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Diagnostic Accuracy of Beta-D-Glucan and Galactomannan as Fungal Markers in the Detection of Positive Fungal Cultures among Immunocompromised Patients

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ABSTRACT

As fungal infections pose a significant threat to immunocompromised patients, necessitating timely and accurate diagnostic approaches for effective management, non-invasive diagnostic markers are direly needed to detect the presence of fungi during the early stage of the infection. **Objectives:** To determine the diagnostic accuracy of Beta-D-Glucan and Galactomannan as fungal markers in the detection of positive fungal cultures among immunocompromised patients. **Methods:** This cross-sectional study was conducted at the Intensive Care Units of Shifa International Hospital, Islamabad, from April 2024 to July 2024. 57 immunocompromised patients of both genders with an age range of 12 to 80 years, who had positive fungal cultures were included in the study via Non-probability, consecutive sampling while patients who were taking any antifungal medications were excluded. Diagnostic accuracy, sensitivity and specificity of Beta-D-Glucan and Galactomannan were calculated by comparing these assay results against the gold standard of CSF analysis of fungal culture. **Results:** The Beta-D-Glucan and Galactomannan assays showed a specificity of a moderate nature for both assays, slightly higher for Beta-D-Glucan (77.78%) than Galactomannan (72.73%), with a high sensitivity of 92.31% for Beta-D-Glucan and 90.20% for Galactomannan. Overall, the diagnostic accuracy was high for both assays, with Beta-D-Glucan at 96.49% and Galactomannan at 94.74%. **Conclusions:** It was concluded that while the Beta-D-Glucan assay is more sensitive, the Galactomannan assay appears to be more specific. Thus the combined use of both tests enhances diagnostic accuracy for detecting fungal infections in immunocompromised patients.

INTRODUCTION

Over the last two decades, there has been a notable surge in fungal infections (FI), especially among immunocompromised patients, such as those with hematological malignancies, organ transplants, or undergoing prolonged immunosuppressive therapy. These infections significantly contribute to increased morbidity and mortality, with rates reported as high as 60% to 90% in these vulnerable populations [1, 2]. The growing burden of FI in healthcare settings highlights the critical need for improved prevention, early detection, and effective treatment strategies. In particular, the prevalence of FI among patients with hematological malignancies has been found to range from 24% to 31%, emphasizing the importance of prompt diagnosis and intervention in this high-risk group [3]. One of the primary challenges in managing FI is the timely identification of the causative fungal species, as delayed or inadequate treatment can lead to fatal outcomes. This challenge is compounded by the often non-specific clinical presentation of fungal infections, which can resemble bacterial or viral infections. As a result, many cases are diagnosed late, leading to poor outcomes. Hence, there is an increasing demand for the development of more reliable and rapid diagnostic methods that can detect fungal pathogens at an earlier

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stage, potentially improving patient outcomes [4]. This is particularly crucial in immunocompromised patients, who are more susceptible to invasive fungal infections (IFIs), where early detection can significantly impact the course of the disease. In recent years, the detection of fungal biomarkers in clinical samples has emerged as a promising diagnostic approach for IFIs. The identification of fungal cell walls components, such as fungal DNA, galactomannan (GM), and 1,3-β-D-glucan (BG), has shown potential in improving diagnostic accuracy. GM, a polysaccharide component of the fungal cell wall, is shed into the bloodstream during fungal growth, making it a valuable marker for invasive aspergillosis. Similarly, BG is a major component of the fungal cell wall that is released during active fungal infection, particularly in deeper fungal layers [5, 6]. These biomarkers can be detected in serum or Broncho alveolar lavage fluid, providing a non-invasive means of diagnosing fungal infections. However, the sensitivity and specificity of these assays can vary widely, depending on the fungal species and the clinical context. The GM assay, for instance, has shown a sensitivity range of 30% to 100% and a specificity of 38% to 98%, depending on the type of patient population and the timing of the test [7]. In contrast, the BG assay has a reported sensitivity and specificity of 70% to 90%, making it a useful tool for the detection of a broader range of fungal infections, including candidiasis and aspergillosis [8]. However, both assays have their limitations, including false positives due to factors such as concurrent use of antibiotics or the presence of other non-fungal conditions that may interfere with the results. Over the past several decades, fungal infection (FI) has been increasing following the growing number of immunosuppressed or immunocompromised patients with its high associated morbidity and mortality up 60–90%. The major obstacle is to recognize which fungus causes the infection and prescribe appropriate therapy. Thus, there is a need for newer diagnostic modalities for FI. Early detection of FI markers as cell wall components and fungal DNA is important for prompt diagnosis. The most extensively evaluated diagnostic marker is the cell wall surface antigen galactomannan (GM), followed by β-d-glucan, present in deeper layers of the cell wall [9, 10]. The increasing incidence of FI, particularly among immunocompromised individuals, underscores the need for novel diagnostic approaches that can improve early detection and guide timely therapeutic interventions.

This study aimed to determine the diagnostic accuracy of Beta-D-Glucan and Galactomannan as fungal markers in the detection of positive fungal cultures among immunocompromised patients. By enhancing the detection of fungal biomarkers, this research hoped to contribute to the growing body of evidence supporting the use of non-invasive diagnostic methods in the management of fungal infections, ultimately aiming to reduce the high morbidity and mortality associated with these infections.

METHODS

This cross-sectional study was conducted at the Intensive Care Units of Shifa International Hospital, Islamabad, from April 2024 to July 2024. The sample size calculated was 57 immunocompromised patients of both gender with age range of 12 to 80 years, who had positive fungal cultures, chosen via non-probability, consecutive sampling. The sample size was calculated via taking expected sensitivity of B- galactomannan as 70% [10]. The confidence level was taken as 90% with 10%, desired precision. Patients who (at the time of taking samples) were taking any antifungal medications were excluded from the study. The IRB approval was obtained prior to the study (070-24). Informed written consent was taken from every participant. Positive fungal cultures were identified by growing fungal organisms in culture media under laboratory conditions, confirming the presence of viable fungi in collected samples. The β-d-Glucan levels were measured using the fungi tell assay, based on the Limulus gametocyte lysate (LAL) method, with a positive result defined by levels exceeding 80 pg/mL. Additionally, the Platelia Aspergillus enzyme immunoassay was employed to detect Aspergillus Galactomannan, with an optical density index greater than 0.5 indicating a positive result. Diagnostic accuracy, sensitivity and specificity were calculated by comparing these assay results against the gold standard of CSF analysis of fungal culture. The Receiver Operating Characteristic (ROC) curve was plotted to evaluate and compare the diagnostic performance of the Beta-D-Glucan (BG) and Galactomannan (GM) assays. The Area Under the Curve (AUC) provided a summary measure of diagnostic accuracy, with higher AUC values indicating better discriminatory ability of the assays. Data were analyzed using SPSS version 25.0. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated.

RESULTS

The mean age was 45 + 18.5 years, the majority being male (61.4%). Most participants belonged to the middle or lower socioeconomic classes (82.5%). The primary underlying conditions were malignancy (43.9%) and organ transplantation (31.6%), with hematologic malignancies being more common than solid tumors. Kidney transplants were the most frequent among organ transplant recipients. A significant portion of patients were on steroids (73.7%) and antibiotics (87.7%), with 52.6% experiencing neutropenia. The average duration of the disease was 12.5 ± 7.8 months, and blood samples were collected after an average of 18 days of hospitalization (Table 1).

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Table 1: Demographic and Clinical Characteristics

Variables	Results
Age	45 ± 18.5 years
Gender	
Male	35 (61.4%)
Female	22 (38.6%)
Socioeconomic Status	
Upper	10 (17.5%)
Middle	25 (43.9%)
Lower	22 (38.6%)
Underlying Disease	
Malignancy	25 (43.9%)
Organ Transplantation	18 (31.6%)
Other	14 (24.5%)
Type of Malignancy	
Hematologic	15 (60%)
Solid Tumor	10 (40%)
Type of Organ Transplantation	
Kidney	12 (66.7%)
Liver	4 (22.2%)
Heart	2 (11.1%)
Use of Steroids	
Yes	42 (73.7%)
No	15 (26.3%)
Use of Antibiotics	
Yes	50 (87.7%)
No	7 (12.3%)
Neutropenia Present	
Yes	30 (52.6%)
No	27 (47.4%)
Duration of Disease/Condition	12.5 ± 7.8 months
Duration of Hospitalization till Collection of Blood Sample	18 ± 10 days

The Beta-D-Glucan (BG) and Galactomannan (GM) assays both demonstrated high sensitivity, indicating their effectiveness in detecting positive fungal cultures among immunocompromised patients. Both assays also showed strong positive predictive values (PPV), with BG at 96.00% and GM at 93.88%, suggesting that a positive result is highly indicative of a true fungal infection. Specificity was moderate for both assays, slightly higher for BG (77.78%) than GM (72.73%). Overall, the diagnostic accuracy was high for both assays, with BG at 96.49% and GM at 94.74%, reinforcing their utility as reliable markers in this patient population (Table 2).

Table 2: Diagnostic Parameters of Beta-D-Glucan (BG) and Galactomannan (GM) Assays

Diagnostic Parameter	BG	GM
Positive	48 (84.2%)	46 (80.7%)
Negative	9 (15.8%)	11 (19.3%)
Positive Predictive Value	96.00%	93.88%
Negative Predictive Value	63.64%	61.54%
Sensitivity	92.31%	90.20%
Specificity	77.78%	72.73%
Diagnostic Accuracy	96.49%	94.74%

The study displays the ROC curves for Beta-D-Glucan (BG) and Galactomannan (GM) assays, comparing their diagnostic performance in detecting fungal infections in immunocompromised patients. The Area Under the Curve (AUC) for BG was 0.94, indicating high diagnostic accuracy, while GM showed a slightly lower AUC of 0.92. Both assays demonstrated strong sensitivity and specificity, but BG outperformed GM in terms of positive predictive value (96.00% vs. 93.88%) and overall diagnostic accuracy (96.49% vs. 94.74%). The ROC analysis confirms BG as a slightly more reliable marker for fungal infections in our study (Figure 1).

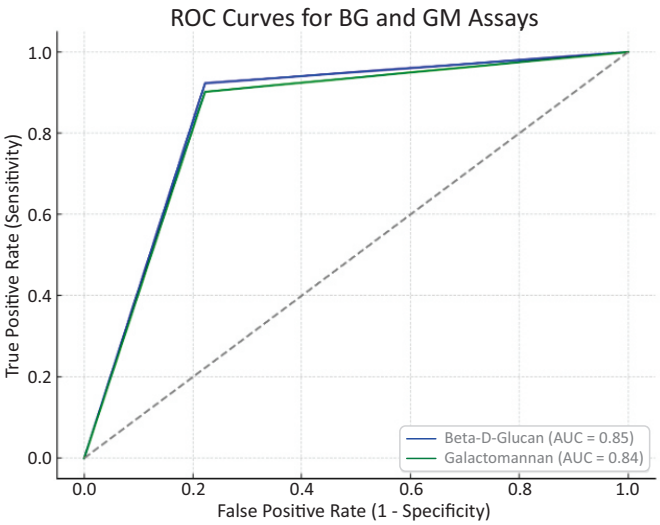

Figure 1: ROC Curve for Beta-D-Glucan (BG) And Galactomannan (GM) Assays

DISCUSSION

Fungal infections (FIs) remain a diagnostic challenge for clinicians and microbiologists due to the imperfections of current diagnostic tools. While fungal culture is considered a superior method, it often takes longer to yield detailed information about the various fungi involved in infections [11]. This technique is regarded as the gold standard for diagnosis through histopathological examination of tissue biopsy specimens [12]. However, in immunocompromised patients, its invasive nature can lead to increased morbidity. As a result, there has been a growing emphasis on less invasive serological assays, such as Beta-D-Glucan (BG) and Galactomannan (GM), for diagnosing fungal infections. In this study, the BG assay returned positive results in 48 of the 57 cases, yielding a sensitivity of 90% and a specificity of 65.56%, with a positive predictive value (PPV) of 96% and a negative predictive value (NPV) of 3% (%p≤0.001). These findings align with previous studies by Ostrosky *et al.*, [13], which reported a sensitivity of 70% and specificity of 87%, and Pazos *et al.*, [14], which found a sensitivity of 87.5% and specificity of 89.6%. The lower specificity observed in our study (65%) indicates that while

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BG is a valuable tool, it may not be suitable for all fungal species. The typical concentration of Beta-D-Glucan in human serum ranges from 10 to 40 pg/mL, with levels exceeding 80 pg/mL considered clinically relevant and indicative of fungal infections. However, this assay does not identify fungi at the genus or species level and serves primarily as a rapid presumptive screening technique. Additionally, false positives can occur due to external factors, such as contamination during specimen collection, particularly when cotton gauze is used. Furthermore, the BG assay's accuracy is highly dependent on procedural conditions that may not be achievable by non-laboratory personnel, impacting the reliability of results—an aspect that highlights significant limitations in this study. Although the assay can specifically identify fungi like Aspergillus, Candida, and Fusarium, it cannot accurately differentiate species lacking BG in their cell walls, such as Zygomycetes and Cryptococcus [15]. In our study, the GM assay demonstrated a sensitivity of 90.2% and a specificity of 72.73%, with 46 positive tests out of 57 cases. In comparison, PCR testing is more sensitive (100%) for diagnosing both fungemia and non-fungemia infections without the interference of masking agents like Voriconazole, serving as a specific backup to culture methods. This aligns with findings from Sims et al., [16] and Cornillet *et al.*, [17], particularly regarding the diagnosis of Aspergillus infections. However, caution is necessary when interpreting GM assay results in patients receiving beta-lactam antibiotics, as these can cross-react with GM and produce false positives [17]. Additionally, the sensitivity of the GM assay may decline in patients who have undergone prior antifungal treatment, particularly with azole medications, as noted by Foy *et al.*, [18]. This underscores the importance of collecting serum samples before initiating antifungal therapy. Dietary factors should also be considered, particularly in young children, as humanized milk can serve as a minor source of Galactomannan and lead to misleading test results. Notably, all patients who tested positive for GM in our study were also positive for BG, indicating that while BG demonstrates greater sensitivity, this specificity necessitates dual assessment. Combining these assays could enhance diagnostic accuracy among high-risk patients, including those receiving antifungal therapy [19, 20].

CONCLUSIONS

It was concluded that our study demonstrates that both Beta-D-Glucan (BG) and Galactomannan (GM) assays are effective for detecting fungal infections in immunocompromised patients. BG exhibited superior diagnostic accuracy at 96.49% and a positive predictive value (PPV) of 96.00%, while GM achieved an accuracy of 94.74% and a PPV of 93.88%. These findings highlight BG as a more reliable biomarker, suggesting its potential to enhance timely treatment and improve patient outcomes. Future studies should focus on optimizing these assays across diverse immunocompromised populations and exploring their combined use to increase diagnostic accuracy.

Authors Contribution

Conceptualization: SA,
Methodology: SA, ZS, MMS, MAA, SR, AA
Formal analysis: SR
Writing review and editing: ZS, MMS, MAA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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