Invasive Aspergillosis in Pediatric Hematology Oncology Ward

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Abstract

Background: Patients with prolonged neutropenia and/or severe underlying immunosuppression are at the greatest risk for disseminated aspergillosis. This study was undertaken to determine the incidence of invasive aspergillosis by Platelia Aspergillus enzyme-linked immunosorbent assay ELISA kit in high risk children admitted to the hematology ward of Dr. Faghihi hospital, Shiraz University of Medical Sciences, Iran.

Materials and Methods: From Oct. 2006 to Jun. 2008, 62 patients with hematologic malignancies were followed and evaluated for invasive aspergillosis in Shiraz. All clinical samples were cultured and a direct microscopic examination was performed. Blood samples were cultured by bedside inoculation to BACTEC medium.

Blood samples were collected prospectively once a week and stored at –20 ˚C until examination. All the collected blood samples were assayed for galactomannan antigen using Platelia Aspergillus ELISA kit. Patients were classified according to the diagnostic criteria set by the European Organization for Research and Treatment of Cancer-Mycosis Study Group.

Results: The female-to-male ratio was 22:40, and mean age of the patients was 9.3 years. The sensitivity, specificity, negative and positive predictive values of the ELISA method were 91% , 90%, 83.3%, and 94.7%, respectively. Galactomannan antigen test was positive in 1 proven, 8 probable, and 2 possible cases. The incidence rate of invasive aspergillosis was found to be 16.7%.

Conclusion: Considering the incidence of invasive aspergillosis and the corresponding morbidity and mortality rates in patients with hematologic disorders, it seems that more efficient methods are in demand for early diagnosis and thereby promoting the patients' survival.

Keywords: Mycoses, Aspergillus, ELISA.

Introduction

Invasive aspergillosis (IA) in children is different from that in adults in terms of signs, symptoms, associated specific findings, and difficulties in early diagnosis. Patients with prolonged neutropenia and patients with severe underlying immunosuppression are at the greatest risk for IA.[1,2] Early detection of IA is difficult due to the lack of sufficiently sensitive and specific diagnostic tools. Conventional diagnosis of IA is dependent on culture and histopathologic examination of the tissue(s) involved.Microscopy and culture of sputum and bronchoalveolar lavage samples, and blood culture are insufficiently sensitive for proper diagnosis. Most IA cases are detected only at autopsy and biopsy from the infected tissue which is not always available in patients with a severe underlying condition. Therefore, diagnosis depends on a combination of clinical and radiologic signs, and clinical experiences. A serum enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of circulating galactomannan (GM), which is a major constituent of Aspergillus cell wall.

This study was undertaken to determine the

incidence of invasive aspergillosis by Platelia Aspergillus ELISA kit in high risk children admitted to hematology ward of Dr. Faghihi hospital, Shiraz University of Medical Sciences, Shiraz, Iran.

Materials and Methods

From Oct. 2006 to Jun. 2008, 62 patients with hematologic malignancies who had received chemotherapy were followed and evaluated for invasive aspergillosis during their hospitalization, in Shiraz. All clinical samples (e.g. cerebrospinal fluid, pleural and abdominal tap, bronchoalveolar lavage, and sputum) were examined for Aspergillus infections during the follow-up. All the samples were cultured on Sabouraud Dextrose Agar (Merck, Germany) with chloramphenicol and direct microscopic examination was performed. Blood samples were cultured by bedside inoculation to BACTEC medium (Becton-Dickinson, Sparks, MD, USA). Serum samples were collected prospectively once a week and stored at –20 ˚C until galactomannan test examination. All the sera were assayed for galactomannan antigen using Platelia Aspergillus ELISA kit (Double –Sandwich ELISA Platelia; Sanofi Diagnostics Pasteur, Marnes La Coquette) according to the manufacture's protocol. Patients were classified according to the diagnostic criteria of the European Organization for Research and Treatment of Cancer-Mycosis Study Group (EORTC/MSG).[3] Sera from 10 healthy volunteers were used as negative control.

The ethics committee of Clinical Microbiology Research Center, Shiraz University of Medical Sciences has reviewed and approved the study regarding the patients' written consents before participation in the study.

Results

A total of 101 sera from 62 patients were examined for GM antigen. The female-to-male ratio was 22:40. Mean age of the patients was 9.3 years (range: 2-16 years). Twelve patients (19.4%) had acute myelocytic leukemia, 29 (46.8%) acute lymphocytic leukemia, and other patients had aplastic anemia, pancytopenia, chronic myelocytic leukemia, and chronic lymphocytic leukemia, who all received chemotherapy. The etiologic agents were A. flavus (3 patients), A. fumigatus (6 patients), and Aspergillus spp. (2 patients). Most of aspergillosis infections occured in patients with acute myelocytic leukemia. None of the patients with IA had a positive blood culture result. Lung was the most infected site followed by sinusitis. Six patients died despite the antifungal therapy they received.

The index of GM detection with platelia test in the study was 0.2 ng/ml. Of the 10 patients with proven and probable aspergillosis, and 9 cases with possible criteria according to EORTC/MSG criteria, GM antigen test was positive in 1 proven, 8 probable, and 2 possible cases. Of the 43 patients with no clinical or mycological evidence of IA, GM antigen was positive in two patients (false-positive test results), therefore, the sensitivity, specificity, and negative and positive predictive values of the ELISA method in patients with proven and probable IA were 91% , 90%, 83.3%, and 94.7%, respectively. The incidence rate of invasive aspergillosis in patients was found to be 16.7%. The results of GM in all of healthy volunteers were < 0.2 ng/ml.

Discussion

Invasive aspergillosis is one of the most important infectious complications in patients with neutropenia.[4] The overall case fatality rate (CFR) for IA was 58% in a review of 1,941 patients from 50 studies, regardless of antifungal therapy.[5] Nosari et al. reported that among 675 acute leukemia patients, the incidence of proven or probable aspergillosis was 7.1%.6 There are a few studies about IA in pediatric patients. Invasive aspergillosis in children is associated with even greater mortality: 68% to 77%.[7-10] The mortality of untreated IA is nearly 100% in some patient groups, and the overall survival rate among the patients treated with amphotericin B is 34%.11,12 A higher mortality rate is seen in those with greater degrees of immunosuppression, particularly after transplantation.[8] In the present study, 54.5% of the patients with IA died in spite of antifungal therapy.

Radiologic evaluation has a foundational role in IA diagnosis; however, there have been recent advances in noninvasive alternate markers of many diseases. In adult series of pulmonary aspergillosis, 50% of cases show cavitations and 40% air crescent formation.[13] In one 10-year review of 27 consecutive

pediatric patients (mean age: 5 years), there was central cavitation in small nodules in 25% of children and no evidence of air crescent formation within any area of consolidation.[14] In the current study, none of the patients presented air crescent formation, but infiltration and cavitations were shown in pulmonary infections.

In some studies, the sensitivity and specificity of the GM test were 88.6% and 97.5%, respectively, in adult patients. The sensitivity increased to 100%, and the specificity dropped to 89.9% in children.[15] This is similar to the sensitivity and specificity rates in the present series of pediatric patients (91%, 90%).

Some aspects of IA care appear to be the same in adults and children (e.g. blood cultures are rarely positive), but there are important basic epidemiologic, pathophysiologic, and therapeutic differences between children and adults with IA. Differences include infective organism, predisposing factors, and site of infection. In our region, the incidence of aspergillosis in adults was 7.2%,[16] and in pediatric oncology ward was 16.7%. There are very few studies calculating the incidence of pediatric IA, thus we are not able to compare ours with them.

Most patients had pulmonary aspergillosis, and lung was the most frequent site of disseminated aspergillosis.[17-19] A large National Institute of Allergy and Infectious Diseases Bacteriology and Mycoses Study Group study reviewed 256 isolates of Aspergillus spp. in patients with IA from 24 medical centers;[20] A. fumigatus constituted 67% of isolates, whereas A. flavus was the second most common isolate with 16% incidence. In our study, pulmonary infections were the most common infection and A. fumigatus was the most frequent etiologic agent.

Conclusion

Considering the incidence of invasive aspergillosis and the corresponding morbidity and mortality rates in patients with hematologic disorders, it seems that more efficient methods are in demand for early diagnosis and thereby promoting the patients' survival. As demonstrated, Platelia Aspergillus EIISA kit for detecting galactomannan antigen can be helpful.

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