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A Study of Dermatophytic Onychomycosis in Patients Attending Dermatology Department in a Tertiary Care Hospital in Eastern India

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Abstract: *The etiology of onychomycosis is only occasionally documented in the eastern part of India. 106 clinically suspected onychomycosis patients were evaluated in this study. Male : female ratio was 1.35:1 and the commonest age group of affliction was 31-40 years. KOH examination revealed 88 (83%) cases, and culture was positive in 86 (81.1%) cases. 66 (62.2%) of them were due to dermatophytes, 8 (7.5%) due to Candida spp. 8 (7.5%) due to Aspergillus spp. and 4 (3.7%) due to Fusarium spp. Among the dermatophytes, Trichophyton was the predominant etiology (90.9%). T. mentagrophytes 14, (21.2%) was the commonest dermatophyte isolated; all the isolates were either anthropophilic or zoophilic. Sensitivity and specificity of KOH mount for diagnosis of dermatophytes in nail samples were 93.9% and 35% respectively. T. verrucosum was associated history of cattle handling and T. mentagrophytes with dog handling. Community based studies are required to reveal the actual burden of dermatophytic onychomycosis patients. Public awareness of the disease is of utmost importance to control and prevent this type of infection.*

Keywords: *Onychomycosis, zoophilic, dermatophytosis, KOH mount, Trichophyton, Epidermophyton*

I. Introduction

Superficial mycoses are one of the most common skin infections found all over the world, especially in the hot and humid climate of tropical countries like India. Dermatophytosis is one of the most common fungal infections seen in man affecting skin, hair and nails with a morbidity of 10-20% [1].

Dermatophytes are keratinophilic fungi which possess keratinase. This enzyme helps in causing infection in the keratin rich tissues like nail [2]. The severity of onychomycosis depends on the type of fungi, the site of infections and the immunological response of the host. The distribution of onychomycosis and their causative agents can vary with geographical region and is influenced by a wide range of factors, like type of population, climatic factors, lifestyle, migration of people, cultural practices and socioeconomic conditions, incidence of peculiar co-morbidities and drug therapy [3, 4]. Onycholysis almost always occurs. Dermatophytic onychomycosis is more common in older adults and in persons with vascular disease, diabetes mellitus, and trauma to the nails [5]. The burden of this infection is increasing in both developed and developing nations as immunosuppressive therapies and immuno-compromised patients are increasing in number.

Proper etiological diagnosis of this infection is necessary, as the lesion, though typical, may be confused with other cutaneous conditions [5]. It is to be noted that the commonly prescribed antifungal agent, fluconazole is ineffective against dermatophytes. Further, etiological diagnosis to genus level is essential in selecting the correct antifungal agents. This study was undertaken to isolate, identify and speciate the different etiological agents of dermatophytes from the clinically suspected lesions of onychomycosis, and to determine the proportion and distribution pattern of the etiological agents of dermatophytic onychomycosis among the patients coming to the dermatology out patients department of a tertiary care hospital in Kolkata.

II. Aim And Objectives

This study was to determine the proportion, distribution pattern of the etiological agents of dermatophytic onychomycosis and their epidemiology among the clinically suspected cases, attending the dermatology OPD of our hospital with specific dermatological complaints.

III. Material And Methods

3.1 Study settings The study was carried on 106 clinically suspected onychomycosis patients attending the dermatology department of RGKMCH, Kolkata from 2011-2012. After taking detailed history, clinical examination of patient was made in broad day light which included site of lesion, number of lesions, type of lesion, presence of inflammatory margin. The following parameters for each patient were recorded in a

Microsoft Excel database: age, sex, and history of contact with animals, presence of fungal elements in the nail samples, species of the dermatophytes identified by microscopy, culture and biochemical tests (urease test). Patients undergoing any types of antifungal therapy for any cause currently, were excluded from the study. Patients having simultaneous bacterial co-infections in the affected sites were excluded from the study. The study was approved by the Institutional Ethical Committee and properly informed consent was taken from the patients.

3.2 Sample collection The affected nail was cleaned with 70% alcohol. Nail clippings of the infected part and scrapings beneath the nail were collected in a sterile petridish. For distal subungual onychomycosis (DLSO), the abnormal nail was clipped proximally and the nail bed and underside of the nail plate were scraped with a no.24 scalpel blade; the outermost debris was discarded. Care was taken to avoid penetration of the nail plate and bleeding. Nail material was obtained from the advancing infected edge closest to the cuticle, where the likelihood of viable hyphae was the greatest. For white subungual onychomycosis (WSO), the white spots on the nail were scraped and the outermost surface was discarded; the white debris directly underneath was then collected. For each patient samples were taken on at least 2 different occasions on 2 different days to rule out the chance of environmental contamination in the cultures.

3.3 Direct microscopic examination The sampled material was divided into 2 portions, one for direct microscopy and the remainder for culture. The specimens were obtained when the patient has been off both topical and systemic antifungal drugs for at least four weeks. Specimens were not kept in moist media to avoid rapid multiplication of bacterial and contaminating fungal spores. Nail samples were kept in vials containing 40% KOH overnight to dissolve the keratin properly and viewed under microscope next day with low power objective, first and then under high power.

3.4 Culture of samples Each sample was inoculated in 3 tubes of Sabouraud's dextrose chloramphenicol agar, SDCA, for aerobic fungal culture at 22°C, 25°C and other in 37°C at room temperature, BOD incubator and aerobic incubator respectively. Another part of the sample was inoculated in the Dermatophyte test media, DTM and incubated at 25°C. The cultures were examined every two days for a period of 6 weeks for the presence of growth. The growth was generally observed starting from sixth day onwards. If no growth was found after 45 days it was considered negative for the growth of fungi. In DTM, growth of dermatophyte was associated with change of colour of the media to red within 3 – 6 days. If no change were seen up to 2 weeks, sample was declared negative.

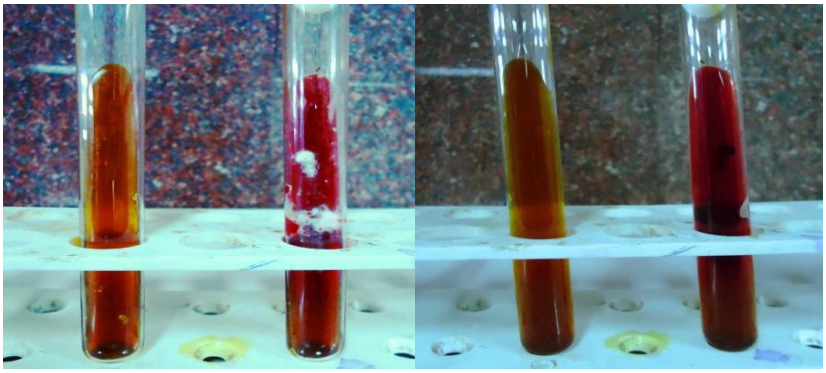

Fig. 1 Culture in DTM, obverse and reverse

3.5 Identification of different species of dermatophytes Identification of a dermatophyte was based on its gross colonial morphology on culture media, biochemical tests, and on its microscopic morphology on LCB mount. In a few cases, slide culture method was also used.

IV. Results And Analysis

Out of 106 clinically suspected cases of onychomycosis, 66 were found to be due to dermatophyte infection, which showed growth of different dermatophytes on culture. Remaining 40 were either contaminants, fungi other than dermatophytes, or did not show any positive finding either in KOH preparation or culture. Nail samples were taken after detailed history and clinical examination, for direct microscopy, culture and biochemical tests. In the different age groups, dermatophytic onychomycosis was frequently found in the age group of 31-40 years (Table 1). The mean age of patients was 38.0 ± 4.4 years.

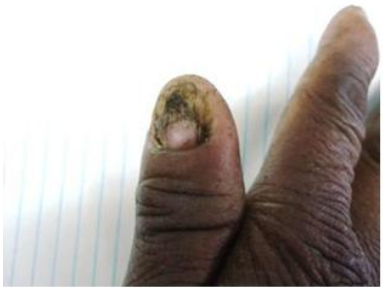

Fig. 2 Dermatophytic onychomycosis of right thumbnail.

Table 1 Age-wise distribution of onychomycosis patients.

Age in years	Suspected cases		Dermatophytic onychomycosis	
0-10	4	3.8%	4	6.1%
11-20	10	9.4%	8	12.1%
21-30	20	18.9%	8	12.1%
31-40	26	24.5%	16	24.2%
41-50	22	20.8%	14	21.2%
51-60	12	11.3%	10	15.2%
> 60	12	11.3%	6	9.1%
Total	106		66	

The male (52.8%) were marginally greater in number than the female (47.2%) patients in the study group. The burden of dermatophytic nail infection was also higher in the males (57.6%) in comparison with the female patients (42.4%) (Table 2). Male : Female ratio of dermatophytic onychomycosis patients was 1.35 : 1.

Table 2 Gender wise distribution of onychomycosis patients

	Clinically suspected cases		Dermatophytic onychomycosis	
Male	56	52.8%	38	57.6%
Female	50	47.2%	28	42.4%

Out of 106 clinically suspected cases of onychomycosis, 76 (71.6%) were samples from affected toe nails and 30 (28.4%) were from fingernails. Fungal elements were demonstrated with KOH mount in 88 cases (83%) by direct microscopy. 62 cases (58.5%) were positive by both microscopy and culture (Table 3). The KOH mount examination for diagnosis of dermatophytosis was found to be very useful with high sensitivity (93.9%) but the specificity was 35%.

Table 3 Comparison of positive and negative direct microscopy by KOH mount, versus positive and negative culture of dermatophytes.

	Dermatophyte culture positive	Dermatophyte culture negative
KOH positive	62(58.5%)	26(24.5%)
KOH negative	4(3.7%)	14(13.3%)

The isolates from the culture of the different samples were diverse. As multiple sampling was done from a single patient on repeated occasions, fungal etiology other than dermatophytes were documented when similar organism was isolated in all the culture tubes in different samples from the same patient. Though dermatophytes were found in 66 (62.2%) of the 106 samples, yeasts like different *Candida* species 8 (7.5%), filamentous fungi such as different species of *Aspergillus* 8 (7.5%) and *Fusarium* spp. 4 (3.7%) were isolated from culture (Table 4), (Fig. 4)

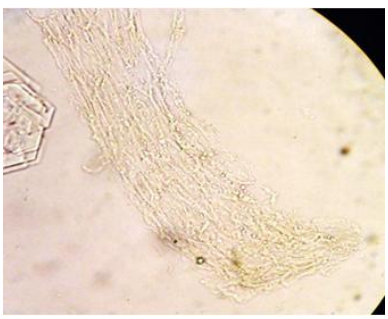

Fig. 3 KOH mount of nail sample showing presence of fungal elements.

Table 4 Distribution of different fungal etiology isolated from culture

Dermatophytes	66	62.2%
Fusarium spp	4	3.7%
A. fumigatus	4	3.7%
A. flavus	2	1.8%
A. terreus	2	1.8%
Candida spp	8	7.5%
Contamination	4	3.7%
No growth	16	15.1%
Total	106	100%

Among 66 dematophytes, 60 were Trichophyton (90.9%) and 6 were Epidermophyton (9.1%). Most common isolate was *T. mentagrophtes* (21.2%), followed by *T. verrucosum* (18.2%), and *T. schonleinii* (15.1%). 2 cases showed isolation of 2 dermatophytes, *T. mentagrophytes* and *T. violaceum*. (Table 5).

Table 5: Distribution of different species of dermatophytes isolated from culture.

Trichophyton rubrum	8	12.1%
Trichophyton mentagrophytes	14	21.2%
Trichophyton verrucosum	12	18.2%
Trichophyton schoenleinii	10	15.1%
Trichophyton soudanense	8	12.1%
Trichophyton tonsurans	4	6.1%
Trichophyton equinum	2	3%
Epidermophyton floccosum	6	9.1%
Trichophyton mentagrophytes + *Trichophyton violaceum*	2	3.%

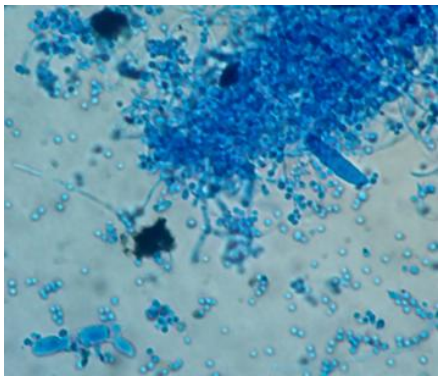

Fig. 4 T. mentagrophytes macro and microconidia.

History of contact with animals was elicited in 40 patients (cats-8, cattle -12. Dog-14, poultry-6), of whom 32 were diagnosed with dermatophytic onychomycosis. Highest association was found with *T. verrucosum* in cattle handlers 10(83.3%) and *T. mentagrophytes* in dog handlers 6 (42.8%).

V. Discussion

Dermatophytic onychomycosis is among the commonest superficial infections affecting Indian population. However its microbiological etiology is seldom documented up to species level. The present study shows that commonest age group affected was 31-40 years (24.2%) which is comparable with studies done by others [6, 7]. The mean age of the patients having this infection was found to be 38.0 ± 4.4 years. The highest incidence in young adults aged 31-40 years may be due to increased physical activity and increased opportunity for exposure. In the present study, males (57.6%) were more commonly affected than females (42.4%), Male to female ratio was 1.35:1, which is comparable with other studies.[8, 9]. Whereas some authors have reported that female cases were more than males [10]. Male predominance in our study may be due to increased outdoor physical activities leading to increased opportunity for exposure to infection, and as well as higher medical awareness among males.

In the present study, out of 106 cases, 88 (83%) were positive for fungi, either by KOH mount or culture or by both, 62 (58.4%) were positive by both KOH mount and culture, 26 (24.5%) were positive by KOH mount and negative by culture; 4 cases (3.7%) were negative by KOH but were culture positive. The

culture positivity rate (62.2%) for dermatophytes was seen to be higher than many of the previous studies [11, 12, 13]. 14 cases (11.9%) were negative by both KOH and culture. These 14 patients highlight the importance of laboratory diagnosis and repeated sampling to confirm a clinical suspicion of onychomycosis. The clinical picture of onychomycosis may mimic different other diseases of non-fungal etiology, which may be the reason behind the KOH negativity of some clinically suspected cases. Though KOH examination can yield false negative results in 5 – 15% cases [14], but it is a great aid for prompt detection of dermatophytes in the clinical sample. The data in the present study was analyzed and it was found that the sensitivity of the KOH mount technique is 93.9%, but the specificity is 35%.

The species distribution among dermatohpytic onychomycosis cases yielded striking results in our study. Among the 66 culture positive cases, Trichophyton was found in 60 (90.9%) occasions and Epidermophyton was found in the rest 6 (9.1%). The difference in proportion can be compared with other studies [15, 16]. Though *T. rubrum* was a common etiology 8 (12.1%), *T. mentagrophyte* 14 (21.2%) was the undisputed leading cause of infection. *T. verrucosum* 12(18.2%) and *T. schoenleinii* 10(15.1%) also emerged as important causative agents. Though in several other studies *T. rubrum* is the leading causative agent [6, 12, 17, 18], but the emergence of *T. mentagrophytes* as the leading dermatophyte causing onychomycosis has also been documented by various authors [7, 8, 14]. The predominance of *T. mentagrophytes*, *T. verrucosum* and *T. schoenleinii* may be due to the geographical distribution of these fungi in our local population. Majority of our cases were due to anthropophilic dermatophytes. However, zoophilic species like *T. verrucosum* and *T. equinum* were also found in some samples. No geophilic species of dermatophyte was found to a causative agent in our study.

History of contact with dogs and cattle was associated with the isolation of *T. mentagrophytes* and *T. verrucosum* respectively. To the best of our knowledge such association has rarely been documented in our part of the country. Zoophilic dermatophytes infect the animals, and from them can infect humans who are in close contact [5, 19].

5.1 Limitation of our study Our study is a hospital out door based study. A community based study to estimate the burden of dermatophyte infection in the onychomycosis patients along with more specific investigations like histopathology or molecular techniques would reveal the actual picture.

VI. Conclusion

Dermatophytosis of the nails are very common form of superficial mycosis in our country where hot and humid climate play an important role in the growth of these fungi. By and large Trichophyton species is the commonest etiological agent of onychomycosis. Without proper treatment, dermatophytic onychomycosis becomes chronic and more difficult to treat; the triazole group of antifungals as fluconazole, are frequently prescribed by the clinicians are ineffective against dermatophytes. Hence proper diagnosis of the etiological agent of onychomycosis is necessary. Surveillance of infections and the awareness level in the common population if increased, the burden of this disease can be reduced to much lower level.

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References

[1]. Noble SL, Forbes RC, Stamm PL. Diagnosis and management of common tinea infections. Am Fam Physician.1998; 58:163–74,177–8.
[2]. Rippon JW. Medical mycology. 3rd ed. Philadelphia, London: WB Saunders Company; 1974.
[3]. Venkatesan G, Singh AJAR, Murugesan AG, Janaki C, Shankar SG. Trichophyton rubrum – the predominant aetiological agent in human dermatophytosis in Chennai, India. Afr J Microbiol Res.2007;9-12.
[4]. Singh S, Beena PM. Comparative study of different microscopic techniques and culture media for the isolation of dermatophytes. Indian J Med Microbiol.2003;21:21-4.
[5]. Longo DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J. Harrison's principles of internal medicine.18thed. New York: McGraw-Hill; 2012.
[6]. Veer P, Patwardhan NS, Damle AS. Study of onychomycosis: prevailing fungi and pattern of infection. Indian J Med Microbiol. 2005; 25:53-6.
[7]. Jesudanam T M, Rao G R, Lakshmi D J, Kumari G R. Onychomycosis: A significant medical problem. Indian J Dermatol Venereol Leprol.2002; 68:326-9.
[8]. Kaur R, Kashyap B, Bhalla P. A five year survey of onychomycosis in New Delhi, India-Epidemiological and laboratory aspects. Indian J Dermatol.2007; 52(1):39-42.
[9]. Rezvani SM, Sefidgar SAA, Hasanjani MR. Clinical patterns and etiology of dermatophytosis in 200 cases in Babol, North of Iran. Casp J Intern Med.2010;1(1):23-6.
[10]. Sivakumar N, Karthikeyan A, Vivek A, Santhamani MD, Aljarari NMH. Prevalence of etiological agents in superficial mycoses with reference to dermatophytoses and pityriasis versicolor: A case study. Lib J Inf Di.2009;3(2):13-8.
[11]. Singh S, Beena PM. Profile of dermatophyte infections in Baroda. Indian J Dermatol Venereol Leprol.2003;69:281-3.

[12]. Shenoy MM, Teerthanath S, Karnaker VK, Girisha BS, Krishna Prasad M S, Pinto J. Comparison of potassium hydroxide mount and mycological culture with histopathologic examination using periodic acid-Schiff staining of the nail clippings in the diagnosis of onychomycosis. Indian J Dermatol Venereol Leprol 2008;74:226-29.
[13]. Grover S. Clinico – mycological evaluation of onychomycosis at Bangalore and Jorhat. Indian J Dermatol Venereol Leprol 2003;69: 284-6.
[14]. Chander J. Textbook of Medical Mycology.1st ed. New Delhi: Mehta Publisher; 1995.
[15]. Ahuja S, Malhotra S, Charoo H. Etiological agents of onychomycosis from a tertiary care Hospital in central Delhi, India. Indian.Indian J Fundamental and Appl Life Sci.2011;1:11-4.
[16]. Bindu V, Pavithran K. Clinico-mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol.2002;68(5):259-61.
[17]. Surjushe A, Kamath R, Oberai C, Saple D, Thakre M, Dharmshale S, et al. A clinical and mycological study of onychomycosis in HIV infection. Indian J Dermatol Venereol Leprol 2007;397-401.
[18]. Kaur R, Kashyap B, Bhalla P. Onychomycosis - epidemiology, diagnosis and management. Indian J Med Microbiol. 2008;26(2):108-16.
[19]. Kwon Chung KJ, BennetJE.Medicalmycology.Philadelphia, London:Lea and Febiger;1992.