

Original Research Article

Dialysate color and its role in presumptive diagnosis of dimorphic fungi in peritoneal dialysis associated fungal peritonitis

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ABSTRACT

Background: Fungal peritonitis is a rare but serious complication of peritoneal dialysis (PD). Dimorphic fungi are described in peritonitis at a much lower percentage than yeasts are, but their involvement in it is growing.

Methods: The study was based on the analysis of available data over a period of 16 years. The sample size was of 421 ESRD patients on CAPD. We retrospectively identified 72 cases of fungal peritonitis. In the present study we assessed the frequency of dimorphic fungi as a single pathogen, its association with the dialysate colour along with the predictors and its impact on the outcome of the patients.

Results: In between January 2000 and October 2016, in present retrospective study, we identified 72 episodes of peritoneal dialysis associated with fungal peritonitis. Among the 72 fungal episodes, 83.3% were *Candida* species, 1.5% were yeast and the remaining 15.2% were dimorphic fungi. The macrolevel observations of dialysate showed greyish, blackish, greenish, pinkish, cloudy, bluish green and cloudy colours that developed due to the pigmentation of fungi and fungal spores. The *Aspergillus* species predominates amongst the dimorphic fungi. Outcome analysis revealed that the loss of life (14.7%) was more frequent in diabetics while the reinsertion of catheter failed in cases of glomerulonephritis. It was also noticed that de novo infections were more frequent among the diabetics and that the previous bacterial peritonitis episode was the strongest predictor amongst them all.

Conclusions: The premise of the current study illustrates the fact that the dialysate colour can be put to use as an early warning system and by combining the macroscopic observations of the dialysate with the results of microscopy and culture, the diagnosis of Fungal peritonitis due to dimorphic fungi will not be missed. Also worth noting is the point that an early assessment of predictors alongside that of an antifungal coverage can lead to a reduction in the rates of morbidity which in turn will shorten the stay in hospital. This will prevent any further nosocomial infection, antifungal resistance and chances of treatment failure.

Keywords: CAPD, Color of dialysate, Dimorphic fungi, End stage renal disease, Fungal peritonitis, Predictors

INTRODUCTION

Fungal peritonitis (FP) is a rare but potentially fatal complication of chronic peritoneal dialysis (PD), associated with high morbidity and mortality ranging between 20% and 30%. Even if it does not lead to death, the inflammatory process usually causes irreversible

damage to the peritoneal membrane with a subsequent dropout from PD therapy.¹ It penetrates the peritoneal cavity through the intraluminal or the periluminal pathways and crosses the intestinal mucosa. It may also enter through the hematogenic pathway due to a distant fungal infection.² The lethality, although variable, remains very high because the fungi form a biofilm on

the surface of the silastic catheters that reduces the penetration of antifungal agents.^{3,4} Most FP cases were caused by yeasts under the *Candida* species, they account for 70%-90% in adults and 80%-100% in the paediatric population. Dimorphic fungi (moulds) such as *Aspergillus*, *Penicillium*, and the other yeasts were much less common and together, they represent about 10% of the cases.¹ Therefore, this retrospective study was carried out to assess the frequency of dimorphic fungi as a single pathogen, its association with the dialysate colour along with the predictors and its impact on the outcome of the patients.

METHODS

It was a retrospective study involving patients undergoing continuous ambulatory peritoneal dialysis (CAPD) at our centre. These patients developed peritonitis over a period of 16 years, from January 2000 to October 2016. Out of 421 patients, 72 end stage renal disease (ESRD) patients developed fungal peritonitis. Aerobic, anaerobic, mycobacterium and polymicrobial peritonitis were excluded from the analysis due to their different outcomes.

As per the peritoneal dialysis related infection recommendations published by ISPD in 2010 and 2016, the patient's exchange bags, containing the effluent dialysate, were received in the microbiology laboratory for a macro level examination, a microscopic examination and simultaneous culturing.^{5,6} From these bags, 100ml of fluid was withdrawn with a sterile needle and syringe under aseptic conditions. The fluid was centrifuged in sterile tubes at a rate of 3000g for 15 minutes and supernatant was discarded, leaving 0.5ml. In the centrifuged deposit, 10ml of sterile distilled water was added and the mixture was shaken vigorously on vortex for 30sec. This mixture was then divided into 4 parts of 1ml, 3ml, 3ml and 3ml each. The 1ml part was further divided for staining characteristic like gram stain, Z.N. stain and lacto phenol cotton blue film while the 3ml part in the FA bottle was used for isolation of aerobes and fungi, the 3ml part in the FN bottle and the remaining 3ml part in the MP bottle were used for the isolation of anaerobes and mycobacterium respectively. These three inoculated bottles were further incubated in BactAlert 3D system following standard protocols. The isolated fungi were re-examined microscopically to ensure the staining and morphologic characteristic. Each positive specimen was inoculated on sabouraud dextrose agar (M286) and sabouraud cycloheximide chloramphenicol agar (M664).

Cultures were routinely incubated at 250 c and 370 c and examined daily for a period of four weeks. The identification of individual fungi was based on standard methods such as microscopy, morphology, colonial characterization, pigment production, rate of growth whereas the identification of the yeast was done using Vitek-2 (Biomeurix, France). Here it is important to note that what took a long time in the above process, it took

comparatively very short spell of time for the same e.g. microscopy became available within 3 to 5 hours and identification along with antibiogram of *Candida* species and yeast was made available within 48 hours while dimorphic fungi was identified within 4-6 days after the clinical diagnosis was made.

The definitions used in the article are as follows. Predictors have been defined as predictors for developing FP. Previous bacterial peritonitis episode suggests that fungal peritonitis appears after the episodes of bacterial peritonitis. Prior antibiotic use means the use of antibiotic for a suspected infectious disease in a period of 30 days, 3 months, or 6 months prior to the development of FP. Prolonged time in the dialysis program means prolonged use of catheter for several years. Prolonged time with the peritoneal catheter inserted means maintaining the catheter after detecting the fungal infection. Use of immunosuppressive agents refers to steroid or immunosuppressive use for at least 2 weeks prior to the diagnosis of FP. Hospitalization means a risk when an infection of nosocomial origin occurs in the 30 days, 3 months, or 6 months prior to development of FP. Co-existence of an extra peritoneal fungal infection illustrates when a patient is suffering from extra peritoneal fungal infection which causes a fungal peritonitis through a haematogenic pathway. De novo means those cases of FP which occur due to direct contamination of dialysis during the exchange procedure, underlying intestinal pathology such as diverticulosis in the host and environmental contaminations.⁷⁻⁹ Data were expressed as mean±standard deviation.

RESULTS

During the period from January 2000 to October 2016, a total of 421 ESRD patients were initially on CAPD. The total number of episodes of fungal peritonitis during the entire period was 72 and the average rate of fungus peritonitis was 2.6 episodes/CAPD year. Their base line and the demographic data were described as follows. Out of the total FP population, the males present were 36.4% and the females present were 63.6%. The mean age of the study population was 58.0±6.46 and the mean duration on CAPD before the development of the fungal infection was 18.00±8.473 months. The predominant causes of ESRD in this group were diabetic nephropathy (54.5%), glomerulonephritis (36.4%) and hypertension (9.1%). Among the 72 fungal episodes, 83.3% were *Candida* species, 1.5% yeast and 15.2% were dimorphic fungi. Non-albicans *Candida* species outnumbered the *Candida albicans* while the *Aspergillus* species predominates among dimorphic fungi.

At the macro level, the observations of 11 cases of FP due to dimorphic fungi were novel and interesting. The bags revealed different colors of dialysate which became darker when kept at room temperature for three days. When these bags were correlated with the fungal colonies, it was found that these grayish, blackish,

greenish, pinkish, cloudy, bluish green and cloudy colors that were present in the dialysate developed due to the pigmentation of fungi and fungal spores. The spectrum of dimorphic fungi, their pigments in dialysate bags, macroscopic pictures and microscopic pictures were illustrated (Figures 1 and 2).

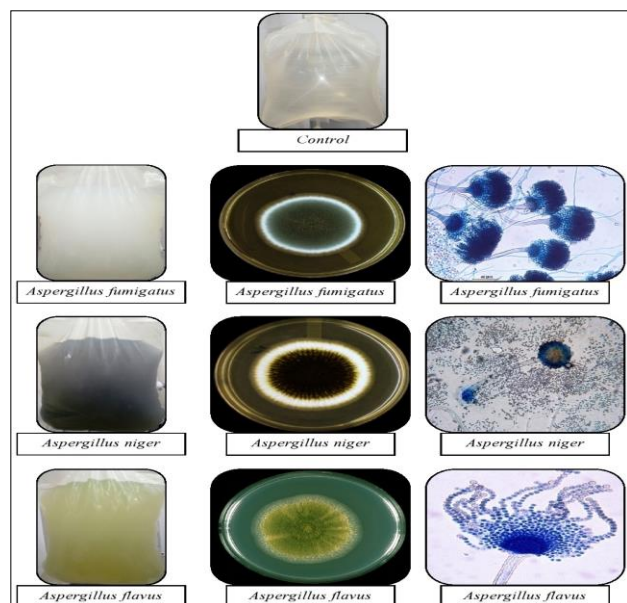


Figure 1: Colour of dialysate with corresponding Macroscopic and Microscopic slides of diamorphic fungi.

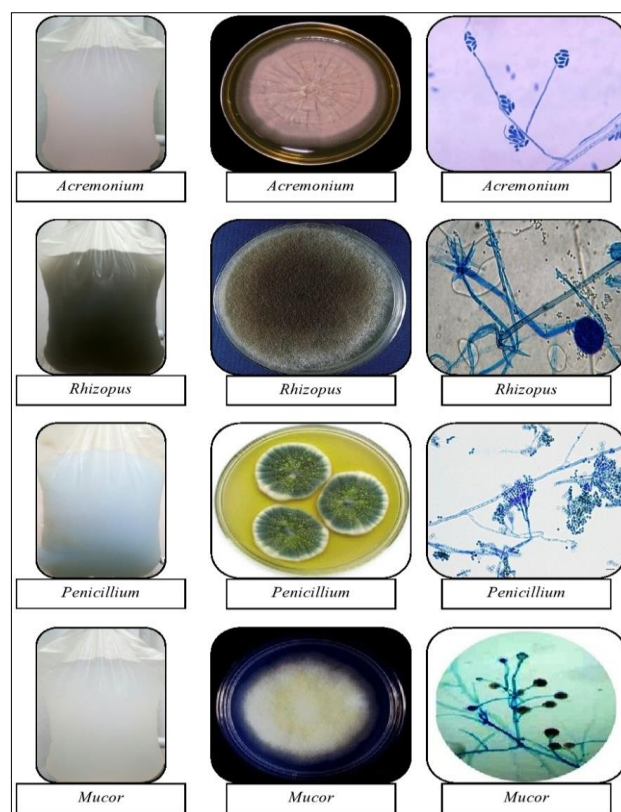


Figure 2: Colour of dialysate with corresponding Macroscopic and Microscopic slides of diamorphic fungi.

Table 1: Complete summary of patients those developed fungal peritonitis due to dimorphic fungi.

Age/Sex	Primary cause of ESRD	Predictors	Fungus	Antifungal regimen	Outcome
46/M	Glomerulonephritis	Previous bacterial peritonitis episodes	<i>Aspergillus flavus</i>	Ampho and It	Catheter removal, Died
52/F	Diabetes	Previous bacterial peritonitis episodes	<i>Aspergillus fumigates</i>	Ampho and It	Catheter removal, Reinsertion failed
45/F	Diabetes	Denovo	<i>Penicillium</i>	Ampho and It	Catheter removal, Died
57/M	Diabetes	Denovo	<i>Acremonium</i>	Ampho and It	Catheter removal, Died
64/M	Glomerulonephritis	Previous bacterial peritonitis episodes	<i>Aspergillus flavus</i>	Ampho and It	Catheter removal, Reinsertion successful
48/F	Diabetes	Wide-spectrum antibiotic treatment in previous months	<i>Mucor</i>	Ampho and It	Catheter removal, Died
37/F	Diabetes	Prolonged time with the peritoneal catheter inserted	<i>Rhizopus</i>	Ampho and It	Catheter removal, Died
51/F	Glomerulonephritis	Wide-spectrum antibiotic treatment in previous months	<i>Aspergillus flavus</i>	Ampho and It	Catheter removal, Reinsertion failed
41/M	Hypertension	Wide-spectrum antibiotic treatment in previous months	<i>Aspergillus fumigates</i>	Ampho and It	Catheter removal, Reinsertion failed
63/F	Diabetes	Previous bacterial peritonitis episodes	<i>Aspergillus niger</i>	Ampho and It	Catheter removal, Reinsertion failed
28/F	Glomerulonephritis	Previous bacterial peritonitis episodes	<i>Aspergillus fumigates</i>	Ampho and It	Catheter removal, Reinsertion failed

Microscopic examination of these 11 cases of FP of the dialysate pellets with lacto phenol cotton blue film revealed conidia, mycelia like structures and in a few cases entangled mass of mycelia. Culture examination revealed that *Aspergillus* was the most frequent fungi isolated. An association between the predictors and the dimorphic fungi revealed 18.2% de novo episodes and the remaining 81.8% episodes were predisposed by different predictors. The strongest predictor to predispose the dimorphic fungi was the previous bacterial peritonitis episode. A Complete summary of fungal peritonitis due to dimorphic fungi is depicted (Table 1).

DISCUSSION

Dimorphic fungi or moulds, like yeasts, are widely found in nature. They are described in peritonitis at a much lower percentage than yeasts are, but their involvement in it is growing and in some series, they comprise of 40% of the cases.¹⁰ Predictors for developing FP could not be clearly determined. Numerous situations were listed which play an important role in the appearance of mycotic infection. The strongest predictors for FP in PD patients were previous bacterial peritonitis, prolonged use of antibiotics, prolonged time in the dialysis program, prolonged time with the peritoneal catheter inserted, use of immunosuppressive agents, hospitalization and co-existence of an extra peritoneal fungal infection.

The novel observation in our study was that we linked the colour of the dialysate with the dimorphic fungi. The different colors of the dialysate were due to the fermentation of sugar present in it and the pigments appeared as either metabolic end product or due to the colour of spores. Hence, it can be said that a macro level observation of the dialysate is equally important in the presumptive diagnosis of fungal peritonitis due to dimorphic fungi. Out of the 11 episodes 27.2% cases were due to *Aspergillus flavus*, and *Aspergillus fumigates* each while 9.10% due to *Aspergillus niger*, *Penicillium*, *Mucor*, *Rhizopus* and *Acremonium* each.

Association among the primary cause of ESRD, pathogens and outcome showed that FP due to dimorphic fungi was more complicated to treat and the cure rate observed was only 60%. The fact that these fungi form a biofilm on the surface of the silastic catheters that reduces the penetration of antifungal agents is why these fungi are more resistant to antifungals and this in turn has sparked a special clinical interest in them. As the data revealed, the loss of life (14.7%) was more frequent in diabetics while the reinsertion of catheter failed in cases of glomerulonephritis. It was also noticed that de novo infections were more frequent among the diabetics.

Wang et al reported 30% of the episodes of fungal peritonitis which were caused by very rare organisms, such as *Trichosporin*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Acremonium*, *Rhodotorula*, and *Cryptococcal* species.¹¹ Yang et al reported 21 episodes

of fungal peritonitis amongst which the *Candida* species (72%) accounted for the most common fungi, the rest 28% episodes were due to filamentous fungi.¹²

Despite the fact that the *Aspergillus* genus is one of the most frequent in clinical practices, the cases of peritoneal infection are not very numerous. *Penicillium* is referred to on several occasions but without listing the species.¹⁰ Episodes of peritonitis due to *Paecilomyces* are detected quite rarely.¹³⁻¹⁴ The so called black yeasts of the *Expholia* genus have also been implicated in peritoneal infection cases as well as *Curvularia* genus which rarely causes disease in man.^{15,16} Zygomycetes are the fungi that are uncommon as peritonitis agents in patients undergoing peritoneal dialysis but are associated with a high mortality rate due to their lack of response to antifungals. Cases of peritonitis due to *Rhizopus* sp. and *Cunninghamella* have been reported.¹⁰

Other filamentous fungi are less common in peritonitis cases in patients undergoing peritoneal dialysis. Some are known as opportunistic pathogens: *Alternaria*, *Bipolaris*, *Aureobasidium pullulans*, *Scedosporium apiospermum*, *Scopulariopsis* sp., *Cladosporium* sp. and *Madurella mycetomatis*.^{9,17-25} Others are less commonly reported as pathogens: *Trichoderma*, *Chaetomium globosum*, *Chrysonilia sitophila*, *Lecytophora mutabilis*, *Hormonema dematioides*, and *Verticillium* sp.²⁶⁻³¹

CONCLUSION

Because of the relative rarity of dimorphic fungi in FP, our total number of episodes remains quite small despite a long follow-up time. Furthermore, the association among the primary cause of ESRD, predictors, dimorphic fungi and the outcome were all limited due to a small sample size. Despite these limitations, from the results obtained in the forging paragraphs, we believe that this study is important considering its unique description of dimorphic fungi in FP. Hence, we over here for the first time in India are reporting the incidence of dimorphic fungi from the dialysate pellets which is unique in itself.

Therefore, the focus of the current study establishes that the colour of the dialysate can be used as an early warning system. If we combine the macroscopic observations of the dialysate with the results of microscopy and culture, the diagnosis of FP due to dimorphic fungi will not be missed. Furthermore, an early assessment of predictors along with an optimum antifungal coverage can lead to a reduction in morbidity, allowing shorter stay in the hospital and thereby preventing further nosocomial infection, antifungal resistance and chances of treatment failure.

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Ethical approval: The study was approved by the institutional ethics committee

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