

COMPARISON OF TRYPSIN TREATMENT METHOD AND STANDARD LABORATORY TECHNIQUE FOR DIAGNOSIS OF DERMATOMYCOSIS

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Abstract. Dermatomycosis is prevalent worldwide. Discrepancy between microscopic examination and culture findings can create problems in the diagnosis of this common infection. In this study, samples from 60 patients were processed after trypsin treatment and examined by neutral red staining to distinguish viable and non-viable fungal elements. The trypsin treatment method was compared with standard laboratory techniques. A higher number of direct-microscopy-positive, culture-negative samples were obtained without trypsin treatment. Trypsin treatment increased the isolation of fungi from clinical samples, and neutral red staining was able to distinguish viable fungal elements.

INTRODUCTION

Dermatomycosis is one of the most common fungal infections of humans. Direct microscopic examination of skin, hair and nail specimens is essential for diagnosis (Moore and Jaciow, 1979; Campbell and Johnson, 1998). Clearing the specimen with 10-20% potassium hydroxide, followed by microscopic examination, is the most widely used method (Moore and Jaciow, 1979; Rippon, 1988). Many other methods have been proposed in the literature, but rarely adopted by dermatologists (Monod *et al*, 1989). Culture is essential after microscopy, as a definitive identification of the etiological agent can be ascertained only by culture (Padhye and Weitzman, 1998). In many instances, the choice of therapy may depend upon the species causing infection. In the diagnostic mycological set-up, specimens that are positive on direct microscopy may fail to grow on culture due to non-viable fungi (Naka *et al*, 1994; Denning *et al*, 1995). This causes difficulties in assessing the therapeutic benefit and prognosis of the disease.

In the present study, we have evaluated the effect treating the sample with trypsin on direct microscopic and culture results.

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MATERIALS AND METHODS

A total of 60 patients coming to the Dermatology Clinic of the All India Institute of Medical Sciences New Delhi, diagnosed clinically with dermatomycosis, were included in this study (39 *Tinea corporis*, 10 *Tinea cruris*, 9 *Tinea unguium*, and one each *Tinea manuum* and *Tinea capitis*). Skin scrapings from these patients were collected from the margin of the lesion, hair samples were taken by plucking infected hair, and nail samples were taken by clipping. All the samples were transported in sterile brown paper envelopes and were processed on the day of collection. For processing of the samples, the following method was followed (Naka *et al*, 1994): 2% trypsin and 0.5% neutral red were made up in phosphate buffer saline. Both the solutions were sterilized by passage through 0.22 µm GS membrane filter.

Part of a scraping was put in 1 ml of 2% trypsin solution and kept at 37°C for 2 hours. The scales were then washed with phosphate-buffered saline (PBS) by centrifuging 3 times (1,500g x 10 minutes). The deposit was then suspended in 300 µl PBS. Two hundred µl of this was cultured on a tube (size 18x150mm) containing Sabouraud's dextrose agar (SDA) supplemented with cyclohexamide alone and SDA with gentamicin (0.026 mg/ml) and cyclohexamide (0.05 mg/ml). One hundred µl of the remaining resuspended deposit was mixed with an equal amount of 1% neutral red (Merck Laboratories) and kept at room temperature for 1 hour. This was then microscopically examined as

a wet mount under high dry objective (40 x) (Naka *et al*, 1994).

The part of the sample that was not treated with trypsin was processed by standard mycological techniques (Moore and Jaciow, 1979; Campbell and Johnson 1998).

All the culture tubes were incubated at 25°C and 37°C for up to 1 month before declaring a negative result. The growth obtained was identified by gross appearance and microscopically. All the yeasts were identified by germ tube test, sporulation on corn meal agar and pigmentation on TTC (2,3,5-triphenyl-tetrazolium-chloride (Moore and Jaciow, 1979).

RESULTS

Skin, hair and nail scrapings from a total of 60 patients were studied. All the samples were examined by direct microscopy and cultured both with and without trypsin treatment. A total of 44 samples was positive by culture. Amongst these, 41 samples had mycelial growth and 3 were yeasts. Among the yeasts, those giving a positive germ tube test, producing chlamydo spores on cornmeal agar test, and cream color on TTC, were identified as *Candida albicans*. The germ tube negative yeast, having giant hyphae, with small oval blastospores at

septa and at terminals on corn meal agar and producing rose pink color on TTC, were identified as *Candida parapsilosis*. In 38 cases mycelial growth occurred within 10-14 days at 25°C. The mycelial growths were carefully observed and identified by their gross appearance and microscopic examination after staining with lactophenol cotton blue (LCB). Most of the colonies were flat; some of them were heaped up, having a red color with velvety surface and a cherry red color on the reverse side of the tube. In the LCB preparation of this growth, microconidia with typical teardrop appearance along the side of the hyphae, and rarely, macroconidia were seen. The macroconidia were thin, smooth-walled, multicelled, and pencil-shaped, with 3 to 8 septa. These isolates were identified, by microscopic characteristics, as *Trichophyton rubrum*. Two of the isolates were violet to purple in color; microscopically, there was no typical arrangement of the microconidia, the hyphae were swollen, and distorted chlamydo spores were noted. These were identified as *Trichophyton violaceum*. One of the isolates grew after 10 days; it was olive-green with an orange-brown color on the periphery, microscopically thin-walled, club-shaped, multiseptate round lipped macroconidia were seen. It was identified as *Epidermophyton floccosum*.

The results of microscopy and culture, both

Table 1
Microscopy and culture results of scrapings from patients without trypsin treatment.

Clinical diagnosis	No. of cases	Microscopy +ve, Culture +ve	Microscopy -ve, Culture -ve	Microscopy +ve, Culture -ve	Microscopy -ve, Culture +ve
<i>T. corporis</i>	39	18	5	15	1
<i>T. cruris</i>	10	1	2	5	2
<i>T. unguium</i>	9	1	5	3	-
<i>T. manuum</i>	1	-	-	-	1
<i>T. capitis</i>	1	-	1	-	-

+ve positive ; -ve negative

Table 2
Neutral red staining and culture results of scrapings with trypsin treatment.

Clinical diagnosis	No. of cases	Microscopy +ve, Culture +ve	Microscopy -ve, Culture -ve	Microscopy +ve, Culture -ve	Microscopy -ve, Culture +ve
<i>T. corporis</i>	39	32	5	1	1
<i>T. cruris</i>	10	4	3	1	2
<i>T. unguium</i>	9	4	4	1	-
<i>T. manuum</i>	1	-	-	-	1
<i>T. capitis</i>	1	-	1	-	-

+ve positive ; -ve negative

with and without trypsin treatment, are shown in Tables 1 and 2, respectively.

Amongst the 44 culture-positive cases, *Trichophyton rubrum* was the most common dermatophyte (38 cases). Two isolates of *Trichophyton violaceum* and one isolate of *Epidermophyton floccosum* was also obtained. Among the yeasts, two were *Candida parapsilosis* and one was *Candida albicans*.

DISCUSSION

Definite diagnosis of dermatomycosis can create problems when there is discrepancy between microscopic findings and culture. Samples positive by direct microscopy may fail to grow on culture if the fungal elements are nonviable or some other structures are mistaken for fungal hyphae. Over the years, several modifications of the classical 10% KOH preparation have been made for more rapid detection of fungal elements in the specimen (Moore and Jaciow, 1979; Monod *et al*, 1989). In one study, neutral red staining was used to evaluate the viability of fungal elements (Naka *et al*, 1994). Modification of the basic method 36% dimethyl sulphoxide (DMSO) to 20% KOH has also been used for clearing a specimen (Denning *et al*, 1995).

Neutral red is a water-soluble vital stain that can differentiate between viable and non-viable fungal elements. It is capable of passing through the intact plasma membrane and is stored in the lysosomes of viable cells. Therefore, the uptake of dye ceases when cell membranes and lysosomes are damaged (Naka *et al*, 1994).

In this study, all the samples were examined microscopically and were cultured. Part of the sample, treated with trypsin, and part without trypsin, were processed by standard protocol. After clearing of the specimen by 10-20% KOH, it was directly cultured on supplemented SDA. Another part was treated with trypsin and examined by neutral red staining before culturing to determine whether non-viable fungal elements can cause a discrepancy between microscopic and culture findings.

We found that, of the 60 samples processed directly, 43 (72%) were positive by direct microscopic examination, but 23 (53%) of these failed to grow on culture, whereas with trypsin treat-

ment and neutral red staining, of the 43 samples that were positive by direct microscopic examination, only 3 (7%) were negative on culture. In four samples, direct examination was negative but culture was positive, both with and without trypsin treatment, which may be due to a scarcity of fungal elements. We also observed a higher number of culture positive samples with trypsin treatment.

Thus, we conclude that trypsin treatment can increase the isolation rate of fungi in patients with dermatomycotic infections and neutral red staining is a very useful technique to distinguish viable and non-viable fungal elements, which can create discrepant microscopic and culture findings. These results correlate with those of Naka *et al* (1994).

Patients on antifungal treatment may still have non-viable fungal elements in their lesions. Therefore this technique can help to increase the isolation rate and to distinguish the non-viable fungal elements. It can also be used to evaluate the efficacy of antifungal agents and the prognosis of the patients.

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