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Original Article

Pathology Section

Utility of Histopathology, KOH Mount and Culture in the Diagnosis of Fungal Infections: A Cross-sectional Study

YASMEEN KHATIB[1], VATSSALA ARVINDANI[2], SNEHA JANJAL[3], JAYASHRI CHAUDHARI[4], PRAJAKTA GUPTE[5], ICCHHA REDKAR[6], SNEHA PATIL[7]

ABSTRACT

Introduction: Fungal infections are caused either by pathogenic fungi affecting the healthy population or by opportunistic fungi affecting the at-risk population. Patients may be asymptomatic, have mild infection or life threatening infection. Hence, a correct diagnosis is necessary for proper management. The rationale of the present study was to compare the utility of various methods used in the diagnosis of fungal infections.

Aim: The aim was to study the combined use of histopathology, Potassium Hydroxide (KOH) mount and culture in improving the diagnosis of fungal infections.

Materials and Methods: The present five years cross-sectional study was performed in the Department of Pathology of a tertiary healthcare centre, Mumbai, Maharashtra, India from January 2019 to December 2023. Sample size was 107 cases (clinically suspected cases of fungal infections which were sent for histopathology, KOH wet mount and culture). All relevant clinical details including patient's age, gender, associated comorbidity, site of infection, histopathological diagnosis and special stain results, KOH mount and fungal culture reports were recorded and analysed by IBM Statistical Package for the Social Sciences (SPSS) version 23.0 (Chi-square test and Fisher's-exact test).

Results: In the present study, the age range was from five years to 78 years of age with maximum cases in the range of 40 to 60 years 49 (46%). The male: female ratio was 1.49 : 1. There were 96/107 (89.7%) cases positive on histopathology for fungus. Of the 107 cases, 37 (34.6%) cases were superficial (skin and appendages) fungal infections, 6 (5.6%) cases were subcutaneous fungal infections and 64 (59.8%) cases were deep fungal infections involving sites like nose and paranasal sinuses, orbit, bones and joints and brain etc. There were 54 (50.5%) cases which showed positivity on KOH mount while 44 (41.1%) cases were positive for fungus on culture. *Mucorales* was the most common fungus detected on histopathology (49/96, 51%).

Conclusion: Histopathology is a valuable investigation which can differentiate contaminants and commensals from pathogenic fungi and it can provide a presumptive diagnosis based on which the treatment can be started. The specificity of culture is higher than that of KOH mount and species identification is possible however it takes longer time than histopathology and KOH mount. All the three modalities can be used as an adjunct to each other for better diagnosis and treatment.

Keywords: Fungal morphology, Inflammatory reaction, Potassium hydroxide, Subcutaneous infections

INTRODUCTION

Fungal infections are very common in tropical and subtropical countries like India [1]. Owing to the distinctive geographical features, hot and humid climate, socioeconomic status, presence of co-morbidities and immune profile, fungal infections are endemic in India [2]. According to a study, fatalities caused due to fungal infections are approximately 1.5 million deaths per year worldwide [3]. With the advent of modern medical interventional procedures, generous usage of broad spectrum antibiotics and immunosupression due to any cause (like neoplasms, Acquired Immuno Deficiency Syndrome (AIDS), steroid therapy, transplants associated infections and autoimmune disorders) there has been considerable increase in morbidity and mortality due to fungal infections [4,5].

Fungal infections are caused either by pathogenic fungi affecting the healthy population or by opportunistic fungi affecting the immunocompromised population [6,7]. Depending upon the site and extent of the fungal infections, they can be broadly classified into superficial, cutaneous, subcutaneous and systemic mycoses [6]. The clinical presentation can vary at times and can mimic other bacterial infections or even malignancy in invasive type of fungal infections. Hence, a correct diagnosis is necessary for proper management especially in immunocompromised patients.

Currently fungal infections are diagnosed by different methods including the clinical features, KOH mount, histopathology and culture using Sabouraud Dextrose Agar (SDA). The less commonly used methods are immunoassay for antigen detection, direct fluorescence for antibody detection etc., [6]. Although KOH mount and culture have their own advantages, the major disadvantage of both methods is that they cannot differentiate between contamination and actual infection as many fungi can be commensals in healthy individuals.

Histopathology is a cheaper and less time consuming procedure as compared to other methods and can also be used for identification of uncultivable fungal infections like *Lacazia loboi*. It helps to identify different morphological forms of fungi like yeast form, hyphae, budding forms and pseudohyphae. The objectives of the current study were to study the types of fungi on histopathology and their inflammatory reactions and to compare the histopathology and microbiological findings in fungal infections.

MATERIALS AND METHODS

The present five years cross-sectional study was conducted in the Pathology department of a tertiary health care centre of Mumbai, Maharashtra, India from January 2019 to December 2023 after Instituitional Ethical approval (IEC no: TP233206162). All clinically suspected cases of fungal infections from all age group whose samples were sent to both Pathology and Microbiology departments were included in the study.

Inclusion and Exclusion criteria: Consecutive samples which fulfilled inclusion criteria were collected over a period of five years and a sample size of 107 cases was obtained (clinically suspected cases of fungal infections from various departments like otorhinolaryngology, dermatology, surgery and orthopaedics were included in the study whose samples were sent to both Pathology and Microbiology Departments). Those samples which were sent to only one department either pathology or microbiology were excluded.

Study Procedure

All relevant data including patient's age, gender, site of infection, associated co-morbidity and microbiological reports (KOH mount and fungal culture) was recorded. The histopathological examination of Haematoxylin and Eosin (H&E) stained sections as well as Grocott Methanamine Silver (GMS) or Periodic Acid Schiff (PAS) stained sections was performed. Morphology of fungus (predominant form, branching, budding, pigment) and associated tissue reactions (inflammation, granuloma, necrosis, invasion) were studied and these findings were compared with microbiological reports.

STATISTICAL ANALYSIS

The data was entered in Microsoft Excel sheet and analysed by IBM SPSS Version 23.0 (Chi-square test and Fisher's-exact test).

RESULTS

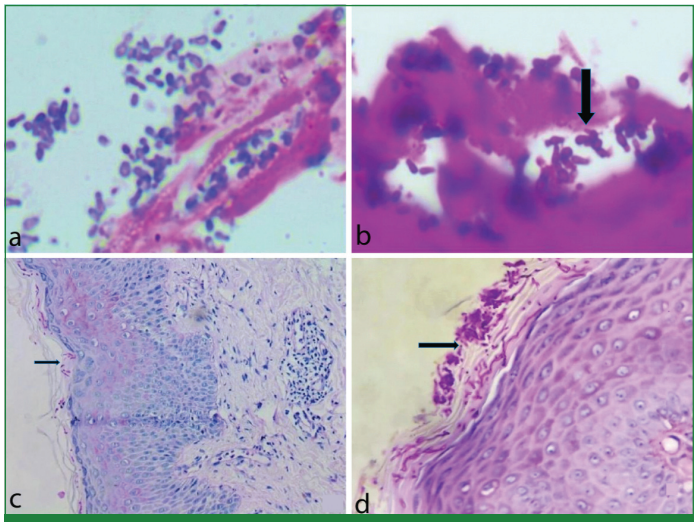
A total of 107 samples from the patients with suspected fungal infection were sent for histopathology, KOH mount and culture. The youngest patient was five years and the oldest was 78-year-old (mean age: 43.5 years). Maximum number of patients were between 40 to 60 years (n=49, 46%). Out of 107 cases, 64 cases were males and 43 cases were females, male to female ratio being, 1.49:1.

Out of the total 107 cases, 41 cases had co-morbidities and the remaining 66 cases had no associated co-morbidities. The commonest co-morbidity was Coronavirus Disease-2019 (COVID-19) infection in 26 cases (24.3%) followed by Diabetes Mellitus (DM) in 21 cases (19.6%). Other associated co-morbidities were hypertension (n=10), tuberculosis (n=3), hyperthyroidism (n=2) and trauma (n=1). Some patients had mutilple co-morbidities.

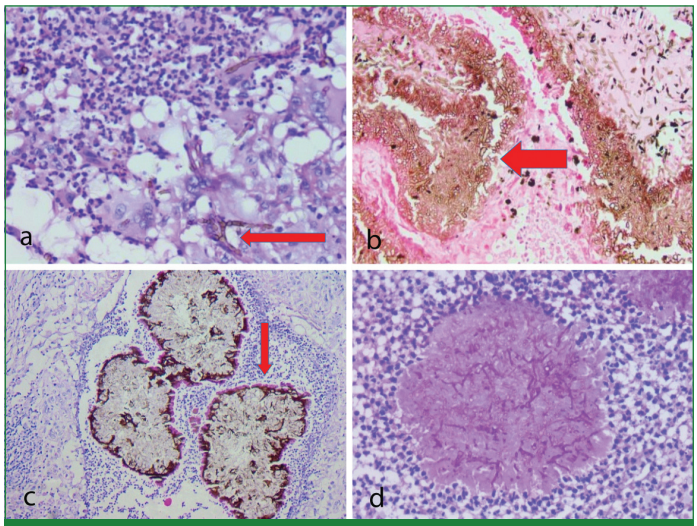
Out of the 107 cases, 96 cases (89.7%) were positive for fungus on histopathology. [Table/Fig-1] shows detailed classification of fungal infections based on the site of involvement and morphology of the fungi with their numbers and percentages. Out of these 96 cases, 30 were negative on H&E and had to be confirmed by PAS/GMS.A total of 37 (34.6%) cases had superficial fungal infection out of which maximum cases were of Onychomycosis or Dermatophytes (n=13). Dermatophytes fungal infections showed slender, uniform hyphae. Out of the six (5.6%) cases of subcutaneous fungal infection, three cases were of Phaeohyphomycosis. A total of 64 (59.8%) cases out of 107 cases were diagnosed as deep fungal infections involving sites like nose and paranasal sinuses, orbit, bones and joints and brain etc. Forty-nine cases (76.6%) out of these 64 cases were diagnosed as Mucormycosis (the predominant form was 'broad aseptate hyphae'). Three cases showed 'thin septate hyphae with acute angle branching' and were diagnosed as Aspergillus. Two cases showed mixed infection (Aspergillus and Mucormycosis). [Table/Fig-2-4], show histopathological images of superficial, subcutaneous and deep fungal infections, respectively. Out of 96 cases positive for fungus on histopathology, 73 cases showed inflammation. Forty out of 73 cases, showed acute inflammation, followed by mixed inflammation (n=21) and chronic inflammation (n=12). No inflammation was seen in 23 cases which were all of superficial fungal infection. Out of 96 cases, nine cases showed giant cell granulomas, 36 cases showed necrosis and 37 cases showed invasion. [Table/Fig-5] show different tissue reactions to fungal infections.

Type	Histopathological diagnosis	n (%)
Superficial	*Candida*	10 (9.3)
	Onychomycosis (Dermatophytes)	13 (12.1)
	Ptyriasis versicolor	1 (0.9)
	Superficialfungal infection of skin (Dermatophytes)	10 (9.3)
	No fungus on HP	3 (2.8)
Subcutaneous	Phaeohyphomycosis	3 (2.8)
	Mycetoma	1 (0.9)
	No fungus	2 (1.9)
Deep	Mucormycosis	49 (45.8)
	Aspergillosis	3 (2.8)
	Mixed (Aspergillosis and Mucormycosis)	2 (1.9)
	Inflammatory pathology of fungal aetiology	4 (3.7)
	No fungus on HP	6 (5.6)
Total		107 (100)

[Table/Fig-1]: Histopathological diagnosis in suspected cases of fungal infections (N=107).



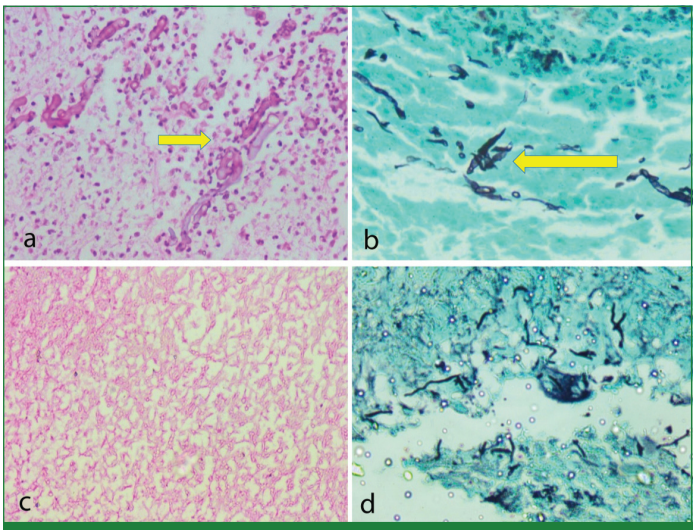
[Table/Fig-2]: Superficial fungal infection: a) Candidiasis of nail plate, (H&E, 40 x); b) Spores of *Candida* of nail plate, highlighted by thick short black arrow, (PAS, 40x); c) Dermatophytes in stratum corneum, highlighted by short thin long arrow, (PAS, 40x); d) Fungal spores and hyphae, in Pityriasis versicolour, highlighted by thin black arrow, (PAS, 40x).



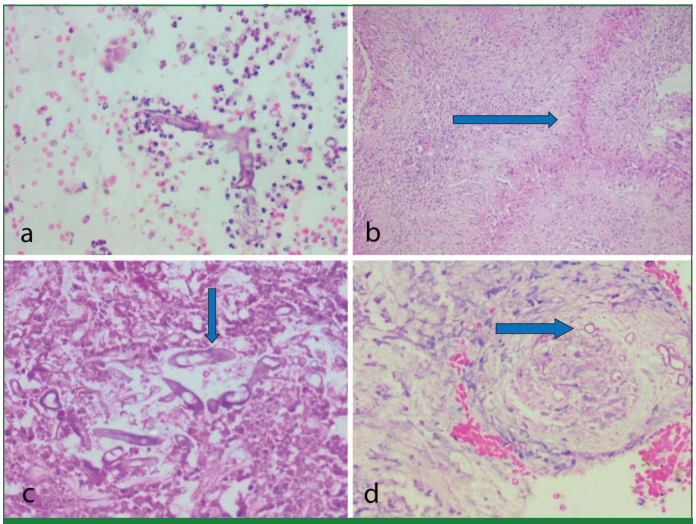
[Table/Fig-3]: Subcutaneous fungal infection: a) Suppurative granulomatous inflammation with pigmented hyphae, Phaeohyphomycosis, highlighted by thin long red arrow, (H&E, 40x); b) Pigmented hyphae, Phaeohyphomycosis, short thick red arrow, (H&E, 40x); c) Sulphur granules and inflammatory infiltrate in Mycetoma, highlighted by thin short red arrow, (H&E, 40x); d) Highlighted fungal hyphae (PAS, 40x).

Fifty four out of 107 cases (50.5%) were positive on KOH mount and 53 cases (49.5%) were negative. Fifty two cases out of the 54 showed hyphae and two cases showed yeast forms. A total of 44 cases (41.1%) were positive for fungus on culture. [Table/Fig-6]

shows distribution of cases based on their detection on KOH mount, culture and histopathology. On fungal cultures, maximum cases showed *Mucorales* (49/96, 51%) followed by *Candida* species and *Aspergillus* species. *Candida* species isolated were *Candida albicans*, *Candida tropicalis* and non-albicans *Candida*. Aspergillus species isolated on culture included *Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus*. Two cases showed mixed fungal growth (Mucormycoses and Aspergillus), one case showed Trichophyton species and one case showed growth of Eumycetoma species on culture and one case was broadly classified as *Zygomycetes*.



[Table/Fig-4]: Deep fungal infection: a) Mucor, broad aseptate fungal hyphae, highlighted by short yellow arrow, (H&E, 40x); b) Mucor, broad aseptate fungal hyphae, highlighted by long yellow arrow, (GMS, 40x); c) Aspergillus, thin septate fungal hyphae with acute branching, (H&E,40x); d) Aspergillus, thin septate fungal hyphae, (GMS, 40x).



[Table/Fig-5]: Histopathological reactions in cases of fungal infections: a) Fungal elements surrounded by acute inflammatory infiltrate, (H&E, 40x); b) Palisading necrosis and granuloma on H&E, highlighted by thin long blue arrow, (H&E, 40x); c) Broad aseptate fungal hyphae and necrosis in Mucormycosis, highlighted by thin short blue arrow (H&E, 40x); d) Angioinvasion, fungal filaments invading the vessel, highlighted by thick short blue arrow, (H&E, 40x).

Histopathology	KOH mount	Culture	n (%)	
Positive	Positive	Positive	32	29.9
		Negative	15	14
	Negative	Positive	6	5.6
		Negative	43	40.2
Negative	Positive	Positive	5	4.7
		Negative	2	1.9
	Negative	Positive	1	0.9
		Negative	3	2.8
Total Samples			107	100

[Table/Fig-6]: Summary of results on Histopathology, KOH mount and culture findings.

Out of the 54 (50.5%) cases positive on KOH mount, 47 cases were positive on histopathology while seven cases were negative. A total of 53 cases were negative on KOH mount out of which only four cases were negative on histopathology while the remaining 49 cases were positive. On culture, a total of 44 (41.1%) cases were positive out of which 38 cases were positive on histopathology while remaining six cases were negative. A total of 63 cases were negative on culture out of which only five cases were negative on histopathology while the remaining 58 cases were positive. The percent agreement between KOH mount and Histopathology is 47.7% (p-value of 0.356) and that between fungal culture and histopathology is 40.2% (p-value of 0.339) as shown in the [Table/Fig-7]. As shown in [Table/Fig-7] the kappa statistic (low agreement between the test, statistically not significant, Calculated using Wilson efficient score method, corrected for continuity).

Tests		Histopathology for fungal infection		Percentage agreement (95% confidence interval*)	Kappa statistic (95% confidence interval)
		Positive	Negative		
KOH mount	Test positive	47	7	47.6 (38-57.5)	-0.055 (-0.163-0.053) p=0.356
	Test negative	49	4		
Culture for fungal infection	Test positive	38	6	40.2 (31.0-50.1)	-0.048 (-0.154-0.058) p=0.339
	Test negative	58	5		
Total		96	11	-	-

[Table/Fig-7]: Agreement between Histopathology, KOH mount and culture findings.
Calculated using wilson efficient method, corrected for continuity

Here, histopathology was considered as the gold standard investigation for the diagnosis of fungal infection. There were 96/107 cases (89.7%) positive on histopathology. As shown in [Table/Fig-8], the sensitivity and specificity for KOH mount alone was 48.9% and 36.3%, respectively. The sensitivity and specificity for fungal culture alone was 39.6% and 45.5%, respectively. (Calculated using Wilson efficient score method, corrected for continuity). There were 53 cases positive by microbiology (either KOH mount or Culture) out of 96 cases positive for fungus. Hence, the combined sensitivity of microbiological investigation (i.e., KOH mount and fungal culture combined) was 55.2%.

Test	Test result	Final diagnosis		Sensitivity (%) (95% confidence interval*)	Specificity (%) (95% confidence interval*)
		Fungal aetiology	Non-fungal aetiology		
KOH mount	Positive	47 (43.9)	7 (6.5)	48.9 (38.7-59.3)	36.3 (12.4-68.4)
	Negative	49 (45.8)	4 (3.7)		
Culture	Raised	38 (35.5)	6 (5.6)	39.6 (29.9-50.1)	45.5 (18.1-71.9)
	Normal	58 (54.2)	5 (4.7)		
	Negative	43 (40.2)	3 (2.8)		
Total		96	11	-	-

[Table/Fig-8]: Sensitivity and specificity of the investigations for diagnosis of fungal infection.
Calculated using Wilson efficient method, corrected for continuity

DISCUSSION

The age range in the present study population was from five years to 78 years. The maximum numbers of cases were seen in the age group between 40-60 years which was similar to study by Chemudugunta R et al., where maximum number of cases, (49.92%) were between 41-60 years [8]. The infections were more in higher age group due to the presence of co-morbidities like DM or other immune-compromised states [7]. The mean age in present study was 43.5 years which is in concordance with the study conducted by Mohanty A et al., (40 years) [9]. The wide age distribution suggests that the fungal infections can affect people of all age groups.

Like the present study, male preponderance was also observed in other studies like Mohanty A et al., Sundaram C et al., and Singh AK et al., with male to female ratio being 1:1.5, 1:1.8 and 1:1.19, respectively [9-11].

A total of 41 (38.3%) out of 107 cases had associated co-morbidities of which the most common co-morbidity was COVID-19 infection followed by DM. This was because the study was conducted during the period of COVID-19 pandemic. In the study conducted by Sundaram C et al., DM was the most common co-morbidity [10]. History of transplant was the most common co-morbidity in the study conducted by Santiago TM et al., as it was a tertiary care institute with the facility of transplantation and their study population being predominantly transplant recipients [12]. Subhasini R et al., included cases with subcutaneous fungal infection and history of trauma was the only risk factor associated in their study [13].

In the present study, the maximum number of cases were from the nose and paranasal sinuses (48/107) which was similar to that reported by Rani Singh G et al., (n=74/77) and Mohanty A et al., (n=122/186) [9,14]. In 30 out of 96 cases, fungus was highlighted by PAS/GMS stain. Baxi SN et al., showed a good fungal detection rate of (70.75%) in their study while other studies like Singh AK et al., (18.4%) and Santiago TM et al., (69.7%) have shown low detection rates on histopathology [11,12,15].

This was probably due to sampling of tissue from the surrounding inflamed area (non-representative) or there were very few fungal elements which could not be visualised on histopathology [11]. Hence, adequate and representative sample is necessary for detection of fungus on histopathology [16]. Guarner J et al., have recommended that PAS/GMS staining should be done in all cases of suspected fungal infection as they stain the fungal wall and clearly delineate fungus on histopathology [7]. Baxi SN et al., found in their study that histopathology (79.78%) had a higher sensitivity than KOH mount (58.51%) and culture (35.11%) and can be relied upon by clinicians for initiating prompt treatment [15].

In the present study, 79.8% cases showed fungal hyphae on histopathology, 13.6% showed yeast form of fungus and 6.4% showed hyphae as well as yeast forms. The studies conducted by Subhasini R et al., and Sunagawa K et al., also reported hyphae as the predominant fungal morphology in majority of cases 81.6%and 70.7% cases, respectively [13,17]. Tissue reactions to fungus depend on its type, virulence and on the host immune response. In case of immunosuppressed patients, there may be no inflammation but fungal invasion of blood vessels can cause thrombosis and necrosis [7]. There is usually minimal or no inflammation in cases of superficial fungal infections [18]. In studies conducted by Rani Singh G et al., Sridevi M et al., and Shobana B et al., the number of cases showing inflammation were 60 (77.9%),18 (38.9%) and 24 (60%), respectively [14,18,19]. In the present study, the percentage of cases that showed granulomatous reaction were 8.4% which is in concordance with the study conducted by Shobana B et al., (12.5%) [19].

Presence of tissue reaction can differentiate between infection and contamination. It is seen only on histopathology and not on KOH mount or culture [7,19]. Detection of vascular involvement and necrosis indicates invasive fungal infection which needs immediate treatment as it can cause morbidity and mortality [7]. In the study conducted by Santiago TM et al., only one case showed necrosis [12]. This was because they included only the cutaneous fungal infections. In the present study, 38.5% of total cases and 63.8% of deep fungal infection cases showed invasion which is in concordance with the study done by Sundaram C et al., (30.8%) [10].

Out of the 107, 50.5% cases were positive on KOH mount. This value is in concordance with that of the study conducted by Baxi SN et al., and Shenoy MM et al., where 51.8% and 53% cases were positive on KOH mount, respectively [15,20]. However, KOH positivity rates in studies by Mohanty A et al., and Singh AK et al., were only 17.2 % and 22.4%, respectively which were less than that of present study [9,11]. They attributed this to incorrect sampling of the tissue or to fungal elements being sparse and entrapped in mucus [11]. As shown in [Table/fig-9] comparison between different studies based on the number and percentage of positive cases on histopathology, KOH mount and culture [11,15,20].

Test positive {n (%)}	Singh AK et al., [11] 2017	Baxi SN et al., [15] 2022	Shenoy MM et al., [20] 2008	Present study 2023
Histopathology	14 (18.4%)	75 (70.75%)	76 (75%)	96 (89.7%)
KOH mount	17 (22.4%)	55 (51.88%)	54 (53%)	54 (50.5%)
Culture	21 (27.6%)	33 (31.13%)	35 (35%)	44 (41.1%)

[Table/Fig-9]: Comparison of number and percentage of cases positive for fungus on Histopathology, KOH mount and Culture among different studies [11,15,20].

In the present study, 44 cases (41.1%) were positive for fungus on culture. Cases positive on culture in studies conducted by Sundaram C et al., Singh AK et al., and Baxi SN et al., were 31%, 27.6% and 31.13%, respectively [10,11,15]. These numbers are slightly less than that of present study. Lower number of culture positive cases can be attributed to the fact that: 1) the sensitivity to identify onychomycosis on culture can be as low as 25%; 2) special culture conditions might be required for growth of certain fungi e.g., *Mucorales*; 3) destruction of delicate fungal hyphae during processing or due to prior antifungal administration; 4) necrotic tissue with few or no fungal elements; 5) entrapped fungal hyphae in the mucus [11,15,20].

In the present study, *Mucorales* were the most common fungi grown on culture which is similar to the findings of Baxi SN et al., [15]. This may be due to the fact that both these studies were conducted during the COVID-19 pandemic. However, Aspergillus was the most common fungus in the studies by Sundaram C et al., and Singh AK et al., [10,11]. In the present study, 15.8% cases had discordance between the fungus diagnosed on histopathology and that grown on culture. The findings were comparable to those of Baxi SN et al., (14% discordance) and Santiago TM et al., (24.2% discordance) [12,15].

There were two cases in the present study where culture yielded Aspergillus but Mucormycosis was reported on histopathology. The histopathology slides of these cases were reviewed but the fungus did not show classical morphology of Aspergillus. This could have been due to swelling and distortion of hyphae within extensively necrotic areas which led to misdiagnosis of *Mucorales*. Also, septations and branching is sometimes not appreciated in folded, crinkled and fragmented hyphae [15]. There were three other cases in the present study where Mucormycosis was diagnosed on histopathology but *Candida* was grown on culture. This could be because of either sporulation failure or sparse fungal elements entrapped in the mucus preventing their contact with the culture media [11,14]. Growth of *Candida* on culture highlights its inability to differentiate between contamination, commensal and infection [18]. Culture may grow commensals which has no clinical implication [21].

Sensitivity of KOH mount in present study was 48.9%. In studies conducted by Mohanty A et al., Baxi SN et al., Shenoy MM et al., and Roy P et al., the sensitivity was 64%, 58.51%, 64% and 90.63%, respectively [9,15,20,22]. The sensitivity for culture in present study was 39.6% which was comparable with the studies conducted by Baxi SN et al., and Shenoy MM et al., where the sensitivity for culture was 35.11% and 42%, respectively [15,20].

Low sensitivity of microbiological investigations can be attributed to the following: 1) tissue samples sent to microbiology and pathology departments were sampled from two different areas; 2) altered fungal morphology during processing; 3) poor growth capacity of *Mucorales* in ordinary culture media; 4) Highly necrotic tissue having few to none viable organisms [15,22].

Limitation(s)

The present study was conducted during COVID-19 pandemic, so the present study showed high number of cases of sino-nasal mucormycosis which is known to be associated with COVID 19 infection. Also, sample size was limited since the cases which were sent to both Pathology and Microbiology department for culture, KOH mount and histopathology were only included in the study.

CONCLUSION(S)

Histopathology is a valuable investigation in detecting fungal infection as it also shows the tissue reactions and presence and absence of invasion. Also, contaminants and commensals can be differentiated from pathogenic fungi. Histopathology, can provide a presumptive diagnosis based on which the treatment can be started. KOH mount is a rapid and cost-effective method for immediate presumptive diagnosis of fungus but cannot differentiate between infection and contamination and has low specificity. The specificity of culture is higher than that of KOH mount and species identification is possible however the results take longer time than histopathology and KOH mount. All the three diagnostic modalities have their advantages and disadvantages and should be used as an adjunct to each other for better diagnosis and treatment.

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PARTICULARS OF CONTRIBUTORS:

1. Additional Professor, Department of Pathology, H.B.T Medical College and Dr. R.N.Cooper Hospital, Mumbai, Maharashtra, India.
2. Ex-Resident Postgraduate, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.
3. Assistant Professor, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.
4. Additional Professor, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.
5. Assistant Professor, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.
6. Laboratory Technician, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.
7. Laboratory Technician, Department of Pathology, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Sneha Janjal,
Assistant Professor, Department of Pathology, Third Floor, H.B.T Medical College and Dr. R.N. Cooper Hospital, Mumbai-400056, Maharashtra, India.
E-mail: drsnehajanjal@gmail.com

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