Original Article

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Clinico-Microbiological Profile of Catheter Related Blood Stream Infections In Patients on Haemodialysis

Microbiology Section

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ABSTRACT

Introduction: Central venous catheter use in Haemodialysis (HD) is associated with a significant risk of blood stream infection. Knowledge of the etiological agents of Catheter Related Blood Stream Infection (CRBSI) is important for patient care.

Aim: To determine the pathogens causing CRBSI and their antimicrobial susceptibility pattern.

Materials and Methods: In this prospective study conducted from July to September 2013, fifty adult patients on HD who developed local or systemic signs of catheter related infection were investigated by swab culture from inflamed site (n=20), culture of blood from catheter hub (n=40), catheter tip (n=20) and peripheral venous blood (n=50).Clinical data were analysed to identify risk factors.

Results: The frequency of Exit Site Infection (ESI) was 24% and the predominant isolates were *Staphylococcus aureus* (50%) and *Staphylococcus epidermidis* (41.7%). Catheter colonisation was detected in 42% of patients. The commonest colonising bacteria were *Staphylococccus epidermidis* (28.5 %) and *Staphylococcus aureus* (23.8%). Methicillin resistance was observed in 66.67% and 50% of *Staphylococcus aureus* isolates associated with ESI and colonisation respectively. CRBSI was diagnosed in 12% of the patients, caused by *Pseudomonas aeruginosa* (50%) *Acinetobacter baumannii* (33.3%)and *Corynebacterium jeikeium* (16.7%). No multidrug resistance was noted.83.3% patients with CRBSI had a precedent catheter colonisation but no ESI. Diabetes mellitus was significantly associated with CRBSI.

Conclusion: Gram positive cocci were the predominant catheter colonisers. Gram negative bacilli caused the majority of CRBSI. No precedence of ESI was noted in a significant proportion of CRBSI. Catheter colonisation was a definite forerunner in all the patients.

Keywords: Chronic Kidney Disease, Vascular access, Blood stream Infection

INTRODUCTION

Chronic Kidney Disease (CKD) is a major health problem which leads to end stage renal disease. Over 1.4 million patients receive Renal Replacement Therapy (RRT) worldwide. The prevalence of CKD in India has been observed to be 17% with approximately 6% having stage 3 or later.The main modality of RRT is Haemodialysis (HD) which depends on long term and effective vascular access [1,2].

One of the main complications of HD is infection which has a significant impact on morbidity and mortality. The high risk for infection in these patients is because of impaired innate and acquired immunity.Vascular access related blood stream infections are not only a major cause of morbidity but also attribute to the increased cost of treatment of End Stage Renal Disease (ESRD) in HD patients [3-6].

Vascular access for an efficient HD can be performed through either a native Arterio Venous Fistula (AVF), ArterioVenous Graft (AVG), or Central Venous Catheter (CVC)-the AVF remaining the best choice due to the higher infectious and thrombotic complications associated with AVG and CVC. CVC is used in situations when urgent HD is required either at the time of start of RRT or when a permanent access becomes dysfunctional [7].

Though, CVC as vascular access are considered inferior to other means of vascular access, the prevalence of usage of CVCs range from 18% to 70% worldwide. This reliance on catheters is attributed to late referrals to nephrologists, delay in access formation or lack of sufficient time for an AVF to mature. Patients with a CVC are at increased risk of infection which is responsible for the removal of about 30%-60% of HD CVCs and face a 41% higher risk of death from infection than AVFs [7,8].

The most dangerous infectious complication is CRBSI, the diagnosis of which is based on clinical and microbiological evidence.The management of CRBSI includes antibiotic therapy, consideration of catheter removal based on the severity of the clinical condition and the pathogen responsible [7,9].

This study was done to determine the microbial aetiology of CRBSI among HD patients in our tertiary care hospital.

The aims and objectives of this study were to identify the aetiological agents causing CRBSI in patients on HD and determine their antimicrobial susceptibility pattern

MATERIALS AND METHODS

This prospective study was conducted at the Institute of Microbiology in association with the Department of Nephrology, Madras Medical College & RGGGH for a period of three months from July to September 2013. Approval from Institutional Ethics Committee and informed consent from the study population was obtained.

Fifty adult patients undergoing HD with a central venous catheter in place with local signs of ESI such as redness, discharge or oedema at the site of insertion of catheter and/or systemic signs of infection such as fever, chills, hypotension, abdominal pain, signs of organ failure were included. Patients with an AV fistula or AV graft were excluded from the study.

Sample Collection, Transport And Processing [9,10]

The following samples were collected from the study population:

a) Swab of the inflamed exit site or swab of the discharge: Two sterile swabs moistened with sterile normal saline were rolled over the inflamed area over the catheter exit site. Serous or seropurulentdischarge if any, was collected and transported immediately.One swab was inoculated onto 5% Sheep blood agar (BAP) and Mac Conkey agar (MAC).The other was rolled over a clean glass slide, air dried and heat fixed and stained by Gram's stain. The presence of pus cells and organisms was noted.

(b) Quantitative culture of blood drawn through catheter hub: One mL of sterile blood drawn aseptically from the catheter was mixed with 19 mL of melted nutrient agar base at 46° C and poured into sterile Petri plates. The plates were rotated for uniform distribution and allowed to solidify. The plates were incubated aerobically at 37° C for 48 hours.

(c) Peripheral venous blood for culture: Under aseptic precautions 10 mL of sterile blood drawn aseptically from a peripheral vein and inoculated into 50 mL of Brain Heart Infusion Broth and incubated aerobically at 37ºC. The bottles were examined for turbidity daily. Subculture was done onto MAC, BAP, Nutrient agar (NA) and Sabourauds Dextrose Agar (SDA) at 48 hours, five days and one week to detect growth.

(d) Catheter Tip: Its culture was performed if the catheter was removed.

Under aseptic precautions the catheter was removed, the tip cut into approximately 5 cm segment using sterile scissors and transported to the laboratory immediately.

(i) Semiquantitative culture of catheter tip by Maki's Roll plate method [9]: The segment was transferred on to the surface of a 5% sheep blood agar plate using sterile forceps and rolled back and forth and incubated at 37ºC for 48 hours. Colonies were identified, enumerated and expressed in Colony Forming Units (CFU).

(ii) Culture of catheter tip by Endoluminal flush technique [9]: One mL of sterile normal saline was flushed in to the lumen of the segment using a sterile syringe and 0.01 ml of the suspension inoculated each onto BAP, MAC and SDA. The plates were incubated aerobically at 37ºC for 24 hours. Colonies were identified, enumerated and expressed in CFU.

(iii) Direct Gram stain: It was performed by rolling the catheter tip on to a glass slide.The presence of pus cells and organisms were noted.

Identification of Bacterial and Fungal Growth

The plates were examined for presence of fungal and bacterial colonies. The bacterial growth was identified by Gram stain and standard biochemical tests. Inoculated SDA plates were incubated at 37° C and 25ºC for upto three weeks. They were examined intermittently for growth of moulds or yeast. Fungal growth was ruled out by Gram stain and lactophenol cotton blue mount [10].

Interpretations [9]

The following definitions were used for diagnosis:

(i) Exit site infection: When exudate at catheter exit site yielded a micro-organism with or without concomitant blood stream infection.

(ii) Catheter colonisation: Significant growth of ≥1 micro-organism in a quantitative (≥ 10^2 CFU per catheter segment) or semi quantitative culture (≥15 CFU per catheter segment) of the catheter tip, subcutaneous catheter segment, or catheter hub.

(iii) Catheter Related Blood Stream Infection (CRBSI): Bacteraemia or fungaemia in a patient with clinical manifestations of infection (e.g., fever, chills and/or hypotension), and no other apparent source for bloodstream infection with a positive result of semi quantitative culture (>15 CFU per catheter segment) or quantitative culture (>10 CFU per catheter segment) whereby the same organism (species) was isolated from a catheter segment or catheter hub blood and a peripheral blood culture.

Antimicrobial Susceptibility Testing (AST)

Antibacterial susceptibility testing was done on Mueller-Hinton agar (Hi-Media laboratories, Mumbai) by Kirby Bauer Disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) recommendations (M100 S23 document, 2013). Isolates of Enterobacteriaceae were phenotypically screened for Extended Spectrum beta-Lactamase (ESBL) production by initial screen test using cefotaxime 30 µg and ceftazidime 30 µg followed by the

phenotypic confirmatory test using cefotaxime 30 µg and ceftazidime 30µg alone and in combination with clavulanate (30/10µg). *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used for Quality Control (QC). *Staphylococcus aureus* isolates was phenotypically screened for mecA-Mediated oxacillin Resistance by disc difffusion method using Cefoxitin 30 µg as surrogate marker. *Staphylococcus aureus* ATCC 25923 was used for QC. Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates were screened for vancomycin resistance on BHI-vancomycin screen agar followed by determination of minimum inhibitory concentration by microbroth dilution method. *Enterococcus faecalis* ATCC 29212 was used for QC [11].

STATISTICAL ANALYSIS

Statistical analysis was done to determine the significance of risk factors namely frequency of dialysis, duration of catheterisation and history of diabetes mellitus by Fisher's exact Test using Open Epi Statistic program (version 3.01). Variables attaining a probability value (p-value) <0.05 were considered to be significant.

RESULTS

The study population consisted of 36 males (72%) and 14 females (28%).The mean age of the study population was 40.3 years (SD=15.06, age range:18-70 years)

The indication for Haemodialysis (HD) was CKD in 58% patients (n=29), among whom the most common clinical diagnosis was diabetes mellitus in 24.13% (n=7), anaemia with renal failure (20.68%, n=6), hypertension (13.79%, n=4), lupus nephritis (10.34%, n=3), hydroureter with nephrosis (3.44%, n=1), Good-Pasteur syndrome (3.44%, n=1) and other diseases comprising the rest of the cases (24.13%; n=7).

Patients with acute kidney Injury formed 42% of the study population (n=21) with acute diarrhoeal disease being the most common cause (33.33%, n=7) followed by drug induced disease (19.04%; n=4), postpartum renal disease (14.28%, n=3), poison induced (4.76%, n=1), renal calculi (4.76%, n=1), acute cortical necrosis (4.76%, n=1) and other diseases comprising 19.04% (n=4) of the cases.

The most common site of catheterisation was the internal jugular vein (92%, n=46). To diagnose catheter related infection, the following samples were collected namely swab from inflamed site (n=20), blood from catheter hub (n=40), catheter tip (n=20) and peripheral venous blood (n=50).

Thirty patients (60%) had systemic signs and symptoms such as fever, hypotension, abdominal pain, persistently abnormal renal function tests without associated local signs around the catheter site insertion.

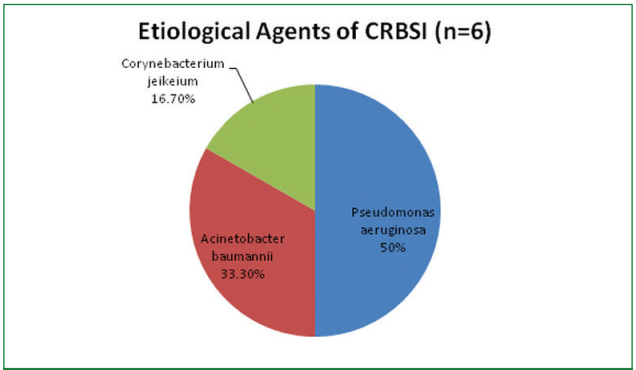
Twenty of 50 patients (40%) had symptoms and signs of local infection such as pain, tenderness, redness crusting, serous or seropurulent discharge over the catheter insertion site. Discharge from the site was cultured using a sterile swab from these patients. ESI was detected in 12 of 50 patients (24%). [Table/Fig 1], (Serial no. I-IV) The organisms isolated were *Staphylococcus aureus* (50%, n=6) followed by *Staphylococcus epidermidis* (41.7%,n=5) and *Corynebacterium jeikeium* (8.3%, n=1) Four of 6 isolates of *Staphylococcus aureus* (66.7%) were Methicillin Resistant which were susceptible to Vancomycin. Among the MRSA strains 75% and 25% were susceptible to Amikacin and Ciprofloxacin respectively.

Catheter colonisation was diagnosed in 21 of 50 patients (42%) with either a positive catheter tip culture or catheter hub blood culture. [Table/Fig-1] (S. no. I, II, V, VI, IX & X). The commonest colonising bacteria were *Staphylococccus epidermidis* (28.5%, n=6), *Staphylococcus aureus* (23.8%, n=6), *Acinetobacter baumannii* (14.3%, n=3) *Pseudomonas aeruginosa* (14.3%, n=3), *Klebsiella oxytoca* (14.3%, n=3) and *Corynebacterium jeikeium* (4.8%, n=1). Methicillin Resistance was observed in 3 of 6 (50%) isolates of *Staphylococcus aureus* (MRSA). All the MRSA isolates were susceptible to vancomycin, amikacin and ciprofloxacin. Among the gram negative isolates, trimethoprim sulphamethoxazole resistance was noted among *Acinetobacter baumannii* and *Klebsiella oxytoca*. ESBL production was observed in the single isolate of *K.oxytoca.* All the gram negative bacterial isolates were susceptible to imipenem.

CRBSI was diagnosed in 6 of 50 patients (12%) with a positive growth in peripheral blood culture and culture positivity of catheter tip and/or catheter hub blood [Table/Fig-1] (S. no.

Serial. no.	Culture of swab/ discharge	Quantitative culture of catheter tip and/or catheter hub blood	Peripheral venous blood culture	No. of patients
I	+	+	+	1
II	+	+	_	8
III	+	_	_	2
IV	+	_	+	1
V	_	+	+	2
VI	_	+	_	1
VII	_	_	+	2
VIII	_	_	_	3
IX	No exit site infection	+	+	3
X	No exit site infection	+	_	6
XI	No exit site infection	_	-	21

[Table/Fig-1]: Sample distribution of study population (n=50).



[Table/Fig-2]: Etiological agents of CRBSI (n=6).

Variable	Total no. of Patients	CRBSI Positive	Frequency of Occurrence of CRBSI	p-value
Frequency of dialysis				
< 10 times	45	5	11.1%	0.487 (not significant)
>10 times	5	1	20%	
Duration of catheterisation				
<20 days	35	3	8.6%	0.245 (not significant)
>20 days	15	3	20%	
History of Diabetes Mellitus	7	5	71.4%	<0.05 (significant)

[Table/Fig-3]: Univariate analysis of risk factors of CRBSI. Level of significance p value < 0.05 by Fisher's exact test.

I, V & IX). The commonest organisms causing CRBSI in the study population were *Pseudomonas aeruginosa* [(3/6)50%] followed by *Acinetobacter baumannii* [(2/6) 33.3%] and *Corynebacterium jeikeium* [(1/6) 16.7%]. [Table/Fig-2]. All isolates of *P. aeruginosa* were sensitive to amikacin, piperacillin-tazobactam and imipenem with 50% of isolates being resistant to gentamicin and ofloxacin. *Acinetobacter baumannii* showed sensitivity to all the antibiotics except trimethoprim sulphamethoxazole. *Corynebacterium jeikeium* was sensitive to penicillin, erythromycin, amikacin, cefotaxime, ciprofloxacin, tetracycline and trimethoprim sulphamethoxazole.

All the six patients with CRBSI (100%) had only catheter colonisation with the same pathogens *(A.baumannii, P.aeruginosa and C.jeikeium)* and 1 of 6 patients (16.7%) had associated ESI *(C. jeikeium)*. Fever and chills were the main symptom in all the six patients. 4 of 6 (66.7%) had associated catheter dysfunction, vomiting and haemodynamic instability. Catheter was removed in 5 of 6 patients (83.3%).Catheter salvage was done in the patient with Corynebacterium infection who was managed with appropriate antibiotics and supportive therapy.

Three of 50 patients (6%) had a positive peripheral blood culture, but had no growth in the catheter tip/catheter hub blood [Table/Fig-1] (S. no IV &VII). These patients were investigated for other causes of bacteremia.

The occurrence of CRBSI was found to be 20% in patients with a frequency of dialysis more than 10 and 11.1% in patients with a dialysis frequency less than 10, but the results were not statistically significant (p=0.487, Fisher's exact test). Among the patients with a longer duration of catheterisation (≥20 days) 20% had CRBSI while 8.6% of patients with a shorter duration of catheterisation (<20 days) developed CRBSI, a difference that was not statistically significant (p=0.245, Fisher's exact test). Of the 7 patients who had diabetes mellitus as an underlying risk factor, 5 patients had CRBSI (71.4%) while only one patient who was not diabetic had CRBSI, which was found to be statistically significant (p<0.05, Fisher's exact test) [Table/Fig-3].

DISCUSSION

The most common replacement therapy for patients with chronic renal failure is HD. In these patients on HD, infection may occur due to the need for intravascular catheters to perform dialysis and depends on the type of vascular access used. ESRD patients demonstrates that septicaemia is a common event for HD patients, requiring hospital admission and occurs in more than 10% of patients over seven years of follow-up. Nearly, 80% of septicaemia accounts for over three fourths of deaths caused by infections [12-14].

Identification of early clinical signs of infection such as ESI is important to initiate measures such as topical antibiotic application which may act as a barrier to contain extraluminal spread of organisms and eventually progression to a blood stream infections. Goulart DB et al., who studied ESIs in a HD unit in Brazil found the overall incidence to 3.5 per 1000 catheter days. The predominant pathogens were Gram negative bacilli in 69% of the patients with *Pseudomonas aeruginosa* and ESBL producing GNBs as the most common agents [15]. Gupta S et al., studied the microbiology of catheter related infections and reported an ESI rate of 7.8% with *Staphylococcus aureus* as the predominant organism. In the present study the frequency of ESI was24% and the most common isolate was *Staphylococcus aureus* (50%) followed by *Staphylococcus epidermidis* (41.7%) [15,16].

Among the 12 isolates associated with ESI, *Corynebacterium jeikeium* was the only isolate which subsequently caused catheter colonisation and bacteraemia. *Corynebacteria* are frequent commensals of the skin, but when isolated from blood cultures are primarily associated with CVCs. They are usually susceptible to antibiotics,though the drug may act poorly on bacteria embedded in biofilms, which is a therapeutic challenge if the catheter is retained. Ghide et al., reported a high frequency of occurrence of Corynebacteria in blood stream infections among patients with intravascular catheters and concluded that systemic therapy with

glycopeptide antibiotics obviates the necessity to remove the CVC [17].

Endoluminal colonisation due to organisms adhering to the luminal wall by biofilm formation can occur in a high proportion of HD patients and often precedes bacteraemia. Preventing colonisation of the exit site and catheter hub has been reported to significantly reduce CRBSI. This includes use of chlorhexidine at the exit site prior to start of HD and scrubbing with 70 % alcohol pad prior to catheter hub manipulation. Our study showed a rate of colonisation of 42%with predominance of gram negative bacterial isolates (42.8%); these patients were managed by close observation and antibiotic therapy. Two patients with *Acinetobacter* spp and all the three patients with *Pseudomonas aeruginosa* colonisation progressed to cause blood stream infection. This was in contrast to a study from a South Indian tertiary care centre reported a frequency of 23.6% of catheter colonisation, *Staphylococcus aureus* being the commonest bacteria (40%) associated [16,18].

The frequency of laboratory confirmed CRBSI in our patients on HD was 12% which was similar to the study by Gupta S et al., who observed a frequency of 15%. The distribution of causative agents of CRBSI varies substantially among various centres [16]. Studies around the globe report Gram positive cocci as the causative agent in 50-80% of the cases with *Staphylococcus aureus* contributing to about 40% of them. Gram negative bacilli contributes to 27-36% of the episodes with *Pseudomonas* spp as the predominant pathogen. Fungi have been reported to be associated with <10% of the BSI episodes [19,20].

In the present study, a predominance of Gram negative bacteria (83.3%) namely *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and the contribution of *Corynebacterium jeikeium as* causative agents of CRBSI was noted. A similar picture was noted by a Gupta S et al., who noted a predominance of Gram negative pathogens (79%) the most common being *Pseudomonas aeruginosa*, over Gram positive cocci (21%). Other studies in Indian literature however report a predominance of Gram positive cocci (57.8%) particularly methicillin resistant *Staphylococcus aureus* and coagulase negative Staphylococci over gram negative bacilli (40%) which included *Pseudomonas aeruginosa* and *Acinetobacter* spp [16,20].

It was interesting to note that among the patients with CRBSI, only one had associated ESI with *Corynebacterium jeikeium* while prior colonisation was observed in all patients.This was also observed by Oliver MJ et al., they found that half the bacteraemia occurred without a precedent or concurrent ESI. The authors also pointed out that endoluminal colonisation was the most important cause of bacteraemia which we also had observed in our study [21].

Several risk factors are linked to the development of catheter related infections in the patients on HD some of which include increased age, Diabetes mellitus, the type of catheter used, the site of placement, duration of catheterisation etc. In our patient group the association of CRBSI with Diabetes was statistically significant which was also noted by other authors [22].

LIMITATION

The incidence of CRBSI was not determined due to the constraint of short duration of the study.

CONCLUSION

The frequency of occurrence of CRBSI among HD patients was 12% among which Gram negative bacteria accounted for 80% of the CRBSI. The role of Gram negative pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in CRBSI was in sharp contrast to the predominance of Gram positive cocci such as Methicillin resistant *Staphylococcus* in catheter colonisation. All the agents of CRBSI were also colonisers of the catheter indicating the need for constant vigil for presence of colonisation. It was noted that precedence of ESI was absent in majority of the patients. Diabetes mellitus which is a known risk factor was significantly associated with CRBSI which requires assessment and accurate management of such co-morbid conditions.

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