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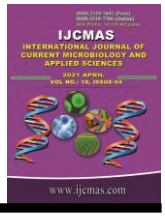

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A Clinico-Microbiological Profile of Peritonitis in Continuous Ambulatory Peritoneal Dialysis Patients

T. U. Jisha[1] and K. L. Sarada Devi[2*]

[1]Department of Microbiology, Mount Zion Medical College, Chayalode, Adoor, Kerala, India
[2]Department of Microbiology, Government Medical college, Thiruvanathapuram, Kerala, India

**Corresponding author*

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A B S T R A C T

The incidence of peritonitis depends on factors such as age, educational and financial background, environment and surrounding climate of the patients but the outcome depends on the organisms isolated. Among the several factors that may contribute to or enhance microbial pathogenicity is the extracellular slime (biofilm) produced by certain organisms on surfaces. All patients undergoing CAPD are included in the study after taking their informed consent. The most common organism isolated was gram negative bacilli (44%) followed by gram positive cocci (29%). 7% of isolates were fungal. A single non tuberculous mycobacterium was also isolated. *A.baumannii* (20.8%) predominated among gram negative bacilli followed by *E.coli* (10.4%). Among the gram positive cocci CoNS (19.2%) was the most common. *S. aureus* was isolated in 6.4% of the dialysates.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is an important form of dialysis treatment with end stage renal disease as compared to hemodialysis. Today, peritoneal dialysis encompasses a closed system of commercially prepared dialysate fluid packaged in plastic bags that are connected by silastic tubing to the Tenckhoff catheter. The effectiveness of CAPD is achieved by hyper - osmolar ultrafiltration across the peritoneal membrane (Von Graevenitz *et al.*, 1992). Major complication of CAPD is peritonitis, which accounts for significant antibiotic use in patients undergoing peritoneal dialysis. There are several potential portals of entry for infection in CAPD patients.

The three most frequent sites associated with CAPD infections are the exit site, i.e., the area where the catheter is connected to lines from the peritoneal dialysate; the tunnel associated with the implant of the Tenckhoff catheter in

the abdominal wall; and the peritoneum itself (Saklayen, *et al.*, 1990).

The incidence of peritonitis depends on factors such as age, educational and financial background, environment and surrounding climate of the patients but the outcome depends on the organisms isolated. Among the several factors that may contribute to or enhance microbial pathogenicity is the extracellular slime (biofilm) produced by certain organisms on surfaces (Piraino, *et al.*, 2005).

According to the International Society of Peritoneal Dialysis (ISPD), antibiotic therapy should be adjusted based on the Gram stain or culture results after empiric initial therapy for peritoneal dialysis related peritonitis. Prolonged and unnecessary broad spectrum antibiotic exposure poses serious concerns due to the potential for inducing and selecting resistance (Piraino B *et al.*, 2011). Therefore a definitive microbiological diagnosis and an appropriate adjustment to therapy are important. Prompt identification and treatment of peritonitis are essential to ensure success of a CAPD program.

Materials and Methods

Study Design

Cross sectional study

Study Population

Patients undergoing CAPD in the Department of Nephrology, Government Medical College Hospital

Operational definition

According to ISPD, at present the most acceptable definition of peritonitis in CAPD patients includes at least 2 of the following criteria

Symptoms or signs (or both) of peritonitis

A cloudy dialysate (effluent with leukocyte count > 100/mm^3)

Positive culture (and/or Gram stain) of the dialysate

Inclusion Criteria

All patients undergoing CAPD are included in the study after taking their informed consent.

Sample Size:125

Processing

From these exchange bags 50mL of fluid was withdrawn with a sterile needle and syringe under aseptic precautions. The fluid was centrifuged in sterile tubes at a rate of 3000g for 15 min and supernatant discarded. The deposit was divided into 3 parts and were subjected to

Gram staining, 10% KOH mount, Ziehl-Neelsen (ZN) staining

Culture for bacteria, fungi and mycobacteria

Antibiotic sensitivity

Results and Discussion

Patients from 5 years of age to 85 years of age were included in the study. Maximum number of subjects in the study group were in the age group 50-59 years (30%) followed by 40-49 years. (24%) CAPD peritonitis can occur immediately following catheter insertion for PD as an intraoperative procedure.

Time of occurrence of the first infection in the present study is given in Table 2. 87 patients (69.6%) got their first infection only after months of initiation of CAPD.

The major clinical symptom was abdominal pain. Others include diarrhea/constipation, fever and nausea/vomiting. Out of 125 patients, 98 patients (78.4%) had abdominal pain.

Organisms were detected indirect smears of only 15.2% of the centrifuged dialysate as represented in Table 6. 84.8% of the centrifuged dialysate showed pus cells only.

Out of the 125 cases studied, 101 cases (80.8%) were culture positive whereas the rest (19.2%) were bacteriologically sterile as shown in figure 8 & table7.

The most common organism isolated was gram negative bacilli (44%) followed by gram positive cocci (29%). 7% of isolates were fungal. A single non tuberculous mycobacterium was also isolated.

A.baumannii (20.8%) predominated among gram negative bacilli followed by *E.coli*(10.4%). Among the gram positive cocci CoNS (19.2%) was the most common. *S. aureus* was isolated in 6.4% of the dialysates.

Of the 9 (7%) fungal isolates 8 were Candida spp. and 1dematiaceous fungi *F.pedrosoi.* Of the Candida isolates 2 were *C.albicans* and 6 were *C.tropicalis.*

In our study including 125 subjects, incidence of gram negative bacteria was more (44%) than that of gram positive bacteria (29%) and lesser percentage of other agents. Gram-negative peritonitis episodes are attributable to fecal contamination and transmural migration of bacteria across the bowel wall.

The rate of decline of gram-positive peritonitis had been attributed to the introduction of the twin-bag system and a reduction in skin contamination with the use of disconnect systems and the "flush before fill" technique.

Although gram-positive organisms are the most common bacteriologic cause of PD-related peritonitis, the incidence of gram-positive infections is falling because of the advances in PD connectology, exit site and nasal chemoprophylaxis. Gram-negative peritonitis may result from touch contamination, exit site infection, or possibly a bowel source such as constipation, colitis, or transmural migration, but the cause is often unclear. Gram-negative organisms now account for 20%–30% of all PD-related peritonitis.

In the study by Szeto *et al.,* the overall incidence of PD-related peritonitis decreased by more than half during the 10-year study period (Szeto *et al.*,2001).When specific organisms were examined, the incidence of peritonitis caused by CONS decreased by about 75%, while the incidence of that caused by *S. aureus* was halved. This declining incidence of Gram-positive peritonitis was not an unexpected finding. It is agreed generally that the incidence of peritonitis decreased markedly as a result of improvements in connection technology. Almost all patients in the present series had used a disconnect system since 1998, and the incidence of Gram-positive peritonitis remained at a low but static level between 1998 and 2002, further supporting the role of the disconnect system. Interestingly, the incidence of Gram-positive peritonitis showed a further significant decrease in 2003. The precise reason for this further decrease remains unknown.

Compared with 1992-1993, the incidence of peritonitis due to a single gram-positive organism decreased significantly after 1994 ($p < 0.05$), whereas that of gram-negative peritonitis increased, resulting in a significant increase in the proportion of gram negative peritonitis after 1994 ($p < 0.05$).This decrease in the incidence of peritonitis due to a single gram-positive organism was due mainly to a

significant decrease in CoNS-induced peritonitis. The incidence of CoNS-induced peritonitis decreased from 0.226 episodes/patient-year in 1992– 1993 to 0.064 episodes/patient-year in 2000 –2001.

In the present study among the gram negative bacteria, *A. baumannii* (21%) was the most common organism identified. Among the 26 cases, 7 (6%) were MDR *A. baumannii*. In the study by Chao *et al., Acinetobacter* species were responsible for 26 PD peritonitis episodes (3.5% of all episodes) in 25 patients.

A. baumannii was the most common pathogen (54%), followed by *A.lwoffii* (35%), with the former being predominant, (86) which is in accordance with our study (Chao *et al.*,2014).

Acinetobacter baumannii, has been identified as a significant cause of antibiotic-resistant infections and has emerged as one of the most troublesome pathogens in health care institutions. Multidrug resistant (MDR) *A. baumannii* is a growing problem world wide, and reports of carbapenem-resistant *A. baumannii* strains are common. Some *A. baumannii* strains have been found to be resistant to all known antibiotics.

In our study, an important observation was that all the gram - negative pathogens were sensitive to third-generation cephalosporins except *MDR A.baumannii. (6%)* which was ESBL-producer and was sensitive to carbapenems.

Our study revealed that *E. coli* caused 10.4% of gram negative peritonitis episodes, which ranked second among other gram negative bacteria. *Escherichia coli* (*E. coli*) is one of the most common organisms that cause gram-negative peritonitis in PD patients. *E. coli* is a bowel flora and is usually of fecal origin. Peritonitis episodes attributable to transmural migration of bacteria across the bowel wall are usually associated with multiple gram-negative organisms. We believe that poor hand washing technique may have been responsible for contamination during the PD exchange procedure. Our analysis shows a trend towards a higher incidence of peritonitis from organisms of fecal origin.

In the present study among the 101 positive samples, 24 cases of peritonitis were due to CONS and *Staphylococcus aureus* accounted for 8 (6.4%) cases.

CONS (19.2%) predominated among gram positive organisms. In the study by Vikrant *et al., CoNS* accounted for 15 (41.7%) episodes of the CAPD peritonitis and *Staphylococcus aureus* was isolated in 8 (22.2%).

About one- fifth of the cases of peritonitis were caused by *CoNS* in this study (Vikrant *et al.,* 2013). Also, there are studies which have reported that the majority of CAPD peritonitis was caused by *CoNS* which may primarily be due to touch contamination or due to the formation of a biofilm.

The resulting decrease in gram positive peritonitis is the result of less touch contamination and better skin care, with occlusive dressings and less trauma to the exit-site in the postoperative period. Occlusive dressings in the postoperative period and exit-site care with mupirocin ointment are given to all patients at our center.

Enterococci may cause between 2% and 6% of PD-related peritonitis episodes and their identification is a hallmark of a gastrointestinal origin of the infection. In our study, 4 (3.2%) *Enterococcus faecalis* were isolated.

Table.1 Age distribution of patients

Age in years	No of cases	Percentage (%)
<20	2	**1.6**
20-29	7	**5.6**
30-39	15	**12**
40-49	30	**24**
50-59	37	**29.6**
60-69	24	**19.2**
70-79	8	**6.4**
>80	**2**	**1.6**

Table.2 Time period of first infection after initiation of CAPD Catheter

Time of occurrence of infection after CAPD initiation	No: of subjects	Percentage (%)
Days	24	**19.2**
Weeks	14	**11.2**
Months	**87**	**69.6**

Table.3 Frequencies of symptoms

Symptoms	Percentage (%)
Abdominal pain	**78.4**
Diarrhoea/constipation	**45.6**
Fever	**43.2**
Nausea/vomiting	**35**

Table.4 Direct smear of the centrifuged dialysate

Staining technique	Type of organism	n(%)
Grams staining	Gram positive cocci	6(4.8)
	Gram negative bacilli	11(8.8)
KOH mount	Yeast cells	1(0.8)
ZN staining	Acid–fast bacillus	1(0.8)

KOH-potassium hydroxide, ZN- Ziehl-Neelsen

Table.5 CAPD Peritonitis - Culture Positivity

Findings	No. of cases	Percentage (%)
Culture positive	101	80.8
Sterile	24	19.2
Total	125	100

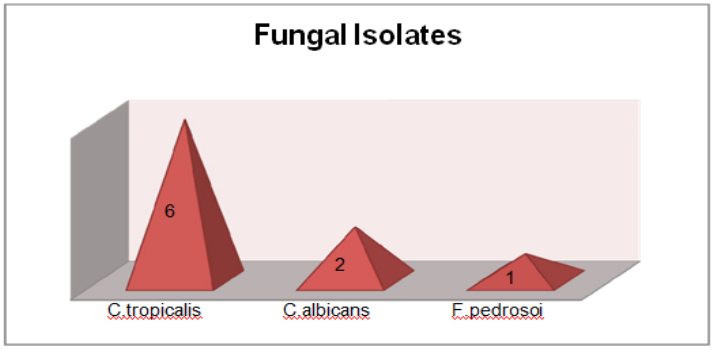
Table.6 Type of Organisms Isolated

Type of Organisms Isolated	N (%)
Gram negative bacilli	**55(44)**
Gram positive cocci	**36(29)**
Fungal	**9(7)**
***Mycobacterial* spp.**	**1(0.8)**

Table.7 Spectrum of Organisms Isolated

Organism	Frequency	Percentage
S.aureus	8	**6.4**
CoNS	24	**19.2**
E.faecalis	4	**3.2**
E.coli	13	**10.4**
K.pneumoniae	5	**4**
E.cloacae	5	**4**
C.freundii	*1*	**0.8**
P.aeruginosa	5	**4**
A.baumannii	26	**20.8**
C.albicans	2	**1.6**
C.tropicalis	6	**4.8**
F.pedrosoi	1	**0.8**
M.abscessus	1	**0.8**
Sterile	**24**	**19.2**

Fig.1 Fungal Isolates



All *E.faecalis* isolates were susceptible to ampicillin and none of them were resistant to vancomycin. In the study by Yip *et al.*, *Enterococcus peritonitis* was rare accouting for 2% of peritonitis of all episodes.

In the recent years, fungal peritonitis complicating CAPD is being increasingly recognized. Recent antibiotic therapy, frequent episodes of bacterial peritonitis, and immunosuppression are the major risk factors of fungal peritonitis which accounts for 1-15% of episodes of peritonitis in various studies. Most fungal peritoneal dialysis-associated infections are due to *Candida* species.(38) In the present study fungal peritonitis accounted for 7.2% of which *C.tropicalis* 4.8%, *C.albicans* 1.6% and *Fonsecaea pedrosoi* 0.8%. The origin of fungal isolates may be from the patients' skin, environment, or from the mucous membranes.

In the study by Bibhasi *et al.*, the incidence of fungal peritonitis (FP) and the fungi that caused FP were evaluated in 422 patients treated with peritoneal dialysis. During a 11-year period, 804 episodes of peritonitis occurred, 46(5.7%) of which were caused by fungi. Treatment was successful for 39 patients. Early diagnosis of FP and prompt therapy decreases morbidity and mortality (Bibhasi *et al.*, 2003).

In our study, *Mycobacterium abscessus* was isolated from a single dialysate. The patient presented with severe abdominal pain fever, cloudy fluid and an elevated leukocyte count in the peritoneal fluid. Patient was reluctant to take antibiotics and succumbed to illness.

Jarzembowski *et al.*, reported that identification of mycobacterial species was a uniformly tedious process involving biochemical tests that could require weeks of subcultures. The development of nonisotopically labeled DNA probes complementary to species-specific rRNA has allowed rapid identification of organisms using aliquots of broth culture or picked colonies. Treatment of nontuberculous mycobacterial infections is difficult, requiring extended courses of multidrug therapy with or without adjunctive surgical intervention (Jarzembowski *et al.*, 2008). In the present study 67% of the patients responded to antibiotics and was doing well with CAPD. Culture negative peritonitis were treated with Inj. Ceftazidime 1gm i/p (intraperitoneal) once daily and InjVancomycin 1gm i/p once in 5 days for 2 weeks. For gram positive organisms, InjVancomycin 1gm i/p once in 5 days for 3-4 weeks.

For gram negative organisms, Inj Ceftazidime 1gm i/p is given for 3-4weeks if third generation cephalosporin is sensitive. If resistant either cefoperazone-sulbactam or amikacin is given based on the sensitivity pattern. 23% had recurrent episodes of peritonitis during the one and a half year study period whereas 10% of the patients succumbed to illness. The cause of death may be due to infectious/ noninfectious complications of CAPD. In 4.8% of patients catheter was removed.

Out of the 9 (100%) fungal peritonitis, CAPD catheter was removed in all patients. 67% were cured with antifungal treatment whereas 33% (n = 3) succumbed to illness.

If fungal peritonitis is suspected, dialysate is sent for fungal culture along with oral flucanazole 100mg once daily is started empirically. If fungus is isolated in culture, catheter is removed and oral flucanazole is continued for 6 weeks (Rosman, 2011). In the present study, out of the 125 patients suspecting peritonitis, 81% (101 patients) were culture positive. Culture negative peritonitis accounted only 19%. (24 patients) Monomicrobial infections predominated

accounting 92% (93 patients) of culture positive peritonitis in the study. 8% of the infections were polymicrobial. Among the polymicrobial peritonitis (6%) were due to gram negative bacteria and fungus and 2% were due to gram positive and gram negative bacteria.

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