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RESEARCH LETTER

Enriched-Culture Polymerase Chain Reaction, a Promising Approach for Diagnosing Tuberculous Peritonitis

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INTRODUCTION

Mycobacterium tuberculosis (TB) peritonitis in patients receiving peritoneal dialysis (PD) carries poor outcomes with reported mortality rates ranging from 15% to 58%.[1–4] Although the 2022 International Society for PD Guidelines recommend antituberculous therapy without mandatory PD catheter removal as the primary treatment of peritonitis caused by *Mycobacterium tuberculosis* (2C), many practices still advocate PD catheter removal and claim favorable outcomes with this approach.[1,2] Unfortunately, diagnosing and initiating early treatment for tuberculous peritonitis in PD pose challenges due to nonspecific clinical symptoms that resemble bacterial peritonitis, characterized by neutrophil predominance (65%–78%).[2,5]

The gold standard for diagnosis, culture for TB, often incurs delays of over 1 month, leading to a prolonged interval from symptoms to treatment initiation.[2,3,6] The GeneXpert *M. tuberculosis* and rifampicin resistance assay, recommended by WHO for initial detection of drug-resistant TB, faces limitations in low resource settings due to sophisticated equipment requirements and high costs.[7] In addition, its sensitivity in detecting extra-pulmonary TB, especially in PD effluent (PDE), is reduced compared to pulmonary TB.[8] The conventional polymerase chain reaction (PCR) is widely available and can rapidly and specifically detect the genetic material of TB bacteria in ascitic fluid.[2,8] Nevertheless, they exhibit lower sensitivity than standard TB culture in samples with a low bacterial load, such as PDE.[2,8] Therefore, we compared PCR assays with and without enriched culture, utilizing samples from PD patients exhibiting culture-positive peritonitis caused by *M. tuberculosis*.

RESULTS

Out of 1843 PD patients with peritonitis from 48 facilities in the MycoPDICS database, 49 episodes (involving 49 participants) met the diagnostic criteria for tuberculous peritonitis. After excluding 10 cases with unavailable specimen for analysis and 2 cases with negative culture for *M. tuberculosis*, 37 episodes involving 37 participants were included in the current study. The median age was 53 (42–62) years, with the average PDE leukocyte count of 420 (250–765) cells/μl and a neutrophil predominance (71%). There were no significant differences in demographics, clinical and *Mycobacterium* characteristics, blood chemistries, and treatment between those who died and those who survived, except that patients who died were more likely to have shorter duration from onset to initiation of anti-TB treatment (Table 1). None had clinical gut perforation or required abdominal exploratory laparotomy.

PD catheters were removed in 28 cases (76%), whereas in 9 cases (24%), they were retained. Notably, among those in whom catheters were removed, 21 cases underwent removal within 5 days after the onset of peritonitis, whereas the remaining 7 cases had catheters removed after 5 days. Patients who underwent PD catheter removal had a higher proportion of cloudy effluent and tended to exhibit higher PDE leukocyte count, % neutrophil, and fever, indicating a more severe clinical presentation of peritonitis. However, it is interesting to note that both groups had a similar death rate, which supports the 2022 International Society for PD approach[2] of obviating the need for removing PD catheters (Supplementary Table S1).

The diagnostic sensitivities of PCR with enrichment were 38%, 46%, 57%, 87%, and 100% on days 3, 5, 10, 15, and 20, respectively, compared to PCR without enrichment (32%, $P < 0.05$). Compared to standard culture, the median time-to-diagnosis following peritonitis onset was significantly shorter for PCR with enrichment: standard culture (32 days, interquartile range [IQR]: 26–35 days), PCR without enrichment (3 days, IQR: 3–4 days), PCR with enriched culture on day 3 (4 days, IQR: 4–4 days), day 5 (6 days, IQR: 6–6 days), day 10 (13 days, IQR: 12–13 days), day 15 (17 days, IQR: 17–18 days), and 20-day enriched PCR (23 days, IQR: 22–23 days); $P < 0.0001$, albeit with similar detection performance. Notably, 10 cases (27%) died before receiving standard culture results, whereas 5 cases (14%) died before obtaining the results of the 15-day enriched PCR (Figure 1).

DISCUSSION

Our findings underscore the potential of PCR after enriched culture as a valuable approach for promptly diagnosing tuberculous peritonitis in patients receiving PD, offering a significantly shorter time from

Table 1. Demographics and patient characteristics

Characteristics	Total ($N = 37$)	Survived ($n = 15$)	Death ($n = 22$)	*P*-value[a]
Age, yr	53 (42–62)	57 (50–61)	50 (41–63)	0.39
Male, %	20 (54)	8 (53)	12 (55)	0.94
BMI, kg/m^2	23 (19–24)	24 (19–25)	22 (20–23)	0.35
Diabetes, %	21 (57)	9 (62)	12 (55)	0.74
PD vintage, yr	1.7 (1.0–3.3)	1.5 (0.9–2.6)	2.0 (1.1–3.4)	0.50
Serum creatinine, mg/dl	8.3 (6.7–11.2)	8.8 (7.5–11.0)	7.8 (4.6–12.0)	0.93
Serum potassium, mEq/l	3.8 (3.2–4.3)	3.9 (3.5–4.3)	3.5 (3.1–4.5)	0.67
Serum calcium, mg/dl	8.4 (7.7–9.0)	8.5 (8.3–9.9)	8.3 (7.5–8.7)	0.13
Serum phosphate, mg/dl	4.0 (2.6–5.2)	3.4 (1.8–4.6)	4.0 (3.4–6.2)	0.19
Serum albumin, gm/dl	2.3 (1.8–2.6)	2.4 (2.2–2.9)	2.1 (1.7–2.4)	0.13
Hemoglobin, gm/l	8.7 (7.1–10.3)	8.3 (6.5–10.3)	9.1 (7.9–11.1)	0.50
PDE cell count, cells/μl	420 (250–765)	560 (309–1497)	302 (220–550)	0.06
PDE neutrophils, %	71 (53–90)	85 (59–98)	67 (49–88)	0.12
Abnormal chest x-ray, %	6 (50)	1 (20)	5 (83)	0.08
Cloudy effluents, %	27 (73)	11 (73)	16 (73)	0.97
Fever, %	8 (22)	4 (27)	4 (18)	0.54
Abdominal pain, %	13 (35)	5 (33)	8 (36)	0.85
Onset of anti-TB treatment, days after peritonitis	36 (22–52)	27 (19–48)	64 (52–78)	0.03
Duration of received anti-TB treatment, days	310 (42–519)	385 (187–524)	38 (10–53)	0.05
Isoniazid resistance	2 (8)	1 (11)	1 (6)	0.63
Rifampicin resistance	0 (0)	0 (0)	0 (0)	
Ethambutol resistance	0 (0)	0 (0)	0 (0)	
PD catheter removal, %	28 (76)	13 (87)	15 (68)	0.20

BMI, body mass index; HD, hemodialysis; PD, peritoneal dialysis; PDE, PD effluent; TB, *Mycobacterium tuberculosis*.
[a]Test of difference using Mann-Whitney U and chi-square test for continuous and categorical measures, respectively.

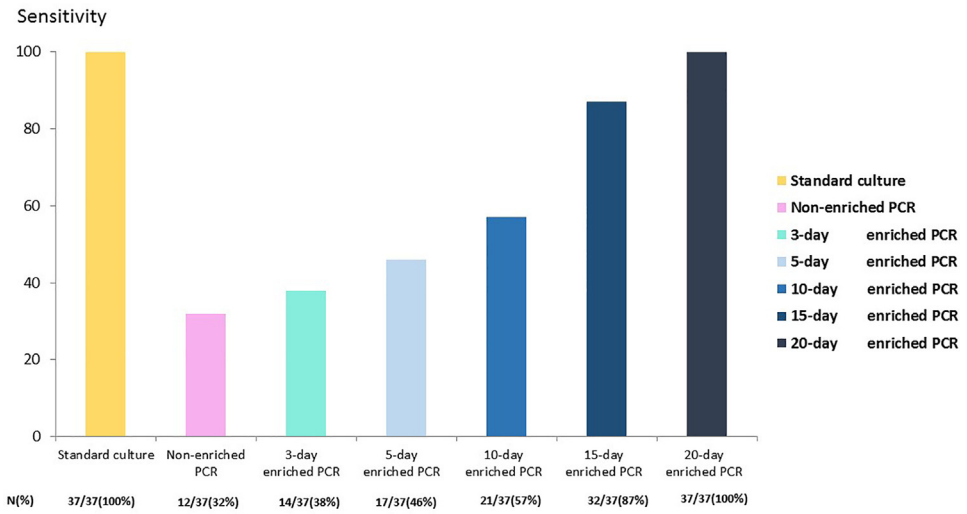

Figure 1. Comparison between sensitivity between conventional versus enriched PCR in detecting *Mycobacterium tuberculosis* from the PD effluents. PCR, polymerase chain reaction; PD, peritoneal dialysis.

peritonitis onset to diagnosis while maintaining diagnostic sensitivity comparable to the gold standard culture technique. Our study confirms the limitations of conventional PCR in diagnosing PD-associated tuberculous peritonitis, with a sensitivity of only 32% in patients on PD.

PCR after enriched culture has emerged as an alternative for diagnosing PD-associated tuberculous peritonitis in resource-limited settings. PCR with enrichment exhibited higher sensitivity for detection of TB in PDE on day 3, matching the sensitivity of the standard culture on day 20 but with a more favorable time-to-diagnosis following peritonitis onset. The barriers to early TB identification, including a small number of acid-fast bacilli diluted in PDE and the less virulent nature of TB, are effectively overcome by PCR with enrichment, thereby showcasing its superiority over conventional PCR (without enrichment).

PD-associated tuberculous peritonitis is associated with significant morbidity and mortality. Our study reveals a mortality rate of 27% among patients who died before receiving a diagnosis from standard culture, emphasizing the crucial need for early diagnosis. The PCR with 15-day enriched culture demonstrated high sensitivity for TB detection in PDE (87%), outperforming modern liquid culture techniques requiring up to 10 weeks for a positive result.[9] Even in cases where the culture alarm was negative, PCR with enrichment at 3 days exhibited higher sensitivity than PCR without enrichment, whereas the enrichment at 20 days matched standard culture for TB detection with a substantially shorter mean time-to-diagnosis following peritonitis onset (23 vs. 33 days). Early detection of PD-associated tuberculous peritonitis offers the opportunity for prompt treatment and may lead to better outcomes compared to cases where diagnosis is delayed. The preferred time point for enriched culture is 15 days, because it had shown high sensitivity (87%) and shortened the time-to-diagnosis from 32 to 17 days. However, it is imperative to reassess and potentially modify the recommended time point for enriched culture based on the specific clinical context and the urgent need for timely decision-making in managing refractory peritonitis cases.

The study possesses notable strengths. First, it is the inaugural proof-of-concept evaluation of the diagnostic efficacy of PCR with enrichment for PD-associated tuberculous peritonitis. PCR with enrichment is practical and requires no sophisticated setup, suggesting a feasible and expedited diagnostic and treatment process for patients on PD with tuberculous peritonitis. Second, the study was conducted across multiple centers, including community-based hospitals, providing insights into real-life practices (Supplementary methods).[S1–S3] Lastly, all recruited participants received antituberculous medications, aligning with the 2022 International Society for PD Guidelines recommendation.[2] Despite these strengths, the study has limitations. First, only 37 patients (79%) with tuberculous peritonitis had specimens available for analysis, potentially reducing the study's statistical power. Further research with a larger sample size is warranted. Second, the study was conducted during the COVID-19 pandemic, resulting in slower recruitment and potential delays in PD catheter removal based on physician justifications. Consequently, the study's outcome results may not be generalizable to situations without pandemic-related challenges. Furthermore, although PCR with enriched culture is helpful for facilitating earlier diagnosis of tuberculous

peritonitis, it is still far from perfect with potential clinical delays in diagnosis leading to significant morbidity and mortality. Further innovative approaches to minimize such delays are needed.

In conclusion, PCR with enriched culture represents a promising alternative for the early detection of PD-associated tuberculous peritonitis, demonstrating performance equal to standard culture in PDE. Given the time-consuming nature of PD-associated tuberculous peritonitis diagnosis, initiating antituberculous treatment, with or without catheter removal, is crucial for achieving favorable outcomes.

APPENDIX

List of The Advisory Board of Peritoneal Dialysis, Nephrology Society of Thailand

Anutra Chittinandana and Duangkamol Wongsawan, Bhumibol Adulyadej Hospital; Chanchana Boonyakrai, Taksin Hospital; Dhavee Siriwong, Khon Kaen University; Guttiga Halue, Phayao Hospital; Monchai Siribamrungwong, Lerdsin Hospital; Pichet Lorvinitnun, Sunpasitthiprasong Hospital; Pornchai Kingwatanakul, Department of Pediatrics, Chulalongkorn University; Solos Jaturapisanukul, Vajira Hospital; Somchai Yongsiri, Burapha University; Surapong Narenpitak, Udonthani Hospital; Tanawoot Limlek, Krabi Hospital; Thanee Eiamsitrakoon, Chulabhorn international College Medicine, Thammasat University; Uraiwan Parinyasiri, Songkhla Hospital; Yuttitham Suteeka, Chiangmai University.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Table S1. Patient characteristics and laboratory findings in individuals with versus without PD catheter removal.

STROBE statement checklist.

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