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ORIGINAL ARTICLE

Role of *Candida Albicans* Germ Tube Antibody Test in Diagnosis of Invasive Candidiasis in Intensive Care Unit at Beni-Suef University Hospital

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ABSTRACT

Background: *Candida species are common human commensals can cause wide range of ailments. The most serious is a spread serious infection, which happens with expanded pervasiveness in emergency unit patients. Authoritative diagnosis relies upon isolation of Candida from blood culture or positive cultures from other normally sterile body sites, yet this may recognize less than half of cases. Improved diagnostics are expected to control inception of antifungal treatment to diminish mortality rate.* ***Objective:*** *The aim of this study was to evaluate the potential utility of the CALB gene recognition PCR and the Candida albicans germ tube antibody test (CAGTA) as biomarkers for quickly identifying invasive candidiasis in patients who were in danger of developing the disease in emergency departments.* ***Methodology:*** *The study included 50 patients from ICU suffering from invasive Candida infection. For all patients, blood culture, CAGTA test and PCR for identification of CALB gene were done.* ***Results:*** *Taking blood culture as the gold standard for diagnosis of invasive candidiasis. The sensitivity of CAGT was 75% and the specificity was 88.1% while the sensitivity of PCR was 100% and the specificity was 83.3%.* ***Conclusion:*** *For the diagnosis of an invasive candida infection, the biomarkers PCR and CAGT have additional value.*

INTRODUCTION

Common human commensals like Candida species can cause a variety of illnesses. A noteworthy concern is the spread of infection, which occurs more frequently in patients in intensive care units [1].

With high rates of morbidity and mortality, invasive fungal infections (IFIs) cause a substantial disease burden. The number of patients who are at risk for IFDs is increasing, and the prevalence of drug-resistant fungal infections is also rising, which exacerbates the issue [2].

Antibacterial agents are overused and widely distributed in emergency units, which disrupts the microbiota and increases candida colonization. The use of intravascular devices, elementary tract operations, and weakened body defenses promote candida invasion[3].

The development of Candida in blood cultures or cultures from other typically sterile sites is necessary for the definitive diagnosis, although this may not be able to differentiate more than half of the cases. Better diagnosis should guide the initiation of antifungal treatment. Therefore, it is essential to have tests that are getting faster, more accurate, and more sensitive in order to accurately and precisely identify candida infections[4].

Promising sensitivity and specificity allow the biomarker *Candida albicans* germ tube antibody test and PCR assays to diagnose invasive candidiasis and candidemia prior to blood cultures[5, 6,7].

The commercially available indirect immunofluorescence test known as the *Candida albicans* germ tube-specific antibody (CAGTA) immunofluorescence assay (IFA) allows the identification of antimycelium IgG antibodies against different species of Candida[8].

Positive serum CAGTA detection is suggestive of profound situated infection in which Candida hyphae attack diverse body tissues. Hyphae contain hpw1 antigens which enhance generation of CAGTA[9].

Although that procedure was originally created for *Candida albicans*, it has been shown that other Candida species, such as *Candida tropicalalis, Candida parapsilosis, Candida glabrata, Candida dubliniensis, Candida guilliermondii, and Candida krusei,* can produce CAGTA to varying degrees [10].

Many DNA-based techniques, particularly PCR, have been developed to enhance diagnosis because of its ease of use, sensitivity, and specificity. The most well-known techniques have concentrated on specific regions of the DNA found in Candida species. This approach has the benefit of being able to differentiate between low candidemia that occurs early in an infection [11].

The aim of this study was to evaluate the potential utility of PCR of *CALB* gene and CAGTA for the quick

identification of invasive candidiasis (IC) in patients presenting to emergency departments.

METHODOLOGY

Patients:

Fifty patients who were suspected of having invasive candidiasis participated in the current study. The patients were enlisted from Beni-Suef University Hospital. Our hospital is a tertiary care hospital, affiliated to Beni-Suef University.

Data confidentiality was preserved according to the **Revised Helsinki Declaration of Bioethics**[12] Informed consent was acquired from all the patients or guardians who consented to participate in the study.

This study was approved by Research Ethical Committee, Faculty of Medicine Beni-Suef University; **Approval No: FMBSUREC/09042023/Kamel on 9th April 2023**

Every patient had a complete history taken, with particular attention paid to age, gender, underlying conditions, and factors that might predispose to an invasive fungal infection (IFI); these include immunodeficiency or neutropenia, burns, recent surgery, hemodialysis, the presence of a central venous line, diabetes mellitus, renal failure, the presence of a urinary catheter, or prolonged stays in the intensive care unit.

Patients received antifungal drugs, were excluded from the study. Patient follow up was done for three months.

The selection of patients is primarily based on the presence of one or more invasive candidiasis (IC) risk factors, such as a prolonged fever lasting longer than seven days, non-responsiveness to antibiotics[13] candidiuria $>10^3$ CFU/ml [14] with or without blood cultures that tested positive for Candida species.

Microbiological techniques:

a- Culture for Urine, sputum, blood and CSF:

Culture of the corresponding sample was performed on blood, chocolate, and MacConkey agar (Oxoid, England) along with blood culture using BACTEC FX 40 (Becton Dickinson, Germany) automated blood culture system, based on the suspected site of infection. *Candida* species identification was performed by colony morphology on Sabouraud dextrose agar (Oxoid, England), Gram staining and germ tube test.

b- Non-cultural techniques:

1- ***Candida albicans germ tube assay (CAGTA)*** Using an indirect immunofluorescence technique, the *Candida albicans* germ tube assay (CAGTA) was performed on serum samples to identify antimycelium IgG antibodies against different Candida species (Vircell Microbiologist S.L., Granada, Spain). The test was conducted as directed by the manufacturer.

Interpretation of results:

The highest dilution at which a positive reaction is found is known as the serum titer. When the mycelium phase exhibits apple-green fluorescence while the yeast phase remains red, the reaction is considered positive. **(Figure 1)**

The reaction was considered negative when red cell pattern over both the mycelium and yeast phases could be identifiable.

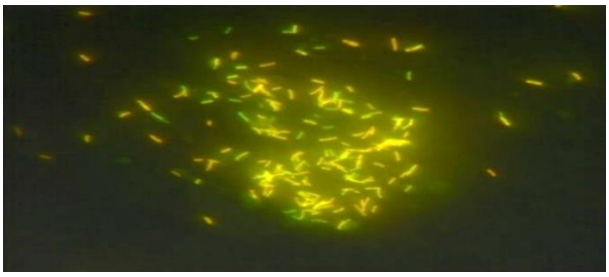

Fig. 1: show postive CAGTA (the green apple apperance)

2- Polymerase chain reaction (PCR) for *CALB* gene:

PCR procedure was done according to [15] on aliquots from each patient's blood culture bottle. That procedure was done in the Microbiology Department for Research and Postgraduate Studies in Faculty of Pharmacy, Beni-Suef University**.**

DNA extraction:

Isolation of yeast genomic DNA from 1.5 ml of an overnight broth cultures was done according to **Harju et al.** [16] Repeated freeze- defrosting of cells in a lysis buffer was used to disturb the cell wall and discharge genomic DNA. Cell lysis was trailed by extraction with chloroform and ethanol precipitation.

DNA amplification and cycling conditions:

- PCR taq master mix: red Taq PCR Master Mix (2X) (Bioline, UK).
- Primer sequence for CALB gene identification: the following primers were used: TTT ATC AAC TTG TCA CAC CAG A and ATC CCG CCT TAC CAC TAC CG (Invitrogen Company, UK) indicated by the method described by[17].
- PCR thermal cycling conditions: DNA thermal cycler (Biometra an Analytic Jena Company, Germany and Sensquest labcycler, Germany.) were used. Thermal cycling conditions were summed up in table 1.

Table 1: Thermal cycling conditions

		CALB
Initial denaturation		94º C for 5 min
Repeated for 35 cycles	Denaturation	94º C for 5 min
	Annealing	54º C for 30s
	Extension	72º C for 30s
Final extension		72º for 5 min

Detection of PCR products:

Using a UV transilluminator and gel electrophoresis, the amplified genes were detected. (Whatman, Biometra, Germany).

The amplified gene product is expected at molecular size of 273 bp [15] as shown in **Figure 2.**

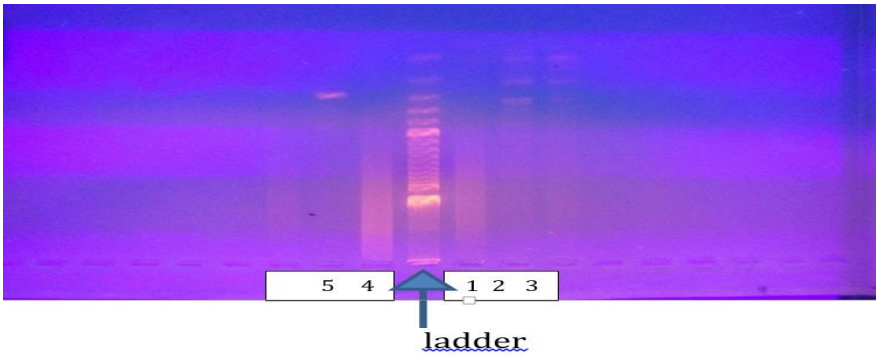

Fig. 2: Gel electrophoresis of PCR amplified *CALB* gene (273 bp)
Lane M was 100 bp DNA ladder.
Lane2,5 considered as positive for *CALB* gene .
Lane 1,3,4 considered negative for *CALB* gene

RESULTS

The age of patients ranged from 1 to 70 years with a mean value 45.6±16.9, the patients were 28 male (56%) and 22 female (44%). The length of stay at ICU ranged from 4 to 24 days with a mean value 11.5±5.0. The clinical data of our patients is shown in table 2.

Table 2: clinical data of patients

	Total Patients (n=50)	Proven invasive candidiasis (positive blood culture) (n=8)	P value
Renal failure or on hemodialysis, renal transplant	17(34)	4(50%)	0.442
Hematological tumour or cancer on chemotherapy	8(16)	0(0%)	0.583
Neutropenia	10(20)	2(25%)	0.664
Long term broad spectrum antibiotic	38(76)	7(87.5%)	0.668
After surgery especially gasrtrointestinal	10(20)	2(25%)	0.664
Unexplained fever	18(36)	2(25%)	0.701
Parentral nutrition	23(46)	6(75%)	0.252
Other sites colonization or Candiduria cc>10*5	11(22)	1(12.5%)	0.987
Antifugal prophylaxis	23(46)	6(75%)	0.252

Bold values are statistically significant
Sig P < 0.05 HS P<0.001 NS P>0.05

It was discovered that none of the risk factors found in our patients was statistically significant for the IFI development.

Our patients were divided into two groups: those with positive blood cultures and proven IC and unproven IC in patients clinically believed to have IC but revealed negative blood culture [18].

We found that 8/50 (16%) of patients had positive blood culture results (proven IC). While 11 positive candiduria cases had colony count $\geq 10^3$ CFU/ml and one of them had blood culture positive. Two sputum cases showed suppressed normal flora with overgrowth of *C.albicans*; these two cases had negative blood culture but positive CAGTA. The number of positive CAGTA test was 11/50 (22%).

For the diagnosis of invasive candidiasis, the *Candida albicans* germ tube antibody test (CAGTA) is considered significant. (*P* value <0.001).

Polymerase chain reaction for detection of *CALB* gene:

Thirteen patients (30%) in our study had positive CALB gene tests.

Considering blood culture to be the gold standard for IC diagnosis [19] the sensitivity and specificity of CAGTA and PCR were calculated as shown in table 3.

Table 3: Sensitivity and Specificity of CAGTA and PCR tests:

	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR
CAGT	75%	88.1%	54.5%	94.9%	6.3%	0.28%
PCR	100%	83.3%	53.3%	100%	6%	0%
Combined CAGT-PCR	56.3%	98%				

PPV=Positive Predictive Values NPV=Negative Predictive Values
PLR =likelihood NLR =negative likelihood
The overall sensitivity is greater than either alone but the specificity is less than either alone.

Patient's outcome:

Twenty-two patients (44%) died. Eight patients of them had positive blood cultures and confirmed invasive candidiasis died.

Table 4: The outcome of all patient and proven invasive candidiasis IC

	Total	Proven IC(+ve blood culture)	CAGTA +ve	PCR+ve
Death	**22(44%)**	**8 (100%)**	**9**	**12**

DISCUSSION

Although fungus infections do not usually occur as primary infections in immunocompetent individuals, they are typically associated with immunodeficiency states or the presence of underlying diseases. Invasive candidiasis infection is often more common in critically ill patients, with higher rates of morbidity and mortality [20]. No risk factor found in our patients was found to be statistically significant for the development of IFI; Martínez-Jiménez et al.'s [21]study revealed similar results.

Conversely, though, Martínez-Jiménez and his colleagues[22] discovered that patients with candidemia had a significantly higher prevalence of traditional risk factors for Candida infection, such as intravenous catheterization, total parenteral nutrition, antimicrobial use, and prior Candida colonization. The different observation in our study might be related to our small sample size.

Because of its high specificity and relatively low sensitivity, fungal culture is the gold standard for diagnosing IC; but it can detect only about 50–71% of cases[19]. Not only is there restricted sensitivity, but growth and identification take a long time. One possible explanation for the negative blood culture is the lack of viable fungal cells or insufficient concentration to be detected [23].

In order to overcome the low detection level of fungal blood culture, nonculture methods were developed. Nonculture techniques have limitations, but they also have some benefits when it comes to identifying cases of IC that are misdiagnosed[5].

Indirect immunofluorescent assay is used to find antibodies made against the *C. albicans* germ tube antigen (CAGTA). According to our research, CAGTA's sensitivity and specificity were 75% and 88.1%, respectively. Parra-Sanchez et al.[24] reported similar outcomes; they compared the CAGTA test with the mono-test automatic VirClia indirect chemiluminescent immunoassay; they reported sensitivity of 69.2% and specificity of 80.3%. However, **Martinez-Jimenez** [22] mentioned lower sensitivity (58.1%) and higher specificity (92%) when used this test alone as a candidaemia biomarker. Because the test relies on the production of antibodies in patients who typically have immunodeficient states or underlying diseases, it has a wide range of specificity and sensitivity. It also can't diagnose other candida infection other than *C. albicans* as reported by Parra-Sanchez et al [24].

In our study, IC was reported in suspected patients using the CALB gene PCR as a tool. The test had an 83.3% specificity and 100% sensitivity. Our findings support those of **Kourkoumpetis et al**[25] who stated that the majority of studies assessing PCR-based assays for blood sample identification of *Candida* DNA report sensitivity that can reach 100% and specificity that may reach 90%.

Nevertheless, Clancy and Nguyen[26] reported a lower PCR specificity of 70%, which might have been influenced by a high-risk control group primarily made up of patients with Candida species colonization or mucosal candidiasis. Because many of the controls were immunocompromised in intensive care units, the authors discovered that some of them had undetected invasive candidiasis and had infection-related signs and symptoms but negative cultures.

The PCR assay method has demonstrated high sensitivity and specificity in certain studies; however, it has certain limitations that indicate its limited applicability in the diagnosis of IC. A small number of cells and complications during sample preparation and DNA extraction led to false negative results. Additionally, because the DNA of fungi and humans is similar, false positive results have occurred. Due to all of these factors, this test is not reliable when used alone to diagnose IC[19].

CONCLUSION

Our research revealed that the polymerase chain reaction (PCR) and the *Candida albicans* germ tube antibody test (CAGTA) performed well in the diagnosis of invasive candidiasis. Because non-culture diagnostics for invasive candidiasis can identify some patients with the disease earlier than cultures, they present challenges for doctors, laboratories, and hospitals. However, these diagnostics can also improve patient outcomes, limit the emergence of resistance, reduce the need for unnecessary antifungal use, and be more affordable. The role of combination testing is also warranted. One crucial point is that for patients who are at high risk of infection, non-culture techniques should be used in addition to blood cultures, not instead of them.

Declarations:
Consent for publication: Not applicable
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