Abstract
Aspergillosis frequently affects respiratory tract. The definitive diagnosis is
difficult. In this study, double immunodiffusion method was developed for detection
of precipitating antibodies to *Aspergillus* species in patients sera by a home-made
battery of reagents, composed of culture filtrate antigens and their homologous rabbit
and *A. terreus* B-985. Three hundred and forty nine sera which comprised of 129
from patients with suspected aspergillosis, 30 from patients with lung cancer, 18
from patients with other systemic mycotic infections, 34 from patients with
meliodosis, 17 from patients with active pulmonary tuberculosis and 121 from
normal individuals were tested. Positive precipitating antibodies by double
immunodiffusion method were found in 27.13% (35/129) of patients with
suspected aspergillosis while false positive results were found in 1.18% (4/220)
in control groups.
Introduction

The most common form of aspergillosis is the infection of the respiratory system.1-4 The disease is caused by inhalation of airborne conidia of Aspergillus species.5-6 The antigens liberated from the conidia will sensitize patients and may contribute to the development of hypersensitivity diseases such as extrinsic asthma, extrinsic alveolitis and allergic bronchopulmonary aspergillosis (ABPA).5 Under some circumstances, such as during immunosuppressive therapy or altered host defenses by severe primary diseases,7-10 the inhaled conidia may germinate and invade the tissue, resulting in invasive aspergillosis. Saprophytic colonizing of the fungus in pre-existing lung cavities is known as aspergilloma3-4,11-22 or progressive course encroaching upon the lung tissue of indolent cavities will be defined as chronic necrotizing pulmonary aspergillosis (CNPA).3,13-14

The non-specific clinical and radiological pulmonary manifestations of aspergillosis create diagnostic difficulty.7,15-16 Demonstration of precipitin antibodies against Aspergillus antigen is widely used17,23 and is very helpful in the diagnosis of aspergillosis.17,24

In this study, a battery of home-made reagents according to the CDC standards had been prepared for detection of precipitating antibodies in patients sera by double immunodiffusion method.

Materials

1. Antigens
Aspergillus fumigatus B-1172 culture filtrate antigen
Aspergillus flavus B-15 CF antigen
Aspergillus niger 107 CF antigen
Aspergillus nidulans B-1390 CF antigen
Aspergillus terreus B-985 CF antigen

2. Antisera
Rabbit antiserum of A. fumigatus B-1172 CF antigen
Rabbit antiserum of A. flavus B-15 CF antigen
Rabbit antiserum of A. niger 107 CF antigen
Rabbit antiserum of A. nidulans B-1390 CF antigen
Rabbit antiserum of A. terreus B-985 CF antigen
All *Aspergillus* species antigens and their homologous antisera were prepared according to the CDC standards and being used as references reagents.

3. Sera

3.1 One hundred and twenty one sera were obtained from normal healthy individuals.  
3.2 One hundred and twenty nine sera were obtained from patients with suspected aspergillosis.  
3.3 Eighteen sera of other systemic mycotic infections  
3.4 Thirty four sera of melioidosis patients  
3.5 Thirty sera of patients with lung cancer  
3.6 Seventeen sera of patients with active pulmonary tuberculosis  

All sera were aliquot and kept at -20 °C until used.

Methods

Double immunodiffusion method was performed according to Coleman and Kauffman.  

Glass slides (1”x3”) were coated with thin film of 1 % purified agar and left dry at room temperature. Three milliliters of molten 1% purified agar was then overlaid on the precoated slide. The gel was allowed to set at room temperature and the wells were punched by a gel puncher, as a seven-well pattern. The microliters of CF antigen was filled into the central well while the 10 µl of either neat rabbit anti-CF homologous or tested sera were added into the peripheral wells. The slide was incubated at room temperature for 24-48 hours in a humid chamber, to allow the precipitation to take place.  

The slide was then washed with distilled water for 10 minutes at room temperature, and dipped in 5% sodium citrate for 45 minutes at room temperature to eliminate non specific bands produced by C-reactive protein. Then the slide was again washed with distilled water for another 10 minutes at room temperature and left in normal saline solution overnight at room temperature. The slide was then washed again with distilled water for an additional 10 minutes. The gel was dried under the soaked filter paper with a blow dryer, stained with Coomassie blue staining solution for 15 minutes and then destained until the background was clear.  

All of the 349 sera were tested by double immunodiffusion method against 5 home-made *Aspergillus* CF antigens. In addition, positive controls from 5 home-made *Aspergillus* rabbit antisera were included in every test. The presence of one or more precipitin lines was indicative of positive results.

Results

All 349 sera obtained from 121 normal individuals, 129 patients with suspected aspergillosis (SUS-ASP), 18 with other systemic mycotic infections (M), 34 with melioidosis (ML), 30 with lung cancer (CA-Lung) and 17 with active pulmonary tuberculosis (TB) were tested with all the five home-made *Aspergillus* CF antigens. The results are shown in Table 1, which can be seen that the precipitating antibody to *A. fumigatus* was detected in 31/129 (24.03%) of the patients with suspected aspergillosis while it was not found in the other groups of patients. Small numbers of positive results to *A. flavus*, *A. niger* and *A. terreus* in this suspected aspergillosis group were found in 1/129 (0.77%), 2/129 (1.55%) and 1/129 (0.77%) respectively.

The precipitating antibodies could not be detected in all the other group, except the melioidosis group in which precipitating antibodies to *A. niger* 4/34 could be detected, false positive results were 4/220 or 1.81%.
### Table 1

<table>
<thead>
<tr>
<th>Group of subjects</th>
<th>Total</th>
<th>Number of sera with positive precipitating antibodies to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>A. fumigatus B-1172</td>
</tr>
<tr>
<td>Normal</td>
<td>121</td>
<td>0</td>
</tr>
<tr>
<td>SUS-ASP</td>
<td>129</td>
<td>31*</td>
</tr>
<tr>
<td>M</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>ML</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>CA-lung</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>TB</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>

* with slight cross-reaction with the home-made CF antigens of A. flavus B-15 5 sera

A. niger 107 1 sera

### Discussion

According to the previous studies performed by many investigators, it was concluded that the demonstration of specific precipitating antibodies in patients' sera with aspergillosis is of value in the diagnosis of pulmonary aspergillosis and can be used together with clinical, cultural, and/or histopathologic investigations for the specific of the disease. In this study, the present of specific antibodies to aspergilli assayed by double immunodiffusion method will be used as one inclusive criteria for laboratory diagnosis of aspergillosis.

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### References