Original Article

Detection of Galactomannan Antigen in the Serum of Iraqi Patients with Suspected Invasive Aspergillosis

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Abstract:

Background: Aspergillosis is a large spectrum of diseases caused by members of the genus Aspergillus. Invasive aspergillosis is a severe infection that occurs in patients with prolonged neutropenia, following chemotherapy,transplantation,or immunosuppressive protocols .Galactomannan (GM) is a molecule ,found in the cell wall of Aspergillus species and is released in the blood during growth .The detection of GM in the blood is used to diagnose the invasive Aspergillosis in humans using ELISA assay.

Objectives: To detect Galactomannan antigen in in the serum of immunocompramized patients suspected to have invasive aspergillosis.

Patients and methods: This study was conducted on 50 patients from the hematology&oncology department,of Baghdad teaching hospital and pediatric oncology wards ,from March 2013 to October 2013. The patients presented with fever that is not responding to antibiotics along with cough and sputum and abnormal chest X-ray findings suspicious of IA. 11 healthy Iraqi individuals served as a control group.

Results: ELISA test was positive in 39 of 50 (78%)while all of the control group individuals were negative(100%).The concentration of galactomannan antigen detected by ELISA was statistically significant when compared to control group(OD 0.658±0.033 vs 0.191±0.020 respectively) ($p<0.05$).

Conclusion: The detection of galactomannan antigen is a very useful and rapid method for the diagnosis of invasive aspergillosis disease in immunocompromised patients.

Key words: Galactomannan antigen,invasive aspergillosis.

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Introduction:

Aspergillus species are second most isolated fungus after candida species in pulmonary fungal infection. Mortality from infections caused by the aspergilla remains extremely high, frequently above 90% in untreated immune-compromised patients(1).

The genus Aspergillus is member of Ascomycetes class of fungi, it has gradually increased to over 200 species (although some are not universally pathogenic)(2). Aspergillus species grow easily and rapidly at a broad range of culture temperatures and on a wide variety of media, although blood cultures are uncommonly positive. Growth of pathogenic species at 37°C is a feature that differentiates the pathogenic from non-pathogenic isolates. Aspergillus species are rapidly growing molds with branching separated hyphae and characteristic arrangement of conidia on the conidiophores. It is widely distributed in nature and found throughout the world, it seems to adapt to a wide range of environmental conditions and heat, resistant conidia are light and able to float in the air by which it spreads and inhaled through upper respiratory tract to colonize and infect the pulmonary tissue.(3)

The most common species causing invasive infection include: Aspergillus fumigatus (historically causes approximately 90% of invasive aspergillosis), A. flavus, A. niger, and A. terreus respectively. (4)

Aspergillus fumigatus was mostly associated with Invasive Aspergillosis in primary hosts with immune deficiencies as febrile neutropenia with neutrophil count less than 1000cell per ml (5). Aspergillus fumigatus infection begins in the bronchial tree and invades the parenchyma of the lung resulting in necrosis and cavitation, manifested as fever and cough tinged with bloody and purulent sputum, sometimes dissemination of the fungus takes place hematogenously to other sites like brain, kidney, skin, and myocardium. (6) A serologic assay was approved by the Food and Drug Administration for the detection of galactomannan in blood, which is a molecule found in the cell wall of Aspergillus species and is released during growth.(7)The detection of galactomannan in blood is used to diagnose the invasive Aspergillus infections in humans(8). This is performed by ELISA assay (9). The assay is most useful in patients who

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have had hematological diseases and those with stem cell transplants (10). A positive result supports a diagnosis of invasive aspergillosis (11).

Patients and methods:

This study included fifty patients, 22 males and 28 females, from which 64 blood samples were studied(some patients had double serum samples tested) for galactomannan .The patients age range was from 2 to70 years, they were the attendant of Hematology Oncology Unit of Baghdad Teaching Hospital and Pediatric Oncology Wards. The patients presented with fever which is not responding to antibiotics, along with cough, sputum and abnormality on chest x-ray suggestive of IA. Eleven healthy individuals were taken as a control group; all were free from any clinically evident disease. The study was conducted from March 2013 till October 2013.

Relevant information from each patient were recorded followed by aspiration of 5 milliliters of venous blood by 5ml disposable plastic syringe and collected in dry plain test tube, allowed to clot for about 2 hours at room temperature 25◦C (for patients and the control group) (12) in the Medical Mycology Unit in Baghdad Teaching Hospital laboratories, the serum was separated from each tube by centrifugation by 3000 cycle per minutes (c p m) for10 minutes at 25◦C, then serum was dispensed into sterile tightly closed Eppendrof tube (1 ml) and stored at -20◦C until assayed by ELISA testing for galactomannan antigens .

The comparison between the mean cutoff values of the galactomannan antigen concentration of the patients group and the control group were compared using student-t test (SPSS software).A p value < 0.05 was considered indicative of statistically significant difference.

Results:

The study shows that 39 out of 50 patients (78%) had positive galactomannan testing. Table 1 shows the results of galactomannan antigen concentration detection by ELISA in the 75 serum studied (11control and 64 patients' serum samples). The difference between the mean cutoff value of the patients and control group was statistically significant (T= 0.135, p value<0.05).

Table 1: Comparison between the optical density values of galactomannan antigen in the sera of patients & control group

Study groups	No	Mean of the optical density± SE
Patients	64	0.658±0.033
Control	11	0.191±0.020
Total	75	P-value < 0.05

The distribution of the study groups according to neutrophil count is shown in table 2 with 10 of 50 (20%) of patients had neutrophilia, 33 of 50(66%) of patients had neutropenia and 7 of 50 (14%) of patients had normal neutrophil count. The entire control group (100%) had normal neutrophil count.

Table 2: Distribution of study groups according to Neutrophil count

Study groups	Normal Neutrophil count		Neutrophilia (>10x10^9/L)		Neutropenia (<1x10^9/L)		Total
	No	%	No	%	No	%	
Patients	7	14	10	20	33	66	50
Control	11	100	\	\	\	\	11
Total	18		10		33		

The detection of galactomannan antigen in acute leukemia patients in the study group was shown in table 3, it shows that 18 out of 50 (36%) of all patients had acute myeloid leukemia (AML), 16 out of 18 AML patients were galactomannan testing positive (88.9%), the association between GM and AML was significant using chi square test to compare with the control group. Also 12 of 50 patients (24%) had acute lymphoblastic leukemia (ALL), with 10 of 12 cases tested positive for galactomannan with also a significant association. The other patients had either lymphoma, chronic lymphocytic leukemia or multiple myeloma, they constitute 20 out of 50 (40%).Thirteen of 20 (65%) were galactomannan testing positive and the association was significant as compared to negative control group.

Table 3: Results of galactomannan testing by disease type

Type of hematological disease		Galactomannan test+ve	Galactomannan test-ve	P value
AML	N=18	16	2	<0.001
ALL	N=12	10	2	<0.001
Others	N=20	13	7	<0.001

Discussion:

Distribution of the study groups according to the concentration of galactomannan antigen done by ELISA showed that 39 out of 50 patients having a high concentration of the galactomannan antigen in their sera above the cut off value which is directly related to their infection with aspergillosis during their state of neutropenia. Positive galactomannan level could be the first proof of invasive fungal infection (13).The patients with negative galactomannan test could have false negative results as seen in local colonization of spores in the bronchial tree rather than angioinvasive Aspergillosis or in non neutropenic patients as opposed to severely neutropenic patients. (14) The blood count results showed that of most of the patients had neutropenia which is the primary defense against infection. Those patients with neutropenia when exposed to the spores of Aspergillus will be more susceptible to develop fungal infection, especially when polymorphonuclear cell level reaches below 1000/ml due to intensive chemotherapy (15,16). This study confirmed that invasive Aspergillosis is most commonly seen in AML because the lung is the most frequent site of invasive aspergillosis and the inhaled spores, in the presence of the low number of polymorphonuclear cells, will be difficult to be eradicated from the body ending in primary colonization and a subsequent invasion of the lung tissue ending in invasive aspergillosis. Twelve cases of ALL also showed a high percentage of infection (10 out of 12) mainly due to defective function of lymphocytes in this leukemia, which is an essential element in cell mediated immunity needed for fungal elimination from the body and clearance of spores, ending in a bad prognosis on the patients infected(17,18).

Conclusion:

The use of galactomannan testing is a very useful and effective way to predict invasive aspergillosis in immunocompromised patients.

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