh	3.66%
Mcloid RS	1992	Leeds area	3.8%
Singh S	1992	M.P.	2.78%
Shakya GR	1996	M.P.	1.64%
Tiwari	2000	M.P.	1.55%
Singh MP	2003	M.P.	1.23%
Manjhi G	2008	M.P.	1.54%
Present study	2018	M.P.	1.01%

Table 2: Common bacteria found in urine culture.

Authors	Year	No. of culture	Urine culture common bacteri	%
Agrawal SL	1972		*E. coli*	62.79
			Klebsiella	9.30
Kumar R	1980	250	*E. coli*	37.05
			Pr. Vulgaris	15.05
			K. Aerogenes	15.05
Kevin A et al	1984	83	*Staphylococcus*	24.09
			E. coli	21.68
Ohawa M et al	1992	-	*E. coli*	21.60
			Klebsiella	4.00
			Proteus	11.55
Singh S	1992	30	*E. coli*	40.0
			Klebsiella	13.33
			Proteus	6.66
Shakya GR	1996	97	*E. coli*	38.14
			Klebsiella	4.12
			Staphylococcus	7.21
Tiwari et al	2000	26	*E. coli*	30.46
			Klebsiella	3.85
			Proteus	3.85
Singh MP	2003	44	*E. coli*	31.82
			Mixed	9.09
			Pseudomonas	4.55
Majhi G	2008	80	*E. coli*	18.75
			Klebsiella	3.75
			Pseudomonas	3.75
			Staphylococcus	3.75
Present study	2018	25	*E. coli*	22.36
			Pseudomonas	2.63
			Staphylococcus	5.26

Table 3: The incidence of bacteria presents in Core of stone.

Authors	Years	% of urinary stone case	Common bacteria	% of culture
Kumar R	1980	14.4	*E. Coli*	16
			K. aureous	6
Jackson E	1984	71.0	*P. Mirablis*	12
			E. coli	2
Takeuchi H	1989	79.0	*P. Mirablis*	-
			Staphylococcus	-
Mitsuo O	1992	70.0	*P. mirablis*	8
			E. coli	5
Shigetta M	1993	-	*E. coli*	7
Rasheed A	1995	17.6	-	-
Hayashi T	1995	60.0	-	20
Gault MH	1995	-	-	18
Shakya GR	1996	20.7	*E. coli*	7
			Staphylococcus	1
Singh MP	2003	38.0	*E. coli*	14
			Staphylococcus	2
Manjhi G	2008	30.0	*E. coli*	18.75
			K. aerogenes	3.75
			Staphylococcus	3.75
Singh P	2016	33.69	*E. coli*	18.57
			Klebsiella aerogenes	2.85
			Staphylococcus	2.85
Present study	2018	11.42	*E. coli*	30.0
			Klebsiella aerogenes	16.0
			Staphylococcus	4.0

In present series the incidence of vesical calculus in relation to total admission and total genitor-urinary system cases was 1.01% and 14.87% respectively. Study clearly indicates that the incidence is progressively increase rural area in this region. This could be due to poor general health in rural area and lower socioeconomic status and in urban region decreasing. This could be due to improved general health and socioeconomic status, better control of infection and dietary deficiencies Table 1.

Urine microscopic and culture

In the present series, urinary tract infection was present in 50.50% cases. Urine culture was done in those cases, where more than 6 pus cells were present per high power field on microscopic examination. The culture was positive in· 26 cases out of 50 cases (52%). In 24 cases single organism was identified and in 2 cases mixed organism were found.

The above Table 2 shows common bacteria on urine

culture. In the present series main organisms responsible for U.T.I. was *E. coli* (22.36%). These findings are corresponding with that of Kevin A et al, Singh S, Kumar R, Ohava M et al, Shakaya GR and Twiari et al who also reported the *E. coli* as the main responsible organism for U.T.I. while Kevin et al, reported differently, that the staphylococcus as a main pathogenic organism.

Culture of stone

Bacteriological study of removed stone from bladder (culture of core of stone) was performed in 25 cases in present study. Culture was seen positive in 15 cases. (30%) *E. coli*. were seen majority of the 15 cases, (16.0%), *Klebsiella* while *staphylococcus aureous* 2 case (4%) and 25 case of stone culture was sterile.

Similar observation has been noted by Jackson E and Takeuchi H. Table 3 shows the incidence of infected stone (bacteria present in core of stone).

CONCLUSION

Our study revealed the following aspects: of the incidence of urinary stones was slightly higher in women compared to men, with a higher rate of infectious stones compared to sterile ones of the preponderant microorganisms found in the stones and urine were *Escherichia coli* and *Pseudomonas. E. coli* (30.0%) was the predominant microorganism cultured from about one-third of crushed stones followed by *Klebsiella* (16.0%). *Staphylococcus aureus*, (4.0%). If We consider more bacteria then we needed a further study to document the importance of the connection between urinary stones and urinary infection.

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