

THE ROLE OF FUNGAL INFECTIONS IN THE PATHOGENESIS OF DIALYSIS-DEPENDENT AND NON-DIALYSIS-DEPENDENT PATIENTS WITH KIDNEY DISEASE

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Abstract

The results of isolation, morphological and microscopic diagnosis, Chromic Agar, Vitik technology and Bact Alert showed that the diagnosis of fungi isolated from blood samples of end-stage renal patients who did not undergo dialysis and those who underwent dialysis was 60 samples for each type. The total number of fungal isolates isolated from people who did not undergo dialysis was 26 pathogenic fungal isolates, with a percentage frequency of 43.33%. In this study, 4 genera of pathogenic fungi were identified: *Candida* spp, *Rhodotorula* spp, *Cryptococcus* spp. and *Aspergillus* spp. The number of *Candida* isolates reached 13 isolates, with a frequency of 50%.

The results also showed that the diagnosed species from the genus *Rhodotorula* was *R. mucilaginosa*, with a frequency of 19.23%, while the diagnosed species from the genus *Cryptococcus* was *C. neoformans*, with a frequency of 7.69%. The results also show that the identified species of the genus *Aspergillus* is *A. flavus*, with an incidence of 23.07%. As for the results of isolation and diagnosis of samples subjected to dialysis, the total number of fungal isolates isolated from patient samples reached 35 pathogenic fungal isolates with a percentage incidence of 58.33%, and the number of *Candida* isolates reached 23 pathogenic isolates with a percentage incidence of 65.71%.

Key word: kidney failure, kidney disease, fungi infection.

Introductions:

One of the most crucial components of the urinary system is the kidneys. The kidneys are situated on either side of the spine, towards the rear of the abdomen., and are surrounded by two layers of fat. Each kidney is connected to the kidney pelvis by the renal artery and the renal vein ((Mader, 2008) Sherwood & Ward, 2019; Hamid et al., 2021). Chronic renal failure (CRF) is characterized by progressive destruction of renal mass with irreversible sclerosis) and an increase in interleukin 6 (Wahid et al, 2006 ; AL-Qadhi& AL-Saadi, 2022 ;Hasan et al.,2021End-stage kidney disease is one of the most well-known worldwide health issues. For those with this illness, dialysis is a vital therapy (Abdul-Jabbar and Kadhim, 2022). and kidney failure impairs cellular motility and immunoglobulins (Saleh, and Albayati, 2017; Al-Naqqash et al., 2021). Inflammation increases

interleukin 6 and other immunoglobulins (Sahib and Al-Shaikely, 2008 ; Ridha& Kadri,2018.;Abed and Mahmood, 2023).

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease that leads to end-stage kidney failure. It is the reason for dialysis in 7 to 10 percent of patients (Altaie et al, 2022). Chronic kidney disease (CKD) is described as kidney dysfunction, i.e. a slow loss of kidney function over time. When the kidneys stop functioning, the patient requires dialysis or a kidney transplant (Balaky et al, 2023). Diabetic nephropathy (DN) is the main cause of microangiopathy and the main cause of disease that occurs at this stage in patients with type 2 diabetes (Hamid et al, 2021). Fungi account for about 7% of all eukaryotic organisms found on earth (Jaloud & Hassan 2009; Mora et al, 2011) and hospitals are full of

pathogenic fungi (Mohammed and AL-Jibouri, 2015; Jasem et al, 2017).

Fungal infections are one of the health problems that accompany patients with chronic kidney disease, especially those who suffer from a weak immune system. Aspergillosis, cryptococcosis and candidiasis are considered the most dangerous fungal species for immunocompromised patients (Rayens et al., 2021), and *Candida* species play an important role in invasive fungal infections (Alsaedi and Al-Hashimy, 2022; Lee et al, 2021). Invasive opportunistic fungal infections are an important risk factor and a source of high rates of kidney disease and increased mortality despite medical and therapeutic intervention (Bongomin et al, 2017). Advances in medicine and the treatments used to save patients' lives have also led to a weakening of the immune system, increasing the number of patients exposed to fungal infection (Liebana-Jordan et al, 2021).

Material and methods

At Al-Imam Al-Kadhimin (peace be upon him) Teaching Hospital in Baghdad, 120 Iraqi patients gave clinical blood samples. They were categorised as having end-stage renal disease and divided into two groups: sixty patients receiving dialysis (twenty-three males and thirty-seven females) and sixty patients not receiving dialysis (twenty-five males). 35 of them were between 6 and 80 years old.

Method of clinical sampling

Additional blood was taken from double-lumen or arteriovenous fistulas in patients who came to the hospital for dialysis in the morning as part of the regular monthly examination. This was done after disinfecting the area with a medical syringe sprayed with a 70% ethanol and 15% iodine solution and before starting dialysis. Each participant and patient (male and female) had 8 milliliters of blood drawn. Each blood sample was then divided into two separate tubes. 4 ml of the sample was added to each gel tube and the tubes were centrifuged at 3000 rpm for 20 minutes. The samples were then placed in the refrigerator.

1- Isolation and diagnosis of fungi and preparation of culture media

All culture media used were prepared according to the manufacturer's instructions. Fungi were grown on

PDA and SDA culture media and incubated at 25°C for seven days and diagnosed morphologically and microscopically according to the taxonomic keys (De Hooge et al., 2005; Deorukhkar and Saini, 2014).

2- Fungal diagnosis with chromogenic agar

The yeast *Candida* was detected in a chromogenic agar medium using the approach of Hemaïd et al. (2021). Vitec2 Compact was also used for the diagnosis of yeasts.

3- Diagnosis of fungi by Bact alert

The fungi were diagnosed in this way according to (Kim et al., (2015), whereby 4 ml of blood was injected directly into the culture medium (Bact alert bottles) and these bottles were then placed in a device. Bact / Alert 3D Microbid identification system / biomérieux for five days at a temperature of 27°C, after which a secondary culture was established on the medium Sabouraud Dextrose Agar (SDA) for four weeks at 37°C.

4- Statistical Analyses:

The Statistical Analysis System tool was used in this study to determine how the variance factors affect the research parameters. In the current work, we used the chi-square test to compare the percentages significantly and we found significant differences at $P \leq 0.001$.

Results

The results of isolation, morphological and microscopic diagnosis showed the diagnosis of fungi isolated from blood samples of patients with end-stage renal disease and patients not undergoing dialysis, which amounted to 60 patient samples. The total number of fungal isolates isolated from patient samples reached 26 pathogenic fungal isolates, with the percentage of occurrence being 43.33% and diagnosed. In this study, 4 genera of pathogenic fungi were analysed: *Candida* spp., *Rhodotorula* spp., *Cryptococcus* spp. and *Aspergillus* spp. The number of *Candida* isolates reached 13 isolates, which were distributed among the following species: *C. albicans* ranked first with 6 isolates and a frequency of 23.07%. In second place was *C. glabrata* with 4 isolates and an incidence of 10.38%, while the number of isolates of *C. parapsilosis*. 3 isolates, with a frequency of 11.53%, as shown in Table (1).

Table (1): shows the types of fungi isolated from the blood of end-stage renal patients not undergoing dialysis, the number of isolates and the percentage of fungal frequencies.

not infected with fungi	Types of fungi	Number of isolates	Frequency percentage	Chi (x2)	P value
	No	/	/	/	/
infected with fungi	<i>C. albicans.</i>	6	23.07%	38.16	<0.0001
	<i>C.glabrata</i>	4	%15.38		
	<i>C. parapsilosis</i>	3	11.53%		
	<i>R. mucilaginosa</i>	5	19.23%		
	<i>C. neoformans</i>	2	7.69%		
	<i>A. flavus</i>	6	23.07%		
Total number of isolates		26	43.33%		

The results of isolation, morphological and microscopic diagnosis showed the diagnosis of fungi isolated from blood samples of end-stage renal patients undergoing dialysis, which amounted to 60 patient samples. The total number of fungal isolates isolated from patient samples reached 35 pathogenic fungal isolates with a percentage of 58.33% diagnosed. The study included 4 genera of pathogenic fungi: *Candida* spp., *Rhodotorula* spp., *Cryptococcus* spp. and *Aspergillus* spp.

The number of *Candida* isolates reached 23 pathogenic isolates, distributed among the following species: *C. albicans* ranked first with 10 isolates and an incidence of 28.57% and *C. glabrata* ranked second with 8 isolates and an incidence of 22.85%, while the number of *C. tropicallis* type isolates was 3 isolates with an incidence of 8.57% and the number of *C. parapsilosis* type isolates was 2 with an incidence of 5.71%. As shown in Table (2).

Table (2): Shows the types of fungi isolated from the blood of end-stage renal patients undergoing dialysis, the number of isolates and the percentage of fungal frequencies.

not infected with fungi	Types of fungi	Number of isolates	Frequency percentage	Chi (x2)	P value
	No	/	/	/	/
infected with fungi	<i>C. albicans.</i>	10	28.57%	43.40	<0.0001
	<i>C.glabrata</i>	8	22.85%		
	<i>C.tropicallis</i>	3	8.57%		
	<i>C. parapsilosis</i>	2	5.71%		
	<i>R. mucilaginosa</i>	2	5.71%		
	<i>C. neoformans</i>	3	8.57%		
	<i>A. flavus</i>	7	20%		
Total number of isolates		35	5833.%		

The fungi recovered from 60 blood samples taken from individuals with advanced kidney disease who are not receiving dialysis are also displayed in Table 3. by age group, as all isolates isolated reached 26 isolates, with a rate of 43.33%, as the number of *R. mucilaginosa* yeast isolates isolated reached 5 isolates. The genus *C. neoformans* occurred in 8.33% of samples less than 20

years old, in 3.84% of samples between 31-40 years old, in 7.69% and in 3.84% of samples between 41-50 years old and 17-80 years old. In samples less than 20 years old and in the 61-70 age group, the rate was 3.84% for both. The results also showed the presence of *Candida* spp. in all age groups except those less than 20 years of age.

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Table (3): displays the fungus found in the blood of renal failure patients who are not receiving dialysis according to their age group.

not infected with fungi	Age	Types of fungi	Number of isolates	Frequency percentage	Chi (χ2)	P value
	For all ages studied	No	/	/	/	/
Patients infected with fungi Those not undergoing dialysis	less than 20	<i>R. mucilaginosa</i>	1	3.84%	36.0	<0.0001
		<i>C. neoformans</i>	1	3.84%		
	21-30	<i>C.albicans</i>	1	3.84%		
	31-40	<i>R. mucilaginosa</i>	2	7.69%		
		<i>C.albicans</i>	2	7.69%		
	41-50	<i>R. mucilaginosa</i>	1	3.84%		
		<i>C.glabrata</i>	3	11.53%		
	51-60	<i>glabrata.C</i>	1	3.84%		
		<i>A. flavus</i>	1	3.84%		
	61-70	<i>C. albicans</i>	3	11.53%		
		<i>A. flavus</i>	2	7.69%		
		<i>C. neoformans</i>	1	3.84%		
	71-80	<i>A. flavus</i>	3	11.53%		
		<i>C. parapsilosis</i>	3	11.53%		
		<i>R. mucilaginosa</i>	1	3.84%		
Total number of isolates			26	43.33%		

The fungi isolated from 60 blood samples from dialysis and kidney failure patients are arranged by age group in Table (4). Thirty-five isolates in all, or 58.33%, were

found. As Table (4) demonstrates, the data also demonstrate the prevalence of the genus *Candida* spp. with 65.71%.

not infected with fungi	Age	Types of fungi	Number of isolates	Frequency percentage	Chi (x2)	P value
	For all ages studied	No	/	/	/	/
Patients infected with fungi Those not undergoing dialysis	less than 20	<i>A. flavus</i>	1	2.85%	32.42	<0.0001
		<i>C. parapsilosis</i>	2	5.71%		
	21-30	<i>C.albicans</i>	2	5.71%		
	31-40	<i>C.albicans</i>	3	8.57%		
		<i>C.glabrata</i>	2	5.71%		
	41-50	<i>C.albicans</i>	1	2.85%		
		<i>C.glabrata</i>	1	2.85%		
		<i>A. flavus</i>	3	8.57%		

	51-60	<i>C. neoformans</i>	2	5.71%		
		<i>C.albicans</i>	3	8.57%		
		<i>C.glabrata</i>	2	5.71%		
	61-70	<i>A. flavus</i>	2	5.71%		
		<i>C. neoformans</i>	1	2.85%		
		<i>C.albicans.</i>	1	2.85%		
		<i>C.glabrata</i>	3	8.57%		
		<i>R. mucilaginosa</i>	2	5.71%		
	80-71	<i>A. flavus</i>	1	2.85%		
		<i>C.tropicallis</i>	3	8.57%		
Total number of isolates			35	58.33%		

Table (4): Relationships between the number of isolates, the proportion of fungal frequencies, and the age of patients receiving dialysis who have advanced kidney disease and the fungal species isolated from their blood.

Regarding the results of blood cultures using a BacT/ALERT bottle, 26 positive (+) isolates with a rate of 43.33% and 34 negative (-) isolates with a rate of 56.6% were found in samples from individuals with kidney disease not undergoing dialysis, as shown. The results of the SDA and PDA culture media identified 26

isolates distributed as follows: 13 isolates from the genus *Candida* spp, 5 isolates from the genus *Rhodotorula* spp., 2 isolates from the genus *Cryptococcus* spp. and 6 isolates from the genus *Aspergillus* spp. Figure (1) and Table (5).



Figure (1): Culture of blood samples in a BacT/ALERT glass bottle.

Table (5): Detection and diagnosis of fungi with Bact\Alert and the media SDA and PDA isolated from individual with advanced (blood) kidney disease not undergoing dialysis.

Type of growing medium	Types of fungi	Number of isolates	Frequency percentage	Ch(x ²)	P value
Bact \Alert	Positive(+)	26	43.33%	0.16	0.05* NS
	Negative(-)	34	56.66%		
	Total	60			
SDA PDA	<i>C.albicans</i>	6	23.07%	41.36	<0.0001**
	<i>C.glabrata</i>	4	15.38%		
	<i>C. parapsilosis</i>	3	11.53%		

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	<i>R. mucilaginosa</i>	5	19.23%		
	<i>C. neoformans</i>	2	7.69%		
	<i>A. flavus</i>	6	23.07%		

Table (6) also shows the detection and diagnosis of fungi with BactAlert and the media SDA and PDA from individual with advanced (blood) kidney disease and dialysis, the number of isolates and the percentage of fungal abundance, as the results of BactAlert indicate the presence of 35 isolates. Positive (+) with a rate of 58.33% and 25 isolates were negative (-) with a rate of 41.66%.

The results of SDA and PDA showed that 35 isolates were diagnosed, distributed as follows: 22 isolates belong to the genus *Candida* spp. 2 isolates belong to the genus *Rhodotorula* spp. and 4 isolates belong to the genus *Candida* spp. *Cryptococcus* spp. and 7 isolates belong to the genus *Aspergillus* spp.

Table (6): Detection and diagnosis of fungi with BactAlert and the media SDA and PDA, isolated from individual with advanced (blood) kidney disease undergoing dialysis.

Type of growing medium	Types of fungi	Number of isolates	Frequency percentage	Ch(x ²)	P value
Bact \Alert	Positive(+)	35	58.33%	8.02	0.05* NS
	Negative(-)	25	41.66%		
	Total	60			
SDA PDA	<i>C.albicans</i>	9	25.71%	75.7	<0.0001**
	<i>C.glabrata</i>	8	22.85%		
	<i>C.tropicalis</i>	3	8.57%		
	<i>C. parapsilosis</i>	2	5.71%		
	<i>A. flavus</i>	7	20%		
	<i>R. mucilaginosa</i>	2	5.71%		
	<i>C. neoformans</i>	4	11.42%		

Discussion:

Fungal infections are caused by opportunistic infections, i.e. infections caused by fungi that coexist peacefully and harmlessly in the host's body and then transform into pathogenic fungi that cause disease when the host's immune system is weakened for some reason, e.g. by taking medication. Immunosuppressive effects caused by

contaminated surgery, contaminated medical devices and equipment, and organ transplants (Verma, 2018).

It has been shown that many complications can occur during dialysis sessions and/or with long-term use of dialysis. In a study conducted by Ali, et al. (2019), he showed that fungal infections associated with catheters are common in double lumen dialysis patients, as out of

(129) patients, the percentage of catheter-related infections was 19 or 14.7% (Ali, et al., 2019).

These results do not agree with those of Shuaib et al. (2020), who isolated 277 fungal isolates from 115 clinical blood samples from dialysis patients with kidney failure and healthy people. He found that the main fungal genera isolated from the blood were: *Alternaria*, *Penicillium*, *A. flauvs*, *Candida* and *Cladosporium*, but they agree with the same researcher regarding the *Candida* species, as the researcher included the species: *C. albicans* and *C. glabrata*.

Candida are yeast fungi that naturally occur in the body, but they become opportunistic fungi, i.e. they infect humans when the immune system is weakened, e.g. by chemotherapy, diabetes or organ transplantation (Muhsen, et al, 2021).

In a study with 120 samples from the mouth, urine and vagina, the percentage of positive samples for *Candida* isolates was 28.67, 4.00 and 1.67 respectively (Alqaysi et al, 2021). In one study, it was shown that the most common types of *Candida* isolated from intestinal patients were five yeast species, namely: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. parapsilosis*, *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Saccharomycosis*, and *Cryptococcus* (Hasan et al., 2023).

This result is also in line with Goffena et al. (2018), who found that the role of the hormone estrogen during pregnancy is to increase the ability of epithelial cells to adhere to the ovaries and that this hormone also plays a role in the transformation of the ovaries from the yeast form to the filamentous form. In a study conducted by Potthuri and Arun (2023), Twenty (46.5%) of the 43 patients receiving dialysis for chronic renal disease were male, and 23 (53.5%) were female. The patients were between the ages of 28 and 86. As for the BacT/ALERT results, the results are consistent with those found by Menchinelli et al. (2019) using the BacT/ALERT bottle in 50 diagnosed cases of *Candida* spp. candidaemia. Only 40% of the BacT/ALERT bottles tested positive within 24 hours. The results of microscopic examination are also in agreement with Majeed (2018) who mentioned all the features seen under the microscope were in the form of spherical or oval shaped cells with bluish-green margins due to the accumulation of dye on the positive wall of the Gram stain. Jasem et al. (2017) found that the *Aspergillus* fungus grew best on Sabouraud's dextrose agar medium.

Conclusion:

This study proves that kidney failure increases the incidence of fungal infections and that gender (male or female), age group and people undergoing dialysis or not have an influence on fungal infections. The BacT/ALERT tests gave results that were consistent with the morphological and microscopic diagnosis, and on chromium Agar.

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الخلاصة

أظهرت نتائج العزل والتشخيص المظهري والمجهري و كرومك أكار و بتقنية الفايترك و من خلال Bact alert تشخيص الفطريات المعزولة من عينات دم مرضى الكلى في المرحلة النهائية غير الخاضعين للغسيل الكلوي والخاضعين للغسيل الكلوي والتي بلغت 60 عينة لكل نوع ، إذ بلغ العدد الكلي للعزلات الفطرية المعزولة من الأشخاص غير الخاضعين للغسيل الكلوي 26 عزلة فطرية ممرضة و بنسبة مئوية للظهور بلغت 43.33 % وشخصت هذه الدراسة 4 أجناس من الفطريات الممرضة هي : *Cryptococcus spp.* ، *Rhodotorula spp.* ، *Candida spp.* و *Aspergillus spp.* . إذ بلغ عدد عزلات الـ *Candida* 13 عزلة وبنسبة 50% كما كشفت النتائج أن النوع الذي شخص من جنس *Rhodotorula* هو *R. mucilaginosa* بنسبة تردد 19.23 % في حين أن النوع الذي شخص من جنس *Cryptococcus* كان *C. neoformans* وبنسبة تردد 7.69 % ، كما بينت النتائج أن النوع الذي شخص من جنس *Aspergillus* هو *A. flavus* و بنسبة تردد 23.07 % . أما نتائج العزل والتشخيص للعينات الخاضعة للغسيل الكلوي فقد بلغ العدد الكلي للعزلات الفطرية المعزولة من عينات المرضى 35 عزلة فطرية ممرضة و بنسبة مئوية للظهور بلغت 58.33 % و بلغ عدد عزلات الـ *Candida* 23 عزلة ممرضة وبنسبة 65.71 %.

الكلمات المفتاحية: الفشل الكلوي ، أمراض الكلى ، الإصابات الفطرية .

دور الاصابات الفطرية في امراضية المصابين بامراض الكلى الخاضعين وغير الخاضعين للغسيل الكلوي