OBSERVATION

A Better Potassium Hydroxide Preparation?

In Vivo Diagnosis of Tinea With Confocal Microscopy

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Background: Traditional diagnostic testing for dermatophyte infection currently requires skin scraping for light microscopy and/or fungal culture or skin biopsy. Immunofluorescent microscopy can also be used with calcofluor stain. All of these tests can be time-consuming to perform, require a waiting period for results, and are invasive. We investigated the use of a real-time, noninvasive, confocal microscope in visualizing dermatophyte hyphae in vivo.

Observations: Confocal microscopic imaging of active tinea can clearly identify dermatophyte hyphae within the upper epidermis after potassium hydroxide application. The hyphae appear as bright linear branching objects not found in uninvolved skin.

Conclusions: It is possible to immediately and painlessly image dermatophyte hyphae in active lesions of tinea by means of a confocal microscope. With further improvement, imaging devices may be available to physicians to instantly and noninvasively evaluate a variety of skin disorders in microscopic detail.

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DERMATOPHYTES are common fungi that often infect the skin. Traditional diagnostic laboratory methods include microscopic examination of scrapings, fungal culture, and skin biopsy.[1] Dermatologists are particularly adept at preparing skin scrapings and detecting hyphal elements with traditional microscopy. Even so, it can be time-consuming and results can vary depending on sampling error. Tests such as fungal culture and skin biopsy also have disadvantages including, but not limited to, waiting several days for results. A possible solution may be diagnosis through the use of noninvasive, real-time imaging with reflectance confocal microscopy as detailed herein.

REPORT OF A CASE

A healthy 28-year-old man presented with a scaly erythematous pruritic plaque on the lateral aspect of his left foot that had been present for 2 weeks (**Figure 1**). The patient was not using any topical or systemic medications. A potassium hydroxide (KOH) preparation was positive for hyphae and confirmed the clinical diagnosis of tinea pedis (**Figure 2**A). A new imaging modality, confocal scanning laser microscopy (CM), that takes in vivo high-resolution cutaneous images in real time was used to further evaluate the eruption. A drop of 10% KOH was applied to the skin before imaging. No changes were seen in the surrounding, apparently normal skin. Images of involved skin demonstrated several linear hyphae in intercellular spaces in the upper epidermis (Figure 2B). The hyphae were seen as highly refractile, linear structures that were brighter than the background keratinized cells. In addition, evaluation of deeper portions of the epidermis and upper dermis showed scattered inflammatory infiltrate. The patient reported that the CM session was painless and without any side effects. The procedure required approximately 45 minutes, mostly spent capturing hundreds of images and visualizing all levels of the skin within the device's range (stratum corneum through upper dermis). A fungal skin culture later yielded *Trichophyton rubrum*.

COMMENT

Dermatologists and primary care physicians diagnose and treat millions of patients with dermatophyte infections yearly.[2]

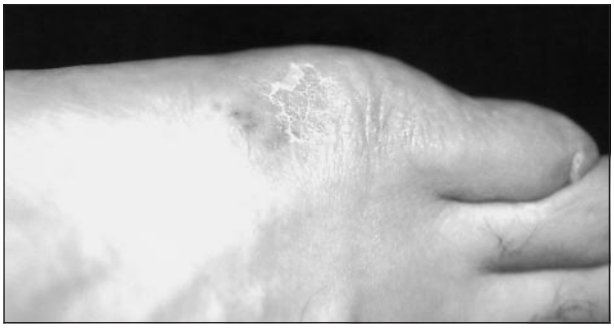

Figure 1. Tinea pedis in a 28-year-old man.

Although diagnosis is often straightforward, some patients are misdiagnosed and treated inappropriately. *Tinea incognito* is the medical term for a dermatophyte infection mistakenly treated with topical or systemic corticosteroids.[3] A missed diagnosis can lead to prolonged suffering, more extensive involvement, and needless expense for physician visits and inappropriate medications.

Current diagnostic methods have some disadvantages. Scraping the skin for microscopy is the most commonly used technique. This involves removing tissue (scales) from the patient's skin for light microscopic detection of dermatophyte organisms. To improve visibility of hyphae, heat, KOH, dimethyl sulfoxide, chlorazol black, or immunofluorescent stain may be used. Sampling error, inadequate KOH hydrolysis, and difficulty "seeing through" the scale are reasons for false-negative results. Occasional fibers (eg, cotton and wool), hair, or "mosaic fungus" (incompletely dissolved hyphae) may result in a false-positive result. Nondermatologists rarely feel comfortable performing such a test and often treat empirically with combination corticosteroid and antifungal creams in a less effective "shotgun" approach.[4] Obtaining a fungal skin culture may delay diagnosis by days to weeks and relies on growth of viable fungi. In addition, a fungal culture may be falsely positive because of contamination and may be misleading. Finally, skin biopsy can be helpful in difficult-to-diagnose cases but is a more invasive procedure that can lead to minor patient discomfort, exposure to blood and needles, and scarring. Although results from skin biopsy return more quickly than do culture results, the diagnosis is still delayed by several days.

Confocal scanning laser microscopy is a noninvasive, painless, real-time, reflectance imaging technique for the skin and other tissues.[5] A confocal microscope contains a low-power laser beam that illuminates the targeted area of tissue.[6] Only the light reflected from a thin "section" within tissue is detected, thus allowing for excellent cellular detail that is comparable to that of histologic examination.[7] We use an 830-nm diode laser with a maximum power of 25 mW in tissue and an ×30 objective lens (0.9 numerical aperture, water immersion), providing en face visual sections, lateral resolution better than 1 µm, and vertical resolution of 3 to 5 µm. High resolution is necessary for imaging dermatophyte hyphae; CM has the highest resolution of any live tissue imaging technique. The illumination power during CM does not produce substantial heating or tissue changes.

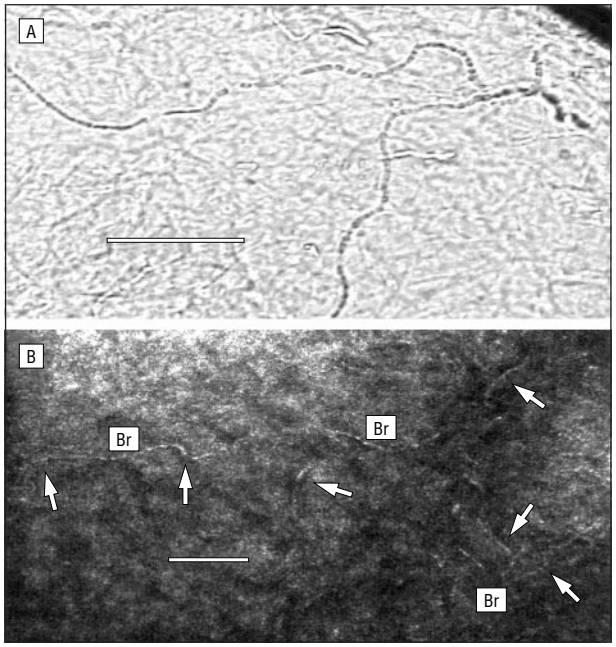

Figure 2. Diagnostic images of dermatophyte hyphae. A, Ex vivo light microscopic image prepared with potassium hydroxide (original magnification ×20). B, In vivo confocal microscopic image of dermatophyte hyphae (arrows) showing branching points (Br). Bars in A and B represent 50 µm.

Several recent publications have documented the appearance of different skin disorders with confocal microscopy, including allergic contact dermatitis,[8] folliculitis,[9] sebaceous hyperplasia,[10] psoriasis,[11] and nonmelanoma skin cancers.[12] In addition, reflectance CM has been able to successfully identify hyphal structures in patients with onychomycosis.[13,14] This is the first report, to our knowledge, of the use of CM to identify hyphal elements in skin in vivo.

We found that CM imaging of active tinea can clearly identify dermatophyte hyphae within the upper epidermis after KOH application. The hyphae appear as bright, thin, linear objects not found in uninvolved skin. Identification was easiest at low power, minimizing image saturation by the surrounding keratinized cell layer. As described above, KOH is commonly used ex vivo in a clinical setting to find hyphae with light microscopy. With CM, isotonic sodium chloride solution is usually applied to the skin to immerse the lens before imaging. We found that application of a drop of 10% KOH in vivo for less than 1 minute before imaging allowed for easier visualization of the hyphae.

Compared with other techniques, the advantages of CM include immediate results, painless noninvasive technique, and easy scanning of multiple areas, thereby minimizing sampling error. In addition, the hyphae and resultant inflammatory infiltrate could also be seen at the same time, minimizing the chance of a false-positive result due to contamination. Follow-up of the same area to assess response to therapy can be performed, as no tissue is removed. Much of the time spent during imaging in this patient was used for academic purposes—taking hundreds of images at different depths, fine-tuning image quality for publication purposes by trying different settings, and carefully evaluating normal skin for com-

parison. A focused physician with a more portable model may need only a few seconds to minutes to successfully image hyphae in the future. The major current disadvantages of CM for diagnosing tinea include expense of equipment, lack of availability, and, in some cases, positioning of the probe. All of these disadvantages may be surmountable in time. A handheld confocal microscope is being developed for more widespread clinical use. In years to come, small, less expensive, handheld skin imaging devices may be routinely available for physicians to instantly and noninvasively evaluate a variety of skin disorders in microscopic detail.

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