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A Retrospective Study of the Risk Factors for Invasive Aspergillosis in Iran

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Abstract

Back ground: Invasive aspergillosis is a well-known complication in immunocompromised patients. Among fungal infections, Invasive Pulmonary Aspergillosis (IPA) is the first cause of death after transplantation, and remains a major complication in curses of leukemia treatment. Despite considerable progress in the management of infections, it remains an important cause of morbidity and mortality, mainly after transplantation. This study was conducted in order to determine the risk factors for *Aspergillus* infections.

Patients and methods: During retrospective investigation of 24 patients with aspergillosis, significant risk factors for invasive aspergillosis have been identified. Diagnosis was confirmed by demonstration of fungi by direct examination of the clinical samples, histopathology and cultures.

Results: All patients were immunocompromised or had one or more predisposing factors. Patients with solid organ transplantation, renal transplant recipients and patients with hematologic malignancy or chronic granulomatous disease were at the highest risk for Invasive Pulmonary Aspergillosis (IPA). Fever unresponsive to broad-spectrum antibiotics was the earliest and most common clinical sign in this study.

Conclusion: The major advances in the management of invasive fungal infections (IFI) have come from the noticing of the risk factors for the development of IFI, from the development of new biological markers of IFI, and also from well-designed therapeutic trails. However, much remains to be done to decrease the rate of mortality due to IFI in high risk patients. A high degree of knowledge and efforts for an early diagnosis may interfere to improve the poor prognosis.

Keywords: Aspergillosis; Risk factors; Immunocompromised patients; Fungal infections

Introduction

Aspergillosis refers to the broad range of disease states caused by members of the genus *Aspergillus* [1]. *Aspergillus* spp. is ubiquitous, commonly occurring in soil, water and decaying vegetation. Most cases of human disease are caused by *Aspergillus fumigatus*, followed by *A. flavus* and *A. niger* in frequency. Infection with *Aspergillus* spp. appears to be the result of both host susceptibility and environmental exposure to the fungus.

There are several forms of aspergillosis: allergic aspergillosis, aspergilloma and invasive aspergillosis. Allergic bronchopulmonary type is an allergic reaction to the fungus that usually develops in people, who already have lung problems or atopic individual. Aspergilloma is a fungus ball that develops in an area of past lung disease or lung scarring, such as lung abscess or tuberculosis. Invasive disease is usually seen in individuals who are weakened immune system due to cancer, leukemia, chemotherapy or broad-spectrum antibiotics. Patients undergoing solid organ or bone marrow transplantation, with associated immune suppression and patients with AIDS, are also at increased risk [1-3]. Patients with acute leukemia and patients with impaired phagocyte immune defense or granulocytopenia longer than three weeks are the major risk factor for developing invasive aspergillosis. In immunocompromised patients, conidia are able to germinate and form hyphae, which invade the lung tissue and initiate an infection [4,5]. In the immunocompetent host, these spores are cleared by phagocytic immune cells [6].

Patients and Methods

During retrospectively investigation, we identified 24 aspergillosis cases, including 13 men and 11 women, aged 7 to 62 years. Sixteen clinical specimens were obtained from the respiratory tract (sinuses and lower respiratory tract), including bronchial washings, tracheal aspirates and sputum from patients with pulmonary disease and tissue biopsies from patients with disseminated disease. Other specimens were collected from brain, heart, aortic valve, subcutaneous, muccocutaneous of superglut.

Specimens were directly examined in 10% potassium hydroxide. Ground tissues and other specimens were inoculated onto primary isolation media, on sabouraud dextrose agar, mycosyl agar, brain heart infusion agar (BHI) agar, and blood agar (BBL) with duplicate; All mediums were incubated at 35C and 25˚C. Microscopic characteristics of the isolates were studied by slide culture preparation. Czapek's agar was also used for *Aspergillus species* identification. All biopsy specimens were histologically assessed using hematoxylin and eosin (H&E), Periodic Acid-Schiff and Gomori methenamin silver stain that were also are helpful in certain situations.

In the cases of culture, positive results of mycological examination was considered as positive only, if direct examination showed the presence of fungal hyphae, and if two successive cultures were positive for the same fungal specie. Cases without these criteria were regarded as negative, and were not included in this study.

Results

We reported 24 cases of aspergillosis in this study. All of our patients had one or more predisposing factor to aspergillosis. *Aspergillus fumigatus* was isolated in 9 cases (37.5%), *Aspergillus flavus* in 8 cases (33.33%), *Aspergillus niger* 2 (8.33%), *Aspergillus terreus* 1 (4.16%), and *Aspergillus species* 4 (16.66%) of specimens.

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In this study, the most common infected site was respiratory tract. The patients comprised 13 male and 11 female, ranging in age from 7 to 62 years, with a mean age of 33.37. In 75% of cases, the infection was localized to the respiratory tract (25% sinuses and 75% lung). Definite IPA were diagnosed in 58.3% of episodes from patients with hematologic malignancy, granulocytopenia, or bone-marrow and renal transplantation; in 16.6% of those with expand surgery, and in 29.16% of those with diabetes mellitus, tuberculosis, systemic lupus erythematous and rhumatoid arthritis. In this study, the solid organ transplantation, renal transplantation and hematologic malignancy were the highest risk factors for developing invasive *Aspergillus* infections. The most frequent infections were isolated from patients with hematologic malignancy, bone marrow and renal transplantation (58.3%).

In current study, 12.5% of aspergillosis occurred in patients with CGD (two cases *A. fumigatus* and one *A. terreus*), and 87% of patients who developed aspergillosis had fever more than 39°C before diagnosis. Chest pain and hemoptysis were observed in 64% and 36% of patients. The most common species were *Aspergillus fumigatus* and *Aspergillus flavus* isolated of 70.8% of specimens. The most and earliest common sign in our patients were fever unresponsive to broad-spectrum antibiotics. Table 1 shows the list of *Aspergillus* species that have been recovered. All cases occurred in immunocompromised patients. Table 2 outlines the different clinical cases and the risk factors.

Discussion

Invasive aspergillosis is a life-threatening complication in immunocompromised patients, particularly in those under going bone marrow transplantation, or receiving intensive chemotherapy for hemotological malignancy [7]. Neutropenic patients with prolonged antibiotic therapy and resistant fever are at high risk. Several organs and surgical procedures may be involved in post operative infections, with *Aspergillus* spp. depending on the surgery performed, including endocarditis [8]. Mortality is high, but it is difficult to determine clearly. It has been estimated at 13 to 15%, with the higher rates in patients with aplastic anemia and recipients of allogenic bone marrow transplants [9]. In present study, most patients had a hematologic malignancy or had undergone stem cell transplantation. Fungal infections remain a major problem for these patients [2].

Risk factors for invasive aspergillosis may be associated with the changes in macrophage and neutrophil function, which may explain why infection mainly affects bone marrow and solid organ transplant recipients, Intensive Care Unit (ICU) patients, post-operative patients, those with chronic pulmonary diseases, patients with AIDS and patients on immune modulating drugs (TNF-α inhibitors), neutropenic patients, or those who have received corticosteroid treatment. Other risk group is patients with chronic granulomatous disease, drug user, patients with sarcoidosis, sever burn patients and alcoholics [10-12]. *Aspergillus* can also develop when no risk factors are present. Thus, cases have been explained of community-acquired pneumonia due to *Aspergillus* in apparently immunocopetent patients [13], but it is likely that the patients had some undetected defect in macrophage and neutrophil function that allowed invasive infection to develop [11,12,14].

A clinical diagnosis may be suspected in high risk patients with prolonged non responsible fever to antibacterial agents, and characteristic single or multiple rounded densities on the chest radiograph [15]. The detection of *Aspergillus* in sputum cultures, from patients with appropriate predisposing conditions, is likely to be of diagnostic importance, and empiric antifungal therapy should be considered.

Blood cultures are not accurate for detecting *Aspergillus* spp. [8], and antibody responses are poorly predictive in immunocompromised patients. The diagnosis of invasive aspergillosis is difficult in the absence of tissue biopsy and histological confirmation. Because of the limitations of the aforementioned diagnostic methods, a nonculture method, based on the detection of the *Aspergillus* antigen galactomannan, has been developed [16].

Sensitive methods that detect significant amounts of *Aspergillus* antigen in body fluids, primarily serum of high risk patients are currently being evaluated, and may provide a noninvasive early diagnostic test that is both sensitive and specific [13].

Histopathologic feature of tissue invasion by fungal mycelia in biopsy specimens may be needed to confirm a diagnosis. Culture of *Aspergillus* spp. from broncho-alveolar lavage specimens, sputum samples or tracheal aspiration may represent colonization, but in conjunction with a clinical diagnosis, positive cultures probably indicate pulmonary aspergillosis [14]. Repeated isolation of *Aspergillus* from the BAL and sputum specimens of our patients clearly indicates pulmonary opportunistic fungal infections. The diagnosis of invasive fungal infections remains challenging. Diagnosis of invasive *Aspergillus* infection can only be obtained by showing invasive aspergillosis in tissue and culture. In many patients, diagnosis obtained only at autopsy [15]. Culture is considered as a useful tool to diagnosis invasive Aspergillosis, but the positive predictive value depends on the prevalence of disease, and on the patient group. The optimal specimen for pulmonary aspergillosis appears to be broncho alveolar lavage (BAL), and culture of BAL contributes to the diagnosis in 30-50% of patients [16-18].

In this study, most common infected organ with *Aspergillus* spp. was respiratory tract. Clinically, the picture consisted of unremitting fever with profound and prolonged neutropenia, cough and dyspnoea in most of patients. We describe hematologic malignancy; granulocytopenia and transplantation were the great risk factor for aspergillosis in this study. High granulocytopenia is the more risk factor related to the host. The greater the risk of invasive disease, the longer the duration of sever granulocytopenia (<1.000 polymorphoneuclear) (leukocytes mm^3) [19]. This fact places patients with autologous bone marrow transplants of great risk, because they tend to be severely granulocytopenic longer than patients with induction chemotherapy or allogenic bone marrow transplants. Moreover, patients with autologous bone marrow transplants may develop graft versus host disease. This requires immunosuppressive therapy that may further increase their susceptibility to invasive aspergillosis. Solid organ transplants receiving patients also are at risk for invasive aspergillosis, as a result of immunosuppression by corticosteroid therapy. However, the use of cyclosporine and FK 506 can helped to increase the degree of immunosuppression of these patients [14-16].

The association of neutropenia and neutrophil dysfunction

Aspergillus species	No	%
A. fumigatus	9	37.5
A. flavus	8	33.3
A .terreus	2	4.17
A. niger	1	8.33
Aspergillus sp	4	16.7
Total	24	100

Table 1: *Aspergillus* species isolated from immunocompromised patients.

No	Sex	Age (years)	Occupation	Site affected-sample	Predisposing factor or associated conditions to aspergillosis	Causative agents
1	M	30	Business	Lung-BAL	TB &SLE	*Aspergillus* spp.
2	F	58	House wife	maxillary sinuses (left) biopsy	Renal transplantation+Immunosuppressive Therapy	*A. flavus*
3	F	35	House wife	Brain abscess-aspiration	Bone marrow malignancy +diabetes	*A. fumigatus*
4	F	37	House wife	Lung-BAL	Diabetes +TB +surgery	*A. niger & c. albicans*
5	F	60	House wife	Lung-BAL	Diabète+ rénal transplantation	*A. niger*
6	F	11	School girl	Thorax-biopsy	C.G.D	*A. fumigatus*
7	F	7	School girl	Head-abscess	C.G.D	*A .fumigatus*
8	F	44	Teacher	Lung-BAL	R.A	*A. flavus*
9	M	45	Farmer	Superglot biopsy	Lymphoma+Smoking	*A .flavus*
10	M	13	School boy	Subcutaneous abscess-aspiration	CGD	*A. Terreus*
11	M	57	Unemployed	Lung-BAL	Multiple myloma	*Aspergillus* sp.
12	M	62	Business	Aortic valve biopsy	Heart open surgery	*A. fumigatus*
13	M	12	School boy	Sinus biopsy	Thrombocytopenia	*A. fumigatus*
14	M	46	Business	Lung–BAL	Multiple myloma	*Aspergillus* spp.
15	F	22	Business	maxillary sinuses (left) biopsy	Renal transplantation	*A .fumigatus*
16	M	19	Business	BAL-Lung	ALL-chemotherapy	*A .flavus*
17	F	31	House wife	BAL-lung	ALL-chemptherapy-citotoxic therapy	*A. flavus*
18	M	42	Worker	Brain abscess-brain	Chronic sinusitis	*Aspergillus* spp.
19	M	20	Army	Sputum-Lung	ALL+BMT	*A .flavus*
20	M	24	Business	BAL-Lung	Renal transplantation	*A .fumigatus*
21	F	60	House wife	Maxillary sinusitis- biopsy	Diabetes mellitus	*A .flavus*
22	M	24	Student	Sinuses biopsy	Nose polypus surgery-corticosteroid therapy	*A. flavus*
23	M	23	Business	BAL-Lung	Renal transplantation	*A. fumigatus*
24	F	19	Student	Sinuses biopsy	Expand surgery	*A .fumigatus*

BAL: Broncho-alveolar lavage
TB: Tuberculosis
SLE: Systemic lupus erythematous
BMT: Bone marrow transplant

Table 2: Characteristics of mycologically positive cases of Aspergillosis.

(in particular, chronic granulomatous disease) has described in to neutrophil-*Aspergillus* interactions. Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease characterized by recurrent life-threatening bacterial and fungal infections. Invasive aspergillosis may be the first manifestation of CGD. Infection usually manifests within the first two decades of life [20]. We have been reported 12.5% of aspergillosis occurred in patients with CGD (two cases *A. fumigatus* and one *A. terreus*). Mamishie et al. [21] reported a 4-month-old girl with CGD, was firstly presented with an anterior chest wall protrusion due to aspergillosis mass. Also Mamishie et al. [22] in 2005 reported a case of osteomyelitis in patient with CGD successfully treated with Amphotericine B and INF-gama. *Aspergillus* produces catalase, an enzyme that changes hydrogen peroxide (H_2O_2) to water and oxygen [23].

Other significant risk factor in this study was diabetes mellitus in 4 patients (16.6%); three of them had more than one risk factor. Common metabolic disorder with significant morbidity and mortality is diabetes. Diabetes is considered as a risk factor of mycoses commonly [2,24,25]. The most common fungal invasions are isolated of the skin, the urinary tract and the respiratory tract [24]. However, the reason for the high susceptibility in diabetic patients is not enough explained.

Aspergillosis occurred in a 30 year-old man with TB and systemic lupus erythematous (SLE) in this study. Risk factors for acquiring aspergillosis in this patient were high grade disease activity, granulocytopenia, steroid therapy and other immunosuppressive treatment and tuberculosis. Only 23 cases have been reported in English language medical literature [26]. Shadzi and Chadeganipour [26] reported that 43 patients with tuberculosis as predisposing factor had 7.3% yeast infection and 0.4% aspergillosis.

In this study, *Mycobacterium tuberculosis* was predisposing factor for aspergillosis in 8.33%. Chadeganipour et al. [27] isolated 36 yeasts (18%) and 7 aspergillosis (3.5%) from 200 patients. Out of 43 patients who had fungal infections, 12 cases were affected with definite tuberculosis.

We observed aspergillosis in a 62-year-old man after operation. Predisposing factor in the patient was expanding heart surgery. Shoar et al. [28] reported an endocarditic infection in 2004 from Iran. Fever is a common problem in neutropenic patients, and should not be considered as an index of aspergillosis [29]. However, in our experience, 87% of patients who developed invasive Pulmonary Aspergillosis (IPA) had fever above 39°C in the days before IPA diagnosis. The other distinguished clinical indicators of IPA are chest pain and hemoptysis [30]. We observed these indicators with a frequency of 64% and 36%, respectively. As described by Gerson et al. [31], the main risk factor of IPA occurrence was the duration and intensity of neutropenia. Corticosteroids can potentiate *Aspergillus* invasion by decreasing intracellular killing of spores by macrophages [32]. Cytotoxic drugs are listed an important risk factors for invasive pulmonary aspergillosis in a number of studies [33,34].

Conclusion

Mortality in invasive aspergillosis is high, early diagnosis allowing an early treatment may improve the prognosis. However, this goal remains difficult to achieve. When diagnosis is confirmed, it is often already too late [35]. This study signifies that clinicians should be alerted of potential fungal etiology in cases of fever, which is unresponsive to conventional medical therapy. Thus, immediate recognition, including respiratory fluid or biopsy specimens and other specimens

processing for fungal culture, should be part of patient's workup. This study suggests that in immunocompromised patients, in *Aspergillus* infections, background assessment and clinical appearance, relationship to clinical symptoms and laboratory examinations should be considered, and investigation of other factors which created the infection will lead us to a clear picture of patients' status [36]. Invasive aspergillosis is a life-threatening disease, so an aggressive approach of high risk patients is required, aimed at identifying patients as soon as possible and initiating antifungal therapy promptly.

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