REVIEW ARTICLE

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Dermatomycosis from the Perspective of Dermatopathology (Version 1.1)

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Dermatomycosis is a skin disease caused by fungi, including dermatophytes and yeasts. Its diagnostic methods include KOH smear, fungal culture test, Wood's lamp test, biopsy, and molecular biology test. Superficial dermatomycosis can already be diagnosed using only KOH smear and culture test, so biopsy has not yet received attention from many clinicians. Nonetheless, biopsy is one of the most basic tests in the field of dermatology, with high diagnostic value for deep and superficial dermatomycoses, which often shows negative findings on KOH smear or fungal culture test. In this study, the histopathologic findings and special chemical staining methods in dermatomycosis are described. This review article is an upgraded English version of the review paper "Dermatomycosis from the perspective of dermatopathology (Korean Version 1.0)" published in 2016.

Key Words: Fungal infection, Histopathology, Special stain

INTRODUCTION

Despite the clinical significance of dermatopathology in the field of dermatomycosis, there are still limited studies about this subject. Therefore, as a response to a number of requests to upgrade and publish the study "Dermatomycosis from the perspective of dermatopathology (Version 1.0)" in English, Version 1.1 (in English) is described in this review article.

Dermatomycosis is a skin disease caused by fungi. In general, it is divided into superficial dermatomycosis and deep dermatomycosis. Superficial dermatomycosis invades the epidermis and skin appendages, while deep dermatomycosis invades the dermis and subcutaneous fat layer¹. The diagnostic methods for skin mycoses include KOH smear, fungal culture test, Wood's lamp test, biopsy, and molecular biology test². Among these, biopsy is widely used to diagnose deep dermatomycosis. However, superficial dermatomycosis can already be diagnosed using only KOH smear and culture test, so biopsy has not yet received attention from many clinicians.

Nonetheless, biopsy is one of the most basic tests in the field of dermatology, and its diagnostic value is high for deep and superficial dermatomycoses. In fact, patients with superficial dermatomycosis often show negative findings on KOH smear or fungal culture test, and it is easy to overlook cutaneous mycoses when clinically nonspecific lesions are formed³. Examples include tinea incognito caused by prior treatments, such as local steroid ointment, and Majocchi granulomas that occur in the dermis or subcutaneous tissue due to the causative agent of superficial dermatomycosis. In these cases, as the typical clinical manifestations of superficial dermatomycosis are not observed, its diagnosis is delayed

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Fig. 1. Carefully examined area in skin biopsy specimen 1) Stratum corneum, 2) Inflammation in dermis, 3) Hair follicle (H&E, ×100)



Fig. 2. Sandwich sign in dermatophytosis patient (H&E, ×400)

or misdiagnosed as another skin disease, resulting in the repetition of wrong treatments. For these cases, the diagnosis can be made through biopsy. When skin biopsy is performed, the diagnosis is made by confirming the presence of hyphae or spores, which are structures peculiar to fungi in tissue sections. If there is no knowledge and recognition about this, fungal infections may be overlooked⁴. In this study, the biopsy findings and chemical staining methods for dermatomycosis are described.

HISTOLOGICAL EXAMINATION OF DERMATOMYCOSIS

The possibility of both superficial and deep dermatomycoses should be considered in the tissue examination of patients with dermatomycosis. Therefore, the areas to be carefully examined when performing a biopsy are the stratum corneum, tissue around the hair follicles, dermis, and subcutaneous fat layer (Fig. 1). The most basic staining method in skin biopsy is hematoxylin-eosin (H&E) staining, which identifies the presence of fungi, including their hyphae or spores in the stratum corneum, parakeratosis, neutrophil invasion in the epidermis and dermis, purulent folliculitis, follicle destruction, and granuloma^{1,2,5}. In particular, the stratum corneum should be examined first, which are commonly invaded by causative fungi of superficial dermatomycoses; sandwich signs are representative during tissue examination⁶. In general, if hyphae exist in the stratum corneum, the diagnosis can be confirmed. However, it is often difficult to distinguish hyphae from normal skin structures in H&E staining, so finding a sandwich sign is helpful in diagnosing superficial dermatomycosis. The sandwich sign is the presence of keratin with

dyskeratosis or thick keratinization in the lower part of the stratum corneum and normal keratinization in the upper part. These findings can be seen when fungal hyphae or spores invade the stratum corneum (Fig. 2). Therefore, if a sandwich sign is visible in a biopsy specimen, confirming the presence of hyphae or spores through special staining is necessary to discriminate mycoses (Fig. 3A, 3B). Other clues to fungal infection include compact orthokeratosis without any other explanations and spongiosis, particularly palmoplantar⁵. The hyphae in superficial dermatomycosis are often found in the hair follicles⁷. When identifying fungi invading the hair follicles, it is helpful to observe whether it is ectothrix or endothrix depending on the location of arthroconidia formation¹⁵. If spores are located inside the hair shaft, they are classified as endothrix, and if they are located outside, they are classified as ectothrix infections. In ectothrix infection, the cuticle is destroyed, and several species show a yellow-green fluorescence on Wood's lamp test. In endothrix infection, the cuticle is normal and does not emit fluorescence on Wood's lamp test (Table 1)¹⁵. In ectothrix infection, *Microsporum canis*, M. audouinii, M. distortum, M. ferrugineum, M. gypseum, M. nanum, and Trichophyton verrucosum can be suspected as causative fungi. In endothrix infection, T. tonsurans, T. violaceum, and T. soudanense are common causative fungi (Fig. 4). Deep dermatomycosis affects the deeper skin layers below the dermis or beyond the muscle layer or other organs. Since it undergoes a long-term inflammatory reaction, it mainly shows granulomatous changes⁸. Therefore, it is helpful to closely observe the surrounding tissues infiltrated with inflammatory cells in tissue findings. In superficial dermatomycosis, substances that cannot be found in normal



Fig. 3. (A) Hyphae and spores are not visible in H&E stain. (H&E, ×400) (B) A number of hyphae and spores in stratum corneum in PAS stain (D-PAS, ×400)

 Table 1. Comparison of ectothrix and endothrix¹⁵

	Ectothrix infection	Endothrix infection
Arthroconidia	On the surface of the hair shaft	Within the hair shaft
Hyphae	Within and out of hair shaft	Within the hair shaft
Hair cuticle	Destroyed	Remain intact
Under Wood's Iamp	Yellow-green fluorescence +/–	No fluorescence

human skin are often a clue. Some fungi form specific vesicles or granules that aid in the diagnosis. For example, in patients with sporotrichosis, asteroid bodies can be observed, and cigar-shaped bodies are rarely seen. In chromoblastomycosis, the causative organism has a characteristic brown color, which are sclerotic bodies or copper pennies (Fig. 5)⁹. This finding is not observed in brownish phaeohyphomycosis, so it helps to differentiate the two. In addition, sulfur granule is characteristically observed in mycetoma, and the causative fungus can be classified according to the color of sulfur granules, which have high diagnostic value¹⁰. In some fungi, their mycelia are branching at a certain angle, so knowing this is helpful in the diagnosis. For example, fungi with branching mycelia at 45° include Aspergillus, Fusarium, and Pseudallescheria boydii. The mycelia of Zygomycetes and *Mucorales* branch at $90^{\circ 8}$ (Fig. 6)⁹. The angle of hyphae



Fig. 4. Ectothrix infeciton - *Microsporum canis*, A number of arthroconidia out of the hair shaft (D-PAS, ×400)

provides necessary insights to make a diagnosis based on H&E stains; the results may be equivocal and may require additional laboratory studies to be confirmed¹¹.

Moreover, while mycotic infections often show closeto-normal pathologic findings, follow-up assessments are warranted if clinical presentations suggest mycotic infections⁵.

CHEMICAL STAINING IN DERMATOMYCOSIS

Unlike human cells, fungi have a cell membrane composed





Fig. 5. Pigmented yeasts (arrow) in chromoblastomycosis resembling "copper pennies" (Fontana Masson, ×400)



Fig. 6. GMS stain of mucormycosis shows blunt angle branching of hyphae. (GMS, ×400)

of chitin and glycoprotein. Using these characteristics, it is possible to distinguish the causative fungal species by performing special staining. H&E staining can also observe hyphae or spores, but it is often difficult to distinguish hyphae from normal skin structures, such as collagen fibers. In addition, if a wrong steroid ointment applied at the beginning of the onset is repeated, the skin would have transformed into an atypical lesion, and it would be easy to misdiagnose the fungal infection¹².

In addition to H&E staining, typical chemical staining, such as periodic acid-Schiff (PAS), Gomori's methenamine silver (GMS), Gridley fungus (GF), mucin (alcian blue, mucicarmine), melanin (Fontana Masson), and acid-fast are also available. In each staining method, fungi are stained with a specific color, so that hyphae or spores can be easily identified¹³.

1. Gomori's methenamine silver stain (GMS stain)

GMS staining, like PAS and GF staining, is mainly used to screen for fungal infections. The cell wall of fungi is stained black, and the surrounding skin tissue becomes dark green (Fig. 7). GMS staining is more sensitive than PAS staining because chromic acid, a stronger oxidizing agent than periodic acid, is used in the staining process. Therefore, degenerated hyphae that are not well-stained with PAS staining are prominently observed in GMS staining. On the other hand, if there is a structure that has a dark color, such as melanin, it is difficult to distinguish it from fungi. In addition, it is difficult to observe inflammatory reactions accompanying the fungal



Fig. 7. GMS stain of dermatophytosis patient (GMS, x400)

infection compared to PAS staining¹⁴. Considering these points, both GMS and PAS staining are useful in screening for fungal infections, but there are differences in their usefulness in some cases. For example, for *Histoplasma* and *Aspergillus* infections and onychomycosis, GMS staining is more sensitive than PAS staining. For phaeohyphomycosis and *Sporothrix, Candida, Blastomyces, Exophiala, Malassezia,* and *Rhinosporidium* infections, PAS staining is advantageous to observe the lesions (Table 2)^{5,13}.

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	GMS stain	PAS stain
Advantages	- More sensitive than PAS	- Demonstrates fungal morphology better
	- Produces better staining contrast	
Disadvantages		- Degenerated fungi are refractory to PAS
	- Not adequate for demonstrating inflammatory response	- Calcific bodies mimic the budding yeasts
	- Mask the natural color of pigmented fungi	- Dead fungi do not always stain
		- The equine fungus Phythium does not stain.
Applications	Superior in visualizing Histoplasmosis, Aspergillosis, Onychomycosis	Superior in visualizing
		Candidaiasis, Blastomycosis, Phaeohyphomycosis

Table 2. Comparison of GMS and PAS stain^{5,13}



Fig. 8. PAS stain of dermatophytosis patient (D-PAS, ×400)

2. Periodic acid-Schiff (PAS) stain

In PAS staining, the glycoproteins in the fungal cell wall are stained red, and it is usually used for screening along with GMS and GF staining (Fig. 8). Compared to other staining, PAS staining is suitable for observing the shape of fungi causing the infection and the surrounding skin structure¹⁰. Mohan et al.¹² said that PAS staining is cheaper, easier, and faster than GMS, which helps in diagnosing dermatomycosis. PAS staining has shortcomings despite its many advantages. It cannot stain phythium and often fails to stain dead fungi, requiring complementary measures, such as GMS staining⁵.



Fig. 9. Mucicarmine staining of *Cryptococcus* (arrow) (Mucicarmine, ×400)

3. Gridley fungus (GF) stain

GF staining dyes the fungal cell walls purple and the surrounding skin tissues yellow. Like PAS and GMS staining, it is mainly used for screening by staining most of the fungal cell walls.

4. Mucin stain (Alcian blue, mucicarmine)

Representative staining include alcian blue and mucicarmine staining, and these are mainly used to diagnose cryptococcosis. When staining with alcian blue, the capsule of *Cryptococcus* is stained blue, and when stained with mucicarmine, it is

stained red (Fig. 9)⁹.

5. Melanin stain (Fontana Masson)

Fungal cell walls and melanin are stained black. It is mainly useful for diagnosing cryptococcosis, phaeohypomycosis, and chromoblastomycosis (Fig. 5)⁹.

6. Bacterial stains (Gram stain and acid-fast stain)

Gram stain and acid-fast stain are mainly used for bacterial infections, but specific fungi can also be stained to aid in the diagnosis¹⁴. For example, *Candida* appears blue on Gram stain, while *Blastomyces* or *Histoplasma* are stained red by acid-fast stain staining.

CONCLUSION

Most dermatomycoses in clinical practice are superficial dermatomycosis. This can be easily diagnosed through KOH smear or culture test, so biopsy is not commonly performed. Biopsy is one of the most basic examinations for diagnosis in dermatology, including the diagnosis of dermatomycosis. The positive rate of biopsy is higher than that of other tests, and its diagnostic value is particularly high in deep dermatomycosis or onychomycosis. In addition, if the results of KOH smear or culture test are negative in patients with superficial dermatomycosis, it is easy to overlook the possibility of dermatomycosis. Therefore, it can be misdiagnosed as other diseases, such as skin tuberculosis, skin bacterial infection, and skin tumors. Thus, it is important to consider dermatomycosis when examining a patient in a clinical setting. If a skin lesion that has a chronic course or a nonspecific skin lesion that recurs repeatedly is observed in a patient with reduced immune function, skin biopsy must be performed to confirm the presence of skin mycosis.

Moreover, since the causative fungi can be identified through appropriate special staining, it is important to keep in mind the kind of staining that is suitable for each fungus.

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CONFLICT OF INTEREST

This thesis is an upgraded English version of a review paper already published on this journal in 2016. Some Tables, images, and texts from the original thesis were re-used with the permission of the editorial board of the journal.

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