ORIGINAL ARTICLE

(1-3)-β-D-Glucan Antigen Detection and PCR for Diagnosis of Invasive Fungal Lung Infections

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ABSTRACT

Background: *Invasive fungal lung infection (IFLI) is a collective term used to describe a range of opportunistic infections, caused by one or more endemic or opportunistic fungi, the most isolated microbes in these infections are Candida species, Aspergillus species and Cryptococcus species. Clinical symptoms typically manifest as fever, cough, dyspnea, chest pain, and hemoptysis, Radiologic examination showed various presentations, few had typical features, such as denseness, cavitation, a halo sign some even showed negative results. For diagnosis; microscopy, fungal culture, galactomannan antigen, and PCR are useful tests. Other tools include imaging facilities and blood serum biomarkers. Antigen detection methods include (1-3)-β-D-Glucan lacks higher sensitivity, PCR hasn't been standardized, in this study, we evaluated serum (1-3)-β-D-Glucan antigen in comparison with PCR for diagnosis accuracy.* ***Objective:*** *This study aimed to evaluate serum (1-3)-β-D-Glucan antigen detection and PCR methods for diagnosis of invasive fungal lung infections.* ***Methodology:*** *The study conducted a cross-sectional comparative study on suspected IFLI patients and a healthy control group. Clinical and radiological criteria were used for patient selection. Blood and bronchoalveolar lavage (BAL) samples were collected for biomarker assessment. PCR and BDG antigen detection were evaluated.* ***Results:*** *Male patients accounted for 78.1%, with no significant gender difference. Hypertension (40%), followed by diabetes (22%), were identified as the most common risk factors. BDG antigen levels were significantly associated with fungal infections. Sensitivity was 92%, specificity 80%, with an AUC of 0.879. BDG levels correlated with treatment outcomes.* ***Conclusion:*** *Elevated BDG levels were associated with cancer and COPD patients. While BDG provides supportive evidence for fungal infection, a multifaceted approach is crucial. Implementing new diagnostic tools and centralizing laboratories of fungal identification in clinical setting are essential as the diagnostic landscape evolves.*

INTRODUCTION

Candida, *Aspergillus*, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, and *Mucormycetes* are some of the more common fungi that can cause IFLI in immunocompromised and critically ill individuals. Their burden and epidemiology are increasing as a result of the increased population of people who have weakened immunity and co-morbidities.[1]

IFLI is difficult to diagnose due to the fact that its clinical and radiological symptoms are not particularly precise. The so-called "gold standard" techniques, microscopy and culturing, are not up to par when it comes to sensitivity, the amount of time it takes to achieve a positive result, and how simple it is to obtain invasive specimens. After a delay of 24 to 48 hours, confirmation can be obtained through culture in fewer than half of instances, and a substantial fungal burden is needed for microscopy to produce a positive result.[2]

In order to start or stop antifungal treatment (AFT) as soon as possible, rapid and reliable diagnosis utilizing methods other than culture is urgently required. However, the use, technical performance, and interpretation of biomarkers for early identification of IFLI are not simple/uniform.[3]

It is crucial to take into account the patient's characteristics (whether they are high risk, non-high risk, adults, or children) as well as the patient's medical manifestations, when blood was taken, the procedures / tests utilized, the cut offs applied, and the concomitant administration of AFT in order to arrive at an appropriate interpretation.[4]

Screening and diagnosing IFLI can be supported by the utilization of fungal biomarkers. The 1, 3, and beta-D glucan test is a promising biomarker for the

screening and finding of invasive fungal infection in high-risk cases who are not on antifungal prescription, as well concerning the recognition of cutting edge contaminations in symptomatic cases. 1-3, β D glucan has restricted clinical use. The particularity of 1-3-βDG tests is worked on by a blend of two positive tests or by two positive tests in addition to GM. [2]

In therapeutic settings, the presence of a carbohydrate biomarker called beta-1-3-D-glucan (BDG) is utilized in order to rule out the prospect of IFLI. The major objective of this research was to evaluate and contrast the diagnostic accuracy of serum BDG and PCR testing for IFLI.[5]

IFLD may be detected using a variety of different molecular-based techniques. Techniques that involved polymerase chain reaction (PCR), which varied by a variety of criteria like primer target (e.g., primers, genus specific panfungal primers), are all to blame for the enormous range of sensitivity & specificity values that have been recorded. In contrast, owing to the work of the International Society of Human and Animal Mycology (ISHAM), fungal PCR has become a large amount more standardized. [6]

Polymerase chain reaction findings might be exposed to an outer quality assessment, and with the accessibility of extensive execution approval information, aspergillus PCR is considered to be genuinely dependable. As an outcome, the EORTC/MSG agreement board chose to include it for the current meaning of IFLI.[7]

The primary objective of the research was to investigate the effectiveness of PCR & serum BDG antigen detection methods in the diagnosis of IFLI.

METHODOLOGY

This study is a cross-sectional comparative study. It was conducted from March 2019 to February 2020 at Chest Department of Mansoura University Hospitals and Medical Microbiology and Immunology Department Faculty of Medicine, Mansoura University. The study was approved by institutional research board (IRB) of faculty of medicine, Mansoura university under code of MS.19.05.650 ,and all participants gave informed consent before enrollment under the number.

Two groups of patients were included, first group of suspected patients of IFLI with inclusion criteria of all or one of the following; fever, cough, dyspnea, chest pain, hemoptysis along with one of the following radiological signs such as: The halo sign, bilateral peri-hilar interstitial, cavitation and pulmonary infarcts, Mass-like or branching opacities, denseness and an air crescent sign, some patients were included with negative radiological investigations.

Control healthy group of 15 blood donors were selected according to: no history of chronic illness or fungal infection and no hospital admission for the past year. This study did not include any patients who were currently undergoing AFT treatment.

Each participant in the study was required to provide a history and undergoes a clinical assessment, history of any chronic illness, drug therapy: antibiotics and immunosuppressive therapy and thorough clinical examinations. A CT examination of the included patients was done.

BAL fluid was collected by bronchoscope. BAL was done from the most affected segment or lobe of the lung, collected samples were taken and transported to the Microbiology Department Laboratories for further assessment for fungal infection.

Venipuncture blood samples from both groups` IFLI patinets were collected for microbiological testing prior to BAL.

Detection of (1-3)-β-D-Glucan antigen:

In order to determine whether or not serum (1-3)-β-D-Glucan antigen was present in the blood samples, an enzyme-linked immunosorbent assay test (ELISA) was performed. The results were obtained by first constructing a standard curve by graphing the average optical density (OD) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and drawing a best fit curve through the points on the graph. This allowed for the calculation of the results. This was accomplished with the use of a computer program that fits curves, and regression analysis was used to decide which fit was the best.

Patients with IFLI had panfungal polymerase reaction performed on their broncho-alveolar lavage fluids and the cut off for statistical significance (p-value) was set at less than 0.05. Significant finding were defined as those with probability value of less than 5% ($p \leq 0.05$).

RESULTS

Fifteen patients were registered in the study and their samples were analyzed at the microbiology diagnostic and infection control unit (MDICU) and the Mycology Unit in the department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University.

Male patients were (78.1%) while female patients were (22%) with no significant difference with the control group. The most identified risk factors among the IFLI group were 40%, hypertension (22%) and Diabetes Mellitus (20 %).

Test results of (1-3)-β-D-Glucan Ag of IFLI were compared to the control group. The test results were strongly associated with the disease with significant variation among the 2 groups as shown in (table 1).

Table 1: Concentration (1-3)-β-D-Glucan Ag in serum by ELISA among patients and control groups

(1-3)-β-D-Glucan Ag	Patients group (n=50)	Control group (n=1٥)	Test of significance	p-value
Median (Min-Max)	51.53 (12.64-246.62)	11.0 (6.1-60)	Z=4.42	<0.001*
Positive (>80) Negative (<80)	11 (22%) 39 (78%)	0 (0.0%) 15 (100%)	χ^2=3.97	0.046*

This study reported increased levels of (1-3)-β-D-Glucan Ag., significantly associated with fungal infection patient with cancer and COPD. As illustrated in (Fig 1 & 2).

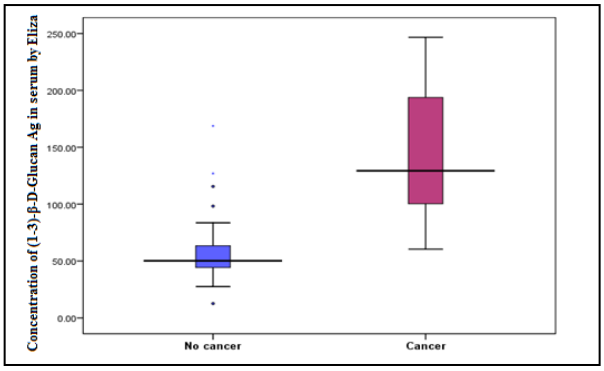

Fig. 1: Box plot for median (1-3)-β-D-Glucan Ag in serum by ELISA among cancer patients

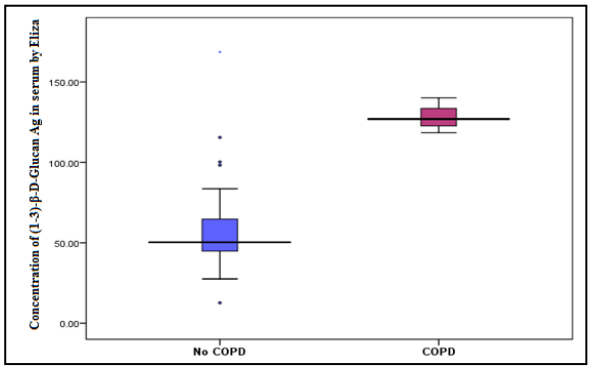

Fig. 2: Box plot for median (1-3)-β-D-Glucan Ag in serum by ELISA among COPD patients

(1-3)-β-D-Glucan Ag, test results showed sensitivity of 92percent, specificity of 80percent. ROC analysis for fungal infected patients generated an area (AUC) of 0.879 (95% CI: 0.756–1.00), as shown in (table 2).

Table 2: Diagnostic accuracy of (1-3)-β-D-Glucan Ag concentration in serum by ELISA in prediction of patients group

AUC	95% CI		Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
	Lower	Upper						
0.879	0.756	1.00	> 33.20	92%	80%	93.8	75	89.2%

AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value

The overall optimal threshold for the diagnosis of fungal infected patients determined by ROC analysis was 33.20 pg/ml, The ROC curve is shown in (Fig. 3).

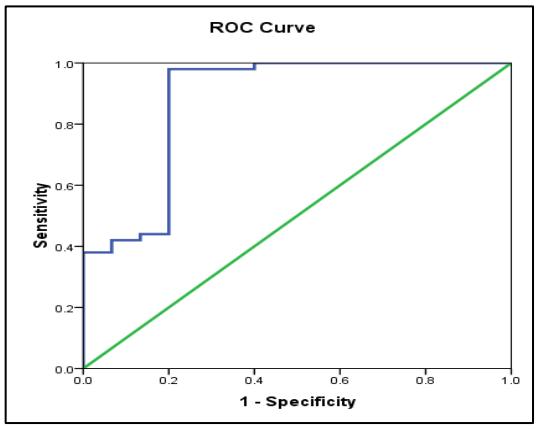

Fig. 3: Receiver operating characteristics curve (ROC) for -(1-3)-β-D-Glucan Ag concentration in serum by ELISA in prediction of patients group

Positive slopes were associated with treatment success (PPV 93.8%), while negative slopes were linked to treatment failure (NPV 75%).

Evaluation of pan-fungal PCR and (1-3)-β-D-glucan Eliza found no statistically significant differences between PCR and (1-3)-β-D-Glucan Table 3 clearly shows it.

Table 3: PCR results among patients group.

	PCR (n=50)	ELIZA (n=50)	p-value
Positive Negative	20 (40%) 30 (60%)	11 (22%) 39 (78%)	0.051

Both tests were evaluated separately for their specificity, sensitivity, positive & negative predictive value. The result shows that sensitivity and specificity of PCR was higher than (1- 3)-β-D-glucan Ag. Table (4)

Table 4: Diagnostic accuracy of ELIZA compared to PCR

	PCR	(1-3)-β-D-Glucan Eliza
Sensitivity	94	92
Specificity	82.5	80
PPV	94.8	93.8
NPV	93	75

PPV: Positive predictive value, NPV: negative predictive value

DISCUSSION

The gold standard for diagnosing IFLI is blood cultures; however, the limited specificity and prolonged turn around times of direct culture methods severely limit their utility in clinical infection diagnosis.[8]

However, the timely administration of appropriate clinical AFT is often delayed, which worsens the patient's condition and ultimately leads to a high mortality rate. According to the statistics, an increase in IFLI was caused by widespread use of broad-spectrum antibiotics, high-dose chemotherapy, glucocorticoidsm & immunosuppressive drugs; however, the statistics do not indicate what caused the increase in IFLI. Patients with IFLI benefit greatly from a rapid and accurate diagnosis for this reason. [9]

BDG has been shown to be a sensitive fungal biomarker across a wide variety of patient populations. This has been demonstrated. As a result, it is one of the criteria utilized to diagnose invasive fungal infections. In spite of the fact that it has been shown that BDG is a good biomarker, there is a relatively low risk of receiving a false positive result because of antigenemia in the lack of an active invasive mycosis. This is due to the fact that BDG is not a biomarker with a very high degree of specificity.[10]

For the diagnosis of IFLI, PCR assays have emerged as a viable alternative to traditional approaches. Due to their increased sensitivity, these assays can detect even trace levels of DNA in clinical samples. Furthermore, the fungal load in clinical specimens can be quantified using those procedures based on quantitative PCR.[11]

Several potential hazards were considered in our study. Smoking, high blood pressure, and type 2 diabetes were the most frequently reported risk factors for greater vulnerability.

One of the benefits of evaluating for (1-3)-β-D-Glucan Ag is that only serum is needed as a specimen. Serum is a convenient specimen that can be obtained from any patient, making it possible to do serial (1-3)-β D-Glucan Ag analysis, which has the potential to greatly improve the clinical performance of the tests. [12]

In patients who had IFLI, the results indicated a sensitivity of 92% and a specificity of 80% for diagnosing fungal infections. This was in comparison to the group that served as the control. In cases with IFLI, significantly higher levels of (1- 3)-β-D-Glucan Ag were found in contrast to the levels seen in the control group.

Using serum (1- 3)-β-D-Glucan Ag tests in individuals with fungal infections, we obtained an AUC of 0.879 (95% CI: 0.756-1.00) for the diagnosis of IFLI, and the overall best threshold for the identification of fungal infections was 33.20 pg/ml, as assessed by ROC analysis.

Previous studies have shown that patients who are suffering from IFLI have a continuous release of (1- 3)-β-D-Glucan Ag into the peripheral circulation. In healthy individuals, the concentration of (1- 3)-β-D-Glucan Ag in the serum was frequently lower than 10 pg /mL. On the other hand, when patients had an invasive fungal infection, the blood level of the fungus dramatically increased and was usually above 20 pg/mL.[13 14]

Positive slopes were associated with treatment failure (NPV 75%) and negative slopes with successful outcomes (93.8%) in our study of (1-3)-β-D-Glucan Ag levels. Consistent findings were provided by Theel [15], who also pointed out that compared to single-time-point testing, performing many tests had significantly higher specificities (76-99%) & NPVs (87-96%) for the presence of IFLI. However, the specificity and sensitivity of the (1-3)-β-D-Glucan Ag tests in the study are still unacceptably poor, even after interval testing.

In spite of the fact that the(1-3)-β-D-Glucan Ag test has a great negative predictive value (NPV), Verma [16] caution that a negative result should not be utilized to rule out the potential of an invasive fungal infection. This is because a positive result does not rule out the possibility of an invasive fungal infection. The reason for this is because the (1-3)-β-D-Glucan Ag assay has a lesser sensitivity compared to other tests that are quite similar. On the other hand, a low sensitivity is quite common among fungal indicators, just like that of (1-3)-β-D-Glucan Ag.

During the course of this investigation, it was found out that the presence of positive results for (1-3)-β-D-Glucan Ag was consistent with the incidence of an invasive fungal infection. This was according to the recommendations that came out of the Third European Conference on Infections in Leukemia, the testing for (1-3)-β-D-Glucan Ag is categorized as a "B II," which means that there is "moderate evidence to support a recommendation for use" in those who have leukemia.[15]

We showed that PCR had a greater value than (1-3)-β D-Glucan Ag as regards of its +ve predictive value, -ve predictive value, sensitivity, & specificity. The findings of this investigation indicated that the difference between PCR results did not approach the level of significance. In another investigation, the use of pan fungal PCR reported concurrently with positive culture results for four patients with an IFLI from 0 to 8 days before the detection of pulmonary infiltrates. This study was carried out from 0 to 8 days before the

diagnosis of pulmonary infiltrates. Their percentage of false positivity was 3%, which is far lower than the data we obtained. Therefore, the identification of any fungal DNA can be an indicator of the presence of an invasive illness. [16]

While there is a debate of (1-3)-β-D-Glucan Ag efficacy & false positive or false negative results rate vary from each study, yet PCR proved more efficient. Negative results of (1-3)-β-D-Glucan Ag could be not conclusive. Negm[17] concluded that a positive (1,3)beta-D-glucan test result has a potential confirmatory for diagnosis of fungal invasisve infection in addition to the blood culture.

For the foreseeable future, the use of such innovative assays in non-specialist settings and impoverished nations is likely to be precluded due to the excessively high upfront costs and the requirement for professional technical and interpretative competence. This is despite the fact that such novel assays may give benefits by lowering the improper use of anti-fungal medications. Therefore, there is an urgent need for diagnostic assays that are both specific and economical for the treatment of IFLI.

CONCLUSION

Patients suffering from COPD and cancer were shown to have a stronger correlation with raised levels of (1-3)-β-D-Glucan Ag, consistent with the findings of our study. Additionally, (1-3)-β-D-Glucan Ag results can be utilized as supportive evidence for the presence of an invasive fungal infection.

When IFLI cannot be definitively confirmed practitioners are required to use a methodology known as "weights of evidence" to analyze host variables, clinical data and mycological information. It is necessary to conduct implementation studies in order to gain an understanding of the most effective methods in which new diagnostic tools can be utilized within therapeutic pathways. The identification of antifungal resistance and the distinction between invasive infections and colonizations continue to be important difficulties. As our arsenal of diagnostic tools grows, it will become increasingly vital to have centralized clinical mycology laboratories and to make efforts to enable access to new diagnosis in settings with limited resources.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.

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