

Studying and Diagnosing the Main Causative Agent of Onychomycosis through Laboratory Procedures

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Abstract– Onychomycosis is a localized infection of the nail caused by pathogenic dermatophytes, yeasts and molds. In this study we diagnose through our laboratory procedures the main causative agent of onychomycosis. Our study included diabetic and non-diabetic patients. We found high incidence of *Candida* species (91.5%) to be responsible for onychomycosis in diabetic patients. There can be some factors involved in *Candida* species being the major causative agent of onychomycosis in diabetic patients in our region. Proper diagnosis is very important before the initiation of antifungal therapy. Treatment of onychomycosis is long and recurrence may occur, therefore; maintenance of good hygiene is very important.

Keywords– Diagnose, Disease, Onychomycosis, Treatment and Diabetic

I. INTRODUCTION

Onychomycosis is a disease caused by the fungal invasion of the nails. The causative agents include dermatophytes (*Trichophyton* species), yeasts *Candida* species and non-dermatophytic molds. It is not self healing disease and may be a source of more widespread fungal lesions of the skin [6]. There are four classic types of onychomycosis [9].

A. Distal Subungual Onychomycosis

It is the most common form of tinea unguium, and is usually caused by *Trichophyton rubrum*, which invades the nail bed and the underside of the nail plate.

B. White Superficial Onychomycosis (WSO)

It is caused by fungal invasion of the superficial layers of the nail plate forming white islands on the plate.

C. Proximal Subungual Onychomycosis

It is a fungal penetration of the newly formed nail plate through the proximal nail fold.

D. Candidal Onychomycosis

It is the invasion of the nails by *Candida* species. This usually happens in individuals who immerse their hands in water for long time. This normally requires the prior damage of the nail by infection or trauma. *Candida albicans* predominates in most yeast-caused onychomycosis cases [1], [6]. They are saprophytes commonly found in air soil and some plants. They are opportunistic fungi. Their pathogenesis in onychomycosis is still controversial; however, their

isolation in nails is becoming more and more frequent [7], [10], [12].

Fungal infections are often uncomfortable conditions and they can have both physical and psychological consequences to the individual. Onychomycosis is characterized by discoloration and thickening of the nail, and thus, the nails are often thick, yellow or brittle. The nail plate may separate from the nail bed (onycholysis) and there may be inflammation of the skin near the nail edge (paronychia inflammation) [3]. Toenails are more frequently involved in onychomycosis than finger nails largely due to the damp conditions associated with the use of shoes. Therefore good foot and hand hygiene is important in preventing onychomycosis.

Diabetic patients are more likely to have onychomycosis than non-diabetic patients. Many studies have been undertaken to assess whether diabetic individuals suffer from a higher incidence of onychomycosis than those without diabetes [5], [2]. Onychomycosis in diabetic patients can become worse if not treated properly. Accurate diagnosis of the agent causing onychomycosis is very important prior to the initiation of the treatment.

There have been many studies from different regions of the world in which particular organism found to be the main cause of the disease. Many more studies have to be done in this regard for the effective and quick treatment of the disease. Diabetic patients are more at risk to develop severe complications after having fungal infection of the nails. Diabetic patients with onychomycosis had an approximately 3 times greater risk of gangrene or foot ulcer compared with diabetic patients without it [2].

Diagnosis of onychomycosis is made by direct microscopy and fungal culture [6], [14], [17], [13]. Other laboratory methods, such as unguis biopsy, PCR, flow cytometry and immunohistochemical techniques, have been used to improve onychomycosis diagnosis; however, these methods are not usually available in common dermatology centers [6], [16], [11].

Treatment of onychomycosis is same in diabetic and non-diabetic patients [15]. Treatment may require many months. To avoid recurrence good hygienic conditions must be maintained. Three drugs are widely used in the prevention and curing of onychomycosis: i.e., Fluconazole, Itraconazole and Terbinafine [18].

II. METHODS AND MATERIALS

Samples were collected from 105 patients having onychomycosis. The patients were then categorized as

diabetic patients and non-diabetic patients. Patients were not given any treatment for onychomycosis prior to the collection of the samples. This study was carried out in a local dermatological center in Karachi, Pakistan in 2010. Male and female adults were included in this study.

III. LABORATORY METHODS

A. Direct Examination

The toe and nail scrapings were placed on the slide and treated with a combination of 10-40% KOH solution and dimethyl sulfoxide. The slides were microscopically examined. Appearance of long, regularly shaped hyphae under the microscopic examination showed the presence of dermatophytes. Presence of yeasts was confirmed by the appearance of budding yeasts.

B. Culture

Part of each sample was placed on Sabraud's Dextrose Agar (SDA), Corn Meal Agar (CMA) with Tween 80 and germination tubes and incubated at 26°C for 7-14 days.

IV. RESULTS AND DISCUSSIONS

Out of 59 diabetic patients 54 (91.5%) of the patients were found to have onychomycosis due to *Candida* species mostly *Candida albicans*. Identification of *Candida albicans* was done by germ tube test. The main causative agents in the remaining 46 non-diabetic patients were *Trichophyton* species i.e., (60.8) of the patients without diabetes. This can be shown in the following Table I:

Table I: Experimental Results

PATIENTS	NO. OF SAMPLES		CULTURE & MICROSCOPIC EXAMINATION
	Positive for <i>Candida</i> species	Positive for <i>Trichophyton</i> species	Positive samples for other organisms
Non-diabetics	46	13 (28.2%) 28 (60.8%)	5 (10.8%)
Diabetics	59	54 (91.5%) 4 (6.7%)	1 (1.6%)

Total no. of samples n = 105

Onychomycosis is a common fungal infection of the nails, accounting for 50% of nail diseases and this percentage has been increasing [7], [8], [4].

In this study we observed high percentage of yeasts isolated from onychomycosis patients having diabetes. Identification of *Candida* Species was done by direct microscopic examination and culture techniques. There may be some factors associated with the isolation of high percentage of *Candida* Sp. in diabetic patients. These factors may include environmental or immunological state of the patients.

Rapid diagnosis of the causative agent of onychomycosis is very important as it may become systemic and can cause complications.

Fungal infection of the nail and toe is chronic and extremely difficult to treat. An accurate diagnosis of the causative agent is of profound importance since the duration of the treatment is very long.

V. CONCLUSIONS

In this study, we have analyzed and diagnosed through our laboratory procedures the main causative agent of onychomycosis. We have mainly focused on diabetic and non-diabetic patients. We have identified through our study that high incidence of *Candida* species (91.5%) were responsible for onychomycosis in diabetic patients. There can be some factors involved in *Candida* species being the major causative agent of onychomycosis in diabetic patients in our region. We conclude that proper diagnosis is very important before the initiation of antifungal therapy. Treatment of onychomycosis is long and recurrence may occur, therefore; maintenance of good hygiene is very important.

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