

Fungal Complications in Patients with Pulmonary Tuberculosis or Other Lung Diseases

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肺結核이나 기타 肺疾患患者에서의 眞菌 合併症

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ABSTRACT: A total of 1,192 patients, who complained a continued chronic cough, sputum or occasional hemoptysis, in spite of successful completion of antituberculous chemotherapy or had some suspected fungal infection, were included. Serum specimens were collected from all the patients studied and sputum or other specimens collected and cultured from the most of the patients. 405(34.0%) cases of the total patients studied showed a positive precipitin reaction to the one or more of the fungal antigens on immunodiffusion tests and 303 cases of them were found to have been infected with *Aspergilli*, of which *Aspergillus fumigatus* was involved in 287 cases, followed by *Aspergillus flavus*(1.7%), *Aspergillus nidulans*(0.3%), *Aspergillus niger*(0.3%) and *Aspergillus nidulans* var. *latus*(0.1%). Precipitin antibodies were produced to *Candida albicans*(8.1%) and *Pseudallerscheria boydii*(0.8%). In the chest radiographs of 186 precipitin positive patients, distinct fungus ball shadows were seen in 47 cases and 45 cases of them were formed by *A. fumigatus*. The isolates from sputum specimens of 724 patients were aspergilli which were consisted of the 46.4% of the total fungal isolates. Identification of 137 yeast like fungi from the sputum specimens of 413 patients revealed that *C. albicans*(64.2%) was a commonest yeast flora.

KEYWORDS: *Aspergillus fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, *Candida albicans*, *Pseudallerscheria boydii*, Antituberculous chemotherapy.

A variety of fungi colonize in the lung cavities, scars or bronchiectasis formed by tuberculosis or other lung diseases or invade lung tissue if the defense mechanisms of the host are not competent(Emmons *et al.*, 1977; Pepys, 1969; Richardson *et al.*, 1982). They distribute world-widely and sometimes show a seasonal or geographical difference in the ubiquity of their airborne spores. Most common species of the opportunistic pulmonary fungal pathogens is *Aspergillus fumigatus*, and many other fungi also infect the lung occasionally(Emmons *et al.*, 1977; Hinson *et al.*, 1952; Pepys, 1969; Richardson *et al.*, 1982; Young *et al.*, 1972).

Increase of the population susceptible to the opportunistic fungal infection urged us to study

ecology or epidemiology of the opportunistic fungal pathogens prevalent in Korea. For this purpose, the patients with pulmonary cavities, scars or bronchiectasis formed by tuberculosis or other lung diseases could be proper subjects. Tuberculosis is still highly prevalent in Korea. In 1985 it has been estimated that there are approximately 189,000 patients with bacillary(tubercle bacilli) pulmonary cavity that is vulnerable to the fungal colonization(MHSA, 1985).

Materials and Methods

Patients

A total of 1,192 patients, who complained a continued chronic cough, sputum, and/or occa-

sional hemoptysis after successful completion of antituberculous chemotherapy or have some suspected symptoms, were included in this study. The fungal flora of the sputum specimens were investigated with 724 patients. Species of yeast like fungal isolates from 413 patients were identified.

Specimens

Serum specimens were collected from all the patients studied and the collection was repeated if necessary for the confirmation of diagnosis or for the follow-up. Sputum specimens were collected from most of the patients and the repeated collection was also performed if necessary. Open lung biopsy specimens were obtained from two patients who received renal transplantation.

Immunodiffusion(ID) tests

Serum precipitin antibody against the fungal antigens were detected by ID as described elsewhere(Kim *et al.*, 1979). ID was based on the one described by Crowle(Crowle, 1961) with following modifications: One per cent Difco noble agar contained 1% sodium azide as the electrolyte. Wells of 3 mm diameter were made on the agar slide by punching out agar blocks at a 3 mm distance between serum and antigen wells. After incubation for 72 hours, the precipitin positive slides were washed with 5% sodium citrate to dissociate precipitates produced by C-reactive protein if any.

Antigen preparation

Mycelial extract(ME) of *A. fumigatus* was described elsewhere(Kim *et al.*, 1979). Antigens of all the other fungi(*A. flavus*, *A. nidulans*, *A. niger*, *Candida albicans*, *Pseudallerscheria boydii*, and *Rhizopus arrhizus*) were prepared from the culture filtrates(CF). The fungus was grown in the dialysate of Sabouraud's dextrose broth medium on an incubator shaker(Lab-Line No.3595. Lab-Line Instruments, Inc., Illinois, USA) at a speed of 100 rpm for 4 weeks at 37°C. The CF was separated from mycelia by filtration through Whatman No.42 paper and concentrated by vacuum dialysis using Visking cellulose membrane(1 cm diameter). The concentrated CF was then centrifuged at 10,000g for 30 min to remove precipitates if any and the supernatant was freeze-dried after exhaustive dialysis against distilled water. The CF antigen of *C. albicans* has been precipitated at 50% saturation of ammonium sulfate and then dialysed against distilled water prior to the freeze-drying.

Isolation and identification of fungi

Sputum or lung biopsy specimens were cultured on the Sabouraud's dextrose agar plates at 28°C and 37°C for up to four weeks. The fungal colonies grown on the medium were subcultured and purified prior to the identification. Identification of the fungal isolates were carried out on the basis of macroscopic and microscopic morphological characteristics of the plate and/or slide cultures. Species identification of yeast like fungi was performed by the API-20 C-Auxanogram(API-Bio-Merieux, Nurtingen)(Dermourmi, 1979).

Results and Discussion

A total of 405(34.0%) cases out of 1,192 patients studied showed a positive precipitin reaction to the one or more of the fungal antigens on immunodiffusion tests(Table I). The fungal infection of three additional cases was found by repeated culture examination of the serial sputum specimens or by demonstrating the fungus in the biopsy specimens although they did not produce detectable antibody even to the antigens prepared with the fungal isolates from their own specimens. The causative fungi to which the patient's serum showed a positive precipitin reaction were isolated from the sputum specimens of 79.4% of the cases studied.

Molds induced precipitin antibody in 317 patients(26.6%) and colonized or invaded in the lung tissues of three cases(0.3%) without inducing antibody. The majority(303 cases or 25.4%) of them were found to have been infected with aspergilli of which *A. fumigatus* was involved in 287 cases(24.1%). *A. fumigatus* infection was found in two kidney transplant recipients by demonstrating the fungus in the open lung biopsy specimens and one of them showed a positive precipitin reaction to the fungus. Of the patients infected with *A. fumigatus*, the fungus was isolated from sputum specimens of 82.2%(Table I), whose sputum cultures had been carried out, while it was isolated from merely 8.7% of precipitin negative reactors(Table III). And approximately 69.6% of culture and precipitin positive reactors expectorated more than 50 colony forming cells per ml of sputum, but sputum specimens from 73.3% of culture positive, precipitin negative reactors yielded less than 50 cells. Sera of

Table I. Immunodiffusion reaction patterns of patients sera to the various fungal antigens

AFG**	Reaction patterns						No. of cases (%)	Percentage isolation of related fungi	
	AFV	AND	ANG	CAC	PAB	RAZ		One	Two or more
+	-	-	-	-	-	-	241(20.2)	79.4	
+	+	-	-	-	-	-	16 (1.3)	85.7	14.3
+	+	-	-	+	-	-	3 (0.3)		
+	-	+	-	-	-	-	3 (0.3)		
+	-	+	-	+	-	-	1 (0.1)		
+	-	-	-	+	-	-	17 (1.4)	30.8	61.5
+	-	-	-	+	-	+	1 (0.1)		
+	-	-	-	-	+	-	4 (0.3)		
-	+	-	-	-	-	-	12 (1.0)	66.7	
-	+	+	-	-	-	-	1 (0.1)		
-	+	-	+	-	-	-	2 (0.2)		
-	+	-	-	+	-	-	2 (0.2)		
-	+	-	+	+	-	-	1 (0.1)		
-	-	+	-	-	-	-	2 (0.2)		
-	-	+	-	-	-	+	1 (0.1)		
-	-	-	+	-	-	-	2 (0.2)		
-	-	-	+	+	-	-	1 (0.1)		
-	-	-	-	+	-	-	88 (7.4)	80.0	
-	-	-	-	+	-	+	1 (0.1)		
-	-	-	-	-	+	-	5 (0.4)	80.0	
-	-	-	-	-	-	+	1 (0.1)		
Subtotal							405(34.0)*	74.9	4.5
Total							1,192(100.0)		

* A total of 405 out of 1,192 patients reacted to the one or more of the fungal antigens. Fungal infection of the three additional cases by *A. fumigatus*, *A. flavus*, or *A. nidulans* respectively, was confirmed by repeated culture examinations and not included here.

** AFG=*A. fumigatus*, AFV=*A. flavus*, AND=*A. nidulans*, ANG=*A. niger* CAD=*C. albicans*, PAB=*P. boydii*, RAZ=*R. arrhizus*

45 *A. fumigatus* reactors cross reacted with other fungal antigens, but only 14 of them seemed to have produced antibodies to the other fungi probably due to the mixed colonization. Seven patients reacted only to the antigen produced with the isolates from their own sputum specimens.

Precipitin antibody to the *A. flavus* antigen was detected in sera of 37 patients, 18 of whom seemed to manifest merely a cross-reaction without actual involvement of this fungus as causative agent. One female patient had a distinct fungus ball shadow in

her left lung and expectorated *A. flavus* for over three years with the absence of detectable antibody to this fungus. Therefore *A. flavus* might have colonized in the lungs of at least 20 patients(1.7%), five of them manifested a mixed infection. *A. nidulans* apparently infected three patients, *A. niger* in three cases, and *A. nidulans* var. *latus* in one case.

Nine patients(0.8%) showed a positive precipitin reaction to the *P. boydii* antigen and the fungus was isolated from sputum specimens of six patients.

Table II. Chest radiographic examination of 186 patients showed a positive precipitin reaction to fungal antigens

Duration of TB history (years)	Number of cases	Absent	Cavity		
			None	Doubtful	Distinct
< 3	14(100.0)	6(42.9)	7(50.0)	0	1 (7.1)
3 - 5	30(100.0)	9(30.0)	14(46.7)	2(6.7)	5(16.7)
6 - 10	54(100.0)	10(18.5)	22(40.7)	5(9.3)	17(31.5)
11 - 15	42(100.0)	5(11.9)	22(52.4)	2(4.7)	13(31.0)
16 - 20	20(100.0)	6(30.0)	10(50.0)	0	4(20.0)
> 20	25(100.0)	6(24.0)	12(48.0)	0	7(28.0)
?	1	1		0	0
Total	186(100.0)	43(23.1)	87(46.8)	9(4.8)	47(25.3)

*Out of 47 cases, fungus balls of 45 were formed by *A. fumigatus*, one by *A. flavus*, and another one by *A. niger*.

Positive precipitin reaction to the *C. albicans* antigen was found in sera of 115 patients, but 97 cases(8.1%) of them seemed to have produced precipitin antibody upon exposure to the unusual heavy colonization of this fungus. Their clinical significance, however, could not be determined.

In the chest radiographs of 186 precipitin positive patients having pulmonary cavities or scars formed by tuberculosis, distinct fungus ball shadows were seen in 47 cases(25.3%) and doubtful shadows in 9 cases(4.8%) as seen in Table II. Fungus balls of 45 cases out of 47 were formed by *A. fumigatus*, one by *A. flavus*, and another one by *A. niger*. The fungus ball shadows were seen more often in the chest radiographs of the patients who had more than five years of tuberculosis history.

A variety of fungi were encountered in the sputum specimens of the patients regardless of whether they had precipitin antibodies to the fungal antigens or not(Table III). A total of 664 strains of fungi were isolated from the sputum specimens of 243(91.7%) out of 265 precipitin positive reactors and of 283(61.7%) out of 459 precipitin negative reactors. Most common isolates were aspergilli that represented 46.4% of the total fungal flora in the sputum specimens. *A. fumigatus* was most numerous represented 27.9% of the total isolates and of course more common amongst precipitin positive reactors than negative reactors. The other aspergilli have been isolated and identified, *A. flavus*(7.5%), *A. nidulans*(0.6%), *A.*

niger(2.9%), *A. ochraceus*(0.8%), *A. oryzae*(0.2%), *A. terreus*(0.5%), *A. versicolor* group(3.8%).

Penicilli(12.3%) were also frequently isolated particularly when the cultures were incubated at the room temperature. They, however, have never involved in the pathogenesis. *Cladosporium* spp.(2.4%), *Fusarium* spp.(1.7%), *Alternaria* spp.(1.2%), and many other identified or unidentified molds were occasionally isolated and seemed not to have colonized in the lungs except for *P. boydii*(0.8%) of which all the isolates were found to have a clinical significance.

Isolates of yeast like fungi were 27.0% the commonest yeast being *C. albicans*(16.0%). As stated before, the number of precipitin positive reactors to the *C. albicans* antigen was considerably high because it is a member of commensal organisms on the mucocutaneum. Identification of 137 isolates of yeast like fungi from 413 patients revealed that besides the commonest yeast, *C. albicans*(64.2%), *C. tropicalis*(5.1%), *C. guilliermondii*(2.9%), *C. parapsilosis*(2.2%), *Rhodotorula rubra*(2.9%), *Saccharomyces cerevisiae*(2.9%), *Torulopsis glabrata*(2.9%), *Trichosporon penicillatum*(1.5%) and many other species were found in few of the sputum specimens(Table IV).

Fungal flora are so much prosperous in the nature that their air-borne spores are frequently inhaled into the human respiratory tract in which many of them can cause the various forms of mycoses in the susceptible individuals. In this study

Table III. Fungal isolates from human sputum specimens

Name of fungi	Isolates from patients whose reaction to fungal antigens was:		Total
	positive(265)	negative(459)	
<i>Aspergillus fumigatus</i>	145(47.1)	40(11.2)	185(27.9)
<i>A. flavus</i>	21 (6.8)	29 (8.1)	50 (7.5)
<i>A. nidulans</i>	1 (0.3)	3 (0.8)	4 (0.6)
<i>A. niger</i>	8 (2.6)	11 (3.1)	19 (2.9)
<i>A. ochraceus</i>	2 (0.6)	3 (0.8)	5 (0.8)
<i>A. oryzae</i>	1 (0.3)	0	1 (0.2)
<i>A. terreus</i>	1 (0.3)	2 (0.6)	3 (0.5)
<i>A. versicolor</i> group	9 (2.9)	16 (4.5)	25 (3.8)
Aspergilli	7 (2.3)	9 (2.5)	16 (2.4)
<i>Alternaria</i> spp.	3 (1.0)	5 (1.4)	8 (1.2)
<i>Aureobasidium</i> spp.	1 (0.3)	2 (0.6)	3 (0.5)
<i>Candida albicans</i>	47 (15.3)	59(16.6)	106(16.0)
<i>Chaetomium</i> spp.	0	1 (0.3)	1 (0.2)
<i>Cladosporium</i> spp.	6 (1.9)	10 (2.8)	16 (2.4)
<i>Fusarium</i> spp.	3 (1.0)	8 (2.2)	11 (1.7)
<i>Monosporium</i> spp.	2 (0.6)	2 (0.6)	4 (0.6)
<i>Mucor</i> spp.	0	4 (1.1)	4 (0.6)
<i>Neurospora</i> spp.	1 (0.3)	3 (0.8)	4 (0.6)
<i>Paecilomyces</i> spp.	1 (0.3)	4 (1.1)	5 (0.8)
<i>Penicillium</i> spp.	24 (7.8)	58(16.3)	82(12.3)
<i>Pseudallerscheria boydii</i>	5 (1.6)	0	5 (0.8)
<i>Rhizopus</i> spp.	0	4 (1.1)	4 (0.6)
<i>Rhodotorula</i> spp.	2 (0.6)	4 (1.1)	6 (0.9)
<i>Scopulariopsis</i> spp.	0	2 (0.6)	2 (0.3)
<i>Trichoderma</i> spp.	2 (0.6)	2 (0.6)	4 (0.6)
Unidentified molds	7 (2.3)	17 (4.8)	24 (3.6)
Unidentified yeasts	9(2.9)	58(16.3)	67(10.1)
Total	308(100.0)	356(100.0)	664(100.0)

* A variety of fungi were isolated from the sputum specimens of 243(91.7%) out of 265 precipitin positive cases and of 283(61.7%) out of 459 precipitin negative patients.

we have isolated a variety of fungi from the sputum specimens of 91.7% of 265 patients whose sera showed a positive precipitin reaction to the one or more of the fungal antigens on ID tests and of 61.7% of 459 ID negative reactors. Of the total isolates, molds were 73.0% and yeast like fungi, 27.0%. The most common molds were aspergilli(46.4%), followed by penicilli(12.3%), *Cladosporium*

spp.(2.4%). *Fusarium* spp.(1.7%), *Alternaria* spp.(1.2%), and many others. This may reflect their ubiquity in the human environment. The commonest species was *A. fumigatus* that was also found to be a most common causative agent of aspergilloma in the lung. *A. flavus* was a next common *Aspergillus* in the sputum specimens, followed by *A. versicolor* group and *A. niger*. None of *A. versicolor* group

Table IV. Species of yeasts like fungi isolated from human sputum specimens

Species	No. of isolates
<i>Candida albicans</i>	88(64.2)
<i>C. tropicalis</i>	7 (5.1)
<i>C. guilliermondii</i>	4 (2.9)
<i>C. parapsilosis</i>	3 (2.2)
<i>C. stellatoidea</i>	1 (0.7)
<i>Cryptococcus terreus</i>	1 (0.7)
<i>Rhodotorula rubra</i>	4 (2.9)
<i>R. minuta</i>	1 (0.7)
<i>R. pilomanae</i>	1 (0.7)
<i>Saccharomyces cerevisiae</i>	4 (2.9)
<i>Torulopsis glabrata</i>	4 (2.9)
<i>T. inconspicua</i>	1 (0.7)
<i>Trichosporon penicillatum</i>	2 (1.5)
<i>T. capitatum</i>	1 (0.7)
Unidentified	15(10.9)
Total	137(100.0)

* () = %

isolates was involved in the pathogenesis, while the other two species occasionally caused aspergilloma.

Out of 1,192 patients studied, 303(25.4%) cases were found to have been infected with aspergilli of which *A. fumigatus* involved in 94.7% of the cases. This figure appears close to those of the other reports in that more than 90% of allergic bronchopulmonary aspergillosis and aspergilloma were caused by *A. fumigatus*(Bardana *et al.*, 1975 ; BTA, 1968 ; BTTA, 1970 ; Longbottom *et al.*, 1964). However in cases of invasive aspergillosis this species was involved some what less frequently showing 59%, but *A. flavus* involved as much as 31%(Young *et al.*, 1972).

Penicilli are ubiquitous in both indoor and outdoor air and have been isolated much more often at the room temperature incubation than at 37°C. They can hardly colonize in the human lungs, probably due to the suboptimal growth temperature and/or the lack of pathogenicity.

Many other molds were seen in few of the sputum specimens. *P. boydii* was rare isolate and all the patients, expectorated this fungus, showed a posi-

tive precipitin reaction. Nine patients were apparently infected with this fungus.

The patients with persistent pulmonary cavities(or scars) after successful completion of antituberculous chemotherapy are vulnerable to the fungal colonization. British surveys(BTA, 1968; BTTA, 1970) revealed that 24.6% of those patients showed a positive precipitin reaction to the *Aspergillus* antigens and 10.8% of them had distinct fungus ball shadows in the chest radiographs. Of the precipitin positive patients, fungus ball shadows were seen in the chest radiographs of 44.0% of the cases(BTA, 1968). In this study 32.9% of the precipitin positive patients with pulmonary cavities had distinct fungus ball shadows.

Precipitin negative reaction to the antigens of the fungi with which two noncompromised patients were apparently infected indicates heterogeneity of antibody response of the hosts.

Several patients reacted to the antigens prepared with the fungal isolates from their own sputum specimens. Whether it indicates heterogeneity in the antigenic composition of the isolates is not known yet.

The yeast like fungi were also common flora of the sputum specimens. *C. albicans* was of course isolated in largest number and many other species were found occasionally. A considerable number of patients had precipitin antibodies to the *C. albicans* antigen, but it was unable to determine the clinical significance of such antibody.

摘 要

성공적인 결핵치료에도 불구하고 계속적인 만성기침 또는 객담이 있거나 간헐적인 혈담 또는 각혈이 있는 환자와 그외에도 진균감염이 의심되는 환자 등 총 1,192명을 대상으로 하여 모든 환자로부터 혈청을 채취하고 대부분의 환자로부터 객담이나 기타 병리가검물을 채취하여 홍부 x선소견과 함께 진균감염 여부를 관찰하였다. 면역확산법검사에 의해 한가지 이상의 진균항원에 대해 양성반응을 나타내는 환자는 전체의 34.0%인 405명이고 그중에서 Aspergilli에 감염된 환자는 303명이었고, 대부분의 환자(287명)가 *Aspergillus fumigatus*에 감염되어 있고 그 다음은 *Aspergillus flavus*(1.7%) *Aspergillus nidulans*(0.3%), *Aspergillus niger*(0.3%),

Asperillus nidulans var. *latus* (0.1%) 순이며 *Candida albicans* (8.1%) 에도 침강항체반응이 나타났으며 *Pseudallerscheria boydii* 도 드물게 분리되는데 9명 (8.1%) 에도 침강항체반응을 나타내었다. 침강항체반응 양성을 나타내는 186명의 흉부 x선사진에서 진균중 음영이 나타나는 환자가 47명이었고 이들 가운데서 45명이 *A. fumigatus* 에 의해 형성되었다.

진균감염 또는 미감염환자 724명의 객담에서 발견되는 진균가운데 46.4%가 *Aspergilli* 였다. 413명의 환자객담에서 분리된 137주의 효모양균주를 분리 동정할 결과 그중 *Candida albicans* (64.2%) 가 가장 많았다.

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