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The Prevalence Onychomycosis in North Western region of Rajasthan

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INTRODUCTION: Onychomycosis is a fungal infection of nail caused by dermatophytes, yeast and mould. Onychomycosis infection continues to spread worldwide and found to persist everywhere. Onychomycosis infection prevalence is continuously increasing and the possible fungal pathogens are also increasing. All nail diseases are not fungal in origin hence laboratory investigations are needed to differentiate accurately between fungal infections and other conditions.

AIM: to find out mycological profile & morphological identification of fungal agent in onychomycosis infection and their prevalence in a part of Rajasthan.

MATERIALS AND METHOD: A total 50 samples of nail clippings were investigated in department of microbiology by using 20% KOH and culture tubes of Sabouraud's Dextrose Agar (SDA) which were mixed with chloramphenicol and cycloheximide.

RESULT: A total of 50 samples (18 male and 32 females) were tested in period of one year (January to December 2016). Among them 28(56%) samples showed fungal growth, the predominant fungal pathogen in present study were Tricophyton spp. 12 (42.86%) followed by yeasts 09 (32.14%) then molds 07(25%). Fungi commonly presented in the middle age, between 31-40 years of age, due to trauma at the work site and in women, due to their wet work.

CONCLUSION: This study highlighted that Dermatophyte T.Rubrum as the main fungal pathogen which caused onychomycosis in the study region. As there are several fungi which cause the infection so it is necessary to perform culture for appropriate treatment. So, it is imperative to diagnose it properly by using microbiological techniques and to treat it properly. For proper management of onychomycosis, diagnosis and accurate treatment play a key role in better outcome.

KEYWORDS: Onychomycosis, Yeasts, Dermatophytes, Middle age

INTRODUCTION

The cutaneous mycoses are most common fungal infection worldwide that affect quality of life in infected patient. Dermatophytosis (tinea) infection include superficial infection of the skin, hair and nail. This infection commonly infects man and animal in developed and developing countries. Dermatophytosis does not cause mortality but it leads to morbidity. In India it is common infection due to variable topography, hot and humid climate. Dermatophytosis infection are mostly seen in summer and monsoon weather.^{1,2,3} The Dermatophytes spp. are mostly distributed in the temperate western countries. Onychomycosis cover 50% of the nail infections.^{4,5}

Invasion of the nail plate by a dermatophyte is referred to as Tinea Unguim, infection of the nail by non dermatophytic fungi is called onychomycosis. Onychomycosis has been traditionally referred to unknown dermatophyte infection of the nails but is now used a general term to denote all fungal infections of nail. Onychomycosis term derived from Greek word "Onyx" a nail and "Mykes", a fungus is defined as

a fungal infection of the nail.^{6,7} Most common cause of Onychomycosis fungal infection is dermatophytes, yeast and mould & sometimes it is a mixed infection.^{8,9} Classification of Onychomycosis can be summed up as Distal Subungual Onychomycosis (DSO), White Superficial Onychomycosis (WSO), Proximal Subungual Onychomycosis (PSO) and Total Dystrophic Onychomycosis (TDO).¹⁰

In nail infection usually begins distally or at the lateral edge of the nail & beneath the nail there is an accumulation of a cheesy epidermal detritus. The affected nails are discoloured, lustreless, brittle, thickened, friable & may become pitted & grooved as a result of paronychia inflammation. In some instances, the top of the nail separates distally leaving it thin, furrowed, ragged & deformed.¹¹

Onychomycosis infection continues to spread worldwide and found persist everywhere. Onychomycosis infection prevalence is continue increasing and the possible fungal pathogens are

also increasing. Onychomycosis prevalence rate is determined by age, predisposing factors, social class, occupation, climate & living environment. There are many fungi which have a tendency to damage the nail, like dermatophytes (50%), yeasts (27%) and moulds (23%).^{9,12} All the nail infection are not caused by fungus but they are also caused by other clinical conditions like trauma, wet work (with the hand submerged in water),¹³ HIV-AIDS,¹⁰ immunodeficiency which is due to organ or bone marrow transplantation¹⁰, old age, psoriasis, atopic dermatitis,¹⁴ diabetes with a predominance of *Candida* spp.,¹⁵ renal transplant recipients¹⁶ etc.

The fungi that commonly infect nail dermatophytes are *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. The yeasts that cause onychomycosis are *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, etc., while the molds have long list of fungi like *Scybalidium* spp., *Aspergillus* spp., *Geotrichum candidum* and *Fusarium* spp. etc. The yeasts (*Candida* spp.) are mainly present in the tropical and the subtropical regions and infect the persons whose hands are usually submerged in water.¹⁷ Presently in the middle age commonly found fungal agents are dermatophytes and yeasts. In the older persons over 60 years of age commonly isolated fungal agent are molds. A saprophytic fungus *Scybalidium* mostly present in water, soil, plants and some decaying material is transmitted by direct contact.¹⁸

In men common fungal agent for foot nail onychomycosis are dermatophytes because men have perspiration, exercise and constant wearing of shoes. In women common agent for fingernail onychomycosis are yeasts because their hands are often submerged in water.

Now a days some patients have nail disease, is a cosmetic issue. These patients do not have medical problems and regularly seek advice for cosmetic reasons. However, it can cause pain, social, emotional and occupational discomfort, permanent damage to the nail for the patient and spread of the infection to other persons.¹⁹

In last few decades, the incidence of

onychomycosis is on increase due to involvement of climate, occupation, socio-economic status, gender, age and genetic and immune factors. So it is necessary to find out mycological profile & morphological identification of fungal agent in onychomycosis infection and their prevalence.

MATERIAL AND METHODS

A prospective study was carried out on 50 patients of onychomycosis attending the department of Skin & V.D. of P.B.M. Hospital Bikaner from January 2016 to December 2016. At the time of taking the samples patients have antifungal therapy were not included in the present study. The detailed H/O patients was taken regarding their sociodemographic factors, presenting complaints, risk factors, predisposing factors and prior use of medication, etc. Total 50 samples of nail clippings were collected over a period in the Department of Microbiology & Immunology, S.P. Medical College, Bikaner.

Sample Collection

The nails of the patients were wiped with 70% ethyl alcohol before the sample collection, to avoid bacterial contamination. Friable material was removed from under the nail or clipping's ans were collected from the distal border with scissors or nail clippers, where dystrophy did not extent to the distal section of the nail and scrapings was collected with a scalpel blade across the affected area.

Sample processing method

All the samples were tested in the following order:
Step 1: Direct microscopy, by using freshly prepared 10/20% KOH to identify the yeast cells, the budding yeast cells, the pseudohyphae, the hyphae and the arthroconidia.

Step 2: The culture was done in four tubes of Sabouraud's Dextrose Agar (SDA), two tubes of SDA with cycloheximide and two tubes without cycloheximide. One tube from each set was kept at 25°C and 37°C respectively. Tubes were examined twice a week for presence of growth and discarded at 4 weeks if no growth was seen.

Step 3: All the culture growths were identified on the basis of the culture characteristics, the Lactophenol Cotton Blue (LCB) test, the germ tube test, the Dalmau method by using corn meal

agar, the sugar assimilation test and the urease test. The germ tube and the sugar assimilation tests were used to differentiate the *Candida* spp, while the urease test was used to differentiate *T. mentagrophytes* from *T. rubrum*.

RESULTS

Table-1 represents the different age groups and the gender which were involved in this study. In the current study, women (64%) had more incidence of infection than men (36%). The mean age of the patients was 36.42 years. Most numbers of patients (34%) were between 31-40 years of age, followed by age groups 41-50 years (22%) and 21-30 years (20%).

A total of 50 samples of nail clippings were tested in the department of Microbiology. Culture was positive in 28(56%) cases, out of this KOH mount was positive in 22(44%) cases and negative in 06(12%) cases. Culture was negative in 22(44%) cases, out of this 14(28%) cases was KOH positive & 08(16%) was both culture and KOH negative.

Table 3 presents the fungus which were isolated from nail samples. The predominant fungus which was identified in our study was Dermatophytes in 12(42.86%) cases of the culture positive samples, which was followed by Non- dermatophyte yeast 09 (32.14%) and molds 07(25%). Among Dermatophytes *Tricophyton* species, *Tricophyton rubrum* showed highest incidence 06(21.42%) cases followed by *Tricophyton mantegrophytes* 04(14.28%) cases and *Tricophyton interdigitale* 02(07.14%) cases. Among yeast most common isolated yeasts was *Candida albicans* in 05 (17.85%) cases followed by even number of samples with *Candida tropicalis* 02 (07.14%) cases & *Candida glabrata* 02(07.14%) cases. Among Non-dermatophyte moulds isolated in 07(25%) cases in which *Aspergillus* spp showed highest incidence that was isolated in 03(10.71%) cases followed by *Rhizopus* spp in 02 (07.14%) cases. *Fusarium* spp. and *Alternaria* spp. was isolated in even number 01 (03.57%) cases.

DISCUSSION

The present study was conducted in an attempt to determine etiological agent, age, sex incidence, mycological pattern in the clinically suspected cases of onychomycosis attending the out patient of

skin & VD at P.B.M. hospital & associated groups of hospitals, Bikaner over a period of 12 months from January 2016 to December 2016.

Recently, the incidence for onychomycosis is on increase and spectrum of causative pathogens have also increased which may be due to awareness among people towards health, cosmetic consciousness, various risk factors like chronic diseases, sport, traumatic injury and other fungal infections of the skin.

In the present study, direct microscopy was found positive in 36 cases whereas fungus was grown in 28 cases. The reason for sterile culture in cases could be that the patients were already on agents that can reduce fungal growth before the samples were taken. In our study 12% samples were identified as false negative (KOH negative and culture positive). False negative findings were also reported in previous reports.^{5,20,21} Thus it is fruitful to perform culture in all clinically suspected cases who are KOH negative as there are chances of recovering fungus in these cases on culture (being 12% in present study).

In our study we found that women have higher incidence rate (64%) than in male (36%), which indicate that onychomycosis was a common disease in women. Women are commonly affected because mostly women are housewives and they constantly submerge of their hands in water. This finding is similar to various other studies.^{7,9,13,22}

In present study, age was also observed as a determinantal factor in onychomycosis. Finger nail onychomycosis was commonly isolated in the middle age. Observations show clear that clinical infection of onychomycosis isolates between the ages of 31-40 years (34%) followed by age groups 41-50 years (22%) and 21-30 years (20%). Six cases even (12%) found in both between 11-20 years & >50 year of age. In the middle age outdoor activity is maximum which decreases with age in elderly and old age. In young age it is also observed that repeated traumatic injuries at work sites like in farms or in industrial occupation are more and farmers have more exposure to soil, dust & moisture. However immunity is comparatively

stronger in young age group but more cases of onychomycosis were observed in this age group.

This shows that infection is more related to personal hygiene rather than status of immunity. We found in our study that fungal infection of nail have low incidence in older persons and teenagers. Similar observations are found by other studies.^{4,5}

In our study we found that Dermatophytes (Tricophyton) are the most common fungal agent isolated in onychomycosis.^{6,19} Dermatophytes are main cause of 90% toe nail and 50% finger nail onychomycosis.⁷ In our study Tricophyton species were present in 12(42.86%) cases, which included 06 (21.42%) cases with Tricophyton rubrum, 04(14.28%) cases with Tricophyton mantegrophytes and 02 (07.14%) cases Tricophyton interdigitae. Dermatophytes and yeasts are have equal incidence in some studies.²¹ Yeasts as a common fungal agent in now days was observed in other studies.^{16,23,24,25,22} These observations are similar to our study. In our study the yeasts was isolated in 09 (32.14%) cases, in which Candida albicans in 05 (17.85%) cases followed by even number of samples with Candida tropicalis 02 (07.14%) cases & Candida glabrata 02 (07.14%) cases. The increasing incidence of yeasts was due to continuous and repeated contact with water, which is mode of transmission of Candida spp.

In our study we found Non-dermatophyte moulds in 07(25%) cases in which Aspergillus spp. was isolated in 03(10.71%) cases followed by Rhizopus spp in 02(07.14%)cases, Fusarium spp. 01(03.57%) cases and Alternaria spp. 01 (03.57%) cases. These studies have similar trend in other studies.^{8,9} Non-dermatophytic molds are mostly isolated in older age. The causative cause in older age may be poor blood circulation, poor personal care, lower immune response and some systemic diseases like diabetes.

Onychomycosis is a fungal infection of finger and toe nails that affect the quality of life. Diagnosis of onychomycosis in clinically is too difficult because inappropriate collection of material for analysis & ineffective treatment make it hard to assess the true profile of such infections.^{7,12,26,27} For proper management of onychomycosis, diagnosis and accurate treatment play a key role in better

outcome. However several newer diagnostic methods have been introduced like PCR based methods and non-invasive methods like optical coherence tomography, confocal laser scan microscopy, matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) and phase contrast hard x-ray microscopy.²⁷ The newer technique may help in early diagnosis and better management, but the spectrum of disease is based on various etiological agents so the culture will remain gold standard in identifying the species. The main issue for such newer methods is that these facilities are available only at some higher centers. For treating onychomycosis different treatment strategies including topical, systemic and surgery are commonly used. Proper and good therapeutic response was unsatisfactory due to therapeutic failure, relapse and reinfection.^{8,12,26,27,28}

To combat poor response newer strategies such as combination, sequential & supplementary therapy have been suggested. The following are the strategies to improve cure rate in onychomycosis.²⁸

- Ensure proper diagnosis.
- Choose most appropriate antifungal drug .
- Ensure bioavailability and compliance.
- Monitor for any possible drug interactions.
- Consider supplemental therapy-in poor prognostic factors.
- Consider surgery in addition to antifungal therapy if required.
- Education regarding risk factors and nail care.
- Ensure treatment of affected contacts.
- Maintain proper hygiene of hand & foot.

CONCLUSION

Our study concludes that prevalence of onychomycosis is moderate to high in south west Rajasthan. The present study highlighted that the dermatophyte T. Rubrum was a predominant pathogen in our region. As there are several fungi which cause the infection so it is necessary to perform culture for appropriate treatment. A high frequency of fingernail onychomycosis was observed among women and so, they were advised to improve their health and personal hygiene. Females are more prone to develop onychomycosis of fingernails due to frequent contact with soap and water. The newer diagnostic methods which are rapid and accurate may

contribute in early diagnosis and management of infection.

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LEGENDS

AGE	MALE		FEMALE		TOTAL	
	No	%	No	%	No	%
11-20	03	06	03	06	06	12
21-30	02	04	08	16	10	20
31-40	06	12	11	22	17	34
41-50	04	08	07	14	11	22
>50	03	06	03	06	06	12
Total	18	36	32	64	50	100

Table 1. Distribution of Patients According to Age Group and Gender

NAME OF METHOD	OUTCOME	No.	FUNGUS CULTURE POSITIVE	FUNGUS CULTURE NEGATIVE
KOH Mount	Positive	36(72%)	22(44%)	14(28%)
	Negative	14(28%)	06(12%)	08(16%)
Total Cases		50	28(56%)	22(44%)

Table 2. Correlation between the Findings of Direct Microscopy (KOH) and Fungal Culture

CATEGORY	FUNGAL SPP.	NUMBERS	PERCENTAGE (N=28)	N=28
Yeasts	C.albicans	05	09(32.14%)	17.85%
	C.tropicalis	02		07.14%
	C.glabarata	02		07.14%
Dermatophytes	T.rubrum	06	12(42.86%)	21.42%
	T.mentagrophytes	04		14.28%
	T.interdigitae	02		07.14%
Molds	Aspergillus Spp.	03	07(25%)	10.71%
	Rhizopus spp	02		07.14%
	Fusarium	01		03.57%
	Alternaria	01		03.57%

Table 3. Distribution of Fungus