The Role of Histological Examination of Nail Clipping in the Diagnosis of Onychomycosis

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ABSTRACT **Background:** Diagnosis of onychomycosis is based on potassium hydroxide (KOH), direct smear, culture, and polymerase chain reaction. Nail clippings are rarely used as a diagnostic tool.

Objectives: To evaluate nail clippings for the diagnosis of onychomycosis and to compare it to KOH smears.

Methods: Nail clipping specimens of 39 patients were collected: 34 with onychomycosis proved by positive culture and 5 from normal nails. The specimens were submitted to histological processing and then stained with periodic acid–Schiff (PAS) and Grocott-Gomori's methenamine silver (GMS) stains. For each nail, KOH smear was also performed. Two pathologists who had no information on the KOH smear and the culture results evaluated the nail clipping histology for the presence of fungal element. Their assessment was compared to the KOH smear and culture results.

Results: Of the 34 specimens that had positive culture, 25 were dermatophytes, 5 were molds, and 4 were candida. Clipping specimens were positive in 30 cases (88%): 23/25 dermatophyte, 4/5 molds, and 3/4 candida. Pathologists were able to classify the pathogens into dermatophytes and non-dermatophytes based on the morphology. PAS stain results were the same as GMS in evaluation of the nail specimen. KOH smear was positive in 29 nails (85%): 20/25 dermatophytes, all 5 molds, and 4 candida. In all five nails where the culture was negative, both clipping and KOH smear did not show fungal elements.

Conclusion: Nail clippings can serve as a rapid, inexpensive, and reliable method for evaluation of onychomycosis, comparable to KOH smear, with the advantage of pathogen group identification.

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KEY WORDS: fungal culture, nail clipping histological examination, onychomycosis, potassium hydroxide (KOH) smear

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Fungal infection of the nails is a very common disorder that is accountable for over 50% of all abnormal nails [1]. The causative agent is mostly dermatophytes but can also be yeasts or non-dermatophyte molds [2]. In approximately 90% of the cases, the cause of onychomycosis is the anthropophilic dermatophyte: *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Non-dermatophyte molds and yeasts are much less common [3]. Predisposing factors for onychomycosis are old age, sex, genetic susceptibility, co-morbidities (e.g., diabetes mellitus, peripheral arterial disease, and obesity), dermatologic diseases (e.g., psoriasis and tinea pedis), trauma, and smoking [4,5].

Signs of onychomycosis include discoloration, subungual hyperkeratosis, and onycholysis. There are seven clinical patterns of onychomycosis. The clinical subtype is derived from the mode and the site of the fungal penetration into the nail plate. The common clinical patterns of onychomycosis are distal and lateral subungual onychomycosis (DLSO), white or black superficial onychomycosis (SO), and proximal subungual onychomycosis (PSO). Additional clinical patterns are endonyx onychomycosis (EO), total dystrophic onychomycosis (TDO), secondary onychomycosis, and mixed pattern onychomycosis (MPO) [6].

Following the clinical diagnosis of onychomycosis, common practice dictates mycological confirmation prior to treatment selection and initiation. Mycological diagnosis is essential to identify the assaulting fungi and offer the most appropriate treatment. Treatment of onychomycosis, especially dermatophytes, is long and requires systemic antifungal agents. The treatment is expensive, including healthcare expenses, and may cause side effects, especially on liver function and drug interactions. Thus, the diagnosis should be made correctly [7,8].

The previous gold standard for the diagnosis of onychomcyosis combined a positive fungal culture and a positive direct microscopy of a smear treated with potassium hydroxide (KOH) that was be correlated with the clinical findings. In recent years, polymerase chain reaction (PCR)-based detection of specific fungal DNA sequences has replaced this combination. The culture requires 3–4 weeks until the causative agent can be con-

firmed. Both culture and smear preparation and interpretation need experienced stuff. If not properly performed, it can yield false negative results. The PCR-based method is mostly used for dermatophytes. It does not detect non-dermatophyte (molds/yeast). It is relatively expensive and takes at least a week for results as pooling of samples is the common practice. [5,7-11]. Some authors recommend nail biopsy when the result is negative, but this can cause permanent damage to the nail plate due to the damaged nail bed [12].

A newer examination method is the ex vivo confocal laser scanning microscopy (CSLM) with similar sensitivity to PCR, but lower specificity. CSLM is faster than PCR but is more expensive [13].

Nail clipping is a procedure that detaches only the distal part of the nail, including the debris underneath, and does not cause any nail bed trauma. It is used for the histological diagnosis of onychomycosis in several laboratories. The sensitivity of this method has been confirmed in several studies and ranges from 81% to 93%, compared to direct microscopy (48%–80%) and culture (26%–70%) [Table 1] [14-23]. In addition, one of the major advantages is the fast results, which can be obtained within 48 hours.

The aim of this study was to evaluate the role of nail clipping specimens in the diagnosis of onychomycosis and compare it to KOH smear, another method in which results can be obtained in a short time.

PATIENTS AND METHODS

This prospective case series included 39 patients who were referred to the dermatology outpatient clinic at the Sheba Medical Center; 34 were referred due to a clinical picture suggestive of onychomycosis and 5 clipping specimens were taken from volunteers with normal appearing nails. After obtaining material for KOH smear and fungal culture from the offended nail, clipping of the distal deformed nail plate was performed and the specimen was submitted for routine histological processing using hair removal cream. First, nail clipping specimens were soften using hair removal cream (Veet®). Next, they were fixed with formalin and embedded in paraffin. We collected 4 µm sections of the formalin fixed, paraffin-embedded specimens, which were stained with periodic acid–Schiff (PAS) and Grocott-Gomori's methenamine silver (GMS) stains [24]. Evaluation by two pathologists who had no information of the KOH and the culture results was performed. Their assessment of the clipping stained slide for the presence of fungi elements and their nature was compared with the KOH direct smear and cultures results. The latter, with the clinical data, served as a definite diagnosis of onychomycosis.

Differences in results between diagnostic methods were tested using Fisher's exact test.

RESULTS

A total of 24 men and 15 women were included in the study. Ages ranged from 3 to 98 years. In general, good correlation was found between the clipping results, the smears, and the cultures. For the five normal appearing nails, both cultures, KOH smears, and nail plate clipping specimen were negative for fungi, resulting in a specificity of 100%. Of the 34 specimens that had positive cultures, 25 were dermatophytes, 5 were molds, and 4 were candida. KOH smear was positive in 20 of the 25 dermato-

Table 1. Comparison of published studies comparing nail clipping histological examination to gold standard diagnostic methods in onychomycosis

Study (year, journal) [reference]	Total number of samples	Histological examiniation (Sensitivity %)	Direct microscopy (Sensitivity %)	Culture (Sensitivity %)
Lawry et al. (2000, *Arch Dermatol*) [16]	63	85%		32%
Gianni et al. (2001, *Dermatology* (Basel) [15]	172	54.6%*	59.3%*	52.9%*
Weinberg et al. (2003, *J Am Acad Dermatol*) [17]	105	92%	80%	59%
Reisberger et al. (2003, *Br J Dermatol*) [18]	387	47%*	40.3%*	25.8%*
Karimzadegan-Nia et al. (2007, *Australas J Dermatol*) [19]	96	80.8%	76.5%	53.2%
Shenoy et al. (2008, *Indian J Dermatol Venereol Leprol*) [23]	101	90%	64%	42%
Wilsmann-Theis et al. (2011, *J Eur Acad Dermatol Venereol*) [14]	1146	82%	48%	53%
Jung et al. (2015, *Clin Exp Dermatol*) [21]	493	A 93.1% B 88.2%	B 55.9%	A 49.5% B 29.4%
Jeelani et al. (2015, *Mycoses*) [22]	216	91.62%	77.09%	70.39%

*% of positive tests from all the nail samples

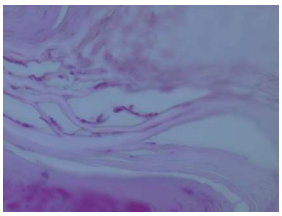
Table 2. Patient characteristics

	Number of patients, positive (n=34)	Number of patients, negative (n=5)	Number of patients, total (n=39)
Sex			
Male	22	2	24
Female	12	3	15
Age, years (missing, n=5)			
> 65	12	1	13
< 65	17	4	21
Potassium hydroxide (KOH) smear	29	0	29
Nail clipping	30	0	30
Nail clipping features			
Septate hyphae	26	-	26
spores	8	-	8
Pseudo/delicate hyphae	7	-	7

phytes, and positive in all 5 molds and four candida with overall sensitivity of 85%. The clipping specimen were positive in 23 out of the 25 dermatophytes and in 4 of 5 molds, and in 3 of 4 candida with overall sensitivity of 88% [Table 2, Figure 1]. For the 25 dermatophytes offended nail, KOH smears were positive in 20 specimens (80% sensitivity) and clipping specimen in 23 (92% sensitivity) (*P*-value = 0.03). Concordance between smears and clipping specimen was observed in 20 cases (80%), in 19 both were positive and in 1 negative. For candida and molds onychomycosis, KOH smears were superior to clipping (all positive for smears vs. 3/5 for molds and 3/4 for candida).

. Nail clipping specimens of dermatophyte showing fungal elements characterized by thick septate hyphae (×400)

[A] Periodic acid–Schiff



[B] Grocott-Gomori's methenamine silver

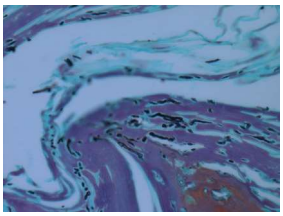
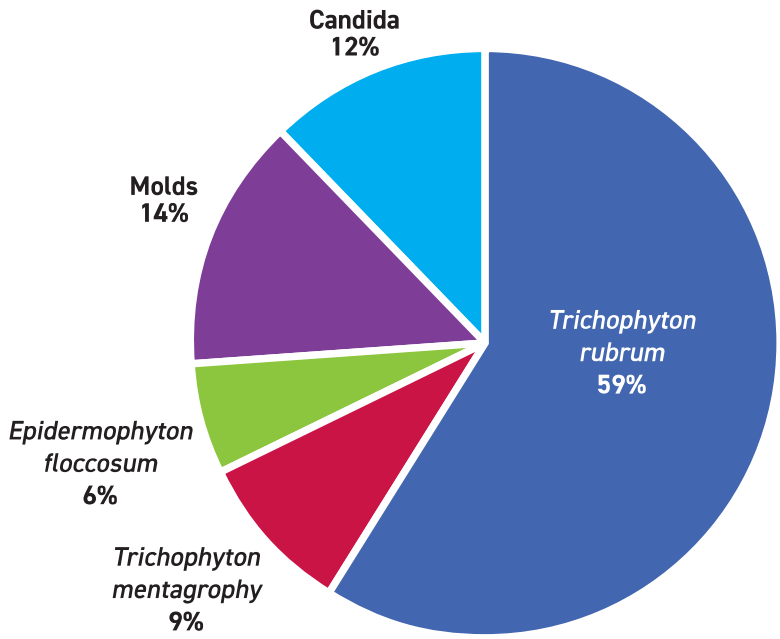


Figure 1. Percentages of the species identified with fungal cultures



In addition, pathologists were able to classify the pathogens based on the morphology of the fungal elements: thick septate hyphae were typical for dermatophytes, pseudohyphae, budding yeasts, and spores for candida and thin septate hyphae with or without spores for molds [Table 2]. Both PAS and GMS stains of the clipping specimens adequately identified the fungal elements.

DISCUSSION

Accurate diagnosis of onychomycosis is of major importance when systemic therapies, which potentially have serious adverse effects, are given for a relatively long period or to exclude other causes of nail changes. Diagnosis is based on the clinical appearance, direct microscopy, and fungal culture. In recent years, detection by PCR amplified specific fungal DNA sequences. Either one of those methods combined with the clinical findings serve as the gold standard for diagnosing onychomycosis in common practice [11]. Both methods require qualified personnel, special equipment, and a long duration until result is obtained (at least one week for the PCR-based method and a few weeks for the culture). To overcome these issues, KOH smear, which is simple and cheap, can be conducted at the point of care, and results are available in less than an hour. Yet, its detection rate is inferior to cultures [17]. Another method to detect fungal elements in the nail is nail clipping histology which is a rapid, inexpensive, reliable, and sensitive method to diagnose onychomycosis. In the present study we compare these two fast and simple methods to diagnose onychomycosis when our gold standard indicated a positive culture. For our cohort, KOH smear and nail clipping material were comparable in diagnosing onychomycosis (85% vs. 88% sensitivity, respectively) and both had a specificity of 100%. Yet, for onychomycosis caused by dermatophytes, nail clipping was clearly superior to KOH smear (92% vs. 80%, respectively). Furthermore, the nail clipping specimen enabled distinction between dermatophyte, candida, and molds, and thus indicated the therapeutic choice.

Our results of a minor superiority of nail clipping to KOH smear in the diagnosis of onychomycosis caused by dermatophytes, non-dermatophyte molds, and yeasts correspond with most of the reports from the literature [14,16-18,21-23] [Table 1]. Notably, unlike our study where cultures were the gold standard (the 100%), in these studies, nails that were clinically suspected for onychomycosis were submitted to at least three methods: KOH smear, nail clipping histology with PAS stain, and fungal culture. Nail clipping specimens showed the highest sensitivity rate (approximately 90%) followed by KOH smears and positive culture. In contrast to these studies, Gianni and colleagues [15] reported that direct microscopy was positive in 59.3% of the cases, whereas nail clipping histological examination was positive only in 54.6% of the cases and cultures were positive in 52.9% of the cases. Karimzadegan-Nia et al. [19] found that sensitivity of nail clipping histological examination was not statistically different from KOH smear (80.8% vs. 76.5% sensitivity, respectively). Thus, all studies note that nail clipping histology is at least comparable, if not superior, to KOH smears. Furthermore, when considering the therapeutic implications of this method, that nail clipping specimens are often positive when either cultures are negative or antimycotic therapies were applied prior to the examination and that its assessment enables distinction between dermatophyte, yeasts, and molds [14,15,18,20,22,23]. There is no doubt that this rapid, inexpensive method has its role in the diagnosis of onychomycosis.

For non-dermatophyte onychomcyosis, both candida and molds, our finding indicated that KOH smear was better (100% vs. 67% sensitivity, respectively). Although this finding may reflect the small sample size for candida and molds, nail clipping specimens should be interpreted with caution because pathogens and combination of both KOH and clipping with PAS stain may be employed as reported by Kang et al [25].

There are some limitations in our study, including the small number of specimens and the absence of a group of onychomcyosis that had negative culture. However, we compared the two fast and inexpensive methods. In addition, the nail histopathological study, KOH smear, and PCR-based method, are limited due to the inability to identify the fungal element viability. The presence of fungi may not be the cause of the nail disease.

CONCLUSIONS

Nail clipping can serve as a rapid, inexpensive, and reliable method for evaluating onychomycosis, which comparable to KOH smear and even better than for dermatophytes. When examined by an experienced pathologist/dermatopathologist, the categorization of the causative fungus is possible, thus enabling the treatment decision.

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Capsule

Neutrophils cast NETs without dying

Neutrophil extracellular traps (NETs) bind to and kill microbes. **Stojkov** and associates found that, contrary to findings from previous studies, agonists that induced NET formation independently of multiprotein complexes called inflammasomes did not require the pore-forming protein gasdermin D (GSDMD) and did not cause cell lysis leading to cell death. Furthermore, canonical inflammasome activation caused GSDMD processing but was not required for NET release. These data suggest that the mechanisms leading to NET formation require further study and should be distinguished from those that lead to cell death.

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Capsule

Off-the-shelf T cell therapy

Multiple myeloma is characterized by the accumulation of malignant plasma cells in the bone marrow. Various B cell maturation antigen (BCMA)–targeted drugs are approved for the treatment of relapsed/refractory multiple myeloma, including an engineered chimeric antigen receptor (CAR) T cell therapy. However, extracting T cells from individual patients to produce autologous CAR T cells presents numerous challenges. ALLO-715 is an allogeneic, off-the-shelf BCMA-targeted CAR T cell therapy. **Mailankody** and co-authors reported interim results from the ongoing first-in-human phase 1 trial of ALLO-715 in 43 patients with relapsed/refractory multiple myeloma. CAR T cell regimens are associated with adverse events that are treatable if managed early. ALLO-715 treatment resulted in grade 3 or higher adverse events in 88% of patients, but 70.8% of patients had a partial response or better with a median duration of 8.3 months. Therefore, ALLO-715 treatment appears to be feasible and could be beneficial for treating multiple myeloma.

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