Molecular analysis of paranasal fungus ball in Japan using formalin-fixed paraffin-embedded (FFPE) sections

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CONCLUSION

PCR technique using FFPE sections was useful for retrospective study to know causative fungi of fungal sinusitis, even in cases which had negative result by culture.
In present study, *Aspergillus* spp. were supposed to be the most common causative fungi of the disease. Whereas concern of contamination should be excluded, it is suggested that *Basidiomycota* is one of considerable causative fungi of non-invasive fungal sinusitis in Japan.

OBJECTIVE

Non-invasive fungal sinusitis, forming fungus balls in paranasal cavities, is the most common type of the fungal sinusitis. The fungus balls are composed by filamentous fungi mostly, but they may not be identified by culture in many cases. And it is difficult to accurately distinguish between *Aspergillus* spp. and other filamentous fungi by Grocott's methenamine silver staining. So we conducted fungus ball using molecular identification to know the epidemiology of non-invasive fungal sinusitis.

MATERIAL AND METHODS

It was extracted 72 specimens of formalin-fixed paraffin-embedded (FFPE) sections of fungus ball removed from paranasal sinuses at Toho University Omori Medical Center in Tokyo, from 2002 to 2016. All 72 were diagnosed as fungal sinusitis of filamentous fungi by histopathological examination. DNA was detected using a conventional polymerase chain reaction (PCR) method using panfungal primer targeting the 18S/ITS1 rRNA gene, and *Aspergillus* primer targeting the 28S rRNA gene. And the resulting amplification products were prepared for sequence analysis and BLAST analysis.

Primers

Specificity	Name of primer	Product size	Sequence
Panfungal 18S and ITS1 rRNA gene Makimura K. et al. J. Clin. Microbiol. 1999.	18SF1	300 bp	AGGTTTCGCTAGGTGAACCT
	58SR1		TTCGCTGCGTTCTTCATCGA
<i>Aspergillus</i> species 18S rRNA gene Hofman V, et al. Diagnostic Pathology. 2010.	ASP nest1	250 bp	TCTTGTTCCGGCATCGAT
	ASP nest2	146 bp	TGACAAAGCCCCATACGCT
	ASP nest3		GAAGAACGCAGCGAAATGC
	ASP nest4		AACACACAAGCCGTGCTTGA

PCR results of the 72 specimens

<u>Panfungal primer</u>		
Positive	39	(54.2%)
Negative	33	(45.8%)
<u><i>Aspergillus</i> primer</u>		
Positive	41	(56.9%)
Negative	31	(43.1%)

Positive by Panfungal or *Aspergillus* primer...57 (79.2%)

BLAST results of the 72 samples (1) (Panfungal primer)

	Number of specimens	(%)
<i>Aspergillus</i> spp.	16	(22.2)
Mushrooms (Basidiomycota)	11	(15.3)
<i>Cladosporium</i> spp.	2	(2.8)
<i>Malassezia restricta</i>	1	(1.4)
<i>Candida rugosa</i>	6	(8.3)
Contamination (plants etc.)	3	(4.2)
Not identified	33	(45.8)

BLAST results of the 72 samples (2) (*Aspergillus* primer)

	Number of specimens	
<i>Aspergillus</i> sp.or <i>Penicillium</i> sp.	15	20.8%
<i>Aspergillus flavus</i>	10	13.9%
<i>Aspergillus fumigatus</i>	6	8.3%
<i>Aspergillus</i> sp.	6	8.3%
<i>Penicillium</i> sp.	2	2.8%
<i>Malassezia</i> sp.	1	1.4%
<i>Mycobacterium</i> sp.	1	1.4%
Not identified	31	43.1%

Case 40 BLAST results: *Aspergillus flavus*

Fungus ball was composed by filamentous fungi with bacterial colonization.

DISCUSSION

PCR technique using FFPE sections enabled retrospective study of causative fungi of fungal sinusitis, even in specimens which had negative by culture. But fragmentation of DNA by formalin-fixation often affects PCR results. In present study, 33/72 (45.8%) and 31/72 (43.1%) were negative for PCR analysis by each primer.
Aspergillus spp. , especially *A. flavus* and *A. fumigatus*, were the commonest fungi involving paransal sinus. 39/72 (54.2%) were supposed to be *Aspergillus* spp. by panfungal and/or *Aspergillus* primer.
And 11/72 (15.3%) showed genes of *Basidiomycota*, that may support the data that fungal sinusitis caused by *Basidiomycota* had been reported frequently because of the humid atmosphere in Japan. But the histological findings of fungi and the PCR results are not always equal. So contamination by environmental fungi or other organisms must be the large problem of PCR technique.

Case 41 BLAST result: *Schizophyllum commune*

Fungus ball with filamentous fungi with some branch and septum.

Histopathological features, showing allergic mucin with degenerated eosinophils and Charcot-Leyden crystals, are that of allergic fungal sinusitis.