

## Detection of Fungal Infection in Patients on Hemodialysis with Double Lumen Catheter by Conventional and Molecular Methods

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### ABSTRACT

**Background:** In hemodialysis patients, tunneled, cuffed, double-lumen catheters are frequently used for long-term venous access. Infections associated with these catheters are one of the major causes of morbidity and resource usage in the hemodialysis population. **Objective:** The aim of the current study is to investigate the frequency and types of fungal infection in patients on hemodialysis with double lumen catheter in Imamein Kadhimein Teaching Hospital and Al-Karama Teaching Hospital, Iraq.

**Material and methods:** For the purpose of detecting a fungal infection, 19 blood samples from hemodialysis patients with double lumen catheters were subjected to both BacT/ALERT system (Blood Culture) and molecular approaches (Multiplex Real Time PCR). **Results:** By using traditional techniques, the fungi *Candida spp.* (2%), and *Cryptococcus spp.* (1%) were detected. The following fungi were identified using molecular techniques: (2%) *R.oryzae*.

**Conclusion:** The nephrologist should be aware of the many fungal infections that patients receiving hemodialysis with double lumen catheters may acquire, including *R.oryzae*, *Candida spp.*, and *Cryptococcus spp.* The fungi mentioned above may be acquired during hemodialysis or may already be present as underlying illnesses. This study established that hemodialysis patients with renal impairment who use double lumen catheters could have several complications.

**Keywords:** Fungal Infection, Hemodialysis, Double lumen, M-RTPCR, BacT/ALERT, Case series, Al-Nahrain University.

### INTRODUCTION

With a rising worldwide burden among solid organ and bone marrow transplant recipients, cancer patients, people living with HIV and those receiving immunomodulatory therapies, invasive fungal diseases (IFDs) represent a serious hazard to human health, especially in the immunocompromised. The pathogens *Candida spp.* and *Aspergillus spp.* are the most frequent causes of IFDs, followed by Pneumocystis and the Mucorales, with the prevalence of IFDs varied by geographic location and patient population from pathogen to pathogen<sup>(1)</sup>. For hemodialysis and IV medicine, central venous catheterization with a double-lumen catheter is frequently used<sup>(2)</sup>. As more permanent vascular access, arteriovenous fistulas, is used usually, but tunneled and cuffed double lumen catheters are being utilized more frequently for acute procedures<sup>(3)</sup>. Infections associated with catheters are a frequent problem and a significant source of morbidity and death in these individuals<sup>(4)</sup>.

Fever, localized redness, and discomfort at the insertion site are less sensitive and specific indicators of catheter-related infection. The most accurate way to identify fungi is by culture, which also enables the assessment of an antifungal's susceptibility<sup>(5)</sup>. To identify a catheter-related infection, a quantitative blood culture collected from a peripheral vein and a culture of the central venous catheter tip can be employed. Samples for

culture should be taken from a catheter and a peripheral vein from patients in whom CRI is suspected<sup>(6)</sup>.

Molecular methods for the precise detection of certain genera or species, as well as broad range (or pan-fungal) assays to capture "all fungi," are available for the direct detection of fungal DNA in clinical specimens<sup>(7)</sup>. Initially, the detection of mucorales DNA from clinical specimens was primarily established for tissue samples as an adjunct to histopathological and microbiological diagnosis<sup>(8,9)</sup>. However, recently, molecular blood and even urine tests were developed<sup>(10)</sup>. The load of circulating mucorales species DNA in serum was shown to be extremely high compared to invasive aspergillosis, possibly due to the angioinvasive character of mucorales infections<sup>(11)</sup>. As a result, blood samples are suitable for therapeutic monitoring and suspicion-free screenings of high-risk patients<sup>(12)</sup>. The aim of the current study is to investigate the frequency and types of fungal infection in patients on hemodialysis with double lumen catheter in Imamein Kadhimein Teaching Hospital and Al-Karama Teaching Hospital, Iraq.

### PATIENTS AND METHODS

A total of 19 patients aged between 13 and 70 years, who were receiving hemodialysis with double lumen catheters in Imamein Kadhimein Teaching Hospital and Al-Karama Teaching Hospital were included in our study. Blood samples from participants were collected from December 2020 to February 2021.

The research was carried out in the Imammain Kadhimain Teaching Hospital and the College of Medicine-Al-Nahrain University's microbiological laboratories. About 5 ml of whole blood was drawn from each patient. The blood sample was divided into two parts; the first of which was placed in EDTA tubes (2 ml) for molecular detection and the second of which was placed in BacT/ALERT bottles (3 ml).

According to “BacT/ALERT 3D Microbial Identification System/Biomerieux” protocol the samples were cultured to detect the fungemia via conventional methods <sup>(13)</sup>.

The Genomic DNA was separated from blood sample according to the protocol of the kit “ReliaPrep™ Blood gDNA Miniprep System kit, Promega”. Thereafter, molecular method (Real-Time PCR) was used for the detection of fungal DNA in the blood sample via the Mic qPCR Cycler, (Bio Molecular System, Australia) <sup>(14)</sup>.

### Ethical consent

The Al-Nahrain University, College of Medicine's Ethical Committee gave its approval to this study on November 17, 2020 (No. 20201080). The research protocol did not interfere with any medical recommendations or prescriptions. Informed consent was obtained from the patient's relatives or the patient himself when he was still conscious with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

### Statistical analysis

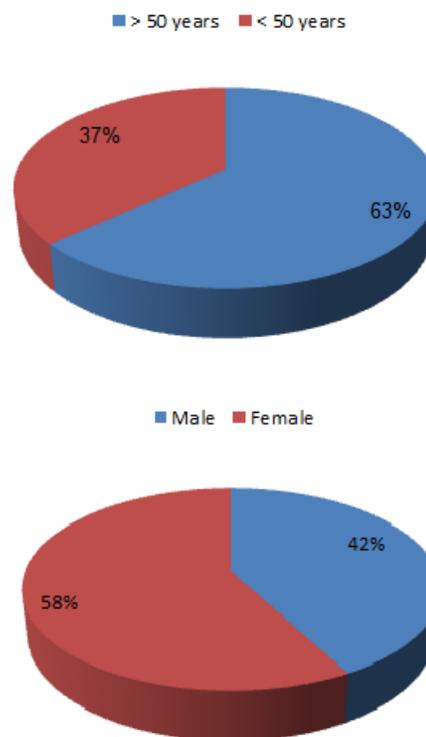
The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 20 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ( $\chi^2$ ) and Fisher's exact test were used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). Independent samples t-test was used to compare between two independent groups of quantitative variables. P value  $\leq 0.05$  was considered significant.

## RESULTS

### Age and Sex Distribution of Hemodialysis Patients with Double Lumen Catheter:

A total of 19 patients with renal failure who are receiving hemodialysis attended Imamein Kadhimein Teaching Hospital and Al-Karama Teaching Hospital in which 11 (58%) were females and 8 (42%) were males. Regarding age of these patients, it was ranging between 13 and 70 years, with mean of 47.32 (SD 19.37). The total number

of the patients were classified into 2 age groups per decade; the first group (>50 years) include (63.0%) of the patients, and the second group (< 50 years) was 37% [Figure 1].



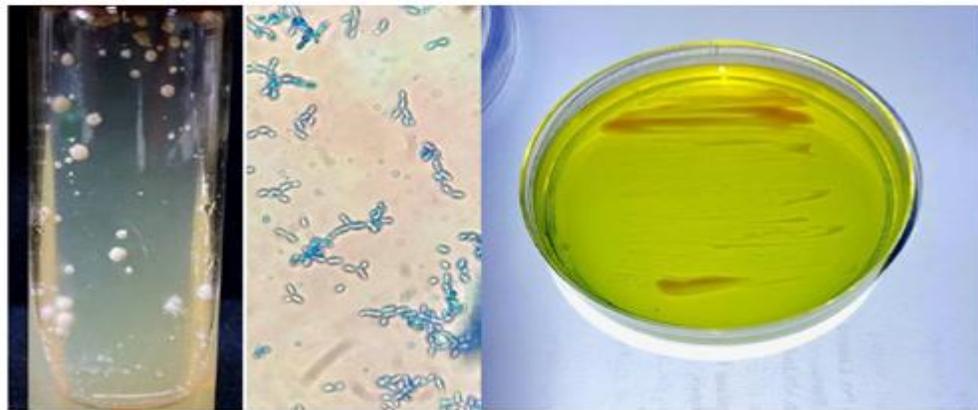
A. B.  
Figure 1: (A) Sex distribution of patients with renal failure on hemodialysis, (B) Age distribution of patients with renal failure on hemodialysis.

### Laboratory Diagnosis of Fungi Infection by Conventional and Molecular Methods:

Regarding blood culturing via BacT/ALERT culture bottle, it was found that 3 (16%) *Candida utilis* (2), *Cryptococcus spp.* (1), of the cases were positive, while 16 (84%) were negative [Table 1, Figures 2 and 3]. Then when it comes to the molecular method (Multiplex Real Time PCR) only 2 (11%) out of the 19 patient were diagnosed with *Rhizopus oryzae* [Table 1, Figure 4].

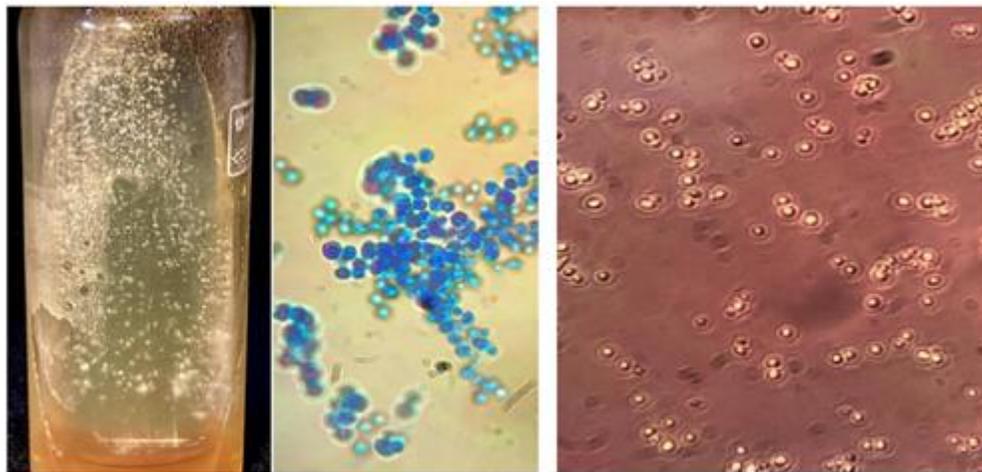
Table 1: Results of the conventional and molecular methods used for the detection of fungal infection among patients on hemodialysis.

Patients	Conventional methods (BacT/ALERT)	Molecular method (MRTPCR)
A.S	<i>cryptococcus spp.</i>	
F.M	<i>Candida utilis</i>	
A.S.J	<i>Candida utilis</i>	<i>Rhizopus oryzae</i>
A.A		<i>Rhizopus oryzae</i>



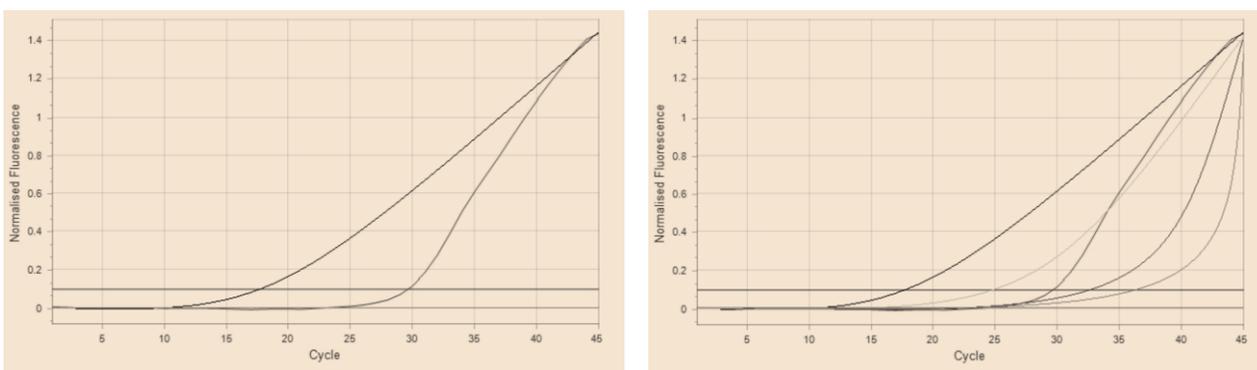
A. B. C.

**Figure 2:** (A) *Candida utilis* on SDA medium at 27 °C. (B) Microscopic observation (400) x, stained with lactophenol cotton blue. (C) *C. utilis* colonies appears pale pink on HiCROME candida differential agar medium at 35 °C.



A. B. C.

**Figure 3:** (A) *Cryptococcus spp.* on SDA medium at 27 °C. (B) Microscopic observation (400) x stained with lactophenol cotton blue. (C) *Cryptococcus spp.* stained smear by india ink (1000) x.



A. B.

**Figure 4:** (A) Amplification plots at the end of M-RT PCR run for the positive and negative controls *Mucor spp.* and *R.oryzae*. (B) Amplification plots at the end of M-RT PCR run for the ITS1, ITS2 gene as positive results, *R.oryzae* and *Mucor spp.* positive controls and negative control.

**Fungal Infection in Relation with Demographic Information among Patients on Hemodialysis with Double Lumen Catheter:**

Regarding the association between age group and fungal infection in hemodialysis patients, it was found that age has no significant association with fungal infection detected by conventional and molecular methods (P= 0.525<sup>NS</sup>, respectively) [Table 2].

**Fungal Infection in Relation with the Causes of Renal Failure among Hemodialysis Patients with Double Lumen Catheter:**

Regarding the relation of fungal infection among patients on hemodialysis and the cause of renal failure, it was found no association with fungal infection detected by conventional nor with molecular method (P=0.352<sup>NS</sup>) [Table 3].

**Fungal Infection in Relation with Hemodialysis Session/ Hours among Hemodialysis Patients with Double Lumen Catheter:**

Regarding the relation of fungal infection among patients on hemodialysis and the hemodialysis session/hour, there is a significant association has seen by both method of detection (P=0.033\*), in case of confidence interval which was (95%) is proved that patients undergoing hemodialysis in the period of time >3 hours are at high risk to mycosis then those <3 hours (P=1.667) [Table 4].

**Fungal Infection in Relation with blood transfusion before One Year among Hemodialysis Patients with Double Lumen Catheter:**

Regarding the relation of fungal infection among patients on hemodialysis and the number of blood transfusion, it was seen no significant association regarding the conventional or molecular methods (P=0.728<sup>NS</sup>) [Table 5].

**Table 2: Fungal Infection in Relation with Demographic Information among Hemodialysis Patients with Double Lumen Catheter.**

Variable		Infection		Total	P value	Value	95% Confidence Interval	
		Positive	Negative				Lower	Upper
Age groups	>50 years	3	9	12	0.525 <sup>NS</sup>	1.143	0.732	1.784
	%	25%	75%					
	<50 years	1	6	7				
	%	14.29%	85.71%					

**Table 3: Fungal Infection in Relation with Cause of renal failure among Hemodialysis Patients with Double Lumen Catheter.**

Variable		Infection		Total	P value	Value	95% Confidence Interval	
		Positive	Negative				Lower	Upper
Cause of renal failure	Chronic disease	4	11	15	0.352 <sup>NS</sup>	0.733	0.540	0.995
	%	26.67%	73.33%					
	Congenital	0	4	4				
	%	0.0%	100%					

**Table 4: Fungal Infection in Relation with hemodialysis session hour among Hemodialysis Patients with Double Lumen Catheter.**

Variable		Infection		Total	P value	Value	95% Confidence Interval	
		Yes	No				Lower	Upper
Hemodialysis session hr.	>3 sessions	4	6	10	0.033 *	1.667	1.005	2.765
	%	40%	60%					
	3 sessions	0	9	9				
	%	0.0%	100%					
	%	21.43%	78.57%					

**Table 5: Fungal Infection in Relation with blood transfusion last year among Hemodialysis Patients with Double Lumen Catheter.**

Variable		infection		Total	P value	Value	95% Confidence Interval	
		Positive	Negative				Lower	Upper
Blood transfusion last year	Yes	1	4	5	0.728 <sup>NS</sup>	0.917	0.073	11.577
	%	20%	80%					
	No	3	11	14				
	%	21.43%	78.57%					

**DISCUSSION**

**Laboratory Diagnosis of Fungi Infection by Conventional and Molecular Methods:**

Infection-related morbidity and mortality are more likely in hemodialysis patients with central venous catheters <sup>(15)</sup>. Regarding the fungal infection results (*Candida utilis*, *Cryptococcus spp.*, *Rhizopus oryzae*) detected by conventional and molecular methods, they agree with previous studies i.e, a study done by **Ali et al.** in (2018) who proved that catheter related infection is common in patients on hemodialysis with double lumen in which out of (129) patients the ratio of catheter related infection was found to be 19 (14.7%) <sup>(16)</sup>.

Regarding another study done by **Parameswaran et al.** in (2010) as a case-control study was conducted over 19 months involving 232 patients showed that 16% patients diagnosed with *Candida spp.* using the BacT ALERT for blood culturing <sup>(17)</sup>. Another study done by **Fujisawa et al.** in (2020) showed that hemodialysis patients are at high risk for mucormycosis due to long term of hemodialysis treatment and the use of certain medications specially in elderly <sup>(18)</sup>.

**Fungal Infection in Relation with Demographic Information among Patients on Hemodialysis with Double Lumen Catheter:**

In the current study, the results disagree with previous studies. A study done by **Loster et al.** in (2016) showed that age factor can increase the risk of fungal infection, in which ( $\leq 50$ ) years old have the higher rate of infection with *Candida spp.* (61.4%), and when it comes to other age groups the rate of infection occurred about  $\geq 50/\leq 60$  year (43.7%),  $\geq 60/\leq 70$  year (49.6%) and  $\geq 70$  year (44.0%) <sup>(19)</sup>. The discrepancies among these results may be due to the number of samples included in each study, racial factor differences.

**Fungal Infection in Relation with the Causes of Renal Failure among Hemodialysis Patients with Double Lumen Catheter:**

The results of the current study disagree with the previous studies done. A study done by **Singh et al.** in (2016) that out of 206 patients, 74 patient were with IFD (invasive

fungal disease) the majority group was nonsurgical/non-trauma (72.9%) with a mortality rate reach to (50%), diabetes mellitus was identified in the multivariate analysis as an independent predictor related with early IFD critically extremely sick individuals<sup>(20)</sup>. The disparities in the quantity of samples used in each study and racial factors may be the cause of the inconsistencies between these results.

**Fungal Infection in Relation with Hemodialysis Session/ Hours among Hemodialysis Patients with Double Lumen Catheter:**

There are several potential for the transmission of infectious organisms because the procedure necessitates vascular access for extended periods of time in a setting where multiple patients receive hemodialysis simultaneously. It has been proven that healthcare workers' hands or contaminated tools, supplies, injectable drugs, external surfaces, or gadgets can transmit infectious pathogens from patient to patient. Additionally, the repeated hospital stays and surgeries that hemodialysis patients must undergo raises their exposure to and risk of contracting infections linked to healthcare <sup>(21)</sup>.

The results of present study agree with previous ones, in which a study done by **Habas et al.** in (2012) who revealed that hemodialysis can improve morbidity and mortality of renal failure but increase the risk of infection, it showed that many complications may occur during the time of dialysis sessions and/or as long-term use of hemodialysis <sup>(22)</sup>.

**Fungal Infection in Relation with blood transfusion before One Year among Hemodialysis Patients with Double Lumen Catheter:**

The results of the present study disagree with the previous once, a study done by **Marr et al.** in (1999) showed that multiple blood transfusion process had a significant impact in increasing the risk of infection caused by *Candida spp.* and *Aspergillus spp.* in many centers worldwide <sup>(23)</sup>.

A study done by **Burghi et al.** in (2011) presented that out of 86 patients with severe sepsis had transfusion of at least 4 volumes of red blood cells, the study showed that (31%

of them were diagnosed with fungemia<sup>(24)</sup>. The discrepancies among these results may be due to the number of samples included in each study, racial factor differences.

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