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EVALUATION OF GALACTOMANNAN FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS AT DIFFERENT CUTOFF VALUES IN PEDIATRIC CANCER PATIENTS

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ABSTRACT: Introduction: Invasive Aspergillosis (IA) can cause significant morbidity and mortality in pediatric cancer patients. The Galactomannan enzyme immunoassay (GM-EIA) has shown promising results to diagnose IA in adult patients. But data on this assay in children are limited by small sample size and conflicting results. We evaluated GM-EIA at different cut off in pediatric cancer patients on chemotherapy with fever and neutropenia and not responding to broad spectrum antibiotics for four days. Aim: To Evaluate Galactomannan at different cut off values in 200 pediatric cancer patients. **Materials and Methods:** It is a hospital based cross sectional cohort study in pediatric cancer patients. Serum specimens were collected either once or twice weekly, during neutropenic periods. Operating characteristics were calculated using the GM- EIA index values (IV) of 0.5, 0.7. 1.0 & 1.5 in serum samples. **Results:** 232 serum samples from 200 febrile neutropenic patients were available for evaluation. Of these 232 samples 168 samples were single samples and 32 were consecutive samples (received twice in a week). GM assay was evaluated in both single and consecutive samples at different cut off IV. For single sample the best cut off IV was found to be 1.5 IV. Sensitivity, specificity, PPV, NPV, PLR and NLR at 1.5 IV were: 78, 86, 79, 83, 4.83, and 0.20 respectively. For consecutive samples the best cut off IV was found to be 0.5 at which the sensitivity, specificity, PPV, NPV, PLR and NLR were: 84.2, 86.46, 83.73, 85.50, 5.82 and 0.18 respectively. **Conclusion:** Role of single sample cannot be ruled out in establishing the diagnosis of IA in pediatric cancer patients especially in resource poor settings where frequent sampling is not possible. The best cut off IV of GM for diagnosis of IA in pediatric cancer patients in single serum sample is 1.5 and in consecutive sample is 0.5.

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KEYWORDS: Galactomannan, Invasive fungal Infection, pediatric cancer patients, Aspergillosis, cut-off value, early diagnosis.

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1.INTRODUCTION

Invasive fungal infections (IFI) are a serious cause of morbidity and mortality in immunocompromised patients, especially in patients with hematological malignancies. [1] The incidence has dramatically increased in recent decades. [2] Various factors account for this increased frequency; these factors are dose-intensive regimens causing neutropenia and anemia, mucosal damage due to chemotherapy and widespread use of broad spectrum antibiotics. Patients with hematological malignancies are at increased risk of developing invasive aspergillosis (IA), which is a major cause of morbidity and mortality in these types of patients.[3]Also placements of indwelling central venous catheters, hematopoietic stem cell transplantation (HSCT) render these children more vulnerable to fungal infections .[4,5]In an effort to standardize the definitions of IFI, an international consensus of the Invasive fungal Infection cooperative group of the European Organization for research and treatment of Cancer (EORTC) and mycoses study group (MSG) proposed three levels of IA: proven, probable, possible and no IFIs based on host factor, and microbiological, histopathological and clinical criteria.[6]. Galactomannan is a hetero-polysaccharide (mannan core and side residues of galacto-furanosyl units) present in the cell wall of Aspergillus spp. and is released into biological fluids during fungal growth in the tissues.[7]Detection of Galactomannan by EIA is widely used to diagnose invasive aspergillosis. Its analytical performance for the diagnosis of invasive aspergillosis is well documented in adult patients but in case of pediatrics cancer patient's data is not sufficient especially for Indian population. This is because of its less use in clinical practice. The degree of antigenemia that indicates IA is a subject of debate in pediatric patients. Typical cut off values of optical density index ODI or index value (IV) of GM range from 0.5 to 1.5. A cut off of 0.5 is currently approved by FDA. [1, 8] Deciding on a clear cut off value for this assay to diagnose IA in pediatric cancer patients will be a big help to pediatricians/ clinical hematologists/ infectious disease specialists. Keeping this in mind the present study was planned to evaluate the Galactomannan Index value (GM IV) of Galactomannan at different cut off values.

2. MATERIALS AND METHODS

The study was conducted in the Mycology division of Postgraduate Department of Microbiology, King George`s Medical university, Lucknow and Pediatric Oncology department, Lucknow. It is a hospital based cross-sectional cohort study. 200 pediatric patients in the age group of 1-15 years with Hematopoietic disorder (n=180) and solid tumors (n=20) were included in the study from period of January 2013 to January 2016.All the patients were admitted in hematology/oncology unit of pediatric department of King George's Medical University, Lucknow, India. Any of the following patient types were included in the study: (1) Persisting neutropenic fever in spite of administration of broad spectrum antibiotic more than five days. (2) Patients undergoing chemotherapy and/or with febrile neutropenia [< 500 neutrophils/mm3]. (3) Patients on corticosteroid for more than 3 weeks. Patients were also classified into proven, probable, possible & no IFI. The patients with proven aspergillosis were those in which septate fungal hyphae were demonstrated in histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy. The probable patients were those who had a host factor, a clinical feature and had a mycological evidence in the form of positive microscopy or culture from samples such as sputum, bronchoalveolar lavage or sinus aspirate. Possible cases were only those cases who had appropriate host factors and sufficient clinical evidence consistent with IFI but for which mycological evidence was absent [9]. Following exclusion criteria were used: Patient confirmed to have bacterial/ parasitic infections, patients on haemo-dialysis, patients receiving immunoglobulins/ albumin or plasma-lyte. Blood samples were collected in pediatrics biphasic fungal blood bottle and serum vials. Serum was separated immediately after collection and stored at -20°C until analysis.2-3 ml of blood were collected in pediatric biphasic blood culture bottle prior to the administration of antimicrobials and were incubated aerobically at 37˚C and checked every day up to 7 days and then twice a week for up to 4 weeks, unless evidence of microbial growth was observed, subculture was done on Sabouraud's dextrose agar (SDA) plates, and incubated at 37°C and 25°C. The blood cultures were considered as negative after four weeks of incubation. [10]. GM levels in serum was measured by using Platelia Aspergillus enzyme immunoassay test (Bio Rad, Platelia, Marnes, La Coquette, France) as per the manufacturer instructions. Results were recorded as indexes relative to the mean OD of the threshold controls. Samples that had GM IV of ≥ 0.5 were considered as positive. Second samples of the patients were considered as independent episode if it was received after 4 week apart from the previous one, with the patient becoming clinically well between this duration. All these samples were also evaluated at 0.7, 1.0 and 1.5 IV. Decision for the start of antifungal prophylaxis like Amphotericin B (AMB), fluconazole (FLU) or caspofungin (CAS) was given based upon persistent fever for 4 days after administration of broad spectrum antibiotics or clinical or radiological suspicion of fungal infection. HRCT chest was carried out in patients showing clinical features of chest infection or patients with persistent fever and not responding to antibiotics for 4-6 days. In some patients HRCT of paranasal sinus was also done.

Ethical approval was taken from ethical committee of the institute. Written consent was taken from the guardian of the patients.

Statistical Analysis

Diagnostic values of the GM assays were calculated using sensitivity, specificity, Positive predictive Value (PPV), Negative predictive value (NPV), Positive likelihood ratio (PLR) and Negative predictive value (NLR). European Organization for research and treatment of Cancer and mycoses study group (EORTC/MSG) [9] 2008 revised guideline was used as reference gold standard to calculate true positive, true negative, false positive and false negative cases. Probable and possible cases of IA were considered as true positive and no IA cases were considered as true negative. GM results were not used to define probable, possible and no IFI cases. Analysis was performed on IBM SPSS Statistics v18 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

During the study period, 232 serum samples for GM EIA were obtained from 200 patients with a median age of 10 years (range: 1-15 years). Demographic pattern and underlying disease condition of the patients who were enrolled in our study were shown in Table / Fig 1. Out of 232 samples, 32 were consecutive samples and remaining were single serum samples. The distribution of patients as per their disease diagnosis were shown in Table / Fig 2. Among 108 patients of ALL, 50 were on delayed intensification stage of chemotherapy, 30 patients were on maintenance, 15 patients were on induction and 13 patients were on consolidation stage of chemotherapy. As per EORTC/MSG 2008 revised guidelines for IFI, out of these 200 patients 2 cases were classified as proven, 21 as possible, 89 as probable and 88 as No IFI. Proven IFI cases were positive for *Candida albicans* and *Candida glabrata* in blood culture. [9] GM IV was also raised (ranges 1.5 to 7.5 GM IV) in some cases of probable and possible IFI. Out of 200 patients, 64 patients had abnormal chest X-ray in the form of generalized infiltrates suggestive of IA. CT-thorax could be done in 68 patients. Out of these, 38 had mass like infiltrates suggestive of IA. Blood cultures were also positive for bacterial growth in 32 cases. Among these 10 (41.6%) had grown Coagulase negative Staphylococcus, 4 (16.6%) *S. aureus*, 4 (16.6%) *E. feacalis*, 4 (16.6%) *Acinetobacter baumanii* and 3 (12.5%) *E. coli*. Amikacin and sulbactum was administered as first line of drug in 85% of episodes. An inadequate response after four days from these drugs was considered as an indication to second line of antibiotics (vancomycin/peptaz/meropenem) and or antifungal therapy. The mean duration of administration of intravenous antibiotics was 12.2 days (range 4-30). Out of 200 patients 78 patients were on piperacillin-tazobactum (PTZ) and none of them were on any antifungal including echinocandin. Out of these 78 patients who were on PTZ, 48 patients were classified into No IA. Sensitivity, specificity, PPV, NPV, PLR, NLR in 168 patients with single serum sample at GM IV of ≥ 0.5 was found to be 92.38, 28.43, 59, 57, 1.86, and 0.36 respectively; at ≥ 0.7 IV it was found to be 88, 45, 63, 68, 2.23, and 0.29 respectively; at ≥ 1.0 IV it was found to be 82, 67, 76, 74, 2.87, and 0.22 respectively; at ≥

1.5 IV it was found to be 78, 86, 79, 83, 4.83, and 0.20 respectively. (Table/ Fig 3). Sensitivity, specificity, PPV, NPV, PLR, NLR in 32 patients with consecutive samples at GM IV of ≥ 0.5 was found to be 84.2, 86.46, 83.73, 85.50, 5.82 and 0.18 respectively; at ≥ 0.7 IV was found to be71.4, 77.8, 71.4, 79, 3.21 and 0.37; at ≥ 1.0 IV it was found to be 79, 84, 80, 84, 4.71, and 0.26 respectively; and at ≥ 1.5 IV it was found to be 71, 85, 77, 79, 4.29, and 0.22 respectively. (Table / Fig 4) Out of 200 patients 41 (20.5%) patients died. These include 13 possible, 15 probable and 13 No IA cases. Five patients died due to proven bacterial infections among which 2 had Coagulase negative Staphylococcus, 1 had E. feacalis and 2 had Acinetobacter grown in their blood culture. All the patients who died also had a high GM IV ranging from 1.5 to 5.

DISCUSSION:

This study suggests important cut off values of GM-EIA which can be used to diagnose IA in cancer patients of pediatric age group. Although the test for GM have been approved by the FDA in 2003 for use with patients with neutropenia and undergoing stem cell transplantation, controversy related to cut off value still exists. [11] We did the diagnostic evaluation of GM assay at different cut off values for both single and consecutive samples. Some authors suggest the use of ≥ 0.7 as cut off in single serum samples.[12] Dinand et al have also suggested that optimal cut off of ≥ 0.7 in single serum sample has excellent negative predictive value (94.5%). But they also found that false positive cases are high at this cut off. [13] Contrary to this in our study cut off of ≥ 0.7 in single serum sample had a relatively low NPV when compared to NPV at cut off of ≥ 1.5 (68 vs. 83). Specificity and PPV was also low for cut off of ≥ 0.7 in comparison to cut off of ≥ 1.5 in our study. Next best cut off in our study was found to be ≥ 1.0 (Table 3). False positive cases were also low at cut off of 1.5 (12%). Similar results have been found by Maertens et al, Kawazu et al and Lai et al. [14, 15, 16]. Cut off values have also been reviewed by Mycoses study group.[17] They suggest that single sample with cut off of 1.0 to ≥ 1.5 has higher specificity but lower sensitivity in comparison to cut off of ≥ 0.5. Lower cut off of ≥ 0.5 was also found to have higher false positive results. We also found similar results in our study. To our best knowledge ours is the single largest study which has evaluated GM IV in single serum samples in pediatric patients especially from India. We therefore suggest a cut off value of 1.5 in single serum samples to diagnose IA in pediatric cancer patients. Evaluation of single serum samples for GM is important and much required as serial sampling for GM is not possible every time. Reason behind this is mostly the high cost of the test or low general condition of the patient. Therefore interpretation of GM in single serum sample becomes important. Value of 1.5 GM IV have also been used for early prediction of mortality by Han et al, and it was found to have good specificity (89.3 %) and sensitivity (61.5 %) [18]. Though the sample size for consecutive samples in our study was small but when we analyzed them the cut off value of 0.5 was found to be the best (Table 3). Our results are in agreement with the study done by Badiee et al [19]. They found the sensitivity of 90% and specificity of 92 % in probable and proven IA cases. Meta-analysis done by

Pfeiffer et al in the year 2006 found pooled sensitivity and specificity of GM in serial samples to be 0.64 (95% CI, 0.54–0.73) and 0.89 (95% CI, 0.88–0.90), respectively [20]. Ghosh et al from Chandigarh, India found that more than 2 samples with GM IV > 0.5 can identify patients having higher probability of harboring IA and such patients also have a high risk of mortality. It can also identify these patients earlier than CT scan and is much easier to perform frequently. [21] One of the Indian study done by Savio et al from Bangalore suggested a cut off value of 0.52 instead of 0.5. In this study with 0.52 cut off they found sensitivity, specificity, PPV and NPV as 75%, 79%, 76%, 82%, however the patients whom they have included were mostly non cancer adult patients [22]. Review done by Mycoses study group also suggests a cut off of 0.5 in consecutive samples. [17] However few studies such as done by Sulahian et al propose a cutoff of 1.5 in consecutive samples. They did their study in 347 hemato-oncological pediatric and 450 bone marrow transplant patients and found that 1.5 cut off has a high sensitivity and specificity (90%, 94% respectively). [23] Another study by Jha et al from north India did a study in 100 febrile samples from 78 hemato-oncological pediatric patients. They found cut off of 1.0 as best with sensitivity of 60% and specificity of 93%. They also report that sensitivity dropped to 40% and specificity to 38% at cut off of 1.5. [24] In our study also the sensitivity and specificity at cut off 1.0 was good but not better than 0.5. However the sensitivity and specificity did not drop much in our study at cut off 1.5 (table 3). False positive cases in our study were low (15.3%) despite 78 patients being on piperacillin/tazobactam. This is justifiable as not all the batches of piperacillin/tazobactam are positive for galactomannan. [25] Study done by Fisher et al evaluated the GM-EIA in urine and serum sample at cut off value of 0.5 and 1.0. They concluded that serum GM EIA does not provide frequent false-positive results as previously reported and they also found that the specificity of this assay in serum and urine sample was 95% and 80% [26]. Depending upon the clinical profile, patients suffering from ALL and AML were given Itraconazole or Amphotericin B prophylaxis intravenously or orally. These antifungal were given when the patients did not respond to i.v. antibiotics for more than 5 days.

Limitations: Sample size for consecutive samples were less in our study. Therefore, studies with equal number of consecutive samples are further needed to validate our results.

4.CONCLUSION

To conclude best cut off value of GM for diagnosis of IA in pediatric cancer patients in single serum sample is 1.5 and in consecutive sample it is 0.5. Role of single sample must be established as in developing countries like India, frequent sampling is not possible either due to poor general condition of the patient or due to high cost of the test.

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SUPPLIMENTARY FILES

Table/Fig 1: Demographic data and underlying disease of patients enrolled in the study

Characteristics	**Type of IFI**			**Total**
	Possible	**Probable**	**No IFIs**	
No. of Episode	23 (11.5%)	89 (44.5%)	88 (44%)	200 (100%)
No. of Death	7	11	23	41 (20.5%)
Age (Range)	0-12 years	2-13	1-15	1-15
Sex	14 Male/ 9 Female	50 Male/39 Female	62 Male /26 Female	126 Male/ 74 Female
No. with disease				
ALL	13	49	46	108
AML	8	30	12	50
NHL	-	3	4	7
HD	2	5	8	15

WT	-	2	2	4
ES	-	-	7	7
Neuroblastoma	-	-	6	6
Rhabdosarcoma	-		3	3
Duration of episode (Days)	30-180	50-235	60-300	60-300
Neutropenia	23	89	88	200
Fever	23	89	88	200
Steroid	23	89	88	200
Anti-cancer Drug	23	89	88	200

Abbreviations: ALL: Acute lymphoid leukemia. AML: Acute myeloid leukemia. NHL: Non-Hodgkin's lymphoma. HD: Hodgkin's disease. WT: Wilm's Tumor. ES: Ewing's Sarcoma

Table/Fig 2. No. of Patients underlying with different Cancer

Sr.no	Types of Cancer	Total Number of Patients
1.	Acute Lymphoblastic Leukemia (ALL)	108 (54%)
2.	Acute Myeloid Leukemia (AML)	50 (25%)
3.	Hodgkin's lymphoma (HL)	15 (7.5%)
4.	Non-Hodgkin's lymphoma (NHL)	7 (3.5%)
5.	Ewing's sarcoma (ES)	17 (8.5%)
6.	Wilm's tumor (WT)	3 (1.5%).

Table/Fig. 3 Sensitivity, Specificity, PPV, NPV, PLR, and NLR of 168 patients with single sample

GM Index	Sensitivity	Specificity	PPV	NPV	PLR	NLR
0.5	92.38	28.43	59	57	1.86	0.36
0.7	88	45	63	68	2.23	0.29
1.0	82	67	76	74	2.87	0.22
1.5	78	86	79	83	4.86	0.20

Table/Fig 4. Sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratio of GM of patients with 32 consecutive at different cut off values.

GM Index Value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	N P V (95% CI)	PLR (95% CI)	NLR (95% CI)
0.5	84.21	86.46	83.73	85.50	5.82	0.18
0.7	71.43	77.78	71.43	77.78	3.21	0.37
1.0	78.75	83.33	79.75	84	4.71	0.26
1.5	71	85	77	79	4.29	0.22