

Research Report**Fungus ball paranasal sinuses:
pattern of histopathology and culture characteristics****Nugroho Suharsono**Department of Otorhinolaryngology-Head and Neck Surgery
St. Vincentius A Paulo Hospital
Surabaya**ABSTRACT**

Background: Fungal infection of the nose and paranasal sinuses is an uncommon condition which is now being increasingly recognized. The clinical presentation is not specific with various symptoms such as nasal obstruction, purulent nasal discharge, facial pain, and chronic cough. Only unilaterality may alert the clinician. **Purpose:** To find the morphological characteristics of the fungus in patients with paranasal sinus fungus ball. **Methods:** A retrospective study of 13 paranasal sinus fungus balls cases which underwent endoscopic sinus surgery at Department of Otorhinolaryngology Head and Neck Surgery St. Vincentius A Paulo Hospital Surabaya from March, 2012 until December, 2013. Age, sex, histopathology and fungal culture were analysed. Histopathologic sections of all the patients were stained with hematoxylin and eosin (H&E), and Gomori methenamine silver (GMS). The specimens were then cultured on Sabouraud dextrose agar plates and incubated at 30°C for 1 month. At the end of the incubation period, the samples were evaluated microscopically to detect fungi and identify their species. **Results:** The age reported of the 13 patients, was ranging from 36 to 63 years old. There was a significant female predominance, 10 female patients (76.92%) and 3 male patients (23.08%). Histopathological examination showed that most causative agents were *Aspergillus* species 92.31% (12/13). Culture test was positive for 69.23% (9/13). *Aspergillus niger* (61.54%, 8/13) is the most frequent fungus reported to cause fungus balls. **Conclusion:** Pattern of histopathologic on HE and GMS is very helpful and sensitive to identify fungi. The most common isolated mould in our study was *Aspergillus niger*.

Keywords: fungus ball, histopathology and culture, *Aspergillus niger*

ABSTRAK

Latar Belakang: Infeksi jamur di hidung dan sinus paranasal merupakan kondisi yang jarang terjadi, namun kini lebih sering ditemukan. Gejala klinisnya tidak spesifik dapat berupa obstruksi hidung, sekret dari hidung, nyeri wajah, dan batuk kronis. Bila terjadi unilateral, patut diwaspadai oleh para klinisi. **Tujuan:** Untuk mengetahui karakteristik morfologi fungus yang didapati pada pasien sinusitis jamur yang kami teliti. **Metode:** Dilakukan penelitian retrospektif pada 13 pasien sinusitis jamur yang menjalani bedah sinus endoskopi di Departemen Otorinolarinologi-Kepala Leher Rumah Sakit St. Vincentius A Paulo Surabaya dari bulan Maret 2012 sampai dengan Desember 2013. Dilakukan analisis usia, jenis kelamin, histopatologi dan kultur jamur. Pewarnaan preparat histopatologi menggunakan Hematoxylin dan eosin (H&E) dan Gomori Methenamine Silver (GMS). Kemudian spesimen diletakkan pada piring agar Sabouraud dextrose, dan dilakukan inkubasi pada suhu 30°C selama satu bulan. Pada akhir masa inkubasi, sampel dievaluasi dengan mikroskop untuk mendeteksi jamur dan spesiesnya. **Hasil:** Didapati usia 13 penderita berkisar dari 36-63 tahun. Wanita lebih dominan sebanyak 10 penderita (76,92 %) dan 3 penderita laki-laki (23,08%). Hasil pemeriksaan histopatologi menunjukkan spesies *Aspergillus* sebagai penyebab utama (92,31%) pada 12 penderita (12/13). Tes kultur positif pada 69,23% (9/13). Jamur yang paling sering menyebabkan bola jamur pada sinus adalah *Aspergillus niger* (61,54%, 8/13). **Kesimpulan:** Pewarnaan preparat histopatologi menggunakan Hematoxylin dan eosin (H&E) dan Gomori Methenamine Silver (GMS) sangat berguna dan sensitif dalam mendeteksi adanya jamur. Jenis jamur yang paling banyak ditemukan pada penelitian kami adalah *Aspergillus niger*.

Kata kunci: bola jamur, histopatologi dan kultur, *Aspergillus niger*

Correspondence author: Nugroho Suharsono. Department of Otorhinolaryngology-Head and Neck Surgery. St. Vincentius A Paulo Hospital, Surabaya. Email: drglinapurnama@hotmail.com

INTRODUCTION

Fungal infection of the paranasal sinuses is an uncommon condition which is now being increasingly recognized. It is a wide spectrum of disease that is classified into several categories. Two main classifications are non-invasive and invasive forms. Non-invasive fungal infection consists of saprophytic fungal infestation, fungus ball, and allergic fungal rhinosinusitis, while invasive fungal infection consists of acute, chronic, and chronic granulomatous.^{1,2} The most prevalent form is non-invasive type.³ Fungus ball is the most common type of fungal infection of paranasal sinuses accounted for 53%.⁴ The incidence of fungus ball in France is 20 cases per year.⁵

Fungus ball is defined as the presence of a dense mass of non-invasive matted fungal hyphae in paranasal sinus.^{2,6} Any findings of hyphae in mucosa, submucosa, vessels, and bone are considered as invasive forms.^{3,7} Fungal infection can happen in both immunocompetent and immunocompromised person.⁸ Fungus ball occurs in immunocompetent patient.⁹

Fungus ball is characterized macroscopically by green, yellow, brown, or black grumous brittle cheesy material that can easily be peeled off the mucosa.³ Fungus ball is mostly caused by *Aspergillus spp.*⁷ *Aspergillus* lacks of keratolytic enzyme, so it could not penetrate undamaged and intact mucus membrane or skin.¹⁰

Most patients present with non-specific complaints or asymptomatic.² The clinical presentation is not specific with various symptoms. The slow development along with asymptomatic or non-specific symptoms

lead to late diagnosis.³ Unilaterality of the symptoms may be the clue for the physician.^{9,11} Sometimes fungal infection is discovered as part of the investigation for chronic rhinosinusitis.⁵ Fungal infection can occur because of multiple pathogenesis process in chronic rhinosinusitis that leads to facilitate the colonization and spreading of fungal infection.¹¹

Patient, who is suspected of having a fungus ball, should undergo a Computed Tomography (CT) scan of the sinuses. CT scan findings for fungus ball are calcifications or metallic densities with opacified sinus cavity.⁵ Bone resorption of the nasal sinus can be suspected for fungus ball as it can be caused by hyphal proliferation.⁷ Although radiologic examination shows some specific features for fungus ball, histological and mycological assessments are still needed to confirm the diagnosis.⁵ Histologic examination only recognizes hyphae in the fungus ball. It can not differentiate which species is causing fungus ball.

The aim of this study was to find the morphological characteristics of the fungus which have not been definitely identified to its particular species.

METHODS

This is a retrospective study conducted at Department of Otorhinolaryngology Head and Neck Surgery St. Vincentius A Paulo Hospital Surabaya using total sampling method. The subjects were all patients with paranasal sinus fungus balls who came to the hospital from March, 2012 until December, 2013. All patients underwent endoscopic

sinus surgery.

Tissues obtained during endoscopic sinus surgery was divided into two section and was put in normal saline (NaCl 0,9%) and formaldehyde solutions respectively. The tissues then were stained with Hematoxylin and Eosin (HE), and Gomori Methenamine Silver (GMS). The histopathology characteristics were examined using microscope. Subsequently, the specimens were cultured on Sabouraud dextrose agar plates and incubated at 30° C for one month. At the end of the incubation period, the samples were microscopically evaluated to detect fungi and identify its species.

All data were analyzed using Statistical Package for the Sosial Sciences (SPSS) software. The data was presented based on characteristics of the patients (i.e. age and

sex), site of infection, histopathological findings, and mycological culture.

RESULTS

There was a total of 13 patients with paranasal sinus fungus balls from March, 2012 until December, 2013. The patients' age ranged from 35 until 64 years old. Most of the patients were 55 – 64 years old (53,85%, 7/13). More than three-quarters of the patients were female (76,92%, 10/13). Combined data shows that the majority of the patients were female in 55 – 64 years old group (46,15%, 6/13). The most frequent site of infection was maxillary sinus (84,61%, 11/13). Two remaining patients each had fungus ball at sphenoidal sinus and multiple sinuses (maxillary and ethmoidal sinus).

Tabel 1. Subjects' characteristics

Characteristics	n
Sex	
Male	3 (23,98%)
Female	10 (76,92%)
Age	
35 – 44 yo	4 (30,77%)
45 – 54 yo	2 (15,38%)
55 – 64 yo	7 (53,85%)

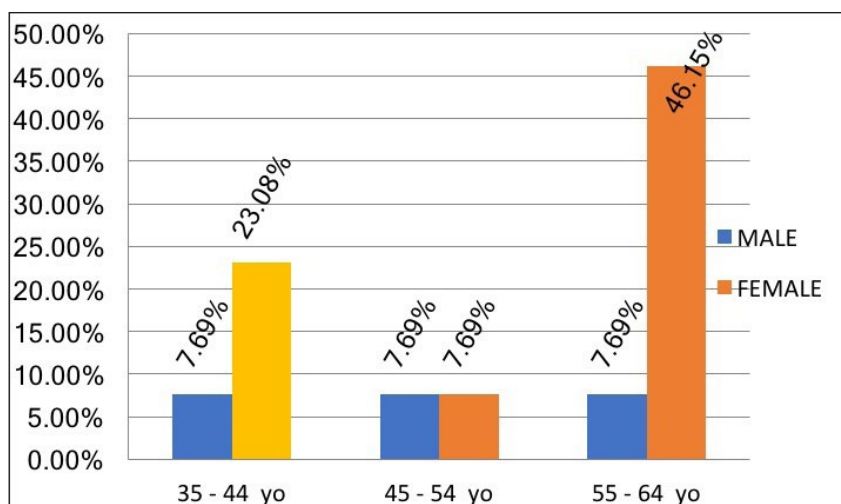


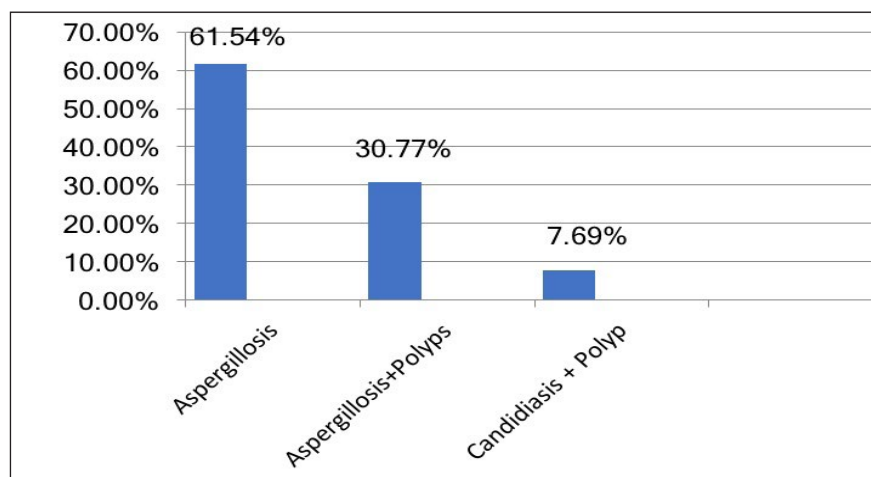
Figure 1. Age and gender distribution

Tabel 2. Paranasal sinuses location findings

Localization	n
Maxillary sinus	11 (84,61 %)
Sphenoidal sinus	1 (7,69 %)
Maxillary & ethmoidal sinus	1 (7,69 %)
Ethmoidal sinus	0 %
Bilateral maxillary sited	0 %
Multiple monolateral	0 %

Tabel 3. Mycological culture findings

Mycological culture	n
<i>Aspergillus niger</i>	61.54 % (8/13)
<i>Aspergillus fumigatus</i>	7.79 % (1/13)
<i>Penicillium spp</i>	0 %
<i>Aspergillus flavus</i>	0 %
<i>Cryosporium</i>	0 %
No Growth	30.77 % (4/13)

**Figure 2. Histopathologic findings**

Aspergillosis was found in 12 patients whilst 1 patient had Candidiasis. We also found polyp involvement in Candidiasis patient and four Aspergillosis patients. Culture test was positive for 69,23% (9/13). *Aspergillus niger* was detected in 61,54% samples (8/13). There were 4 samples that failed to develop and showed no growth at all.

DISCUSSION

There were 13 cases of paranasal sinus fungus ball from March, 2012 until December, 2013 at St. Vincentius A Paulo Hospital Surabaya. The patients' age ranged from 35 to 64 years old. Several studies found similar wide range of patients's age from young

adult to older age, starting from 26-62 years old,¹¹ 19-91 years old,⁷ 25-79 years old.¹² There was no pediatric case that have ever been reported.^{3,7} Dufour,⁵ found fungus ball case in a 14 years old girl but the patient was already pubescent. There was a significant female predominance, 10 female patients (76.92%) and 3 male patients (23.08%). Other studies also reported female predominance in paranasal sinus fungal infection.^{5,7,9,12,13} Combined data shows that the majority of the patients were female in 55 – 64 years old group (46.15%, 6/13). It shows that fungus ball most likely to occur in older female.

Maxillary sinus infection was found in 84.61% cases. Several studies revealed that the most frequent site of infection is maxillary sinus, followed by sphenoid sinus.^{5,7,9,11-13} Fungus ball can develop in multiple sinuses as well. We found one case of multiple sinuses involvement in maxillary and ethmoidal sinus. Nomura,¹² found 5.8% cases of multiple sinuses involvement, and Pagella,⁷ found 6.2% cases involving multiple monolateral sinuses.

Blockage of the sinonasal passages leads to development of fungus ball.¹ There are two proposed pathogenesis for fungus ball, the aerogenic pathway which a lot of fungus spores enter the sinus via nasal ostium, and the odontogenic iatrogenic pathway which fungal colonization in maxillary sinus happened secondary to overfilling of the dental canal.³ Materials used for root canal sealants may affect the mucociliary clearance, thus preventing fungal spores to be eliminated.³ Dental sealers consist of zinc oxide and eugenol. Zinc oxide has the ability to promote fungal development, while eugenol inhibits fungal growth. Nevertheless, inhibiting feature of eugenol wears off over time thus allowing zinc oxide to continue promotes fungal growth.³

However, the said proposed pathogenesis have not been confirmed yet. Most studies found that maxillary sinus was the most

frequent site. This finding does not support the aerogenic pathway because sphenoid sinus would likely to be the most frequent site due to position of its ostium.⁵ The odontogenic iatrogenic pathway could not apply to fungal infection located in remote sinuses such as sphenoid sinus or frontal sinus.³ Previous studies showed various results. Pagella,⁷ found that 60% patients had previous history of endodontic care and 81% of them had maxillary sinus involvement. Dufour,⁵ had opposite result with overfilling was found only in 18 of 173 cases. Our study did not include previous history of dental care. Therefore, the odontogenic iatrogenic pathway could not be investigated in our cases.

Histopathology examination showed *Aspergillus spp.* as the most dominant species (92.3%, 12/13). Our study shows similar result with Dufour,⁹ Khan,¹⁰ and Nomura,¹² that found *Aspergillus* in 93.1%, 94.2%, and 82.61% cases respectively. Pagella,⁷ revealed the presence of a high number of hyphae. Histologically, fungus ball appears as tightly packed hyphae with layered appearance surrounded by dense inflammatory infiltrate.^{1,3,4} The adjoining mucosa showed mixed inflammation with no fungal invasion.^{3,4} *Aspergillus* is recognized by its septate hyphae with 45° branch angle.^{3,8}

Although the presence of fungus can be demonstrated with routine H&E stain, a special stain is needed to identify *Aspergillus* species.^{3,6} Silver impregnation stain such as GMS is very helpful to identify *Aspergillus*.^{3,6} GMS had high sensitivity (93.6%) to identify *Aspergillus* species.⁵ The specimens should not be considered negative if silver stain has not been performed.³ Both our study and Dufour,⁹ used GMS, while Khan¹⁰ used Potassium Hydroxide (KOH), and Pagella,⁷ used Periodic Acid-Schiff (PAS), Chromotrophe anilineblue and H&E. PAS is considered to have less sensitivity than silver stains because senescent fungal cells may not be stained.³

In our study, positive culture result accounted for 69.33%, which surprisingly higher than other studies. Other studies had low culture sensitivity ranging from 16.7% to 34.5%.^{5,7,12,13} Low sensitivity in culture test is probably because of fungal's poor viability.³ However, despite of low sensitivity, culture test is still needed to find the causative fungal species.

Culture test revealed that *Aspergillus niger* is the most frequent fungus found in our patients (61.54%, 8/13). Our result differ with previous studies. Pagella,⁷ and Dufour,⁵ found *Aspergillus fumigatus* as the most common isolated species. Al-Bhlal,⁸ and Dincer,¹³ showed different result with *Aspergillus flavus* as the most common fungi. Although most studies revealed *Aspergillus spp.* as the most common species in fungus ball, other species can also be the causative agent in fungal infection. Other species found in previous studies were *Candida spp.*,¹² *Penicillium spp.*,^{7,13} and *Cryosporium*.⁷ However, these species were only sporadically described.

No clear reason why there are differences for the most frequent causative species. It is reported that fungal infection have regional characteristics.¹² Dufour,⁵ reported that 120/173 patients lived in rural area. Indonesia is a tropical country with hot and moist climate. Environmental and host-related factors must be considered as influencing factors.⁸

Pattern of histopathologic on HE and GMS is very helpful and sensitive to identify fungi. The most common isolated mould in our study was *Aspergillus niger*. The hot and moist climate might explain the epidemiology of fungus ball in Indonesia.

REFERENCES

1. Montone KT. Pathology of Fungal Rhinosinusitis: A Review. *Head and Neck Pathol.* 2016; 10: 40-6.
2. Deutsch PG, Whittaker J, Prasad S. Invasive and Non-Invasive Fungal Rhinosinusitis—A Review and Update of the Evidence. *Medicina.* 2019; 55: 319.
3. Grosjean P, Weber R. Fungus balls of the paranasal sinuses: a review. *Eur Arch Otorhinolaryngol.* 2007; 264: 461-70.
4. Navya BN, Vivek TG, Sudhir, Kariappa TM, Shwetha VP, Ahalya R. Role of Histopathology in the Diagnosis of Paranasal Fungal Sinusitis. *IOSR-JDMS.* 2015; 14(1): 97-101.
5. Dufour X, Kauffmann-Lacroix C, Ferrie JC, Goujon JM, Rodier MH, Klossek JM. Paranasal sinus fungus ball: epidemiology, clinical features and diagnosis. A retrospective analysis of 173 cases from a single medical center in France, 1989-2002. *Medical Mycology.* 2006; 44: 61-7.
6. Hathiram BT, Khattar VS. Fungus Balls of the Paranasal Sinuses. *Otorhinolaryngology Clinics: An International Journal.* 2009; 1(1): 33-5.
7. Pagella F, Matti E, Bernardi FD, Semino L, Cavanna C, Marone P, et al. Paranasal Sinus Fungus Ball: Diagnosis and Management. *Mycoses* 2007; 50: 451-6
8. Al-Bhlal LA. Fungal Infection of the Nasal Cavity and Paranasal Sinus: Review of 26 Cases. *Ann Saudi Med.* 1996;16(6):615-21.
9. Dufour X, Kauffmann-Lacroix C, Ferrie JC, Goujon JM, Rodier MH, Karkas A, et al. Paranasal sinus fungus ball and surgery: a review of 175 cases. *Rhinology.* 2005; 43: 34-9.
10. Khan MA, Rasheed A, Awan MR, Hameed A. *Aspergillus* Infection of Paranasal Sinuses. *J T U Med Sc.* 2010; 5(2): 60-5.
11. Gumussoy M. Frequency of non-invasive fungal sinusitis in patients undergoing surgery for chronic rhinosinusitis. *Int Med.* 2019; 1(4): 180-4.
12. Nomura K, Asaka D, Nakayama T, Okushi T, Matsuwaki Y, Yoshimura T, et al. 2013. Sinus Fungus Ball in the Japanese Population: Clinical and Imaging Characteristics of 104 Cases. *International Journal of Otolaryngology.* 2013 (731640): 4. Doi: <http://dx.doi.org/10.1155/2013/731640>.
13. Dinçer E, Yazır M, Günaldı A, Pınar E, İmre A, Songu M, et al. Paranasal Sinus Fungus Ball: Retrospective Analysis of 37 Patients. *Eur J Rhinol Allergy.* 2018; 1: 70-2.