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Original article

A study to compare and classify etiological agents of onychomycosis

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ABSTRACT

Background: Onychomycosis, the condition that was always regarded as merely a cosmetic problem, now has become an important medical issue. Etiological prevalence of onychomycosis varies subject to the population demography, geographical location or the mycological technique used for the diagnosis.
Aim: This study aims to identify and classify the fungal etiology of onychomycosis from a tertiary care hospital of north India over a duration of two years.
Methods: The study included 169 clinically suspected patients of onychomycosis. The fungal etiology was determined by direct examination in 20% KOH after overnight incubation at 37 °C and culture on Sabouraud's Dextrose Agar.
Results: 135 (79.9%) out of the total patients were confirmed cases of onychomycosis as per the diagnostic criteria. Hyaline hyphomycetes were the most common group of fungi isolated (50.0% of all fungal isolates) with only 15.4% isolates of the classical dermatophytes. A positivity of 18.3% and 78.7% by KOH examination and culture respectively was reported. .A sensitivity and specificity of 21.48% and 100% was found for KOH positivity; though culture was 98.52% sensitive and 82.35% specific.
Conclusions: Hyaline hyphomycetes are a common cause of onychomycosis. KOH examination can not be relied upon for diagnosis of onychomycosis, for which fungal culture appears to be an appropriate choice along with KOH examination.

1. Introduction

Onychomycosis remains an extremely obstinate and frequent fungal infection of the toenails or fingernails that can hugely impact the patients' physical, social or emotional quality of life. The condition, that was always regarded as merely a cosmetic problem unworthy of any significant medical attention; now has become an important medical issue due to recurrence, chronicity, probability of transmission, therapeutic difficulties or association with immunosuppression [1]. Infected nails also are a potential reservoir of fungi which can spread to other sites of the patient [2].

Though the prevalence of onychomycosis varies subject to the population demography, geographical location or the mycological technique for diagnosis, an estimated 3–26% of the global population are affected by this significant health problem which accounts for approximately half of all onychopathies [3,4]. In a previous study done at our tertiary care centre the prevalence of onychomycosis was confirmed in as high as 45% of the analyzed patients [5]. Dermatophytic filamentous fungi by far remain the predominant etiology in most of the cases of onychomycosis, though yeasts and non-dermatophyte molds (NDM) are now implicated as the other common causes accounting for approximately 8–11% and 1.45–17.6% cases respectively [3,6,7,8].NDMs are increasingly reported as secondary or emerging causes of onychomycosis triggered by various factors like traumatic nails, comorbidities leading to compromised circulation or immunocompromised states [9].

Despite optimal management by the current antifungal regime, clinical as well as mycological cure is often unattainable in all onychomycosis cases [10]. The reasons for the limited success in cure rate could be many, some of which are incorrect identification of the fungal pathogen, co-existent co-morbidity, inherent characteristics of the nail which worsen the response to treatment or a plethora of host related factors [1]. Several non infectious causes of nail dystrophies mimicking onychomycosis further complicate the diagnosis [11]. Onychomycosis can be divided into several categories based upon the site involved, clinical pattern, mode of invasion or fungal etiology [12]. Such a classification aids in initiating a correct diagnosis and predicting the response to treatment and its prognosis. A laboratory confirmed clinical

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diagnosis with the identification of the underlying fungal pathogen further helps to exclude non fungal etiology or a mixed infection and hence guide appropriate antifungal treatment especially in less responsive cases of onychomycosis. Onychomycosis continues to be a diagnostic dilemma for a dermatologist and a microbiologist as the etiology spectrum is diverse and the techniques for diagnosis are still evolving. This study aims to identify and classify the fungal etiology of onychomycosis from a tertiary care hospital of north India over a duration of two years.

2. Materials and methods

This was a retrospective study spanning a period of two years (January 2014–December 2015) conducted in the Mycology Laboratory, Department of Microbiology of a tertiary care hospital in New Delhi, India. The study included 169 patients, who presented with the signs and symptoms representative of the fungal infection of either toe or finger nails (onychomycosis). The affected area was cleaned with 70% ethanol and allowed to dry. Nail clippings along with the deep sub-ungual material were collected from the most affected nails. The specimens were transported to the Mycology laboratory as soon as possible in a dry, sterile, screw capped container.

Nail specimens were incubated overnight at 37 °C in 20% KOH; next day wet mounts were prepared and observed under 100 x and then 400 x light microscopy magnification to look for presence of fungal hyphae and/or yeast cells. "Specimens were inoculated onto two sets of sealed tubes: (i) Sabouraud's Dextrose Agar and (ii) Sabouraud's Dextrose Agar with chloramphenicol (0.05 mg/ml); one tube of each set was incubated at 25 °C and another at 37 °C." The culture tubes were examined twice a week for any suspected fungal growth. Tubes showing no growth were discarded after a period of 6 weeks. Isolates were identified by the morphologic characteristics of the colony growth, pigment production and microscopic examination of the Lactophenol cotton blue (LPCB) preparation. In case identification was not possible, slide cultures were put up using corn meal agar to stimulate sporulation and incubated at 25 °C for 4–7 days. Subsequently, LPCB preparations were made and observed under light microscope.

Onychomycosis was diagnosed when fungal elements were seen on 20% KOH preparation, and/or repeated cultures or growth in multiple tubes yielded same fungal agent. Repeat samples were collected and processed when direct KOH wet mount was negative and fungal growth was obtained in a single tube, raising suspicion of contamination. Other fungal growths were labeled as contaminants and treated as false positive cultures for statistical calculations.

2.1. Statistical analysis

The data was presented as percentages and proportions. 95% confidence intervals were calculated wherever applicable. Fischer's exact test was used for testing the statistically significant differences; p values of <0.05 were considered statistically significant. All calculations were done using SPSS version 23.0.

3. Results

A total of 169 patients were involved retrospectively in the study, including 98 (58.0%; 95% CI = 50.45%–-65.17%) males and 71 (42.0%; 95% CI = 34.83%–49.55%) females. Majority of patients were adults (>18 years of age), with only 11 (6.5%; 95% CI = 3.55%–11.40%) subjects being under 18 years of age; maximum patients belonged to 20-30 years of age group (Fig. 1). Majority of patients (60.4%) had fingernail infection, 37.8% had toenail infection, while 1.8% patients had infections of both toenail and fingernail.

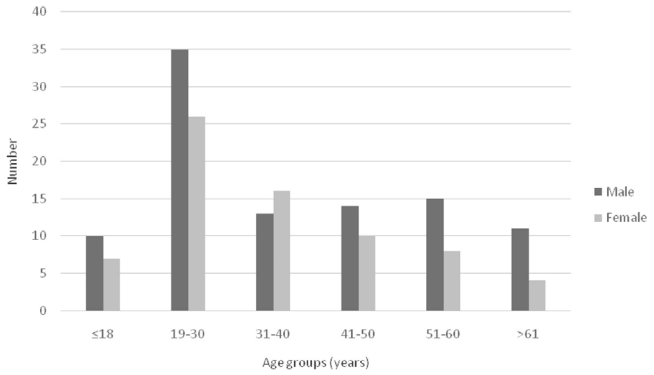

Fig. 1. Age-wise distribution of male and female patients.

KOH wet mount of the nail clippings and material collected deep under nail bed were prepared; out of 169, a total of 31 (18.3%; 95% CI = 13.19%–24.90%) specimens showed presence of fungal elements (fungal hyphae and/or yeast cells). Out of these 31 specimens, 29 turned out to be positive on culture; while 2 showed no growth even after 6 weeks of incubation (Table 1). Twelve KOH positive specimens yielded growth of dermatophytes, while remaining showed non-dermatophytic growths. A total of 133 (78.7%; 95% CI = 71.89%–84.23%) specimens showed growth on SDA, some showing co-infections of two or more fungi; with a total of 156 fungal isolates. Thus, 135 (79.9%) patients were diagnosed as cases of onychomycosis. KOH mount preparation had a low sensitivity of 21.48% as compared to the culture method (98.52%). As per the criteria, six cultures were labeled as contaminants/false positives.

Isolated fungi were classified as shown in Table 2. Hyaline hyphomycetes were the most common group of fungi isolated, constituting 50.0% (78/156) of all fungal isolates. *Aspergillus niger* and *A. flavus* were the most common fungi in this group as well as among all groups. Twenty-four (15.4%) isolates of the classical dermatophytes were cultured, *Trichophyton spp. (T. schoenleinni, T. verrucosum, T. mentagrophytes* and *T. rubrum*) being the most common. Members of Class Zygomycetes (now Class Glomerulomycetes) and the dematiaceous fungi were relatively less common, constituting 13.5% and 9.6% of fungal isolates, respectively. *Rhizopus spp.* and *Mucor spp*. (9 isolates of each) were common fungi of Class Zygomycetes. Dematiaceous fungi were least common of all the groups, constituting 9.6% of all fungal isolates. Among yeast-like fungi, non-albicans *Candida* (14 isolates) were isolated more frequently as compared to *Candida albicans* (3 isolates). A single isolate of *Geotrichum spp.* was also identified. Though, there were differences between males and females with respect to rates of isolation of these fungi (dermatophytes and yeast-like fungi being more common in males), the differences were not statistically significant (Table 2).

Fig. 2 depicts the month-wise rates of isolation of various groups of fungi; it is easily discernible that these rates were highest during the months of July to September. Highest isolation of hyaline hyphomycetes, dermatophytes and zygomycetes were seen during the months of July, August and October, respectively.

Twenty two specimens showing simultaneous growths of two fungi were considered as co-infections. The details are presented in Table 3.

4. Discussion

Onychomycosis is increasingly being recognized as a common problem in dermatological practice. Dermatophytes, yeasts as well as molds have all been implicated as potential pathogens in

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