Diagnosis of aspergillosis by double immunodiffusion method with home-made *Aspergillus* antigens and antisera

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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 antisera of *A.fumigatus* B-1172, *A.flavus* B-15, *A. niger* 107, *A. nidulans* B-1390 and *A. terreus* B-985. Three hundred and fourty 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.

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Aspergillosis มักจะพบก่อโรคในระบบทางเดินหายใจ การให้การวินิจฉัย ที่จำเพาะโดยวิธีการเพาะแยกเชื้อหรือย้อมสีโดยตรงจากสิ่งส่งตรวจที่ได้จากรอยโรค มักกระทำไม่ค่อยได้ในทางปฏิบัติ เพราะฉะนั้นในการวินิจฉัยจึงต้องอาศัยข้อมูลต่างๆ ประกอบกันนอกเหนือไปจากผลการเพาะแยกเชื้อ เช่น ภาพถ่ายรังสีทรวงอก รวมทั้งวิธีการตรวจน้ำเหลือง ในการศึกษาครั้งนี้ได้พัฒนาวิธีตรวจหาแอนติบอดีชนิดตกตะกอน

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ได้ ที่มีความจำเพาะต่อเชื้อในสกุล *Aspergillus* ในสิ่งส่งตรวจเป็น serum โดยวิธี double immunodiffusion โดยการเตรียม reference reagents ไว้ใช้เองเป็นชุด อันประกอบด้วย culture filtrate antigens และ rabbit antisera ที่เตรียมจากเชื้อ *A. fumigatus* B-1172, *A. flavus* B-15, *A. niger* 107, *A. nidulans* B-1390 และ *A. terreus* B-985 และใช้ในการทดสอบ serum จำนวน 349 ตัวอย่างตรวจ ซึ่งได้จากผู้ป่วยกลุ่มต่างๆ อันประกอบด้วย serum จากกลุ่มผู้ป่วยที่สงสัยโรค aspergillosis 129 ตัวอย่าง, serum จากกลุ่มผู้ป่วยที่เป็นมะเร็งปอด 30 ตัวอย่าง, serum จากผู้ป่วยที่เป็นโรคติดเชื้อราระบบอวัยวะภายในชนิดอื่นๆ 18 ตัวอย่าง, serum จากผู้ป่วย melioidosis 34 ตัวอย่าง, serum จากผู้ป่วยวัณโรคปอดระยะ active 17 ตัวอย่าง และ serum จากบุคคลปรกติ 121 ตัวอย่าง จากการศึกษาพบผลบวกโดยวิธีตรวจหาแอนติบอดีที่ตกตะกอนได้ 27.13 % (35/129) ในกลุ่มผู้ป่วยสงสัยโรค aspergillosis และพบผลบวกลวง 1.81% (4/220) ในกลุ่ม control *(MJS 1997 ; 1 : 7 - 11)*

Introduction

The most common form of aspergillosis is the infection of the respiatory system.[1-4] The disease is caused by inhalation of air borne 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 colonizating of the fungus in pre-existing lung cavities is known as aspergilloma[3-4,11-22] 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 used[17,23] and is very helpful in the diagrosis of asperogillosis.[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 (CF) antigen

Aspergillus flavus B-15 CF antigen

Aspergillus niger 107 CF antigen

Aspergillus nidulans B-1390 CF antigan

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.[25-26]

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 ataining 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%.

Group of subjects	Total	Number of sera with positive precipitating antibodies to				
	No.	*A. fumigatus* B-1172	*A. flavus* B-15	*A. niger* 107	*A. nidulans* B-1390	*A. terreus* B-985
Normal	121	0	0	0	0	0
SUS-ASP	129	31*	1	2	0	1
M	18	0	0	0	0	0
ML	34	0	0	0	0	0
CA-lung	30	0	0	0	0	0
TB	17	0	0	0	0	0

Table 1 The number of subjects who gave positive results for precipitating antibodies to the home-made CF antigens of *Aspergillus* species by double immunodiffusion method.

* 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 doudle immunodiffusion method will be used as one inclusive criteria for laboratory diagnosis of aspergillosis.

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