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Original Research Article

A study on non-dermatophyte moulds as pathogens of onychomycosis

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Abstract

Introduction: Onychomycosis, a fungal infection of nails is ordinarily caused by dermatophytes non dermatophytes and yeasts. The isolation of non-dermatophyte moulds in onychomycosis specimens are being reported increasingly in different geographic regions. Therefore aim of this study was to determine the role and pattern of non- dermatophyte moulds as causative agents of onychomycosis.

Materials and Methods: The study was carried out from September 2017 to September 2018 and 70 samples were included in the study. KOH examination and culture have been carried out in all the samples. Moulds were identified based on colony morphology, microscopic examination with Lacto phenol cotton blue stain (LPCB) and slide culture & Yeasts were identified by Gram staining germ tube test, urease test and using Chrome agar.

Results: Out of 70 samples 42(61%) showed growth in culture, among this most common isolate belongs to non- dermatophyte fungi. Fusarium species (45%) was the most common among the non dermatophyte isolates followed by candida species. Curvulera, Acremonium sps, Fonsacea and Trichosporon species were the other non-dermatophyte moulds isolated.

Conclusion: Non-dermatophyte moulds should be considered as important pathogens with a high index of suspicion in evaluating the patients with culture negative for dermatophytes or those subjects ending up in treatment failure.

Keywords: Non- dermatophyte moulds, Dermatophytes, Onychomycosis, Yeast, Fusarium.

Introduction

Onychomycosis is defined as a fungal infection affecting finger and toe nails. It is a common nail infection caused mainly by two groups of pathogenic fungi, dermatophyte and yeast.[1] There is a tremendous increase in the isolation of Non-dermatophyte moulds (NDM) belonging to different genera and species as etiological agents of onychomycosis.

Predisposing factors such as ageing population leading to increase in chronic health problems such as diabetes, poor peripheral circulation, immunosuppressive therapies, antibiotics, sports participation leading to increase use of health clubs, communal swimming pools and occlusive foot wear exercise etc. lead to rise in onychomycotic infections.

Onychomycosis can be a source of pain and discomfort although it is not life threatening. Psychological and occupational problems can be generated by onychomycotic infections and can impair patient's quality of life.[3] Approximately 10% of the general population, 20% of the population aged >60 years, up to 50% of people aged >70 years and up to one-third of diabetic individuals have onychomycosis. Care should be taken for the accurate diagnosis and timely treatment of toe nail onychomycosis to prevent complications.[4]

The three major types of fungi—dermatophytes, saprophytes, and yeasts associated with onychomycosis are distinct in their morphology, physiology, and reproductive behaviors. The differences between the fungal species result in various treatment options for physicians and show different response to antifungal agents.

The current FDA-approved treatment of onychomycosis include oral medications, such as itraconazole, terbinafine, and griseofulvin, and topical medications, such as ciclopirox. Proper identification of the causative agent in the laboratory is required for initiating proper treatment. As the treatment of onychomycosis caused by dermatophytes may require long term therapy with an oral antifungal medication with potential side effects, it is essential to diagnose the infection correctly.[16]

Several studies indicates a continuous change in the epidemiological and mycological characteristic of onychomycosis in the same population. Causative agents of onychomycosis changes according to geographic differences. Therefore for making adequate strategies for the prevention and treatment of infection, awareness about these changing patterns and causative fungi is important.

Several clues about the infecting organisms can be obtained from the clinical presentation of onychomycosis, however at times, appearances caused by different fungal species may be indistinguishable. Therefore therapies are more effective only with correct identification of the causative fungus before initiating treatment.

Materials and Methods

The study was conducted in the Microbiology laboratory of a Tertiary care hospital setting for a period of one year from September 2017 to September 2018. Nail clippings, subungual debris and nail scrapings collected from proximal end of abnormal nails were used in this study. All samples were collected after thorough cleaning of the nail area with 70% alcohol to remove contaminant fungi. A total of 70 samples were received from Dermatology department in

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properly labeled sterile containers and all were examined by microscopy and culture.

Patients on treatment for nail abnormalities, patients under other antifungal treatment and pediatric age group patients were not included in the study.

Sample processing

The specimens were examined microscopically by KOH preparation. A part of this sample was dissolved in 20% potassium hydroxide (KOH) and examined directly under a light microscope for fungal hyphae/ elements/ yeast cells. The remainder was inoculated on (i) Sabouraud dextrose agar (SDA) supplemented with cycloheximide and chloramphenicol and (ii) SDA without cycloheximide supplement. Specimen was inoculated into the media using sterile loop by furrowing into the media with as much material as possible. These culture tubes were incubated at 37° C and 25° C respectively for 6 weeks. Culture tubes were examined daily for any growth. Moulds were identified based on colony morphology, microscopic examination with Lacto phenol cotton blue stain(LPCB) and slide culture & Yeasts were identified by gram staining,germ tube test, urease test and using Chrome agar.

Observation and Results

50 out of 70 samples examined microscopically using KOH preparation showed fungal elements. Of the 50 KOH positive samples, 11 yielded no growth in culture. Fungal growth were obtained in 42 samples (60%) and 27 samples yielded no growth in culture. Non-dermatophytic moulds were the common (52.3%) isolate as shown in Table 1. Among the non-dermatophyte isolate, Fusarium species were the most common followed by Aspergillus species and Curvuleria as shown in Table 2. One case with mixed infection of Fusarium and Curvuleria were also isolated.

Aspergillus *niger* was the predominant Aspergillus species isolated. Among the Dermatophytes,5 were Trichophyton *rubrum* and 2 were Trichophyton *mentagrophyte*.

Candida *albicans* was the predominant species of Candida isolate. From one case Trichosporon species was isolated.

Table 1: Isolate pattern obtained in the study

Isolates n=42	Number
Dermatophytes	7(17%)
Non-dermatophyte moulds	22(52.3%)
Yeast	13(31%)

Table 2: Non dermatophyte mould isolates

Isolates N=22	Number
Fusarium species	10(45%)
Aspergillus species	7(32%)
Curvuleria species	2(9%)
Acremonium species	2(9%)
Fonsacea species	1(5%)

Table 3: Yeast isolates

Isolates	Number
Candida *albicans*	4
Candida *parapsilosis*	6
Candida t*ropicalis*	2
Trichosporon species	1

Discussion

Fungal infections should be diagnosed timely for starting appropriate antifungal therapy. Inaccurate clinical diagnosis can prolong symptoms and discomfort of the patient and can also results in economic drain due to expensive antifungal therapy.

It is difficult to ascertain the role of Non-dermatophyte moulds as primary pathogens of the nails but studies are required to evaluate their ability to invade intact healthy nails. Non-dermatophyte moulds may colonize nails that are damaged by occupational related trauma especially in young age group, immunocompromised conditions, poor peripheral circulation and peripheral neuropathy. Definitive identification of non-dermatophyte as causative agents may require the isolation of the agent from successive specimens from the infected region.

Of the 70 specimens examined microscopically 50 showed fungal elements and fungus was isolated only in 39 samples. Non viability of the fungal hyphae in the distal portion of the nail plate may be the reason for culture negative but positive microcopy results, in some cases of onychomycosis.

In this study non-dermatophytes, accounted for 83% of total fungal isolates, including Yeasts, out of which Non-dermatophyte moulds constitute 52.3%. Among the Non dermatophyte moulds, Fusarium was the most common isolate (45%). The isolation of dermatophytes from cases of onychomycosis in this study was quite low accounting for only 17% of the total isolates.

The prevalence of Non-dermatophyte moulds varies considerably in different studies reported in the literature. In a study conducted in 2006 in Egypt on 32 patients with different mail abnormalities it was found out that NDMs were isolated from 59% of the total culture positive cases and most of them were recovered from specimen taken from housewives with Aspergillus species being the commonest. A study conducted in Rawalpindi in 2007 showed that among the non-dermatophytes Alternaria *alternate* was most common followed by Scytalidium *dimidiatum* and Penicillium *marneffei*. A study conducted in Italy revealed Fusarium species as the most common NDMs followed by Scopulariopsis, Acremonium and Aspergillus species. Similarly studies in Argentina, Srilanka Columbia and Pakistan yielded fairly large percentage of Fusarium species. Majority of patients in this study is in 21-60 years age group, females being affected more than males. Predominance of onychomycosis in female gender may be explained by their domestic wet work and also field work.

In order to determine the fungal species associated with abnormal nails epidemiological investigations should be performed.

Conclusion

Non-dermatophyte colonization in nails was found to be increasing. The role of non-dermatophytes as pathogen is more in immunocompromised individuals, those using broad spectrum antibiotics or those subjected to localized trauma. A high index of suspicion should be considered in case of Non-dermatophyte moulds as causative agents, in evaluating the patients who were culture negative for dermatophytes or for those subjects ending up in treatment failure. Use of appropriate diagnostic techniques including direct microscopy and fungal culture is important to ensure correct diagnosis and treatment of onychomycosis.

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Conflict of Interest

None.

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