Use of Commercially Available Multiplex Polymerase Chain Reaction in Detection of Organism in Culture Negative Peritonitis in Peritoneal Dialysis

Sir,

Peritoneal dialysis (PD) is an underutilized mode of dialysis therapy worldwide. Despite the reduction in peritonitis rate, peritonitis continues to be the main cause of technique failure. In addition, it is an important cause of morbidity and mortality in patients in PD. The most common organisms associated with PD peritonitis reported worldwide in descending order are coagulase-negative Staphylococcus species, *Staphylococcus aureus*, Streptococci, Enterobacteriaceae, non-fermenting gram-negative bacilli (GNB).[1] Paradoxically in India, GNB is the major cause of peritonitis. The incidence of culture-negative peritonitis (CNP) in India varies between 18.2 and 64.7% [Table 1].[2-4]

It is believed that this CNP is due to gram-positive cocci. Polymerase chain reaction (PCR)-based detection of organisms in continuous ambulatory peritoneal dialysis (CAPD) peritonitis is gaining popularity due to rapid detection of organisms than what the conventional culture techniques can offer. However, it is largely restricted to research settings. Commercially available multiplex PCR has been used in early diagnosis of meningitis, neonatal septicemia.[5] Commercially available multiplex PCR-based methods are seldom used in the diagnosis of peritonitis in India.

In a prospective study done between January 2014 and June 2016 in our hospital, all the PD effluent from all the patients with suspected PD peritonitis was subjected to lysis centrifugation (25 ml) and inoculated in blood agar, McConkey agar, chocolate agar, thioglycolate broth, BACTEC blood culture bottle (10 ml) and identification was done by Vitek-2 identification system. With the consent of the patients commercially available mPCR-based detection (Xcyton (R)) was done. The technology comprises rapid multiplex amplification and accurate identification

Table 1: Culture negative peritonitis in India

	Gram-negative bacteria	Gram-positive bacteria	Culture-negative
Abraham *et al*.[2]	47.8 (41/85)	36.7 (31/85)	64.7 (156/241)
Prasad *et al*.[3]	29.4 (89/303)	33.7 (102/303)	18.2 (55/303)
Prasad *et al*.[4]	42.45 (45/106)	28.3 (30/106)	36.9 (62/168)

of the virulence-associated genes of the causative agents. The genetic material of causative agent is isolated from the CAPD effluent and there is a simultaneous amplification of the specific signature genes of all probable agents, followed by syndrome-specific hybridization. Syndrome-specific hybridization is a kind of DNA amplification technique. Gene amplification allows higher sensitivity and renaturation of amplified signature gene to its chemically identified complementary gene sequence on the syndrome evaluation system, which allows for higher specificity of test.

Of the 85 prevalent patients, eight patients (9.4%) died during the study period. After an episode of peritonitis two (2.3%) of those patients died within 2 months. The catheter was removed in 7 (8.2%) patients. The mean patient survival was 30 ± 2 months and mean technique survival was 24 ± 3 months. There were 49 episodes of peritonitis in 38 patients at a rate of 0.15 episodes/patient-year. Out of 49 cultures, 36 (73.5%) were positive. The mPCR was sent in 27 episodes and consent was denied in 22 episodes of peritonitis and mPCR was not sent. Out of these 27 episodes where mPCR was done, an organism was isolated in 24 cases. The sensitivity of mPCR in detecting an organism in the setting of a clinical CAPD peritonitis was 88.8% (24/27). The mPCR identified an organism in additional 8 (16.3%) of the cultures. By using both conventional culture technique and mPCR technique, 44 out of 49 cultures (90%) were positive. Thus, the CNP rates could be brought down to 10% instead of 26.5%. The GNB were seen in 23 out of 44 episodes (52%) and gram-positive bacteria were seen in 12 out of 44 episodes (27%), aspergillosis in 3 out of 44 episodes (7%) and atypical mycobacterium was seen in 1 out of 44 episodes (2%) [Table 2]. Both the conventional culture technique and mPCR-based technique identified same organism in 10/12 [83%] of instances. However, both the techniques showed different organism in three instances.

It has been shown by Prasad *et al*.[6] and Kim *et al*.[7] that broad-range PCR-based detection can identify 100% cases

Table 2: Organisms causing peritonitis in patients undergoing peritoneal dialysis

Organism	Routine culture technique (*n*=49)	Multiplex PCR (XCyton)* (*n*=27)	Number of total (%)
Gram-negative	*P. aeruginosa*	*P. aeruginosa* (4#)	9 (18.4)
	E. coli	*E. coli* (3#)	5 (10.2)
	K. pneumoniae	*K. pneumonia* (2#)	3 (6.1)
	E. faecium	*E. faecium* (1#)	2 (4.1)
	S. paucimobilis[1]	*S. aureus**	1 (2)
	E. species	*Enterococcus*	1 (2)
	E. cloacae	Aspergillosis*	1 (2)
	B. diminuta[2]	Not detected	1 (2)
	Not detected	*E. coli*	2 (4.1)
	Not detected	*A. baumannii*	1 (2)
Gram-positive	*S. epidermidis*[3]	Not detected	4 (8.2)
	S. aureus	*S aureus* (1#)	2 (4.1)
	S. hemolyticus[3]	*K. pneumoniae**	1 (2)
	S. gallolyticus[3]		1 (2)
	S. species		1 (2)
	S. lutetiensis		1 (2)
	Not detected	*S. aureus*	1 (2)
	Not detected	*S. species*	1 (2)
Fungus	A*spergillosis*		2 (4.1)
	Not detected	Aspergillosis	1 (2)
Tuberculosis	Atypical *Mycobacterium*[4]	Not detected	1 (2)
Culture-negative	Routine culture technique only		13 (26.5)
Culture-negative		Routine + multiplex PCR	5 (10.2)

Xcyton is not designed to detect unusual organisms like ([1,2]), coagulase-negative *Staphylococcus* sp. (3) and atypical mycobacterium ([4]). #Cases which were positive for organism by mPCR. All these cases were positive in both conventional and mPCR techniques. *The conventional and mPCR techniques grew different organisms in three instances. These positive cultures were treated as additional positive detection of organism by mPCR technique in analysis. We did not include one patient who grew *E. cloacae* by conventional culture and Aspergillosis by mPCR. He was lost to follow-up, and because we don't know which of the report is correct, we did not include that case in the analysis. Both the conventional culture technique and mPCR-based technique identified same organism in 10/12 (83%) of instances. However, both the techniques showed different organism in three instances. *P. aeruginosa: Pseudomonas aeruginosa, K. pneumonia: Klebsiella pneumonia, E. faecium: Enterococcus faecium, S. aureus: Staphylococcus aureus, S. species: Streptococcus species, S. paucimobilis: Sphingomonas paucimobilis, E. species: Enterococcus species, E. cloacae: Enterobacter cloacae, B. diminuta: Brevundimonas diminuta, A. baumannii: Acinetobacter baumannii, S. epidermidis: Staphylococcus epidermidis, S. hemolyticus: Staphylococcus hemolyticus, S. gallolyticus: Streptococcus gallolyticus, S. lutetiensis: Streptococcus lutetiensis,* PCR: Polymerase chain reaction

of peritonitis [Table 2]. However, lack of commercially available mPCR technology had limited their use to only research facilities as in the above two studies. We have shown that any PD unit can use the commercially available mPCR (Xcyton (R)) and reduce their CNP rates to <10%. In stable patients and in centers with high culture positive rates by the existing conventional culture techniques, the role of mPCR may be limited to only cases with CNP.

Conclusion

Centers with >20% culture-negative peritonitis can consider mPCR-based tests in addition to improving their culture techniques. The mPCR technique yields result in 24 h, and we recommend its use only in cases which are culture negative by routine technique.

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Conflicts of interest

There are no conflicts of interest.

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