DOI: 10.7860/JCDR/2013/5969.3378

Original Article

Microbiology Section

A Study on Clinico-Mycological Profile, Aetiological Agents and Diagnosis of Onychomycosis at a Government Medical College Hospital in Kashmir

RUBEENA LONE[1], DEEBA BASHIR[2], SHABIR AHMAD[3], ARSHI SYED[4], SYED KHURSHID[5]

ABSTRACT

Background: Onychomycosis is a major public health problem with a high incidence, associated morbidity and a long lasting treatment with anti-fungal agents. This study was carried out to know the clinico-mycological pattern of onychomycosis, which could help in the control of this infection.

Aims: The aim of this study was to determine the prevalence of various causative agents of onychomycosis and to study the clinical and mycological patterns of onychomycosis.

Material and Methods: This was a prospective study which was carried over a period of one year, from 1st February 2011 to 31st January 2012 on samples from 150 patients with clinically suspected nail infections, who attended the Dermatology Department of SKIMS Medical college, Kashmir, India. The nails were evaluated clinically and the nail samples were subjected to direct microscopy and culture.

Results: 60% samples were found to be positive by direct microscopy and culture. Males were infected more than females. The commonest age group which was infected was the 21-30 years age group. Finger nails were affected more frequently than toe nails and distolateral subungal onychomycosis was the most common clinical type of infection which was seen in 64.44% patients. The aetiological agents were dermatophytes (61.66%), Non–Dermatophyte Moulds (NDM) (31.66%) and yeasts (6.66%). Among dermatophytes, *T. rubrum* was the commonest aetiological agent.

Conclusion: Although dermatophytes were the main causative agents, NDM and yeasts were also not uncommon aetiological agents of onychomycosis. This study also emphasized the need of performing both a direct examination and culture to improve sensitivity. Since onychomycosis can cause physical, psycological and occupational problems, the clinico-epidemiological data can be helpful in development of preventive and diagnostic strategies.

Key Words: Onychomycosis, dermatophytes, non-dermatophyte moulds, yeasts

INTRODUCTION

Onychomycosis is a term which is used to describe a fungal infection of one or more of nail units and it can be caused by dermatophytes, yeasts or non-dermatophyte moulds. It represents upto 30% of mycotic cutaneous infections [1]. Clinically, onychomycosis is classified into various types; Disto–Lateral Subungal Onychomycosis (DLSO), Superficial White Onychomycosis (SWO), Proximal Subungal Onychomycosis (PSO), Endonyx Onychomycosis EO), Candidal Onychomycosis (CO), and Total Dystrophic Onychomycosis (TDO) [2-4]. The prevalence of onychomycosis is determined by age, predisposing factors, social class, occupation, climate, living environment and frequency of travel [5]. The worldwide incidence of onychomycosis is increasing and a number of factors contribute to its rise, like an immunocompromised status which is caused by HIV, immunosuppresive therapy and cancer chemotherapy or increased antibiotic usage [6]. Although onychomycosis is often regarded as merely a cosmetic problem which is rarely life threatening, its high prevalence and the associated morbidity makes it an important public health problem [1]. Onychomycosis resembles several diseases in the field of dermatology and medicine, so it is necessary to diagnose the infection with some laboratory evidence before treatment with anti-fungal agents, whose duration of treatment is long and may have some serious side effects [7]. The incidence of onychomycosis is high in Indian sub-continent because warm and humid climate, poverty, overcrowding and lack of medical facilities contribute to high prevalence of disease. Since the patients with dystrophic nails who seek medical advice is increasing, the present study was carried out to determine the prevalence of various causative agents of onychomycosis, to identify the clinical pattern of this disease in our part of world and to analyze the potential risk factors.

MATERIAL AND METHODS

Study Population and Period

This study was conducted over a period of one year, from Ist Feb 2011 to 31st Jan 2012, on samples from 150 patients with clinically suspected nail infections, who attended the Dermatology Out-Patients Department of SKIMS Medical College, Srinagar, India.

Inclusion Criteria

All patients with a clinical diagnosis of onychomycosis were included in the study.

Exclusion Criteria

The samples of patients who had taken anti fungal drugs were excluded from the study.

Detailed history of patients was taken. The clinical pattern and location of disease was also documented.

Sample Collection and Processing

The specimens were collected for microbiological analysis, on the basis of the results of clinical evaluations. Samples from clinical abnormal nails were collected by vigorously scraping the distal portion of the nail, the nail undersurface as well as nail bed, after cleaning the area with 80 % alcohol, to remove contaminants with

a no. 15 scalpel blade. The specimens were analyzed by direct microscopy and culture.

Direct Microscopy

Specimens were placed on slides and one drop of 20% Potassium Hydrooxide (KOH) was added to each slide. A microscopic examination was carried for the presence of fungal elements after incubating the slides for two hours or until digestion of specimens occurred [8].

Culture

Culture was done by using: 1. Sabouraud's dextrose agar without antibiotics and 2. Sabouraud's dextrose agar with 5% Chloramphenicol and cycloheximide. Both media were used in duplicate and they were kept at 25°C and 37°C. They were examined daily for six weeks before they were declared as negative. The growths were noted for colony characteristics in the form of rate of growth, texture of growth, surface colour, and colour on reverse and diffusible pigments. For microscopic morphology, tease mounts, cellophane tape mounts and slide cultures were done [9,10].

Yeasts were identified on the basis of germ tube tests, microscopic morphologies on corn meal agar and colour production on CHROMAGAR candida culture medium (Becton Dickinson).

The criteria for reporting NDMs as pathogens were positivity on direct microscopy and isolation of same fungi in second samples which were obtained some days later.

RESULTS

Out of 150 patients, 90 (60%) showed positive results on direct examination and/or culture. Direct examination was positive in 84 (56%) patients and fungal culture was positive in 60 (40%) patients [Table/Fig-1].

	No of Samples	Percentage
Samples positive by KOH and/or culture	90	60
Total KOH positive	84	56
Total culture positive	60	40
Positive by both	54	36
Positive by KOH negative by culture	30	20
Positive by culturte negative by KOH	6	4
Both Negative	60	40

[Table/Fig-1]: Shows Results of direct microscopy and culture (Total no. of samples = 150)

Among 90 patients with onychomycosis, 55 (61.11%) were males and 35 (38.88%) were females, with a male to female ratio of 1.57: 1

Age Group	Male	Female	Total And % Age
0 - 10	2	-	2 (2.22)
11 – 20	5	4	9 (10)
21 – 30	25	16	41 (45.55)
31 – 40	14	9	23 (25.55)
41 – 50	6	4	10 (11.11)
51 – 60	2	2	4 (4.44)
> 60	1	-	1 (1.11)
Total	55	35	90

[Table/Fig-2]: Shows age wise distribution of patients with onychomycosis.

The mean age of patients with onychomycosis was 34.5 years (range 8-75 years). Highest number of patients (45.55%) was seen in the age group of 21-30 years, followed by those who were in the age group of 31-40 years (25.55%) [Table/Fig-2].

The finger nails were involved in 48 (53.33%) patients, whereas toe nails were involved in 24(26.6%) patients. Both finger and toe nails were involved in 18 (20%) patients [Table/Fig-3].

Distolateral subungal onychomycosis was the commonest clinical pattern which was seen in 58 (64.44%) patients, followed by proximal subungal onychomycosis 14(15.55%) patients, superficial white onychomycosis 8(8.88%) patients, total dystrophic onychomycosis 6(6.66%) patients and paronychia 4(4.44% patients. In our study, no case of endonyx onychomycosis was seen.

Pattern	Fingernails	Toenails	Both	Total
DLSO	31	14	13	58
PSO	8	5	1	14
SWO	5	3	-	8
TDO	-	2	4	6
Paronychia	4	-	-	4
Total	48	24	18	90

[Table/Fig-3]: shows distribution of patients showing morphological patterns of onychomycosis.

Culture	KOH-positive	KOH-negative	Total and %age
T. rubrum	21	-	21 (35%)
T.mentagrophytes	16	-	16 (26.66)
A.niger	6	3	9 (15)
Alternaria	8	0	8 (13.33)
Penicillium	0	1	1 (1.66)
Curvularia	0	1	1 (1.66)
Candida albicans	3	0	3 (5)
Candida parapsilosis	0	1	1 (1.66)
Total	54	6	60

[Table/Fig-4]: Shows distribution of etiological agents on the basis of KOH and cultural characteristics.

The most common organisms which were isolated in culture were dermatophytes 37 (61.66%), followed by Aspergillus *spp* 9 (15%), Alternaria 8 (13.33%), *Candida albicans* 3(5%), *Candida parapsilosis* 1 (1.66%) Penicillium 1(1.66%) and Curvularia 1(1.66%). The most common dermatophyte which was isolated was *Trichophyton rubrum,* followed by *Trichophyton mentagrophytes* [Table/Fig-4].

DISCUSSION

In the present study, 60% samples were positive by direct examination and/or culture. In studies which were conducted by Kaur et al., Das et al., Jesudanam et al., and Aghamirian et al., 54.5 %, 51.76%, 45.53% and 40.2% samples respectively were found to be positive by direct examination and/or culture [11-14]. In our study, direct microscopy with KOH mount and mycological culture showed positive results in 84 (56%) and 60 (40%) patients respectively. In our study, direct microscopy was positive in more cases than culture. The results were in accordance with the findings of study which was carried by Manjunath Shenoy et al., which showed positive results in 53% and 35% cases by direct microscopy and culture respectively. However, in the study which was conducted by Das et al, direct microscopy was positive in only 32.94% cases, while culture was positive in 49.4% cases [7,12].

In our study, 61.11% patients with onychomycosis were males and 38.88% were females, with a male female ratio of 1.57:1. Although many reports had shown a greater susceptibility of females to this infection [15,16], in our study, males were dominant. In the study which was conducted by Garg et al., and Veer et al., males were infected more than females [17,18]. The increased prevalence of onychomycosis in men could be due to nail trauma and more common use of occlusive footwear. In the present study, highest numbers of patients (46%) were in the age group 21-30 years, followed by those in age group of 25-45 years (25.55%). Adhikari et

al., also found a higher prevalence of onychomycosis in similar age groups [19]. In contrast, Velez et al., and Mercantini et al., reported higher prevalences among adults who were over 50 years of age [16,20]. The increased prevalence of onychomycosis at young ages could be because of occupation related trauma, cosmetic awareness and shoe wearing habits. In elderly, prevalence may be higher than what was observed, but as the disease is asymptomatic they are not mostly bothered about it.

In our study, distolateral subungal onychomycosis was the commonest clinical pattern in 64.44% cases, followed by proximal subungal onychomycosis (15.55%), as was found in many other studies [14,21]. The present study showed that finger nails were involved more often than toe nails. Although toe nails have been reported to be more commonly involved [1], our finding was in accordance with those of many other studies in which finger nails were found to be more frequently affected [14,21]. The lower incidence of toe nail onychomycosis could be because of lesser cosmetic awareness on their disfigurement.

In our study, the most common organisms which were isolated in culture were dermatophytes (61.66%), NDM (31.66%) and yeasts (6.66%). This finding was in accordance with those of many studies, which had demonstrated a greater prevalence of dermatophytes as the aetiological agents of onychomycosis [11, 14, 22] and it was in contrast to those of other studies which had found yeasts as the most common agents [23,24]. Among the dermatophytes, *T. rubrum* was the most common aetiological agent which was found in our study, followed by *T. mentagrophytes*. Although some studies had reported T. *mentagrophytes* as the most common dermatophyte [11], our finding was in concordance with those of many other studies which had found *T. rubrum* as the most common dermatophyte which was responsible for onychomycosis [15,18]. The increased prevalence of *T.rubrum* could have been due to increased virulence and better adaptation to hard keratin of nails. Among the NDM, *A. niger* was the commonest isolate which was obtained. Kaur et al., and Grover et al., also found A. niger to be the most common NDM which was responsible for onychomycosis [11,21]. *Candida albicans* accounts for a majority of cases of onychomycosis which are caused by yeast. *Candida papapsilosis*, *Candida tropicalis* and *Candida krusei* account for the remainder of the cases [25]. In our study, *Candida albicans* was isolated from 3 (5%) cases and *Candida parapsilosis* was isolated from 1(1.66%) samples.

CONCLUSION

In our study, dermatophytes were the most common aetiological agents of onychomycosis; the roles of NDM and yeasts in causing infections were also demonstrated. In our study, the combined sensitivity of direct microscopy and culture was greater than those of direct microscopy and culture alone. This emphasizes the need of performing both tests. In the present study, men were more commonly infected and the age group of 20-40 years was more commonly involved. Distolateral subungal onychomycosis was the common clinical pattern. The clinico-epidemiological data can be helpful for creating public awareness and for the development of diagnostic, preventive and treatment strategies.

REFERENCES

[1] Kaur R, Kashyap B, Bhalla P. Onychomycosis-epidemiology, diagnosis and management. *Indian J Med Microbiol.* 2008; 26(2): 108-16.
[2] Scher RK. Onychomycosis: a significant medical disorder. *J Am Acad Dermatol.* 1996; 35: S2-S5.
[3] Midgley G, Moore MK. Nail infections. *Dermatol Clin.* 1996; 14: 41-49.
[4] Baran R, Hay RJ , Tosti A, Haneke E. A new classification of onychomycosis. *Br. J Dermatol.* 1998; 139: 567-71.
[5] Williams HC. The epidemiology of onychomycosis in Britain. *Br J Dermatol.* 1993; 129:101-9.
[6] Proceedings of International Summit on Cutaneous Antifungal Therapy and Mycology Workshop. San Francisco, California, October 21-24, 1993. *J Am Acad Dermatol.* 1994; 31: S1-116.
[7] Shenoy MM, Teerthnath S, Karnaker VK, Girisha BS, Krishna Prasad MS. Pinto J. Comparison of potassium hydroxide mount and mycological culture with histopathologic examination using periodic acid-shiff staining of nail clippings in the diagnosis of onychomycosis. *Indian J Dermatolol Veneorol Leprol.* 2008; 74:226-9.
[8] Kurade SM, Amladi SA, Miskeen AK. Skin scrapings and a KOH mount. *Indian J Dermatol Venereol Leprol.* 2006;72:238-41.
[9] Milne L J R Fungi. In Colle JC , Duguid JP Fraser AG , Marmion BP, editors. Practical Medical Microbiology. 13 ed. Edinburg: Churchill Living Stone; 1989; 676-7.
[10] Koneman EW, Allen SD, Janda WM, Schreckberger PC, Winn WC. Color atlas and text book of Microbiology. 5th Ed. Philadelphia: JB Lippincott; 1997; 983-1053.
[11] Kaur R, Kashyap B, Bhalla P. A five year survey of onychomycosis in New Delhi, India: epidemiological and laboratory aspects. *Indian J Dermatolol.* 2007; 52: 39-42.
[12] Das NK, Ghosh P, Das S, Battacharia S, Dutta RN, Sengupta SR . A study on etiological agents and clinico-mycological correlation of fingernail onychomycosis in eastern India. *Indian J Dermatol.* 2008;53(2) 75-79.
[13] Jesudanam TM, Rao GR , Lakshmi DJ, Kumari GR. Onychomycosis: A significant medical problem. *Indian J Dermatol Venereol Leprol.* 2002; 68:326-9.
[14] Aghamirian MR, Ghiasian SA. Onychomycosis in Iran: epidemiology, causative agents and clinical features. *Jpn J Med Mycol.* 2010; 51: 23-29.
[15] Alvarez MI, Gonzalez LA, Castro LA. Onychomycosis in Cali, Colombia. *Mycopathologia* 2004; 158: 181-86.
[16] Velez A, Linares MJ, Fernandez- Roldan JC. Study of onychomysis in Corboda, Spain. Prevaling fungi and pattern of infection. *Mycopathalgia.* 1997;137:1-8.
[17] Garg A, Venkatesh V, Singh M, Pathak KP. Onychomycosis in Central India: a clinico etiological correlation. *Int J Dermatol.* 2004; 43(7) 498-502.
[18] Veer P, Patwardhan NS, Damle AS. Study of Onychomycosis: prevailing fungi and pattern of infection. *Indian J Med Microbiol*. 2007; 25: 53-56.
[19] Adhikari L, Gupta AD, Pal R, Singh T. Clinico-etiologic co-relates of onychomycosis in Sikkim. *Indian J Pathol Microbiol.* 2009 ; 52: 194-7.
[20] Mecantini R, Marsella R, Moretto D . Onychomycosis in Rome, Italy. *Mycopathalgia.* 1996;136:25-32.
[21] Grover S. Clinico-mycological evaluation of onychomycosis at Banglore and Jorhat. *Indian J Dermatolol Veneorol Leprol.* 2003; 69: 284-6.
[22] El Sayed F, Ammoury A,Haybe RF, Daybi R. Onychomycosis in Lebanon: a mycological survey of 772 patients. *Mycoses.* 2006; 49:216-219.
[23] Gupta AK, Jain HC, Lynde CW, Macdonald P, Cooper EA, Summerbell RC. Prevalence and epidemiology of onychomysis in patients visiting physicians offices: A multicentre Canadian survey of 15000 patients. *J Am Acad Dermatol.* 2000; 43: 244-8.
[24] Koursidou T, Devliotou Panagiotidou D, Karakatsanis G, Minas A, Mourellou O, Samara K. Onychomycosis in Northern Greece during 1994-98. *Mycoses.* 2002; 45: 29- 37.
[25] Daniel CR 3rd. The diagnosis of nail fungal infection. *Arch Dermatol.* 1991;127: 1566,

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, SKIMS Medical college and Hospital, Srinagar, J&K-190018, India.
2. Senior resident Department of Microbiology, SKIMS Medical college and Hospital, Srinagar, J&K-190018, India.
3. Consultant Medicine SDH Kangan, J & K Health Services-191202, India.
4. Lecturer, Department of Microbiology, SKIMS Medical college and Hospital, Srinagar, J&K-190018, India.
5. Associate Professor, Department of Microbiology, SKIMS Medical College and Hospital, J&K-190018, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rubeena Lone,
Assistant Professor, Department of Microbiology, SKIMS Medical College Bemina Srinagar, J&K-190018, India.
Phone-9796177262, E-mail: deebabashir@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 02, 2013**
Date of Peer Review: **May 13, 2013**
Date of Acceptance: **Jun 04, 2013**
Date of Publishing: **Sept 10, 2013**