Histopathology of Nail Clippings in Onychomycosis

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Abstract

Objective: To demonstrate histopathologic findings of nail clipping specimens in patients with onychomycosis.

Material and Methods: Patients with clinically suspected onychomycosis were enrolled in this study. After cleansing affected nails with 70% ethyl alcohol, the most proximal part of abnormal nail and subungual keratinous debris were collected by sterile nail clippers and scalpel blade scraping. Each specimen was fixed in 10% formalin solution and processed for routine histologic examination. Hematoxylin-eosin (H&E), and special stainings for fungus by periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS) were applied. Slides were examined for hyphae using light microscopy. Concomitant fungal culture was performed in each case.

Results: 75 nail specimens were evaluated. Histologically, 30 specimens (40%) were positive for fungus demonstrated by PAS and GMS stainings showing fungal elements lying between laminae of nail plates.

Conclusion: Histopathologic examination of nail clippings can be used to diagnose onychomycosis. Procedure is painless, simple to perform and can be used as screening test of early diagnosis in cases of KOH-negative, clinically suspected onychomycosis before culture results are achieved.

Introduction

Onychomycosis is the fungal infection of the nails. It is very common and is up to 50% of all nail diseases.¹ The causative organisms include dermatophytes, yeasts, or non-dermatophyte molds.² Onychomycosis may affect the patients' emotion, social, and occupational functions, and can cost considerable health care expense.³,⁴

Onychomycosis especially due to dermatophytes needs long-term duration of systemic antifungal treatment. It is not only expensive but also carries potential side effects and it is necessary to diagnose the infection correctly. Patients with onychomycosis may present as onycholysis, subungual hyperkeratosis and/or change in nail color. However, it should be kept in mind that many nail diseases may clinically mimic onychomycosis, including psoriasis, lichen planus, and contact dermatitis⁵ which needed to be excluded. For these reasons, laboratory investigations for confirmation of mycosis are necessary.

Conventionally, direct microscopic examination using potassium hydroxide method (KOH) and fungal culture are used to diagnose onycho-
mycosis. These methods are technically difficult even when performed by experienced hands, and may yield false-negative results. Culture requires at least 4 weeks to confirm the causative agents, especially in nondermatophyte mold infection. In cases of which the common mycological examinations are negative but the clinical features are highly suggestive of onychomycosis, nail unit biopsy is recommended by some authors. However, this procedure is painful and can cause permanent damage to the nail plate. The nail clipping of detaching distal portions of the nails including the subungual keratinous debris can be performed without any surgical traumatization, and has now been used for histological diagnosis of onychomycosis in many laboratories. Several studies confirmed its high sensitivity and specificity, in comparison to those of the direct microscopy and the fungal culture.

Aim of this study is to demonstrate the histopathologic findings of nail clippings in patients with onychomycosis.

Materials & Methods

Patients of either sex from the inpatient and outpatient departments of Thammasat University Hospital with nail dystrophy clinically suggestive of onychomycosis, evaluated by a dermatologist (M.A.), were enrolled in this study. Normal nail samples were included as the negative controls.

After cleansing of the affected nails with 70% ethyl alcohol, the full-thickness nail clippings were obtained with sterile standard dual-action nail clipper. Each specimen was clipped at the most proximal point of the nail’s separation from the nail bed. Subungual keratinous debris, if any, was collected by scalpel blade scraping. Histologic examination and fungal culture were performed in each sample.

For histologic examination, nail clippings were placed in 10% formalin solution and processed for routine histology. After embedded in paraffin blocks and sectioned at 4 μm, the sections were stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS) and Gomori methenamine silver (GMS), following the standard staining protocols. Each histologic slide was examined for fungal elements under light microscope by a pathologist (D.S.) who was blinded to the clinical findings.

For microbiologic studies, nail clipping specimens were plated on Sabouraud’s dextrose agar, Sabouraud’s dextrose agar plus cycloheximide, and Sabouraud’s dextrose agar plus chloramphenicol and incubated using standard mycological technique. Culture specimens were checked periodically for growth for at least 4 weeks by a microbiologist who was unaware of the results of the other tests. Cultures were considered positive if a dermatophyte grew. Cultures yielding non-dermatophyte molds or yeasts were considered positive if the same organisms growing on a second culture were obtained from the portion of the original specimens reserved in the sterile container. Species were identified for each positive culture by means of light microscopy and subculture, if required.

Results

75 nail clippings obtained from 68 patients were enrolled in the study. Among these, 15 specimens were from the clinically normal nails, submitted as the negative controls. Histologically, 30 specimens (40% of all, 52% of clinically abnormal nails) were positive for fungus. The fungal hyphae or yeasts were visible by H&E stain in few cases. In all positive cases, PAS and GMS stainings demonstrated fungal elements lying between laminae of the nail plates (Fig. 1-3). These fungal filaments stain bright pink by PAS staining and stain black in sharp contrast to the green background by GMS staining. In some cases, the included subungual keratinous debris contained the same-appearing organisms as in the nail plate tissue (Fig. 3B).
Fig. 1 Presence of brightly pink fungal filaments invading the nail plate. The culture in this case was positive for \textit{Aspergillus niger}. (PAS x 200)

Fig. 2 The hyphae stain black in contrast to the greenish nail lamina. (GMS x 400)

Additionally, coccal and short rod bacteria were found in the nail anfractuosities in 3 cases (Fig. 4). However, their cultures for bacteria were negative. There were 14 cases yielding bacteria on cultures but histology-negative. Among these, concomitant growth of molds was found in 5 cases, and 4 with concomitant yeasts.

22 specimens were positive for fungus by both histology and culture. The identified organisms were summarized in Table 1. Our results showed a significantly larger proportion of nondermatophyte fungi (21 cases, 96%). Among these, 18 cases (73%) were nondermatophyte molds and 5 (23%)
**Table 1** Organisms identified from the histology-proven positive samples

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of Specimens</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus species</em></td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Non-sporulate hyaline mold, NOS</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td><em>Candida non-albicans</em></td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Dematiceous fungus</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>22</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

NOS, not otherwise specified

were yeasts. Only 1 specimen yielded dermatophyte (*Trichophyton rubrum*).

All 15 samples of the clinically-normal nails were negative for fungus by both histology and culture techniques.

**Discussion**

Onychomycosis needs accurate diagnosis in order to avoid the inappropriate treatments, such as unnecessary, long-term systemic antifungal therapy, which is not only expensive but also carries the potential side effects. Direct microscopy of the KOH preparation and fungal culture are their essential diagnostic tests. In cases of clinically suspected onychomycosis with negative routine-test results, nail unit biopsy has been recommended as the adjunctive diagnostic tool. Various techniques of nail unit biopsy have been described, including punch biopsy, distal nail avulsion, and nail plate biopsy, depending on the part of the nail unit affected. These techniques require local anaesthesia and application of tourniquet at the base of digit to achieve complete homeostasis. Because these procedures are time-consuming, possibly painful, and potentially disfiguring, they have not been widely used.

Processing of distal nail clipping for detection of invading fungi was first described by Jillson and Piper in 1957. Their studies suggested that the small, detached pieces of distal part of nail provided equivalent histopathological information regarding the existence of mycotic nail infections when compared to the samples from entire nail. The procedure is painless and requires only simple equipment, such as nail clipper, scalpel blade, and curette. The yielded samples can be used for fungal culture as well. Several studies confirmed that, for diagnosing onychomycosis, histology of nail clippings yielded the highest sensitivity and specificity, in comparison to those of the direct microscopy and the fungal culture. Some institutes now use it as the routine investigation for onychomycosis.

In our study, although H&E can demonstrate the fungi occasionally, its sensitivity is very low. In the literature regarding evaluation of nail histology for diagnosing onychomycosis, PAS was the most common useful staining to identify the organisms. As seen in the histologic study of any other tissues, the fungal elements in the nail plate and in subungual debris stain brightly pink (Fig. 1, 3). The other stain, GMS can demonstrate the fungi in nail clippings as well. As far as we could determine, ours is the first study to apply the GMS stain for detecting fungi in nail clippings. The silver salt stains fungi black, in
contrast to the greenish nail tissue background (Fig. 2).

By fungal cultures, not only the causative but also contaminant organisms can be grown, resulting in the low specificity and high false-positive rate. In contrast, the histological examination can demonstrate the distribution of the fungi in nail tissue providing evidence of their invasiveness, in addition to the morphology, of the organisms.

The bacteria on nail plate tissue, as seen in Fig. 4, are generally considered as contamination in spite of the fact that bacteria can constantly associate with fungi, particularly in nondematophytes. These findings may suggest a possible synergistic effect between these two groups of microorganisms. It is also recommended that, before nail clipping, cleansing with alcohol to eliminate as many bacteria as possible is very important. Nevertheless the organisms may still localize in the anfractuous nails, as in our study.

The limitation of nail histopathological study is the inability to identify the species of pathogens which could affect the treatment, as in cases of nondematophytes resistance to certain drugs. However, it can be used as a screening test, particularly in the cases of KOH-negative, clinically suspected onychomycosis while waiting for the culture results.

**Conclusion**

Histologic examination of nail clippings can be used to diagnose onychomycosis. Special stainings as PAS and GMS stainings highlight the fungal elements lying between laminae of the nail plate. Results are quickly obtained and can be applied to any clinical situations without delay, before the culture results are available. The procedure is painless, and can be performed with simple equipment. It can be used as a screening test, especially in the cases of KOH-negative, clinically suspected onychomycosis while waiting for the culture results.

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**References**

บทคัดย่อ

ลักษณะทางกายภาพของขี้มอ่อนในโรคขี้ขาวที่ไทย

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วัสดุและวิธีการ:
เพื่อศึกษากลยุทธ์ทางกายวิทยาของขี้มอ่อนผู้ป่วยโรคขี้ขาวที่ไทย

วัสดุและวิธีการ:
เก็บตัวอย่างขี้มอ่อนจากกลุ่มตัวอย่างในกลุ่มที่มีการติดเชื้อ ซึ่งถูกใช้ตกค้างสกุลของตัวเย็นสูง
และขี้มอ่อนที่มีการติดเชื้อ การติดเชื้อในผู้ป่วยที่มีการติดเชื้อ ซึ่งถูกใช้ตกค้างสกุลของตัวเย็นสูงสุดสิบ
โดยผลการวิเคราะห์รุ่น 10% ความรู้ทางกายวิทยาทางกายวิทยา โดยข้อมูลที่ได้จากการติดเชื้อ
และข้อมูลเชื้อ คือ PAS และ GMS แล้วตรวจหาเชื้อหัวขาดกลุ่มที่รังสีในทุกครั้งต่อ 1-2 เดือน

ผลการวิจัย:
จาก 75 ตัวอย่างมี ตัวเย็นของโรคติดเชื้อ 40% ท้อง PAS และ GMS

สรุป:
การตรวจขี้มอ่อนสามารถยืนยันได้ว่ามีการติดเชื้อที่ไทย การตรวจไม่เจอความเจ็บป่วย และไม่
มีการติดเชื้อในกลุ่มตัวอย่างกลุ่มโรคขี้ขาวที่ไทย การตรวจไม่พบความเจ็บป่วย และไม่
มีการติดเชื้อในกลุ่มตัวอย่างกลุ่มโรคขี้ขาวที่ไทย การตรวจไม่พบความเจ็บป่วย และไม่
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