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Original research article

Histopathological Features of Fungal Infections in Sinonasal, Oral and Orbital Sites Associated with COVID-19 Patients.

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Abstract

Background: The COVID- 19 infections are associated with wide range of bacterial and fungal co-infections. They may be associated with various comorbidities. Definite diagnosis requires demonstration of fungi in tissue sections or in culture. Yield of organism in culture is suboptimal. Hence histopathology plays critical role in establishing the diagnosis and provide evidence of tissue invasion.

Objectives: To study the histopathological features of fungal infections in sino nasal, oral and orbital area associated with COVID-19 patients.

Material and methods: One hundred twenty cases of fungal infections involving sinonasal, oral and orbital area in laboratory confirmed COVID-19 positive patients between June-September 2021 were taken for study. Clinical data was recorded, histopathological examination was done along with periodic acid Schiff stain and culture report was obtained.

Result: The study included 92(76.6%) males and 28(23.3%) females with age ranging from 13 to 78 years. The tissues included debridement, biopsy and excision specimen. Acute inflammation was seen in 8(6.66%) cases, chronic inflammation in 112(93.33%), granulomas in 25, thrombosis in 14, necrosis in 104, angioinvasion in 13, perineuritis in 10 and bone invasion in 18 cases. Mixed fungal infection was seen in 11cases. **Conclusion:** Histopathology remains the mainstay in diagnosis of invasive fungal infections especially when culture is negative.

Keywords: Covid-19, fungal infection, histopathology, mucormycosis

Introduction

Coronavirus disease 2019 (COVID-19) is an infection caused by severe acute respiratory syndrome corona virus-2 (SARS-COV-2). The first case was detected in Wuhan, China in December 2019(1).The COVID-8 infections are associated with wide range of bacterial and fungal co-infections. They may be associated with co morbidities like diabetes mellitus (DM), lung diseases, and ventilator associated pneumonia(2). Critically, ill patients in intensive care units, prolonged hospital stay (3,4) and widespread use of glucocorticoids (1,2,5) and broadspectrum antibiotics (2, 5) are more likely to develop fungal infections.

The fungal infections are on rise which are rarely reported in the beginning of the pandemic COVID-19 especially mucormycosis(6). The cases are reported in COVID-19 patients in the course of disease or after recovery. India contributes to 40% of global burden of mucormycosis(7). Our institute, a tertiary care hospital has seen a sudden surge in fungal infections cases involving noses, sinuses, and eyeball from May to August 2021.

Early diagnosis reduces the need and extent of surgical resection and improved survival in fungal infections. Clinical features and imaging studies are nonspecific. Definite diagnosis requires demonstration of fungi in tissue sections or recovery of organism in culture. Yield of organism in culture is suboptimal. Hence histopathology plays critical role in establishing the diagnosis and provide evidence of tissue invasion(8). Only limited number of cases have been reported on this topic. Hence, we present our recent experience of histopathological features of fungal infections associated with COVID-19 patients. It also helps in diagnosing co infection with other fungi and to rule out culture contamination (9).

Material and methods:

The present observational study was done in department of pathology, Belagavi Institute of Medical Sciences, Belagavi over a period of four months from June 2021 to September 2021. One hundred and twenty cases of fungal infections involving sinonasal, oral and orbital area in laboratory confirmed COVID-19 positive patients operated in ENT department were taken for study. The cases were either COVID-19 positive or had recovered from COVID-19 infection. The objective of the study was to assess the histopathological features of fungal infections in sinonasal, oral and orbital area associated with COVID-19 infection. All cases showing fungi in tissue section were included in study. Laboratory confirmed COVID-19 infection and the cases without fungi in tissue sections were excluded. Institutional research and ethical approval were taken. Demographic data, clinical details were recorded and radiological finding were noted when available. Samples received were grossly examined and sections were given. They were stained with hematoxylin and eosin (H& E). They were studied for type of inflammation, vasculitis, thrombosis, necrosis, angio and perineural invasion. Fungal morphology was identified with addition of periodic and Schiff (PAS) stain. Mixed infection if any were noted. Culture report was obtained from microbiology department in all 120 cases. Statical analytic was performed using SPPS software version 22 and basic data was presented in percentage and proportions.

Result

The study included 92(76.6%) males and 28(23.3%) females with age ranging from 13 to 78 years (Median 50 yrs.). Multiple overlapping predisposing factors were identified in 91(75.8%) patients which included diabetes mellitus in 84(92.3%) patients, on steroids 64(70.3%), HIV 4(4.3%) and other predisposing factors seen in 19(20.8%) cases, whereas 29(24.16%) cases with no any predisposing factor developed fungal infection (**Table no.1**). Eye affected in 6, nasal cavity in 26 and oral cavity in 7 and multiple site affected in 81 cases, out of which ethmoidal sinus infection are most common and least affected is frontal sinus. The tissue submitted for histopathology included debridement, biopsy, and excision of eyeball (**Table**

no.2,3). Acute inflammation was seen in 8(6.66%) cases, chronic inflammation in 112(93.33%) cases. 25 number of cases showed granulomas. Vasculitis was seen in 26 cases. Thrombosis in 14 cases, necrosis in 104 cases. Angioinvasion was seen in 13 cases and perineural invasion in 10 cases. And bone invasion in 18 cases. Sporangia, sporangiophores, splendore hoeppli material in calcium oxalate crystals seen in 2 aspergillus cases (**Table no.4**). Mucor mycosis seen in 104, Aspergillus in 2, candida in 3 cases, mixed fungal infection was seen in 11 cases. Mixed infections caused by the candida and aspergillus in 4 cases, mucor and aspergillus seen in 3cases, and mucor candida seen in 4cases (**Table no 5**). Culture report was available in 120 cases (**Table no.6**).

Table 1: Predisposing factors in fungal infections in COVID 19 cases (N=120)

Predisposing factors	**Percentage of cases out of 91 cases (75.83%)**
Diabetes mellitus	84
Steroids	64
Other comorbidities	19
HIV	4
Healthy	**29/120 (24.16%)**

Table 2: Site affected by fungal infections (N=120)

Site affected	**In number and percentage**
Sinonasal	81 (67.5%)
Only nasal	26 (21.66%)
Oral	7 (5.833%)
Eye	6 (5%)

Table 3: Multiple site affected by fungal infections (N=81)

Sinonasal sites	**In number and percentage**
Ethmoidal sinus	43 (53.08%)
Maxillary sinus	17 (20.9%)
Frontal sinus	5 (6.1%)
Nasal	16 (19.71%)

Table 4: Microscopic features of fungal infection

Microscopic features of fungal infection	**Number of cases**
Acute inflammation	**8**
Chronic inflammation	**112**
Granuloma	**25**
Vasculitis	**26**
Thrombosis	**14**
Angioinvasion	**13**
Perineural invasion	**10**
Bone invasion	**18**
Sporangia	**2**

Table 5: Types of fungal infections on histopathology (N=120)

Fungal infection	In number and percentage
Mucor mycosis	104 (86.6%)
Mixed	11(9.16%)
Aspergillus	2(1.66%)
Candida	3(2.5%)

Table 6: Types of fungal infections on culture (N=120)

Fungal infections	Type of fungal in number and percentage
Mucor mycosis	32 (26.6%)
Aspergillus	9 (7.5%)
Candida	4(3.33%)
Rhizopus	3(2.5%)
Other	6(5%)
No growth	66 (55%)

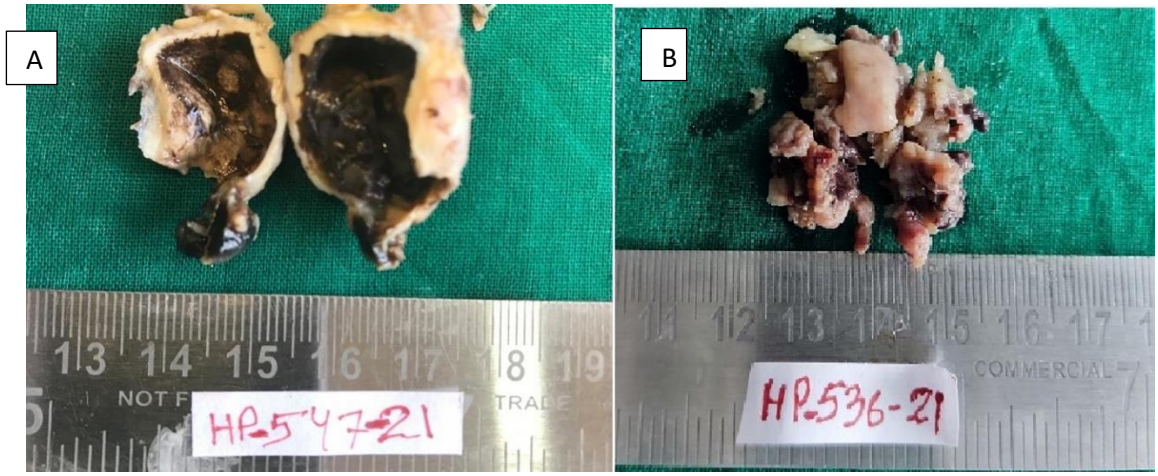

Fig1. Gross photograph A. Cut section of eyeball showing loss of architecture B. Biopsy tissues of Maxillary sinus.

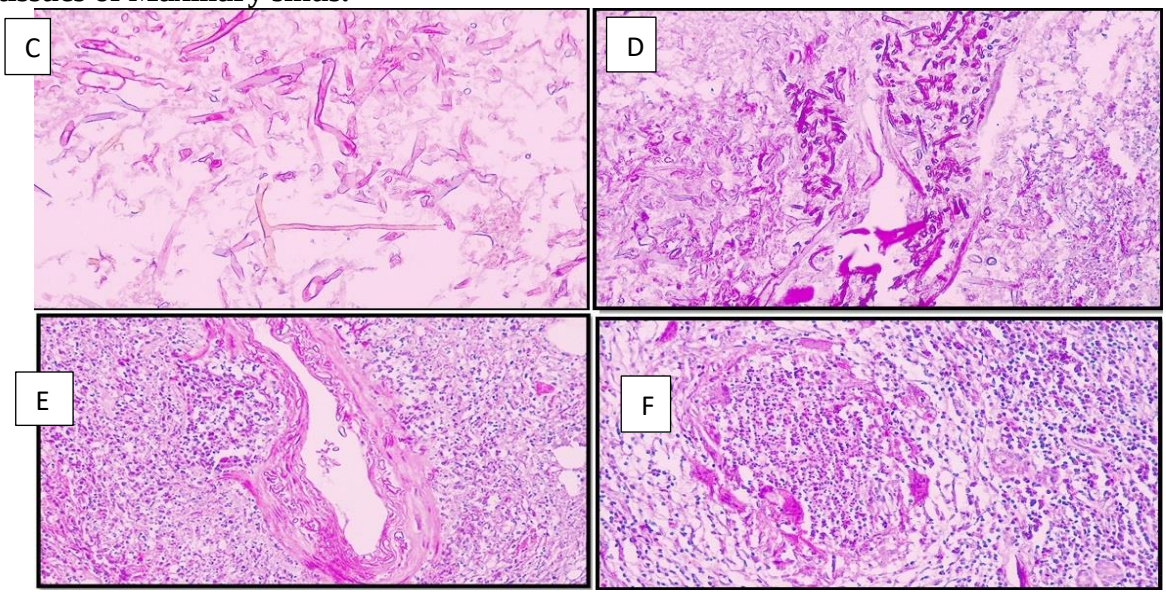

Fig2. Microphotograph of Mucormycosis C. (H&E X400). D (PAS X400), E. Microphotograph showing perivasculitis (H&E X400), F. Micrograph showing Granuloma (H&E X400).

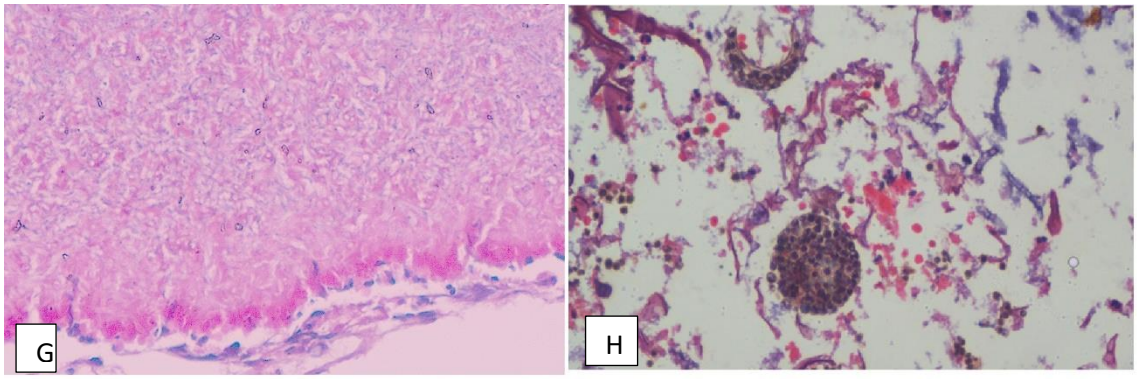

Fig3. G. Splendore hoeppli material. H.Microphotograph sporongiospores in mucormycosis (h&EX400)

Discussion

Since the first case of COVID-19 infection was detected in December 2019, there have been various changes in terms of its pathophysiology, diagnosis management and squealed and complications(1). Mucormycosis emerged as a life threatening complication of COVID-19 infection affecting sinonasal and orbital region.it typically affects immunocompromised individuals which includes DM,AIDS, hematological malignancies and organ transplantation (1). In developed countries it is less common and is seen in patients with hematological malignancies. In developing countries it is common in patients with uncontrolled DM and trauma(4,9,10). The mortalities rate of mucormycosis in patient with uncontrolled DM is 40 to 80%(8). In review by Garg et al DM and steroids play a major role(5). In india DM is the main factor for mucor mycosis(6,10). In the present study all the patients had history of steroids use and DM in the main co-morbid condition.

Rhinocerebral mucormycosis is the most common form of the disease. (8) it may present with headache, fever,painful eyes, nasal congestation and loss of vision(6). Mucormycosis enters through respiratory tract and couse remarkable affinity for arteries causing thrombi leading to necrosis of tissue (6,8). The affected patients showed overexpression of inflammatory cytokines which causes diffuses alveolar damage, impaired cell mediated immunity with decreased CD4+ T and CD8+T helper cells indicating susceptibility to fungal infections. (1,2,3,4). Ethmoidal sinus is the most commonly affected sinus followed by maxillary sinus(1). In our study ethmoidal is the commonly affected sinus.

Hallmark of mucormycosis is tissue necrosis (9,10). In study done by sravani et al necrosis with or without infarction was seen in all samples but angioinvasion in 83.33% of cases (8). In our study chronic inflammation was the common finding. Inflammation may be absent in immunosuppressed individuals (4).

	Present study	**Vaghasiya et al study**	**Singh et al study (N=76)**
Mucormycosis	104	23	-
Apergillosis	2	6	64
Candidiasis	3	3	-
Candida and aspergillosis	4	3	-
Aspergillous and mucormycosis	3	6	-
Alteriaria	1	-	4

In our study CT,MRI was done in all the cases to know bone and soft tisuue invasion. Bone involvement seen in 18(15%) cases in histopathology compared to (15/50) cases in the stud (11).
Sporangia and sporangiophore was seen in 3 cases. Brownish sporangiospore are suggestive of Rhizopus microspores (5), which is an unusual finding. Sporangia are almost never seen in tissue section in preCOVID19 studies (13).

Fungal infections	Type of fungal in number and percentage
Mucor mycosis	32 (26.6%)
Aspergillus	9 (7.5%)
Candida	4(3.33%)
Sporangiophore	2

The hyphae of mucormycosis are thin, nonseptate, ribbon shaped branching at 90 degrees. It is very commonly seen in environment(13). Even when fungal hyphae are seen in histopathological sections, fungal culture are only positive in 50% of the casesof the cases (9,10). It may be due to grinding or homogenization of tissue specimrn (9,10) or due to the presence of genera which require special culture conditions (9). When culture is negative, serological, immunohistochemical and molecular methods help in final diagnosis. (4) in india Rhizopus species is the most common fungus (4,10) compared to Europe where mucor is 27%. Aspergillous colonies show uniform, tubular, narrow, hyphae with septation and show regular progressive dipotamous branching at acute angles. Calcium oxalate crystals common in aspergillous colonies. In the present study 2 cases showed calcium oxalate crytals. In aspergillous culture yield is low(2). In study done by Singh et al aspergillosis is the common organism (12). Candida is normal flora of oral cavity, upper respiratory tract infection, gastro intestinal, and vagina. (13). In present study candida hyphae were seen in 4 number of cases embedded in necrotic material admixed with spores. Splender Hoople material was seen in one cases. In these cases culture was negative in 66 of cases. Culture may be negative due to extremely low concentration or deep fungal infection (3). The incidence of invasion fungal infection varies in between geographic region. In study ehite et al aspergillosis is the common organism (1,2) In Indian study candida infection is more common (2). The Chinese studies pathogen for fungal coinfection in severe COVID 19 patients aspergillosis and candida with mucor and crypto as infreguent pathogen (3). In present study mucor is the most common fungal pathogen similar to study done in Germany(4). Culture was negative in 55% cases in present study which may be due to limitation sampling in biopsy. The mortality rate of mucormycosis with comorbidities is 66% (4). With early medical and surgical management survival rate is now thought to be esceed 80% (6).

Conclusion:

Fungal infections are long term complication in patients withCOVID19 infection. DM and extensive use of steroids play major role in development of fungal infections. Biopsy remains the mainstream in diagnosis invasive fungal infection when culture is negative and it also helps in differentiating from contamination. It also helps in identify coexisting fungal infections.

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