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FLUORESCENCE OF FUNGI IN SUPERFICIAL AND DEEP FUNGAL INFECTIONS

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ABSTRACT

Background: When H&E stained sections are examined under a fluorescent microscope, fluorescence of several fungus is observed. Theoretically, this phenomena might help in the identification of cutaneous and widespread fungal infections without the time-consuming wait involved with specific stains. To ascertain the practical applicability of this approach, 76 cases of superficial and deep fungal infections as well as 3 cases of protothecosis were examined.

Results: Fluorescence was occasionally observed, but it was rarely strong. The age of the specimen did not correspond with the fungi's fluorescence. Most of the time, ordinary light microscopy made it easier to identify organisms in H&E stained sections than fluorescent microscopy.

Conclusion: According to this study, it is difficult to identify fungal species using fluorescence microscopy on skin samples that have been stained with H&E.

I. INTRODUCTION

It has been suggested that the technique is helpful in identifying deep fungal species in tissue. Many pathogenic fungus have been demonstrated to exhibit fluorescence when H&E stained tissue sections are examined under a fluorescent microscope. The goal of the current investigation was to ascertain whether or not fungi's fluorescence contains information that can be used in clinical settings.

- There are three genera of dermatophyte species: Epidermophyton, Microsporum, and Trichophyton. They are further classified into three categories based on natural environments (humans, animals, and soil).
- Skin, hair, and nails are all keratinized tissues that are infected by dermatophytes.
- To confirm a dermatophytosis, microscopic inspection, culture, Wood's light analysis, and histology may all be helpful.
- The most prevalent species that is isolated in the US is Trichophyton.
- Dermatophytoses can be effectively treated with a number of topical medications (imidazoles and allylamine) and oral medications (griseofulvin, itraconazole, fluconazole, and terbinafine).
- A superficial dermatophyte infection called tinea nigra may resemble an acral lentiginous melanoma.
- Piedra is a superficial fungal infection of the hair shaft that is asymptomatic and comes in both white and black forms.
- Tinea capitis describes dermatophyte infectionof hair and scalp, typically caused by Trichophyton and Microsporum species, with exception of Trichophyton concentricum.
- Aspergillosis is an infection caused by Aspergillus, a common mold (a type of fungus) that lives indoors and outdoors. Most people breathe in Aspergillus spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to Aspergillus. The types of health problems caused by Aspergillus include allergic reactions, lung infections, and infections in other organs.
- Folliculitis is a common skin condition that happens when hair follicles become inflamed. It's often caused by an infection with bacteria. At first it may look like small pimples around the tiny pockets from where each hair grows (hair follicles).
- The condition can be itchy, sore and embarrassing. The infection can spread and turn into crusty sores.
- Mild folliculitis will likely heal without scarring in a few days with basic self-care. More-serious or repeat infections may need prescription medicine. Left untreated, severe infections can cause permanent hair loss and scarring.

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- Certain types of folliculitis are known as hot tub rash and barber's itch.

II. MATERIALS AND METHODS

A fluorescent microscope was used to analyze Seventy Six H&E stained slices from superficial and deep fungal infections as well as 3 cases of protothecosis. The specimen was processed without the use of any immune-system reagents. All specimens have been kept in formalin and processed in a standard manner, including being embedded, sectioned, and stained. All were inspected using an Olympus Provis microscope equipped with a dichroic mirror and filters, as well as fluorescent light sources (334 and 365 nm) (DM-40 – DM 455). The specimens were anything from a few hours and more than 20 years old.

III. RESULTS

Most organisms showed some degree of fluorescence. Chromomycosis, sporotrichosis, and aspergillosis-related organisms were among those that sporadically displayed fluorescence. There was no fluorescence in the single cases of blastomycosis or tinea nigra that were investigated. In the study's Cryptococcus's cases, fluorescence was absent in 50% of the cases. There were both strong and mild foci of fluorescence in invasive phaeohyphomycosis. The intensity of the fluorescence did not seem to be related to the type of colored fungus. In H&E stained sections, phaeohyphomycosis was more easily recognized using standard light microscopy.

Instances of protothecosis and superficial dermatophyte infection (Figures 3 and 4) showed the most prominent fluorescence. Even in these circumstances, the approach proved of limited value in identifying fungus. Majocchi's fungal folliculitis was the only instance in which fluorescence was remarkably helpful in identifying the fungus (Figures 5 and 6). The stratum corneum fluoresced strongly in every case of onychomycosis, tinea versicolor, and candidiasis that was examined, overpowering the fungi's fluorescence. In Table 1, the study's findings are enumerated.

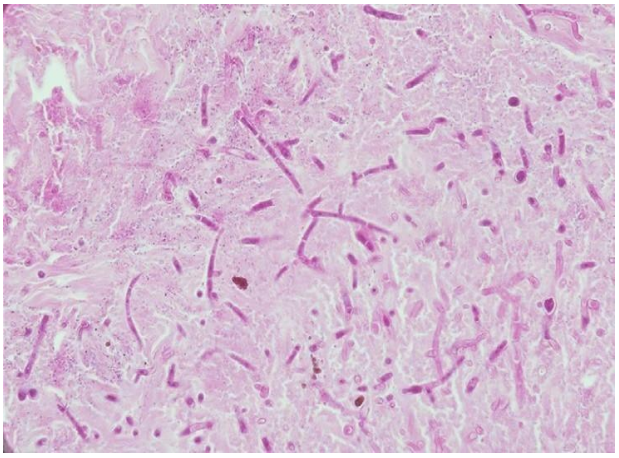

Figure 1

Aspergillosis: organisms clearly seen in H&E sections.

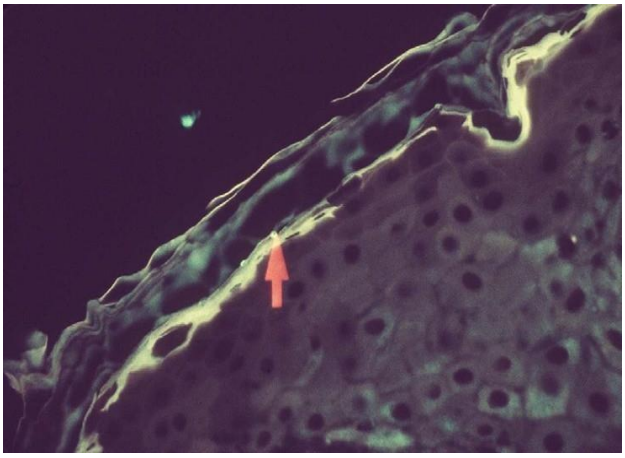

Figure 4

Tinea: Highlighted by fluorescence

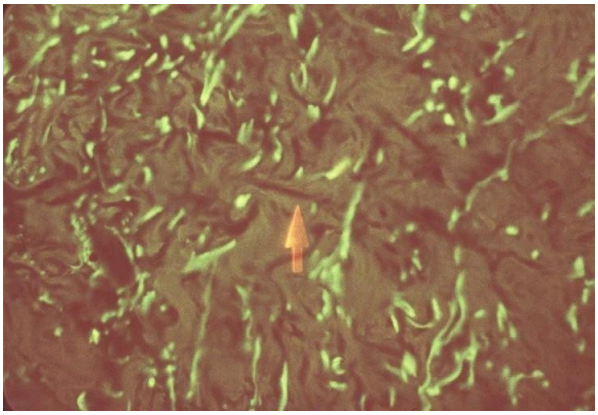

Figure 2

Aspergillosis: Fungi are less visible with fluorescence micros-copy.

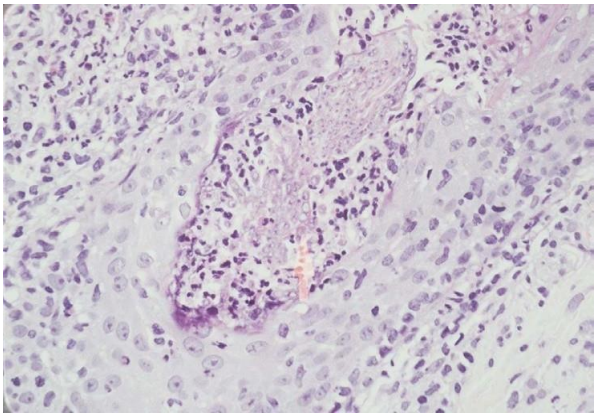

Figure 5

Fungal folliculitis: H&E

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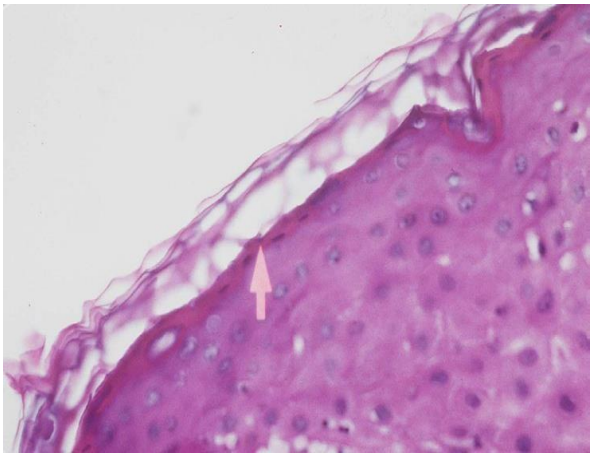

Figure 3
Tinea: H&E

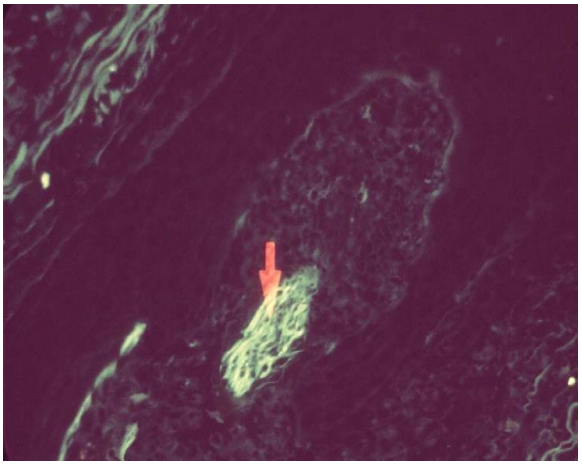

Figure 6
Fungal folliculitis: Fungi much more easily visualized with fluorescence microscopy

Table 1

Fungal organism	Total number	Negative	Weak	Moderate to Strong
Coccidioidomycosis	5		3	2
Cryptococcosis	10	5	3	2
Chromomycosis	6	6		
Histoplasmosis	5	4		1
Phaeomycotic cyst	1			1
Invasive Phaeomycosis	3		2	1
African Histoplasmosis	2	2		
Mucormycosis	5	4		1
Candidiasis	4	?	1	
Lobomycosis	2		2	
Rhinosporidiosis	2	1	1	
Tinea nigra	1	1		
Fusariosis	1	1		
Eumycetoma	1			1
Dermatophytosis	11	4		7
Onychomycosis	3	?		2
Tinea versicolor	3	1	1	1
Sporotrichosis	2	2		
Majocchi's	5	2		3

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Granuloma				
Aspergillosis	3	3		
Prototheсosis	3			3
Blastomycosis		**1**		**1**

IV. DISCUSSION

According to several accounts, diagnosis could be made using luminous fungi in H&E stained sections inspected under a fluorescent microscope without the use of specific stains. According to the study's findings, skin infections rarely benefit much from fluorescence microscopy, with a few notable exceptions. The approach may be useful in some cases of dermatophytosis and protothecosis because of the fluorescence, however even in these situations, fungi were typically not visible at scanning magnification. The clinical appearance of each case of dermatophytosis shows that the organism in question was most likely Trichophyton rubrum, despite the fact that no cultures were performed in any of the dermatophytosis cases.

The intense stratum corneum fluorescence observed in all cases of tinea versicolor and candidiasis is an intriguing finding. Dermatopytosis never shown this characteristic. If the fluorescence of the stratum corneumis useful in differentiating dermatophytes from yeast infections in skin specimens, more research will be required to ascertain this.

The results of earlier publications conflict with the lack of fluorescence microscopy sensitivity mentioned in this article. Margo reported a case of periocular blastomycosis that was initially misdiagnosed as squamous cell carcinoma both clinically and histologically [1]. In H&E stained sections, fungi fluorescence showed species that were not apparent at first glance. Hettlich described the aspergillus' fluorescence in cytological samples stained with the Pa- panicolaou stain [2]. The method, they observed, was useful in identifying morphological traits that set aspergillus apart from other filamentous fungi. A fluorescent microscope, according to Mann, makes it simple to identify fungi at scanning power [3]. This was not the case in my opinion. Even intense dermatophyte fluorescence was better visible at greater magnifications (at least 4X). Mann added that the method was useful when working with specimens that had few organ-isms. I discovered that this was exclusively true in instances of dermatophytosis and protothecosis. Two older cases of candidiasis and coccidiodomycosis, according to Mann, had little fluorescence. Strong fluorescence was observed in specimens that were 20 years old, and there was no correlation between the age of the specimen and my findings. Several specimens processed the same day showed no fluorescence. The emergence of histoplasmosis, aspergillosis, and blastomycosis were recognized by Mann. In the current investigation, these organisms did not exhibit fluorescence. In solid Parenchymal Organs, Graham claimed that the approach was particularly useful for detecting coccidiodomycosis, candidiasis and aspergillosis [4]. The technique's usefulness could vary depending on the tissue. Although even a high fluorescence of Candida would have been obscured by the intense fluorescence of the stratum in skin, I did not find the procedure to be effective with these organism. Graf observed that the fluorescence pattern of Candida albinos and Aspergillus was affected by the light's wavelength, fixation technique, and mounting medium [5]. Strong fluorescence was produced by acetone fixation with green excitation. Although it is possible that some of the variations between the results of the current investigation and those of past studies could stem from slight differences in mounting media, I feelthis is improbable. Formalin, not acetone, is the standard for fixing biopsy specimens. Over a period of years, the slides under examination were processed in 15 different laboratories. There was no lab that showed a higher incidence of fluorescence.

V. CONCLUSION

In conclusion, the present study raises questions about the usefulness of the approach even if other studies had suggested that fluorescence of H&E stained sections would be helpful in cases of cutaneous fungal infection. Although frequently present, fluorescence was typically minimal. With the exception of a single case of fungal folliculitis, the technique was less sensitive than routine light microscopy in the setting of skin biopsy specimens.

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