Effects of Modified Sample Collection Technique on Fungal Culture Yield: Nail Clipping/Scraping versus Microdrill

H.S. Qureshi, A. H. Ormsby (Department of pathology, Henry Ford Hospital, Detroit, Michigan, USA)
N. Kapadia (The Department of Medicine and Dermatology, The Aga Khan University Hospital, Karachi)

Abstract

Objective: Onychomycosis requires accurate diagnosis but fungal culture yield is frequently low by routine sampling techniques. The aim of this study was to investigate the utility of nail plate/subungual microdrilling as an alternative to conventional nail clipping/subungual scraping.

Methods: Patients with clinical evidence of onychomycosis (n=46) were prospectively evaluated for fungal potassium hydroride (KOH) microscopy and culture comparing two sampling techniques: nail clipping versus microdrilling.

Results: Fungal cultures were positive in 48% with 2 additional cases detected by combining both methods. KOH microscopy was positive in 17% cases. Specimen obtained via the microdrill technique gave consistent heavier fungal growth on culture media. Candida species were the most common isolates (82.7% of cases) and were negative on KOH microscopy in 95% of culture proven cases. The microdrill technique yielded consistent heavier growth on culture media.

Conclusion: Microdrill technique improves laboratory diagnosis and ultimately treatment of onychomycosis, particularly in patients with repeated KOH microscopy and culture failure despite strong clinical suspicion (JPMA 54:301;2004).

Introduction

Onychomycosis is the most frequent and treatable cause of nail disorders with a prevalence varying from 3%-20%. Effective treatment requires long-term systemic therapy with antifungal agents at considerable cost to the patient with diverse adverse effects and the possibility of long-term treatment resistance. Moreover, with the advent of more effective systemic therapy, increasing numbers of clinicians are treating clinically suspected onychomycosis. Only 50% of dystrophic nails have a fungal cause, therefore it is imperative to establish a correct diagnosis by microscopy and culture before a patient is treated with a systemic antifungal agent. Although positive microscopy is often used as a criteria to initiate therapy, cultural identification of an offending
organism is desirable and can have important treatment implications. Fungal culture, however, is frequently negative despite positive microscopy and a high index of clinical suspicion. The reasons for culture failure include the presence of non-viable fungi in the distal nail, inadequate or improper sampling and the presence of possible interfering substances or microorganisms. Clinicians, therefore, should be aware of the need to collect an adequate amount of suitable clinical material. Numerous methods of obtaining specimens for culture in cases of distal and lateral subungual onychomycosis have been described. Most frequently used methods to obtain nail and subungual debris include nail clipping, scraping and subungual debris. These collection methods, however, may not result in adequate yield in proximal onychomycosis, disto-lateral onychomycosis and in severely dystrophic nails. Moreover, fungi usually grow most actively at the proximal part of the affected nail, which at times is difficult to sample particularly in disto-lateral onychomycosis and in dystrophic nails. To increase specimen yield, Epstein suggested using a microdrill, while English and Atkinson used a microdrill with a suction nozzle attached. Using these techniques, the Atkinson and Epstein showed that it is possible to obtain a specimen from a definitive area of the nail with a resultant increased frequency of successful mycological culture despite a low rate of fungal hyphae detection by direct microscopy. Since then, many have advocated the use of the microdrill technique to obtain specimens in all types of onychomycosis with special emphasis on proximal and dystrophic onychomycosis. To our knowledge, there have been only few comparative studies of the nail clipping and drilling methods. The aim of this study was to investigate the utility of nail plate/subungual microdrilling as an alternative to conventional nail clipping/subungual scraping and to directly compare the frequency of KOH microscopy and fungal culture yielded by both methods.

Materials and Methods
Patient Selection Forty six clinically suspected cases of onychomycosis from the clinics of dermatology at The Aga Khan University Hospital, Karachi Pakistan and outside referral patients from various hospitals of the city were prospectively evaluated over a 15 month period (from March 1998 to June 1999). Patients on topical antifungal therapy within the last 1 week or systemic antifungal therapy within the last 4 weeks were not included in the study. A detailed patient history, clinical examination, and relevant investigations were recorded on a pre-designed Performa at the screening visit. Specimen Collection The most severely affected nail was selected as the target nail for sampling for direct microscopy and culture by two different methods: clipping and drilling. When both toe and finger nails were affected, specimens were collected from both sites after selecting the target nails. The nail was first cleaned with 70% alcohol, after which the most distal part of the nail was removed and discarded. In the clipping technique, several small pieces of the affected nail were taken with the nail cutter and nail was scraped with a scalpel; in addition, debris from beneath the nail was scraped. In the drilling technique, a microdrill (Figure 1) without any suction device attached was used. The needle of the microdrill was autoclaved in between each patient. The nail was drilled to a considerable depth at the growing edge as well as the center of the affected area to obtain a well
represented specimen. In the same specimen, debris from beneath the nail was also carefully drilled. To obtain comparable nail specimens, these were taken from the same most affected nail, clipping first followed by drilling. Figure 2 demonstrates a post nail clipped and microdrilled patient’s nail. Microscopic Examination and Culture

Microscopic examination of the collected nail samples was performed using 20% potassium hydroxide (KOH) solution under the light microscope. Fungal culture was performed using three test media: 1) Sabouraud's dextrose agar medium with 0.5% chloramphenicol without cyclohexamide (at 250C and 370 C); 2) 5% Sheep blood agar (at 370 C) and 3) Dermatophyte test medium (at 370 C). Both nail clippings and drilled powder were inoculated separately on to the media through a scalpel blade. The culture Petri dishes were incubated for 1-4 weeks. All moulds and dermatophytes were identified by gross colony morphology and microscopy examination with lactophenol cotton blue preparation. All yeasts were identified using Twin80 agar. Positive culture was defined as the presence of pure growth of either a dermatophyte/non-dermatophyte fungus or yeast at more than 5 out of 20 or 20% of the inoculum's site with or without corresponding positive microscopy. 25 Statistical Analysis The results of KOH microscopy and culture by nail clipping/scraping and microdrill were statistically analyzed using Fisher's Exact test (2 sided). A P value of <0.05 was considered statistically significant.

Results

The demographic data and site of involvement of onychomycosis in all patients are summarized in Table 1. Female outnumbered males. Four of 46 (9%) patients were children between 3-6 years of age. Housewives dominated over other patient's population. Finger nails were the most common site of involvement. None of the patients were HIV positive. One patient had poorly controlled type II diabetes mellitus and another patient had recently completed chemotherapy treatment for multiple myeloma.

KOH Microscopy On microscopic examination (Table 2), 8 of 46 (17%) specimens were positive by combining the results of nail clipping and microdrill. Of the 8 KOH microscopy positive cases, 4 (50%) were positive by both method, 3 cases were positive by microdrill alone, while 1 case was positive by nail clipping/scraping alone. The difference in the number of positive KOH microscopy using nailclipping/scraping and microdrill methods was not statistically significant (P=0.379). Of the 7 specimens positive for microscopy by drilling, one case grew Trichophyton rubrum, 2 cases grew Trichophyton

Table1. Patients Demographics and sites of involvement of onychomycosis in all (46) patients.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>Rabge</td>
<td>3-74</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>36-4</td>
</tr>
</tbody>
</table>
Table 2. Microscopy and fungal culture results by type of specimen collection (Nail clipping and Microdrilling).

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housewives</td>
<td>13</td>
</tr>
<tr>
<td>Students</td>
<td>7</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>11</td>
</tr>
<tr>
<td>None</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Number and Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finder nail</td>
<td>24 (52%)</td>
</tr>
<tr>
<td>Toenail</td>
<td>18 (39%)</td>
</tr>
<tr>
<td>Finger and toe</td>
<td>4 (8%)</td>
</tr>
</tbody>
</table>

Clipping (46)  
Positive 5  
Negative 41  
Microdrill (46)  
Positive 7  
Negative 39  
Clipping and Drilling (46)  
Positive 8  
Negative 38  
(results combined)

mentagrophyte and one case grew Candida parapsilosis. Of the 5 cases positive for microscopy and clipping alone, 2 cases grew Trichophyton mentagrophyte and one case grew Candida parapsilosis. Nineteen of 38 (50%) microscopy negative specimens were culture positive. All microscopy negative but culture positive cases grew yeast organisms. Mycological Culture Mycological cultures (Table 2) were positive in 48% cases by combining the results of microdrill and nail clipping (Table 1). Of the 22 positive cultures cases, 18 were positive by both methods while 2 were positive by the clipping technique alone and 2 were positive by the drilling technique alone. Hence, by combining the results of both techniques two additional cases were detected compared with clipping or drilling techniques alone. Organisms isolated are listed in Table 3.

Table 3. Organisms isolated from fungal culture in onychomycosis in order of frequency.

<table>
<thead>
<tr>
<th>Organisms isolated</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Dermatophytes</td>
<td>20</td>
<td>87</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>9</td>
<td>39.2</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>6</td>
<td>26.1</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td>Dermatophytes</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>1</td>
<td>4.3</td>
</tr>
</tbody>
</table>
dermatophytes, including 2 cases of Trichophyton mentagrophyte and 1 case of Trichophyton rubrum. Of the yeasts, Candida parapsilosis was the most common isolate (47%). Of the 22 positive cultures, 14 cases (64%) were obtained from finger nails (yeast n=13, mould n=1), 7 cases (32%) were obtained from toe nails (yeast n=5, dermatophyte n=2) and 1 case (4%) involved both the finger and toe nails (dermatophyte). Overall, the growth of yeast from the microdrill powder specimen was considerably heavier, in the order of 8 to 10 times the quantity seen in conventional nail clipping/scraping culture, in over two thirds of the fungal culture plates (Figure 3). One case of Candida parapsilosis also grew Aspergillus terreus.

**Discussion**

Many studies in the past have advocated the use of the microdrill technique with documented success in obtaining high fungal culture yeild. The microdrill instrument is inexpensive, readily available and its use requires little practice and no special expertise. The most important risk using the instrument is transmission of infection including Human-Immuno Deficiency virus (HIV), Hepatitis B and Hepatitis C virus. This risk can be minimized by autoclaving the drilling needle after each use and by adjusting the speed and depth of the instrument, and drilling at an obtuse rather than an acute angle, so as to avoid bleeding. The drilling itself is painless. However, it can at times be time consuming especially when the nail is relatively hard; drills slowly, taking an appreciable amount of time to collect an adequate amount of powder. In the present study patients tolerated the procedure well. In the study by Heikkila, which is the most recent evaluation to directly compare fungal culture by nail clipping to microdrill, 47% of the cases were culture positive and 50% were microscopy positive. Eighty-threepercent of the culture positive cases grew dermatophyte species, using both specimen collection methods. Yeast and moulds yielded positive cultures with similar frequencies using both clipping and microdrilling. However, 7 additional cases of dermatophyte were isolated by nail clipping. Almost all dermatophytes grown on fungal culture (94%) were detectable on microscopy, in contrast to only 44% of yeasts/molds. The difference in the rate of culture positivity using both methods in the present study versus the study of Heikkila may reflect the difference in prevalence of yeast infection in the study populations. The increased dermatophyte culture yield by nail clipping over microdrilling in Heikkila study was not observed in the present study. However, the number of dermatophytes in the present study were too small (only 3 cases) to draw any firm conclusions. All dermatophytes grown from culture in the present study were positive by microscopy while all yeasts, with the exception of one case, were associated with negative microscopy. The low frequency of positive microscopy (17%) in the present study reflects the high prevalence of candidal infection (82.7% of all culture positive cases) which is less frequently detected on microscopic examination. These findings reinforce the importance of performing fungal culture in addition to microscopy so as to increase the diagnostic yield in the event of a yeast infection of the nail, which is likely to be missed by performing microscopy alone. One may expect to increase the KOH microscopic detection by microdrilled specimen as it is assumed to be a better representative specimen quantitatively as well as qualitatively. However, no statistically significant difference in microscopy was noticed between the two methods. A possible
explanation for this may be the excessive destruction of the mycelial forms of the organism produced as a result of tissue grinding thus resulting in difficult identification at microscopy. An important finding in the present study was the heavy growth of organisms in specimens collected by microdrilling. Possible explanations for heavy fungal growth from microdrill specimens include the following: 1) the microdrill specimens are larger than the nail clippings, so grow more fungi; 2) if a piece of nail that contains viable fungi is cut into small pieces, each piece will give rise to a fungal colony. Therefore, the more pieces it is cut into, the more colonies will grow from it. The more colonies grow from it, the heavier the growth will appear on an agar plate. The maximum number of pieces will be obtained by grinding it into a fine powder. Culture of a fine powder will therefore result in heavier growth than culture of nail clippings and 3) by using the microdrill technique, fine powder is obtained from the edge of the growing infection as well as from the deeper proximal layers of the nail plate where fungi grow most actively. 17,19-21 Hence it provides a more comprehensive sampling of the specimen being collected and theoretically should yield an increased quantity of organisms. This was confirmed in the present study which demonstrated a consistent and marked increase in the quantity of organisms on fungal culture plates with microdrill specimens. Onychomycosis is mainly caused by dermatophytes, but non-dermatophyte moulds and yeasts do play a role in its etiology. 28,29 In our study, yeasts were the dominant isolate with Candida parapsilosis being the most common. This is similar to studies conducted in Asia27,30, Australia31 and Saudi Arabia32 where Candida is also a frequent cause of onychomycosis. The reason for this could be ritualistic washing of hands and feet which is a common religious practice in the Middle East and Asia, as well as the hot and humid weather in these parts of the world. Moreover, in the Middle East and Asia the process of washing clothes is commonly undertaken by hand thus predisposing to the development of onychomycosis, especially among women. These cultural practices may explain the high proportion of females presenting with onychomycosis (65% of cases) in this study, as well as the increased proportion of cases involving the finger nails (61% of cases).

References