Original Article

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Microbiology Section

Revisiting the Utility of Histopathological Examination of Biopsy: A Necessity in Microbiology

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ABSTRACT

Introduction: Culture is the gold standard, while potassium hydroxide mount is simplest technique used for diagnosis of fungal pathogens. Histopathological examination is the only definitive means to identify certain uncultivable fungi.

Aim: To analyse role of histopathological examination and potassium hydroxide (KOH) mount for diagnosing fungal infections by correlating them with culture.

Materials and Methods: In this nine year retrospective study, all biopsy specimens submitted for microbiological examination were included. Histopathological examination of biopsies of cases with positive microbiological findings on either KOH mount or culture was carried out. Any discrepancy between histopathology interpretation and microbiology KOH or culture results, taking culture as the gold standard, were noted.

Statistical Analysis: Open Epi software was used for statistical analysis. Comparisons between groups were made by using the chi-square test. A p-value < 0.05 was considered statistically significant. Cohen's Kappa coefficient (κ) was calculated as a measure of agreement between different variables.

Results: Concurrent pathology specimen could be obtained in 70 samples positive for fungal elements in either KOH or culture. Thirty-two cases were positive for fungi in culture, of which 16 were correctly identified by histopathological examination. Histopathological examination was strongly associated with culture result. KOH mount was in good agreement with positive culture result for yeast. Eleven culture negative but KOH and histopathology positive cases included seven samples with hyphae suggestive of zygomycosis, and two cases of rhinosporidiosis. Allergic mucin was strongly associated with *Aspergillus* species. KOH mount and detection of allergic mucin on histopathological examination were found to be excellent complementary tools for diagnosing *Aspergillus* species. Necrosis was highly specific for fungal growth in culture and had good positive predictive value.

Conclusion: We advocate using histopathology, culture and KOH examination in an integral manner to avoid potential lapses in patient management.

Keywords: Culture, Fungal infections, Potassium hydroxide mount

INTRODUCTION

The occurrence of fungal infections is rising due to growth in the susceptible population and utilization of treatment modalities that empower these patients to survive for a longer time [1]. Fungal culture is deemed the gold standard for speciation of most of the fungal pathogens [1,2]. Histopathology is a rapid and cost effective method of providing a presumptive diagnosis of an invasive fungal infection [1]. Histopathological examination can offer expeditious interim identification of fungal pathogens and continues to be the only definitive means to identify certain uncultivable fungi like *Rhinosporidium seeberi* and *Lacazia loboi* [3]. Direct microscopic examination of the specimen is the simplest and the most cost effective technique used for the diagnosis of fungal infections. The microscopic examination is usually done by potassium hydroxide (KOH) mount [4]. A number of studies have established improved fungal detection by histopathological examination in a few conditions, but none to our knowledge have performed a comprehensive assessment of the diagnostic utility of both histopathological examination and KOH mount in biopsies obtained for diagnosis of fungal pathogens [5-8]. Hence, this study attempts to analyse the role of histopathological examination and KOH mount for diagnosis of fungal infections by correlating results of histopathological examination and KOH mount with concurrent fungal culture outcomes.

MATERIALS AND METHODS

This was a nine year retrospective study for the period January 2007 to December 2015. All specimens obtained under the category of biopsy in Mycology Division of Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India, were included in the study. For this type of study formal consent is not required. Specimens obtained from autopsy cases were excluded. Histopathological examination of biopsies of the cases with positive microbiological findings on either KOH mount or culture was carried out. Of the concomitant study subjects, those with known documented prior fungal diagnosis or those with a reported clinical history of a specific fungal infection were excluded to avoid any potential bias in the histopathological diagnosis. Any discrepancy between histopathology interpretation and microbiology KOH or culture results (culture was established as the "gold standard" for this study) based on standard identification criteria, were noted [9].

STATISTICAL ANALYSIS

Data entered in MS Excel were analysed with Open Epi software Version 3.01. Frequency distributions were obtained and percentages were calculated. Comparisons between groups were made by the chi-square test. A p-value < 0.05 was considered statistically significant. Cohen's Kappa coefficient (κ) was calculated as a measure of agreement between different variables.

RESULTS

A total of 348 biopsy samples were received in the Mycology laboratory during the study duration. Around 45% (158) tested positive for fungal elements in at least KOH or culture, of which 70 had a concurrent pathology specimen. Thirty-two samples

were positive for fungi in culture and 38 were negative. Among the 32 culture positive cases, 16 (50.00%) were correctly identified by histopathological examination. The findings of the culture positive cases on KOH mount and histopathological examination are depicted in [Table/Fig-1]. Histopathologic examination was strongly associated with culture results with p-value of 0.0055. Histopathology showed fair agreement with culture as indicated by Cohen's kappa coefficient of 0.2953. Accuracy as per the Rand index and specificity of histopathologic examination were 68.57% and 78.95% respectively.

Fungal pathogen isolated in culture	Total No.	No. (%) positive for fungal elements on KOH mount	Findings on histopathological examination		
			No. (%) positive for fungal elements	No. (%) positive for allergic mucin	No. (%) positive for necrosis
Aspergillus spp.	19	17 (89.47%)	8 (42.11%)	13(68.42%)	2 (10.53%)
Rhizopus spp.	4	4 (100%)	4 (100%)	0	2 (50%)
Candida spp.	4	4 (100%)	2 (50%)	0	2 (50%)
Cunnighamella spp.	1	1 (100%)	1 (100%)	0	1 (100%)
Fusarium spp.	2	2 (100%)	0	0	0
Mucor spp.	1	1 (100%)	1 (100%)	0	0
Alternaria spp.	1	0	0	0	0
Total No. (%) (p-value)	32	29 (90.63%) (0.1413)	16 (50%) (0.0055)	13 (40.63%) (0.000006680)	7 (21.88%) (0.5853)

[Table/Fig-1]: Findings of the culture positive cases on potassium hydroxide (KOH) mount and histopathological examination.

KOH mount was found to have overall sensitivity of 90.63%. Accuracy in terms of Rand Index was 44.29%. KOH was 100% sensitive and had 100% negative predictive values for yeast infection, although histopathological examination could detect only 50% of them. KOH mount was in good agreement with positive culture result for yeast as demonstrated by Cohen's kappa coefficient of 0.63. In case of moulds, the sensitivity of KOH was 89.29%. Nonetheless, KOH could detect fungal infection in the two *Fusarium* spp. cases though histopathological examination was negative in both.

Histopathological examination correlated with 16 culture positive cases belonging to six fungal genera: *Rhizopus* spp. (n=4), *Aspergillus* spp. (n=8), *Candida* spp. (n=2), *Cunnighamella* spp. (n=1), *Mucor* spp. (n=1). Sixteen cases were positive for fungal growth on culture, but had negative histopathology findings. These included *Alternaria* spp. (n=1), *Aspergillus* spp. (n=11), *Candida* spp. (n=2), *Fusarium* spp. (n=2). Eleven (28.95%) out of the 38 culture negative but KOH positive cases were strongly positive for fungi in histopathology. The 11 cases included seven (63.64%) samples with broad aseptate hyphae suggestive of zygomycosis on histopathology and KOH mount, as well as two cases of rhinosporidiosis. Rest of the two cases showed non-specific hyphal forms in histopathology. The samples containing *Rhinosporidium seeberi* though negative on culture, on KOH mount displayed multiple sporangia filled with endospores in various sizes and stages of development morphologically resembling sporangia of *Rhinosporidium seeberi*. On histopathology, haematoxylin and eosin stained sections revealed globular cysts representing sporangia in different stages of development surrounded by a mixed inflammatory infiltrate. Rest of the 27 KOH positive, culture negative cases were negative in histopathological examination as well.

Out of the 19 *Aspergillus* spp. isolated in the present study, 13 (68.42%) were positive for allergic mucin on histopathology, while hyphae could be seen in only eight (42.11%) cases. Allergic mucin was strongly associated with *Aspergillus* spp. grown in culture with a p-value of 0.00005, specificity and positive predictive value of 100%, and Cohen's kappa coefficient of 0.63 indicative of good agreement with culture result. On KOH mount, hyphae were seen in 17 (89.47%) cases of the 19 *Aspergillus* species. Out of these 17 KOH positive cases, six and 12 cases were positive for hyphae and allergic mucin respectively on histopathology. The two KOH negative cases were positive for allergic mucin on histopathological examination. Hence, KOH mount and detection of allergic mucin on histopathological examination collectively serve as excellent complementary tools for diagnosing *Aspergillus* species.

Necrosis was seen in histopathological examination in eight cases, of which seven were positive for fungal growth in culture. Necrosis was highly specific (specificity= 96.15%) for fungal growth in culture and also had good positive predictive value of 87.50%. Necrosis showed moderate agreement with culture in terms of Cohen's kappa coefficient (κ=0.5343).

In case of the six zygomyecetes (*Rhizopus* spp., *Mucor* spp. and *Cunnighamella* spp.) isolated, both histopathology and KOH were positive in all of them. The only *Mucor* spp. isolated in the present study was positive for fungal hyphae in both KOH mount and histopathology.

DISCUSSION

In this retrospective study, we found that identification of fungi in histopathological specimens showed strong association with culture results and manifested an overall accuracy of 68.57% considering culture as gold standard. This is lesser than that reported by Sangoi AR et al., (79%) [5]. The discordance between histopathology and culture reports with negative histopathological findings but positive cultures can be attributed to the following: (1) modification of fungal characteristics because of antifungal agents or host immune responses; (2) inexperience of the pathologist in fungal identification; (3) alteration of fungal morphology caused by fragmentation of the fungal elements during tissue processing; (4) inflammatory response of the host concealing fungal morphology; (5) fungus growing in the cultures was actually a colonizer and not a pathogen or may be a laboratory contaminant; (6) tissue samples sent to microbiology and pathology laboratories were sampled from two different areas [1,10].

It must be noted that the actual pathogens invade tissues as seen in histopathology while colonisers and contaminants do not do so. For instance, *Candida albicans* colonizes the human oropharynx and vagina, and a small number of viable organisms can be cultured from these surfaces. In these circumstances, it is essential to know the pathology that was encountered in the specimen and the location and number of colonies found in the plates. When only one or two colonies are seen at the area of inoculation of the plate and there is negligible pathology in the tissue sections, it is logical to think that the fungus present in the culture may be a colonizer. When only one or two colonies are observed away from the area of primary inoculation of the plate and there is minimum pathology in the tissue sections, it is reasonable to think that there was contamination of the fungal culture [1].

Direct KOH mount is an uncomplicated, rapid economical point of care test requiring minimum technical assistance [11]. The sensitivity of KOH mount was observed to be 90.63% in the present study. This is more than that reported by Dass SM et al., who found the sensitivity of KOH mount microscopy to be 83.02% [4]. We also found KOH mount to be valuable for detection of yeasts and different moulds.

Approximately 30% (11) of the culture negative but KOH positive cases (N=38) were strongly positive for fungal elements on histopathology. Most (7, 63.64%) of these cases contained broad aseptate hyphae indicative of zygomycosis on histopathology as well as KOH mount. *Muorales* have a poor growth capacity in ordinary fungal culture media; in such cases histopathological examination may be the only proof of a fungal infection. Possible explanations for this phenomenon are: (1) homogenization of

tissue. For example, if an original specimen containing molds belonging to *Mucorales* genera is homogenized too aggressively, the hyphae may be destroyed and culture may not grow. The hyphae of zygomyecetes, being long and wide, are extremely prone to destruction [9]; (2) non viable fungal organisms in the specimen, as encountered in immunocompetent hosts with a chronic walled off solitary nodule caused by an endemic mycosis, such as cryptococcosis or histoplasmosis, where yeasts may be observed in tissue sections but culture may be negative as the yeasts are non-viable; (3) collecting samples from two different areas; and (4) the use of preservative containing local anaesthesia before specimen collection [1,10,12]. In the matter of slow growing fungal pathogens like *Histoplasma* spp. and *Paracoccidiodes* spp., histopathological diagnosis may be ready earlier than culture results thereby providing enough information for clinicians to start treatment [1].

The culture negative but KOH positive cases also included two cases of Rhinosporidiosis. Though Rhinosporidiosis is distributed universally, it is endemic in south Asia, particularly southern India and Sri Lanka [13-16]. The two cases of Rhinosporidiosis detected in this study were negative on culture but positive on KOH mount and histopathology for sporangia of *Rhinosporidium seeberi*, in accordance with other studies [13-17]. There are no reports of successful *Rhinosporidium seeberi* in vitro culture and sustenance in subculture [17,18]. Nevertheless, histopathology with the identification of the pathogen in its diverse stages instead of stromal and cellular responses of the host is the only and definitive way to diagnose *Rhinosporidium seeberi* infection [3,17].

Allergic mucin was significantly associated with *Aspergillus* spp. growth in culture and demonstrated 100% specificity and 100% positive predictive value. The presence of allergic mucin was congruent with culture isolation of *Aspergillus* spp. in terms of Cohen's kappa coefficient as well (κ=0.63). Allergic mucin was found to be stronger predictor of *Aspergillus* spp. compared to presence of hyphal forms on histopathology. Allergic mucin is commonly seen in allergic fungal rhinosinusitis caused by *Aspergillus* spp. [1,19,20]. However, several different species of fungi are associated with this finding like *Cryptococcus neoformans*, *Blastomyces dermatitidis* and *Rhinosporidium seeberi* [3]. Though, we isolated fungal pathogens belonging to diverse classes in this study, significant association was present between allergic mucin and growth of only *Aspergillus* spp. on culture.

In this study, necrosis was found to be highly specific and had good positive predictive value and Cohen's kappa coefficient for correlation with fungal growth in culture. A variety of fungi, including *Aspergillus* spp., *Fusarium* spp., the zygomycetes, *Candida* spp., *Cryptococcus* spp., *Histoplasma capsulatum, Penicillium marneffi,* and *Paracoccidiodis brasiliensis*, possess the propensity to invade through blood vessel walls and cause tissue necrosis [1,19].

LIMITATION

Limited number of samples is the limitation of this retrospective study.

CONCLUSION

Histopathological and direct KOH mount examination can deliver expeditious provisional identification of fungal pathogens. However, histopathological examination is the only available reliable method to identify certain uncultivable fungal pathogens. We recommend using histopathology, culture and KOH examination in a complementary manner so that potential lapses in patient management can be prevented. Though, for this study we established microbiological culture as the gold standard, we affirm that a true gold standard entails clinical evaluation of the fungus as true pathogen, colonizer or contaminant.

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