ORIGINAL ARTICLE

The role of (1,3)-beta-D-glucan (BDG), as noninvasive technique for detection of invasive fungal infection (IFI)

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ABSTRACT

Background: *BDG is found in the cell walls of most fungi (eg, Candida, Aspergillus, Fusarium, Pneumocystis jirovecii). Serum BDG levels is high in the presence of a fungal infection. Its high serum levels may be detected before development of clinical symptoms and even prior to isolation or identification of the fungal organism via routine laboratory methods.* ***Objectives:*** *evaluate the performance of serum (1.3) beta -D glucan BDG in comparison with blood culture for diagnosis of the invasive fungal infections with Candida species.* ***Methodology:*** *The study was conducted on 50 patients divided in 3 group first group 20 patients with prolonged stay in ICU receiving broad spectrum antibacterial treatment, and second group 20 patients with diabetic foot and third group 10 patient healthy subjects with matched sex and age. All patients were subjected to full history taking, clinical examination and laboratory investigations Blood was examined with conventional methods (, Gram stain and culture on fungal media) and serum (1,3) beta -D-glucan was determined by ELISA.* ***Result:*** *The average serum concentration of BDG was higher in group II (121.25±88.9 pg/ml than group I (115.5±90.9 pg/ml), however there is no statistically significantly difference between group I and groupII, the mean value of serum (1.3 beta D glucan) in patient with positive blood culture were significantly higher than those with negative blood culture there is significant relation between blood culture and the level of(1,3)beta D-glucan in serum. Blood culture could be considered as marker for detection of invasive fungal infection with sensitivity, specificity, PPV and NPV of, 46.43%, 100%, 100% and 11.76% respectively however (1.3) D glucan can detect invasive fungal infection with sensitivity, specificity, PPV and NPV of, 88.46%, 100%, 100% and 40%.* ***Conclusion:*** *Our results suggest that a positive (1,3)beta-D-glucan assay could be a superior test for diagnosis of candidemia in addition to the blood culture*

INTRODUCTION

Invasive fungal infections (IFI) are a significant cause of morbidity and mortality due to opportunistic fungal pathogens, especially among significantly immunosuppressed patients such as transplant recipients, solid organ transplant recipients, and those with immune deficiencies[1]. The most frequent predisposing factors for development of invasive fungal infections are prolonged stay in ICUs, broad spectrum antimicrobial agents, prolonged use of corticosteroids, chemotherapy and radiotherapy, immunosuppression and disruption of mucous membranes[2].

Candida species are ranked on the 4th position as agents of nosocomial septicemia in many studies across USA, and cause approximately 9-12% of all septicemias and on the 6th or 7th position as causes of nosocomial septicemia in many European studies[3,4].

Blood cultures are sensitive at detecting viable *Candida*. The sensitivity of blood cultures in diagnosis of viable *Candida* is equivalent or superior to that for methods such as PCR[5]. Blood cultures are positive in most patients if samples are collected during active candidemia. However, patients with candidemia complicated by deep-seated infection, blood culture are positive only in 40% of them as *Candida* have been cleared from the bloodstream, so the sensitivity of blood cultures is 50% across the spectrum of invasive candidiasis[6,7]

The earliest non culture diagnostics for invasive candidiasis were serum assays for *Candida* antigens and anti-*Candida* antibodies[8]. Most *Candida* antigens are limited as diagnostics by low serum concentrations and rapid clearance from the bloodstream and varied from 42% to 96% and 54% to 100%, respectively[9,10]

BDG is a polysaccharide composed of glucose monomers linked by beta 1-3 glucosidic bonds and it makes up a large proportion of the cell wall of several types of fungi, including *Aspergillus*, *Candida*, and *Fusarium* which is clinically important fungi and also high abundance of BDG is found in cellulose containing plants[11,12]. Among possible culture-independent serum markers, Mannan and 1,3beta D-glucan (BDG) are successful targets in diagnosing IFI as it is abundant

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constituents of the cell wall. Candida species can be present early in the blood and fluids from patients suffering from IFI [13, 14]

Serum beta -D-glucan concentrations show a constant rise before clinical symptoms and microbiological evidence of infections, then decrease, and eventually become negative if patients respond to antifungal therapy[15, 16].

It helps earlier diagnosis of IFI, which is otherwise feasible with traditional methods. The aim of this study was to prospectively evaluate the performance of serum (1,3)- beta -D-glucan (BDG) in comparison with blood culture, for diagnosis of invasive infections with Candida species.

METHODOLOGY

Patients

We recruited three groups of patients the first group 20 patients with prolonged stay in ICU receiving broad spectrum antibacterial treatment, the second group 20 patients with diabetic foot and the third group 10 patient healthy subjects with matched sex and age. Invasive fungal infection(IFI) was recognized according to the definitions by the EORTC/MSG (European organization for research and treatment of cancer/mycoses study group) consensus group [17,18], the protocol of this study was approved by the ethics committee of the institution involved, and informed consent for studies was obtained from all the subjects.

Laboratory assessments

Blood culture was performed with sub culture on selective Sabouraud agar and nutrient media followed by gram staining Identification of *Candida* species was performed by this method. Serological (1,3)- -D-glucan detection was performed with ELISA kit using Sandwich-ELISA.

We prepared all reagents before starting assay procedure. We set standard wells, testing sample wells ,we added 50µl of the 6 standards in the first six wells then 10µl of samples added in the other wells then 40µl of sample diluent added to testing sample well; 100µl of HRP-conjugate reagent was added to every well and then covered with an adhesive strip and incubated for 60 minutes at 37°C. Washing was performed by auto washer and these washing process was repeated four times. After the final wash, we removed any remnant wash solution by decanting. The plate was inverted and blotted against clean towels then 50µl of chromogen A and 50µl of chromogen B solution were added to each well, gently mixed and incubated for 15 minutes at 37°C. Finally 50µl of Stop Solution were added to each well. Optical Density (O.D.) was read at 450 nm using a microtiter plate reader within 15minutes. The standard curve was generated by plotting the O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the concentration on the horizontal (X) axis. First, the mean O.D. value for the standards and samples were calculated. Standard curve calibration curves were made using standards (0–160 pg/ml), provided with the kit. The amount of (1,3)- -D-glucan in the serum samples was determined by extrapolating OD values using the calibration curves. Interpretation of BDG values was done as follows: <60 pg/ml, negative; 60 to 79 pg/ml, indeterminate; ≥80 pg/ml, positive.

RESULTS

We examined socio-demographic characteristics of the studied patients and the control groups as shown in Table1. The mean age was 43.55±4.8 for group 1 with prolonged stay in ICU receiving broad spectrum antibacterial treatment, 43.8±5.4 for second group with diabetic foot and 38.9±4.2 years old for control group. No significant difference was detected among the studied groups regarding gender distribution or age.

Table 1: Socio-demographic characteristics of the studied groups

Socio-demographic characteristics	Group I (No.=20)		Group II (No.=20)		Group III (No.=10)		ANOVA	P value
Age (years)								
Mean±SD	43.55±4.8		43.8±5.4		38.9±4.2		0.197	P =0.197
Range	36 – 55		32 – 52		35 - 50			
Gender	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Male	12	60.0	9	45.0	6	60.0		
Female	8	40.0	11	55.0	4	40.0		

We had compared the studied groups regarding EORTC/MSG classification showed that patients with a proven fungal infection was equal in those diabetic foot and those with prolonged stay in ICU receiving broad spectrum antibacterial treatment (30%) (table 2). Those with a probable fungal infection were higher in patients diabetic foot and patients (45%) than with prolonged stay in ICU receiving broad spectrum antibacterial treatment (40%)

Table 2: Comparison between the studied groups regarding EORTC/MSG criteria classification

Studied parameters	Group I (No.=20)	Group II (No.=20)	Group III (No.=10)	X2 and P value
Proven	6(30%)	6(30%)	0 (0%)	X2= 0.07
Probable	8(40%)	9(45%)	0 (0%)	P Value 0.79
Possible	6(30%)	5(25%)	10 (100%)	

The blood culture was positive in 45% patients in group I, 30% in group II and no one in group III and the statistical analysis confirmed that positive blood culture was a significantly higher frequent finding in patients prolonged ICU stay with antibiotic treatment, compared to patients with diabetic foot as in table 3.

Table 3: Comparison between the studied groups regarding blood culture

Blood culture	Group I (No.=20)	Group II (No.=20)	Group III (No.=10)	X2 and P value
Positive	9(45%)	6(30%)	0 (0%)	Test=9.70 P=0.04
Negative	11(55%)	14(70%)	10 (100%)	Test=11.3. P=0.03

In table 4, the concentrations of the (1-3)-β-D-glucan marker (BDG) are presented. The average serum level of BDG was higher in group II (121.25±88.9 pg/ml than group I (115.5±90.9 pg/ml), statistically significantly higher concentration of BDG was obtained in group I, II compared to group III (p=0.004) however there is no statistically significantly difference between group I and group II

Table 4: The mean value of (1,3) D-glucan among the studied groups

Studied parameter	Group I (No.=20)	Group II (No.=20)	Group III (No.=10)	Kruskal Wallis Test and P value
Serum level (1,3) D-glucan	115.5 ± 90.9	121.25 ± 88.9	62.5 ± 25.9	Test=22.70 P=0.004

In table 5, patients were divided according to EORTC/MSG criteria classification in to three groups proven, probable and possible. Significant relation between EROTC/MSG Criteria classification and serum level of (1.3) D-glucan was detected

Table 5: Relation between serum level of (1.3) D beta glucan and EORTC/MSG criteria classification

Studied parameter	Proven 12	Probable 17	Possible 21	"Z" of MWU test	p
Serum level (1,3) D-glucan	147.5	117.95	77.85	2.7	0.02

According to EORTC/MSG criteria classification patients were divided in to three groups proven, probable and possible. Significant relation between EROTC/MSG Criteria classification and blood culture was found as in table (6)

Table 6: Relation between blood culture and EORTC/MSG criteria classification

EORTC/MSG criteria classification	Blood culture positive 14	Blood culture negative 36	Total	X 2 P value
Proven	7	5	12	X2=12.4
Probable	6	11	17	P value 0.02
Possible	1	20	21	

In table(7) we divided the patients in two groups according to the result of blood culture, the mean value of serum(1.3 D glucan)in patients with positive blood culture were significantly higher than those with negative blood culture there is significant relation between blood culture and serum level of(1.3) D-glucan

Table 7: Relation between 1,3 D beta glucan and blood culture among the studied groups

Studied parameter	Blood culture Positive 14	Blood culture negative 36	"Z" of MWU test	p
Serum level (1,3) D-glucan	125.35	94.72	2.2	0.007

Blood culture could be considered as a marker for detection of invasive fungal infection with sensitivity, specificity, PPV and NPV of, 46.43%, 100%, 100% and 11.76% respectively however (1.3) D glucan can detect invasive fungal infection with sensitivity, specificity, PPV and NPV of, 88.46%, 100%, 100% and 40% as shown in table 8

Table 8: Evaluation of blood culture and (1.3) D glucan in detection of invasive fungal infection

	Sensitivity	Specificity	PPV	NPV
Blood culture	46.43	100	100	11.76
(1.3) D glucan	88.46	100	100	40

DISCUSSION

Non culture tests have the potential to identify patients with invasive candida infection who are currently unrecognized and to shorten the time for diagnosis. Early diagnosis is crucial to initiate antifungal agents early. Unfortunately, clinical and radiological signs are often unspecific. In our work in this study we have evaluated the performance of a (1.3 beta D-glucan (BDG) as a diagnostic tool for invasive fungal infections**.**

In this study according to EORTC/MSG we detected that patients with proven fungal infection was equal in those with diabetic foot and those with prolonged stay in ICU receiving broad spectrum antibacterial treatment (30%) and we reported that those with a probable fungal infection was higher in patients with diabetic foot (45%) than with prolonged stay in ICU receiving broad spectrum antibacterial treatment (40%)which comes in agreement with another stud which reported that *Candida species* had the highest frequency among (68.8%) diabetes mellitus which were the most frequent underlying diseases among IFI-confirmed patients [19] and also with a study done in ICU which recorded that factors leading to *Candida albicans* invasive infections in ICU patients were prolonged ICU stay ,Diabetes mellitus and treatment with corticosteroids. [20]

[21] Held J et al reported that the most sensitive means of diagnosing a fungal infection is generally considered to be the isolation of the infecting agent on culture media. However, false-negative cultures are well documented in the face of disseminated fungal infection which agreed with our study which showed that blood culture was positive in 45% patients in group I, 30% in group II and no one in group III and that positive blood culture was a significantly higher frequent finding in patients with prolonged ICU stay with antibiotic treatment, compared to patients with diabetic foot[22].

In our study the average serum level of BDG was higher in diabetic foot patient was 121.25±88.9 pg/ml which is higher than those with prolonged stay in ICU receiving broad spectrum antibacterial treatment (115.5±90.9 pg/ml) and there is significant relation between EROTC/MSG Criteria classification and serum level of(1.3) D-gluca which agreed with Fontana [23] et al who reported that the BDG serum levels of the 46 patients helped in diagnosing 24 cases of IFI: 18 patients with probable IFI, 3 patients with proven IFI and 3 patients with possible IFI. Finally in proven IFI the BDG serum level was strongly positive

Also, another study reported that the median BG-level in patients with proven and probable IFD was significantly higher than in patients without IFD (P < .0001). In total, he found that 80% of the titrated samples from patients with IFD were higher than 800 pg/mL[24]

In our study the patients were divided in two groups according to the result of blood culture. The mean value of serum(1.3) beta D glucan in patients with positive blood culture was significantly higher than those with negative blood culture, there is significant relation between blood culture and serum level of(1.3) beta D-glucan which comes in agree with study done in neanatal intensive care unit who worked on 52 patients[25]. They reported 41 patients with negative blood cultures and 11 with positive blood cultures while the results of the BDG concentration in those patients were negative in 32, and positive in 20 cases. Finally he reported that the definite fungemia group was significantly associated with positive BDG results when compared with the no fungemia group

Jerry et al [26] reported 15 patients with blood cultures positive for yeast, thirteen of them (86.7%) were positive for BDG and two negative specimens. So blood culture was significantly associated with positive BDG

In our study blood culture could be considered as a marker for detection of invasive fungal infections with sensitivity, specificity, 46.43%, 100%, respectively, however (1.3)beta D glucan can detect invasive fungal infection with sensitivity, and specificity 88.46%, 100% so (1.3) beta D glucan is superior to blood culture in

detection of invasive fungal infection which comes in accordance with another study which reported that (1.3) D glucan can detect IFI with sensitivity of 71% and specificity of 98%[27].

On the other hand different result obtained by Alexander and Pfaller [28] who worked on 41 specimens from patients with proven or probable IFI with the lung transplant, they found that the BDG sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 64%, 9%, 14%, and 50%, respectively; and (92%) of these patients not diagnosed with an IFI and the sensitivity of the BDG test is border line and its significance as a tool for diagnosis of IFI is doubtful, the explanation for this different results may be due to hemodialysis and colonization with mold which significantly affected mean BDG levels.

Other studies [29] reported that serum concentration of BDG was used in the diagnosis of invasive candidiasis (IC) in adult patients with a sensitivity of 57–97% and a specificity 56–93% among adult patients and also another study [30] reported serum BDG works well in detection of neonatal IC and in evaluating the antifungal therapy efficacy with sensitivity of 72 % specificity of 62% [31].

CONCLUSION

We conclude from these findings that a positive (1,3)--D-glucan (BDG) assay could be a superior test in addition to the blood culture for diagnosis of candidemia and may also be useful in the evaluation of patients at high risk of IFI.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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