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Research Article

TO ASSESS THE UTILITY OF 1,3-BETA–D-GLUCAN ASSAY AS AN AID IN EARLY DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES AT A TERTIARY CANCER CARE CENTRE

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ABSTRACT

To Assess the Utility Of 1,3-Beta–D-Glucan Assay as An Aid in Early Diagnosis of Invasive Fungal Infections in Patients with Haematological Malignancies at A Tertiary Cancer Care Centre.

Introduction: Fungal infections, once considered rare, have become significant causes of morbidity and mortality, especially among immunocompromised patients.

Aim: To assess the utility of 1,3 beta D-glucan assay as an aid in early diagnosis of invasive fungal infections in patients with haematological malignancies from a tertiary cancer care center at Bikaner.

Methodology: This hospital-based cross-sectional study was conducted in the Department of Microbiology and the Department of Medical Oncology at Sardar Patel Medical College and Associated Group of Hospitals, Bikaner, from October 2023 to March 2024, or until the desired sample size was reached. Blood samples registered and processed further at Microbiology lab for Kinetic ELISA (SPECTRA MAX™*). FDA-approved serum test for detecting invasive fungal infections by measuring (1→3)-β-D-glucan The Fungitell® assay used. Interpretation of result values <60, 60 to 79 and ≥ 80 pg/ml interpreted as negative, intermediate and positive respectively. All statistical analysis done by using the SPSS Software. The data represented as number as well as mean and median for quantitative variables. All proportions compared using the Chi-square test and result with P <0.05 were taken as statistically significant

Result: We found, β-D glucan (BDG) had a sensitivity of 100%, specificity of 90.66%, positive predictive value (PPV) of 56.25%, and negative predictive value (NPV) of 100%. The study found consistent result with those of previous studies, which report variable sensitivity and specificity of BDG assays across different specimen types, with generally high specificity but lower sensitivity.

Conclusion: The study concludes that the 1,3-beta-D-glucan (BDG) assay is a valuable, non-invasive biomarker for early diagnosis of invasive fungal infections in haematological malignancy patients, enhancing clinical decision-making and improving outcomes, with further research needed to refine its specificity.

KEYWORDS: β-D Glucan, Haematological Malignancies, Invasive Fungal Infections, The Fungitell Assay.

Introduction

Among the opportunistic infections, fungal infections have emerged as an important etiological agent. The fungal infections are now recognised as one of the significant causes of morbidity and mortality among the human beings. Invasive fungal infections were regarded as rare disease about a few decades ago. Since then there has been a steady increase in the number of patients suffering from life threatening fungal infections. Opportunistic fungal infection is a frequently encountered complication especially in immune compromised patients.

The systematic study of these organisms is about 150 years old. In the past the fungi have caused only a small proportion of infectious disease, but with increasing control of infections due to bacteria and viruses through sanitation, education, immunization and chemotherapy, the fungal infections are becoming more important.

India is a vast country with more than one billion people and spread over an area of 3.3 thousand million square kilometers. The geofigical and environmental conditions vary in different places of the country. Located in the tropics with heavy annual monsoon, the climatic conditions are favorable for the fungi to grow in many parts of the country. All of these factors together favour widespread occurrence of both common and unique mycotic infections. The climatic conditions of India are favorable for the fungi to grow in many parts of the country. The rise in the incidence of invasive fungal infections (IFI) has increased dramatically since the early 1980s and parallels the increase in the population of persons who are immunocompromised[1,2,3]. These infections are associated with high morbidity and mortality, ranging from 60% to 90%, especially in hematologic malignancy patients in the setting of neutropenia and hematopoietic stem cell transplantation[4,5,6].

The frequency of IFI is 5-30% in patients with malignancy[3,8], 2-42% in organ transplant recipients,20-25% among AIDS patients and 10-15% among Diabetes mellitus (DM) patients[3,9].

IFI in immunocompromised patients are difficult to diagnose, but successful treatment requires early intervention with antifungal drugs[7]. Delay in instituting appropriate antifungal therapy contributes to the high death rate, which may approach 50% and 80% for invasive Candida and Aspergillus infections, respectively[8,9,10]. The precise prevalence of disease is not known but population-based surveillance estimates it as 12–17 per 100 000 population[11].

Several reasons favoring IFIs include usage of anti neoplastic and immunosuppressive agents, broad-spectrum antibiotics, patients with uncontrolled diabetes mellitus, burns, neutropenia, HIV infection, prolonged intensive care unit (ICU) admission and aggressive surgery[7].

Traditional methods of culture and histopathologic examination of tissue biopsies require an invasive procedure. Culture requires several days and may be negative in up to 50% of patients who have systemic Candida or Aspergillus infections. Also, invasive procedures necessary to obtain biopsies may be precluded in critically ill patients. Without reliable diagnostic information, antifungal drugs are often administered empirically[12].

Treatment may be prolonged for IFI and is often associated with drug toxicities. The high cost, limited availability, poor compliance are factors favouring emergence of antifungal resistance especially in the settings of immunosuppression[4]. As antifungal susceptibility testing is labour intensive and expensive it is not routinely performed in all laboratories. The treatment therefore rests on empirical antifungal therapy with no available data on antifungal susceptibility testing and no monitoring on emergence of antifungal resistance.

Accurate diagnosis of invasive fungal infection remains a problem in immunocompromised patients, in whom signs and symptoms are nonspecific or develop late in the course of illness due to the defective immune response[13]. Rapid and accurate diagnosis of IFI is essential, but challenging due to non-specific clinical-radiological findings, need for invasive sampling and a low sensitivity of conventional culture and histopathology techniques. Prompt recognition and treatment of life-threatening IFIs in patients with immunosuppression such as those with haemato-oncological malignancies, congenital or acquired immunodeficiencies, transplant recipients and immunosuppressive medication are crucial for improved outcomes. Non-culture-based detection of fungal biomarkers in serum and high-resolution radiology techniques have improved our diagnostic approach. (1→3)-β-d-glucan (BG) is a component of the cell wall of fungi except Cryptococcus neoformans and Zygomycetes[14,15].

Its presence in the bloodstream correlates with IFI.[16] Assays for BG are based on the activation of the horseshoe crab coagulation cascade[17]. Liposaccharide and BG initiate the coagulation cascade by activating different serine protease zymogens, factors C and G. Liposaccharide specifically activates factor C, while BG activates factor G. Limulus polyphemus amebocyte lysates are made specific for BG by removal of factor C.

The detection of 1,3-beta-D-glucan (BDG) has been evaluated for IFI diagnosis and is included in the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) revised diagnostic criteria for IFI[17].

The performance of these assays has been evaluated extensively in immunosuppressed patients in developed countries, and there is substantial evidence supporting their use for IFI diagnosis[13]. However, studies evaluating their role in the Indian scenario are limited[18,19].

No study has evaluated how BDG contributes to the diagnosis of IFI in selected patients with hematological malig-

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nancies admitted to the ICU. These patients usually have organ dysfunctions, which may affect BG performance[20]; and bacterial infections, which complicate the interpretation of BG assay results[21,22]. Furthermore, over 90% of critically ill hematology patients receive antibacterial agents and up to 50% antifungal agents, which have been reported to modify BG values[23].

We studied to assess the utility of the FDA approved Fungitell BDG assay as an aid in the diagnosis of IFIs in immunocompromised patients admitted at our centre, a tertiary cancer care hospital. This to the best of our knowledge was the first study in north west Rajasthan.

Aim and Objectives

We assessed the utility of 1,3-beta-D-glucan assay as an aid in early diagnosis of invasive fungal infections in patients with haematological malignancies.

And studied the demofigic profile and prevalence of IFI in probable cases of IFI in patients with haematological malignancies, sensitivity of 1,3 beta D glucan assay in IFI and compared the result of 1,3 beta D glucan assay with conventional mycological culture methods.

Material and Methods

We conducted a hospital-based cross-sectional study in the Department of Microbiology and with the collaboration of the Department of Medical Oncology at Sardar Patel Medical College and Associated Group of Hospitals (spmc), Bikaner, from October 2023 to March 2024. We aimed the high-risk haematological malignancy patients admitted in the medical oncology department.

We included probable cases of invasive fungal infection (IFI) according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) diagnostic criteria for haematological malignancies, along with patients who were willing to participate. And the immunocompromised individuals, others than haematological malignancies, healthy individuals, and patients unwilling to participate excluded. Convenience sampling was employed for participant selection. Patients selected under the study were those on chemotherapy for malignancy.

A minimum sample size of 90 Probable cases of IFI in haematological malignancy cases at 80% study power and alpha error 5% was taken with prevalence of IFI in haematological cases is 17.00% as per reference.

Based on the inclusion criteria, the patients were screened and selected from the medical oncology department at Sardar Patel medical college. After informed consent and ethical approval from Institutional Ethical Committee (IEC) of SPMC all relevant history was taken in a pre-formed proforma. Blood sample was taken from patients under aseptic precautions. After collecting the specimen, the container was opened in a biosafety cabinet. Then Samples was registered in the laboratory register and processed further at Microbiology lab for Kinetic ELISA (SPECTRA MAX™*). Second sample was taken according to site of IFI like sputum, blood, skin etc. and processed for culture for identification. The samples then processed for KOH mount and fungal assay and finally as gold standard fungal culture. The fungal culture done on sabouraud dextrose agar (SDA) and results were noted after 2 weeks of aerobic incubation at 25° celcius temperature. FDA-approved serum test for detecting invasive fungal infections by measuring (1→3)-β-D-glucan The Fungitell® assay used. The Fungitell® test results used as an aid in the diagnosis of invasive fungal infection. The results expressed in pg/mL of serum and range from non-detectable (<31 pg/mL) to >500 pg/mL and are printed out by the software or read from the standard curve. Accurate values above 500 pg/mL require that the sample be diluted with RGW and retested. Interpretation of result values <60, 60 to 79 and ≥ 80 pg/ml interpreted as negative, intermediate and positive respectively.

All statistical analysis done by using the SPSS Software (Stastical Package for the Social Sciences Inc., Chicago. IL,USA, Version 22.0 for Windows). The data represented as number as well as mean and median for quantitative variables. All proportions compared using the Chi-square test and result with P <0.05 were taken as statistically significant.

Results

In our study, 84 cases studied, among them 50 male and 34 female. Maximum 52.38% were of 26 – 45 yr age group followed by 30.95% were of 46-65 yr whereas minimum 30.95% were in 6 – 25 yr age group. Mean age of study population was 45.03 ± 6.54 yrs (Table 1).

We included maximum 60.71% cases were of AML followed by ALL in 17.86%, Non-Hodgkin's lymphoma (9.52%), Hodgkin's lymphoma (4.76%), Multiple Myeloma (2.38%), CML (3.57%). And CLL (1.19%) (Figure 1). In our study maximum 60.71% samples were of sputum followed by 32.14% of skin and minimum 7.14% of BAL were screened (Table 2). As shown in Table 3, Maximum 75.00% cases had β d glucan <60 pg/ml whereas 5.95% had 60 – 79 pg/ml followed by 19.05% had ≥80 pg/ml. According to culture of specimen, 10.71% were culture positive, 7.14% had candida sp.Growth whereas minimum 3.57% had aspergillus species and 89.29% had no growth (Table 4). Table 5, clearly explained that our study showed sensitivity of β d glucan 100%, specificity of β d glucan was 90.66%, PPV was 56.25% and NPV = 100%. In the study, among IFI-positive cases, 50% were in the 46–65year age group with a mean age of 41.06 ± 15.12 years, while in culture-positive cases, 44.4% were in the same age group with a mean age of 34.88 ± 14.58 years, and a higher proportion of males were affected in both groups (Figure 3). Among IFI positive cases maximum 10 cases had AML, 4 cases had ALL and 1 case each of NHL and CLL (Table 6). According to culture positive cases out of

9 cases maximum 6 cases had AML and 3 cases had ALL (Table 6 and Figure 2). According to IFI positive cases out of 16 cases maximum 8 cases (50%) were in 46 – 65 yr age group followed by 5 cases in 26 – 45 yr with mean age of 41.06 ± 15.12 year of age and culture positive cases out of 9 cases maximum 4 cases were in 46 – 65 yr age group followed by 3 cases in 26 – 45 yr with mean age of 34.88 ± 14.58 year , p-value = 0.96 (Figure 2). Among 16 IFI positive cases 11 were male whereas 7 females. And culture positive cases out of 9 cases maximum 6 cases were male and 3 cases were female.

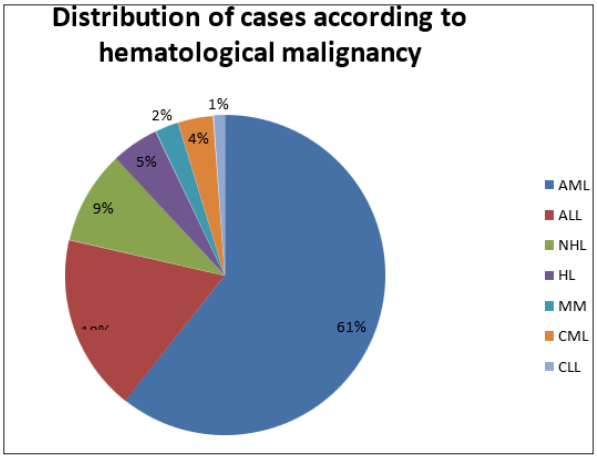


Figure 1: Distribution of probable cases of IFI according to haematological malignancy

Table 1: Distribution of probable cases of IFI according to Socio demography

Age group	No. (n=84)	%
6 – 25	14	16.67
26 – 45	44	52.38
46 - 65	26	30.95
Mean ± SD	45.03 ± 6.54	
Gender		
Male	50	59.52
Female	34	40.48
Residence		
Rural	50	59.52
Urban	34	40.48

Table 2: Distribution of probable cases of IFI according To Specimen for culture

Specimen	Case (n)	%
Skin	27	32.14
Sputum	51	60.71
BAL	6	7.14
Total	84	100.00

Table 3: Distribution of Cases according To β d glucan

β d glucan (pg/ml)	Case (n) No.	%
<60	63	75.00
60-79	5	5.95
≥80	16	19.05
Total	84	100.00

Table 4: Distribution of Cases according To culture

Culture	Case (n) No.	%
Aspergillus Sp.	3	3.57
Candida Sp.	6	7.14
No growth	75	89.29
Total	84	100.00

Table 5: comparision of culture with serum β d glucan Assay (IFI)

IFI	Culture positive (n)	Culture negative (n)
β d glucan (>80pg/ml)	9	7
β d glucan (<80pg/ml)	0	68
Sensitivity = 100% , Specificity = 90.66% , PPV=56.25% , NPV = 100%		

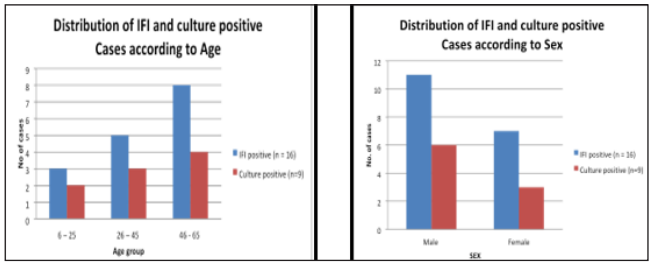


Figure 2: Distribution of IFI culture positive cases according to Age and Sex

Table 6: Distribution of IFI and culture positive Cases according to Malignancy

Malignancy	IFI positive (n = 16)	Culture positive (n=9)
AML	10	6
ALL	4	3
CLL	1	0
NHL	1	0
P value	1.000	

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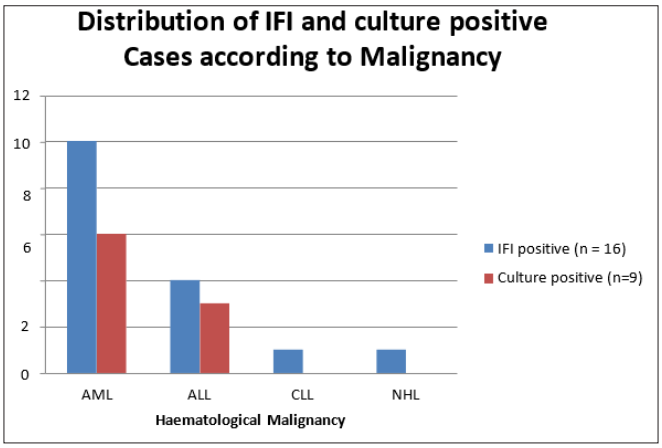


Figure 3: Distribution of IFI and culture positive Cases according to Malignancy

Discussion

We evaluated fungal serum biomarkers and BDG for the diagnosis of IFI patients. Our study revealed that the BDG assay has a higher sensitivity and specificity for the detection of IFI. Limitations of traditional mycological culture, the need for invasive sampling and non-specific radiological findings make the diagnosis of IFI challenging. Evaluation of non-invasive, rapid tests for IFI diagnosis is essential, particularly in immunocompromised patients. Although various studies worldwide have evaluated the use of BDG as a biomarker of IFI in such patients, there is scant data regarding their diagnostic potential in Indian patients.

In a recent study, BDG assay, GM detection and pan-fungal polymerase chain reaction (PCR) were compared for the diagnosis of IFI in pediatric cancer patients[24]. They described GM detection with PCR as the best diagnostic combination while a sensitivity of 88% and a specificity of 59.5% were recorded for the BDG detection assay (Fungitell).

The present study was a hospital based cross sectional study conducted on 84 cases of probable cases of IFI according to EORTC- MSG criteria in hematological malignancy admitted at at medical oncology department at Sardar Patel Medical College and Associated Group of Hospitals, Bikaner from October 2023 to March 2024.

In our study, Maximum 52.38% were of 26 – 45 yr age group followed by 30.95% were of 46-65 yr whereas minimum 30.95% were in 6 – 25 yr age group. Mean age of study population was 45.03 ± 6.54 yrs with 59.52% were male whereas 40.48% were female. Similar findings have been recorded by Kontoyiannis et al showed median age of the patients with IFI was 49years and 60% were males[25]. Elham A. Negm et al. (2021)[26] found that the mean age was 43.55±4.8 for group 1 with IFI, and 38.9±4.2 years old for control group. No significant difference was detected among the studied groups regarding gender distribution or age.

In our study, maximum 60.71% cases were of AML followed by ALL in 17.86% followed by Non-Hodgkin's lymphoma (9.52%), Hodgkin's lymphoma (4.76%), Multiple Myeloma (2.38%), CML (3.57%). whereas minimum was CLL (1.19%). Maximum 75% samples were probable cases of IFI followed by 20.24% of possible cases and minimum 4.76% of proven cases. Similerly Maria Siopi et al. (2022)[19], found a total of 60 adult haematology patients were screened for IA, 13 with probable IA, 19 with possible IA, and 28 with no IA. Also Barbora Weinbergerova et al. (2020)[18] observed that Proven and probable IPA were documented in

13.4% of the episodes. Also Juliette Guitard et al. (2016)[17] found that 8 proven and 4 probable IFIs, and 7 possible IFIs were diagnosed in 9 and 7 patients. Siok-Ying lee et al reported a prevalence of IFI in hematological patients in Singapore of 30.8% proven, 48.7% probable and 20.5% possible IFI[27]. Kontoyiannis et al, studied on patients with IFI for a period of 5 years reported 56% of cases were proven and 44% were to be probable IFI[25].

In our study maximum 60.71% samples were of sputum followed by 32.14% of skin and minimum 7.14% of BAL were screened. Similerly Maria Siopi et al. (2022)[19] found that Serum specimens (n = 166) were collected twice.

Our study states maximum 75.00% cases had β d glucan <60 pg/ml whereas minimum 5.95% had 60 – 79 pg/ml followed by ≥80 pg/ml (19.05%). Similerly Elie Azoulay et al. (2016)[16] found that BG concentrations (pg/mL) were higher in patients with than without IFI (144 (77–510) vs. 50 (30–125), < 0.0001). Also Elham A. Negm et al. (2021)[26] found that the average serum level of BDG was higher in group II (121.25±88.9 pg/ml) than control group. (p=0.004)

In our study, according to culture of specimen, 7.14% had candida sp. Growth whereas minimum 3.57% had aspergillus species and 89.29% had no growth. Similarly Elie Azoulay et al. (2016)[16] observed that IFIs were documented in 78 (10.6%) patients (invasive pulmonary aspergillosis, n = 54; Pneumocystis jirovecii pneumonia, n = 13; candidemia, n = 13; and fusarium infections, n = 3). Our study finding is very similar to Kauffman et al[28] and Chakrabarti et al[1]. who showed Invasive Candidiasis as the most common invasive mycotic infection across India

But, our results are in contrast to the observation made by Wald et al[29] and Marr et al[30] who reported Aspergillus spp as the most common etiological agent isolated followed by Candida sp.

In our study, shows sensitivity of β d glucan 100%, specificity of β d glucan was 90.66%, PPV was 56.25% and NPV = 100%. Similarly Barbora Weinbergerova et al. (2020)[18] found that Sensitivity (SEN), specificity (SPE), positive and negative predictive value (PPV; NPV), and diagnostic odds ratio (DOR) of the BDG assay using standard (80 pg/ml) cut-off for BALF were: 56.5%; 83.2%; 34.2%; 92.5%, and 6.5, respectively, and for serum were: 56.5%; 82.6%; 33.3%; 92.5%, and 6.2, respectively. Also Juliette Guitard et al. (2016)[17] observed that Sensitivity and specificity increased from 75% and 56% to 100% and 91.1%, respectively. Also Elie Azoulay et al. (2016)[16] With 80 pg/mL as the cutoff, sensitivity was 72%, specificity 65%, and area-underthe-curve 0.74 (0.68–0.79). Assuming a prevalence of 10%, the negative and positive predictive values were 94% and 21%.

Conclusion

We concluded that the utility of the 1,3-beta-D-glucan (BDG) assay as a valuable biomarker for detecting invasive fungal infections (IFIs) in patients with haematological malignancies. The findings indicate that the BDG assay offers a non-invasive, rapid, and sensitive method for early diagnosis, which is crucial in this vulnerable population where timely intervention can significantly impact outcomes. The assay's ability to detect a broad range of fungal pathogens, including those difficult to diagnose with conventional methods, enhances its clinical relevance.

The study emphasizes the BDG assay's role in enhancing IFI diagnosis when used alongside imaging and culture, enabling timely antifungal therapy and potentially reducing morbidity and mortality. Further research is needed to improve its specificity and establish standardized thresholds for clinical use. Overall, the BDG assay is a significant advancement in managing IFIs in haematological malignancy patients, improving timely and effective care.

Limitations

Hospital based study

Sample testing for serum BDG assay is costly thus not feasible.

The sample size is less due to financial burden.

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Conflict of Interest

The authors declare that they have no conflicts of interest, financial or otherwise, relevant to the content of this article. No financial support, honoraria, or free diagnostic kits were received from any private pharmaceutical or commercial diagnostic entities for this study.

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