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Characterization of Bacterial Species associated with Urinary Tract Infections among Sudanese Chronic Renal Dialysis Patients

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Abstract

Background: Patients with chronic renal failure (CRF) have an increased susceptibility to bacterial infection. The urinary tract constitutes a frequent source of infection in these patients. Causes of CRF are still unknown because most patients present with terminal conditions requiring immediate dialysis. Only few patients with end stage renal failure are receiving hemodialysis. Hypertension and kidney infection are on top of the blamed causes list.

Objective: To characterize bacterial species associated with urinary tract infections (UTIs) among Sudanese chronic renal dialysis patients.

Materials and methods: Midstream urine specimens were collected from 200 Sudanese patients and streaked on cysteine lactose electrolyte-deficient (CLED) agar and incubated aerobically at 37°C. Growth was considered significant if 10^5/mL bacteria were present. Bacterial species were identified and characterized according to the standard conventional bacteriological methods. Kirby-Bauer method was used for testing the antibiotic susceptibility of the isolated organisms.

Results: A total number of 200 patients presenting with chronic renal failure (CRF) were enrolled in this study. Patients studied were in the age range 10-77 years. 106 (53%) of patients studied were males, and 94 (47%) were females. 53 (26.5%) patients were found to have urinary tract infection. The most commonly bacterial species isolated was *E.coli* (35.8%). 35 (66.7%) of total isolates were sensitive to ciprofloxacin. Significant pyuria was detected in 69 (34.5%) of CRF patients. Sensitivity and specificity of bacterial urine cultures and pyuria associated with UTI were calculated.

Conclusion: E.coli was the commonest organism causing UTIs. Pathogenic organisms isolated were highly susceptible ciprofloxacin and chloramphenicol. Pyuria had a high sensitivity and specificity in detection of significant bacteriuria and it can be used as a tool for screening CRF patients suspected of having UTIs.

Key words: Bacterial species, Urinary tract infections, Chronic renal dialysis.

Introduction

In term of renal failure among Sudanese, estimated to be about 70-140 per million populations per year, hypertension and inflammation of kidneys are in the top list of blamed causes. The most important approach to reducing the incidence of chronic renal failure is to control epidemics and infections; and to manage individual infections when they occur. Another important cause of CRF is the development of kidney stones (urolithiasis), which are observed even in children. However hypertension and diabetes mellitus are the main cause of CRF in Urban population[1].

Urinary tract infection (UTI) is one of the most common infections and it accounts for about 1-2% of all consultation[2].

It has been estimated that more than six million out-patient visits and 300,000 hospital stays every year are due to UTIs [3, 4, 5].

Approximately 10% of humans will have a UTI at some time during their lives[6].

UTIs can cause serious and permanent renal damage in patients with underlying urinary tract abnormalities, diabetes mellitus, pregnancy, immunosuppression or sickle cell disease[7].

In addition, UTIs are a leading cause of Gram negative sepsis in hospitalized patients and are the origin of about half of all nosocomial infections caused by urinary catheters[6].

Patients with CRF are more susceptible to develop UTIs, because of altered immunity, uraemia, low urinary flow rate and urinary concentration defects, all of which favour growth and multiplication of bacteria[8].

Furthermore, UTIs can lead to more deterioration of renal function in patients with CRF due to many factors including septicaemia, fluid depletion leading to hypotension, and reduced cardiac output with reduced renal perfusion and obstruction of the urinary tract by sloughed papillae, stones or plugs of pus[9].

Patients with renal failure are prone to UTI due to decreased immunity, poor nutrition, uremia, and renal dialysis. This study aims to explore the prevalence, aetiology and laboratory diagnosis of UTI in chronic renal failure patients. Chronic renal failure patients are more susceptible to UTI caused by pathogenic organisms.

Materials and methods

This was a quantitative, descriptive, cross-sectional study. It was a facility based study carried out at Haemodialysis Centres in Khartoum State. The study population was 200 patients suffering from CRF and diagnosed as end-stage renal disease and treated by haemodialysis. The study was conducted during the period from April 2002 to October 2005. The inclusion criteria for selection of patients were patients with an established CRF, confirmed by various investigations including blood urea, serum electrolytes, serum creatinine and abdominal ultrasound. The patients selected should have the ability to provide urine samples for analysis; i.e. excluding anuric or severe oliguric patients. The patients should not be on antibiotic therapy for at least two weeks. The patients excluded were patients with acute renal failure (ARF), patients with end-stage renal failure treated by peritoneal dialysis, and kidney transplanted recipients.

Data was collected by a direct interviewing questionnaire designed to collect and maintain all valuable information of each case examined. Approval to run this study was taken from Al-Neelain University, Khartoum (Sudan). Permission to collect specimens was granted by all hospitals and centres involved in the study. A verbal consent was obtained from all patients included in the study. Results of specimens collected were donated to all patients enrolled in the study. Data were analyzed by a computer system using the Statistical Package for Social Science (SPSS) program.

Sampling selected was a non-probability, convenience type in which the patients were chosen on the basis of accessibility. 200 specimens of clean catch mid-stream urine specimens were collected from haemodialysis patients in sterile, screw capped, leak proof disposable plastic containers. Each specimen had reached the laboratory within one hour from the collection time. In the laboratory, first the colour and turbidity for each specimen was observed. After mixing, each specimen was inoculated on cystine lactose electrolyte deficient (CLED) medium, using a 0.001 microliter, sterile standard loop. All cultures were aerobically incubated at 37˚C for 24 hours. Also, on a clean dry slide, 2-3 drops of urine were placed and covered with a cover glass. The slide was then examined microscopically using the low and high power field lenses to detect presence of any pus cells, red blood cells, epithelial cells, yeast cells, crystals, casts, ova, or other materials[10].

The presence of 10^5 colony forming units (cfu) or more of bacteria per millilitre of urine (100 or more colonies on the culture medium) was interprtated as significant bacteriuria. A count less than 10^5 cfu / ml were considered as insignificant[10]. The significant colonies were then morphologically examined for size, colour, shape, and ability to ferment lactose sugar. Smears were prepared, heat-fixed, and stained by Gram's stain. They were examined microscopically and Gram reaction was recorded. Then isolates were subjected to a variety of tests for full identification. Gram negative bacilli were identified by oxidase test, Kligler iron agar test, indole test, citrate utilization test, urease production test, and motility test. Identification of Gram-positive cocci was performed by catalase test, coagulase test, DNAse test, and mannitol fermentation. The analytical profile index (API) biochemical tests were used for the identification of enterobacteria.

Antimicrobial susceptibility tests of all pathogenic organisms were performed using the agar disc diffusion Kirby-Bauer method. Using a sterile wire loop, colonies of the isolated organism were emulsified in 3 ml sterile peptone water. The turbidity of the suspension was matched to the turbidity of Mc Farland turbidity standard. A sterile swab was soaked in the test organism suspension and excess fluid was removed by pressing the swab against the side of the tube. The swab was streaked over the surface of a Muller Hinton agar (Mast Group Ltd, UK). Streaking was done into three directions, and then the plate was allowed 3-5 minutes to dry up. Using a sterile forceps the antimicrobial discs were placed in an even distribution on the inoculated plate. The antimicrobial discs used were ciprofloxacin, gentamicin, norfloxacin, amikacin, cefoxitin, ampicillin, nitrofurantoin, nalidixic acid, and chloramphenicol. The plate was inverted and incubated aerobically at 37ºC for 24 hours. After overnight incubation the control and test plates were examined to ensure that growth was confluent. A ruler was placed on the underside of the plate to measure the diameter of each zone of inhibition[10]. Using the Clinical Laboratory Standards Table, the inhibition zones were interprtated to determine the organism’s susceptibility.

Results

A total of 200 patients presenting with chronic renal failure (CRF) attending dialysis centers in Khartoum State were enrolled in this study. Patients studied had an age range between 10-77 years, with an average of 44 years. 10 patients (5%) were less than 20 years, 46 (23%) were less than 50 years, and 5 (2.5%) were less than 80 years.
106 (53%) of patients studied were males, and 94 (47%) were females. Also 89 (44.5%) of the patients were in their first year of dialysis, 58 (29%) were in their second year of dialysis, and 53 (26.5%) were on dialysis for more than two years.

53 (26.5%) patients were found to have urinary tract infection. Of these, 15 patients (27.5%) were symptomatic and 38 (72.5%) were asymptomatic. Major symptoms were burning micturition (43.8 %), increased urine frequency rate (39.7%), fever (27.3%), and loin pain (12.3%).

37.5% of the causes of renal failure in patients studied were unknown. However, hypertension, diabetes mellitus, kidney stones, malaria, and urinary tract infections were found to cause probably 24%, 10%, 5%, 5%, 4% respectively of renal failure in these patients. In addition, both hypertension and diabetes mellitus were found to cause probably 7.5% of cases.
78 (39.1%) of the CRF patients studied had past history of one or more episodes of UTIs. The bacterial species isolated from these patients were *E.coli* (35.8%), *Staph. saprophyticus* (22.6%), *Staph. aureus* (18.8%), *Staph. epidermidis* (7.5%), *Klebsiella pneumoniae* (5.6%), and *Proteus mirabilis* (4.3%). While the frequency rate of isolation for each of *Pseudomonas aeruginosa*, *Citrobacter freundii*, and *Enterobacter cloacae* was 1.8%.

Bacterial growth was detected more in females (73.6%) than in males (26.4%). Positive urine cultures were more detected (50.9%) among patients suffering from CRF for one year duration, 26.4% among patients suffering from CRF for two years duration, and 22.7% for those suffering from CRF for more than two years duration.
Significant pyuria was detected in 69 (34.5%) of CRF patients; while among 131(65.5%) patients no pyuria in urine was detected (Table 1).
Sensitivity and specificity of bacterial urine cultures and pyuria associated with UTI were calculated according to the method of Galen and Gambino[11] as follows:
* Sensitivity = True Pos.x100/(True Pos.+False Neg.) = 45x100/(45+8) = 84.91 %
* Specificity = True Neg.x100/(True Neg.+ False Pos.) = 123x100/(123+24) = 83.7%
* % of False Negative = False Neg.x100/(False Neg.+True Pos.) = 8x00/8+45 = 15.1%
* % of False Positive = False Pos.x100/(False Pos.+True Neg.) = 24x100/24+123 = 16.3%
From these calculations, it is clear that from the 200 cases studied: 45 cultures were true positive; 123 cultures were true negative; 8 cultures were false negative; and 24 cultures were false positive. Hence the sensitivity of the bacterial urine cultures was 84.91%, and their specificity was 83.7%.
Pyuria gave a sensitivity of 81.8% and a specificity of 74% in detection of significant bacteriuria (Table 1).

Table (1) Association of pyuria and urine culture with urinary tract infections

Pyuria	Significant urine culture	Insignificant urine culture	Total
Significant	True + ve 45	False + ve 24	69
Insignificant	False – ve 8	True – ve 123	131
Total	53	147	200

On the other hand, 35 (66.7%) of total isolates were sensitive to ciprofloxacin, 33 isolates (63.6%) were sensitive to chloramphenicol, and 24 isolates (45.5%) were sensitive to both amikacin and cefoxitin. Also, 18 organisms (33.3%) were sensitive to nitrofurantoin, and 16 organisms (30.3%) were sensitive to gentamicin, norfloxacin and nalidixic acid. Only 6 isolates (12.1%) were sensitive to ampicillin (Table 2).

Table (2) Sensitivity pattern of pathogenic organisms isolated

Bacteria isolated	Number of organisms sensitive to:								
	CP	AM	CF	CL	NT	GT	NR	NA	AC
Escherichia coli	14	8	8	14	7	5	5	7	2
Klebsiella pneumoniae	2	1	-	2	1	-	-	1	-
Proteus mirabilis	2	1	1	1	-	1	1	-	-
Pseudomonas aeruginosa	1	-	-	-	-	-	-	-	-
Enterobacter cloacae	1	1	1	-	-	1	-	1	1
Citrobacter freundii	1	1	1	-	-	1	-	1	-
Staph. aureus	5	4	4	6	4	2	3	3	-
Staph. saprophyticus	7	7	9	9	6	6	7	3	3
Staph. epidermidis	2	1	-	1	-	-	-	-	-
Total	35	24	24	33	18	16	16	16	6

CP: Ciprofloxacin AM: Amikacin CF: Cefoxitin CL: Chloramphenicol
NT: Nitrofurantoin GT: Gentamicin NR: Norfloxacin NA: Nalidixic acid
AC: Ampicillin

Discussion

Urinary tract infections (UTIs) are one of the most common infections that cause serious and permanent renal damage in chronic renal failure (CRF) patients. In addition, patients with CRF are more susceptible to develop UTIs[10]. Because of this many studies had been conducted to determine the prevalence and aetiology of UTIs in CRF patients.

The frequency rate of asymptomatic UTIs cases observed in 72.5 % of total CRF patients is similar to the frequency rate reported by Gauba and his colleagues in India (1997)[12] who stated that the majority of UTIs were asymptomatic.

There are several systemic diseases and infections that can lead to impaired kidney function and ultimately to renal failure. In our study, the most common cause of CRF was hypertension (24%), followed by diabetes mellitus (10%), kidney stones (5%), and malaria (5%). This was different from that reported in 1999 by the United States Renal Data System[13] where diabetes mellitus accounted for 33% of prevalent cases, followed by hypertension (24%), glomerulonephritis (17 %), and cystic kidney disease (5%).

On the other hand, the Center for Disease Control (USA) reported that diabetes mellitus is the leading cause of end-stage renal disease in the United States, accounting for 44% of new cases in 2002[14].

In this study, 39.1% of the patients investigated had past history of one or more episodes of UTIs. This was explained by the increased risk of urinary tract infections among CRF patients. No strong relation was found between the past history of UTIs and the significant growth on urine culture. No data were cited in literature on previous episodes of UTIs in CRF patients to explain this relation.

In the present context, *E.coli* was the commonest organism isolated from CRF patients where 35.8% of all significant bacterial growths were caused by this organism. This was followed by *Staph. saprophyticus* (22.6%), *Staph. aureus* (18.8%), and *Staph. epidermidis* (7.5%). This result was similar to the findings of Saitoh and his colleagues[8] in 1985 who found that the commonest cause of UTIs in CRF patients was *E.coli* accounting for 50% of cases, followed by *Enterococcus faecalis* (27%), streptococci (12%) and *Staph. epidermidis* (9%).

Also, Mamoun[15] in a study performed at Soba University Hospital in 1990, found that *E.coli* as the commonest cause of UTIs and accounting for 60% of cases. This was followed by coliforms (8%), *Klebsiella* (8%), and *Proteus* (6%).

Furthermore, this study revealed that most bacteria isolated were sensitive to ciprofloxacin and chloramphenicol with a frequency rate of 66.7% and 63.6% respectively. This was followed by amikacin and cefoxitin (45.5% each). In his study, Mamoun[15] found the best antibiotics with high sensitivity rates were nalidixic acid, gentamicin and nitrofurantoin; however, Mamoun[15] did not include ciprofloxacin in his study in 1990. These variable findings may be due to the genetic mutation of bacteria, inadequate drug therapy in repeated UTIs, and abuse of antimicrobial agents by a wide range of patients leading to production of drug resistance.

This study also showed that females were more affected by UTIs than males. This finding was similar to that reported by Wanger and his colleagues[16] in 1996 who found similar results.

Also, in this study it was noticed that the duration of CRF had an association with the frequency rate of UTIs. For duration of one year, the frequency rate of UTIs was 50.9%, and for duration

of more than 2 years the frequency was 22.7 %. Davison and his co-authors[2] in 1992 reported that UTIs frequency rate may increase when the duration of CRF becomes longer. The decrease in the frequency rate of UTIs in more than 2 years duration was due to small sample size in this group (22/10.9%) compared with that among patients in 1 year group (125/62.7%). Furthermore, in the present study the frequency rate of significant bacteriuria among CRF patients was 30% and the significant pyuria was 42.7%. These results were slightly higher than those detected by Saitoh and his colleagues[8] in Japan (1985) who found a significant bacteriuria frequency rate of 27% and a significant pyuria frequency rate of 38%.

In this study, the frequency rate of bacteriuria and pyuria among haemodialysis patients was 29% and 34.5% respectively. These findings were higher than those detected in America (1993) by Chaudry and his co-workers[17] who reported frequency rates of 25% and 31% respectively. However, these workers reported a frequency rate of UTIs as 26.5% and this finding was similar to the frequency rate reported by Kolendo and his co-authors[18] on investigating 112 patients on haemodialysis and found a UTIs frequency rate of 27.7%. The increased findings of our study may be explained by the increased deterioration of renal function and the increasing stone formation among the patients studied, since these factors are considered predisposing factors leading to a higher rate of UTIs. The increase rate may also be explained by increased susceptibility in immuno-deficient patients.

On the other hand, in this context the sensitivity of pyuria was 81.8% and its specificity was 74% for detection of significant bacteriuria. These results are almost similar to that detected in 1995 by Bailey[19] who studied urine cultures and found that the sensitivity of urine pyuria was 81.6% and its specificity was 65.1%. Another study performed in Israel (1999) by Waisman and his colleagues[20], to detect the validity of urine screening for early detection of UTIs, showed that the sensitivity of pyuria in urine analysis was 88.6% and its specificity was 88.4%. Therefore pyuria can be used as screening tool to detect UTIs in CRF patients.

From this study it may be recommended that antenatal care of pregnant ladies should include direct interviewing and urine cultures to reveal UTI. Practical programs for the management of CRF should be established in Sudan, and the population should be health educated to minimize hazards of UTIs. Routine urine analysis and urine culture are mandatory for patients with CRF to detect the causative organism of UTIs and to assess the response to treatment. Pyuria can be used as screening marker for suspected cases of UTIs in patients with CRF. Establishment of data bank for all renal failure patients in Sudan to develop an informative map for all regions in order to pinpoint high-risk areas.

Conclusion: The frequency rate of significant bacteriuria and pyuria were 30% and 42.7% respectively. *E.coli* was the commonest organism (35.8%) causing UTIs. Pathogenic organisms isolated were highly susceptible to ciprofloxacin. Pyuria had a high sensitivity and specificity and can be used as a screening tool of CRF patients suspected of having UTIs.

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