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Research Article

A clinicomycological study of onychomycosis in a rural hospital in Central India

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ABSTRACT

Background: Onychomycosis; fungal infection of nails account for about half of the nail diseases. Common site of involvement is toenails. Various etiological agents including dermatophytes, yeasts and non-dermatophytic moulds (NDM) are responsible. It is difficult to treat onychomycosis as compare to other dermatophytic infections because of the inherent slow growth of the nail. Aim: To diagnose etiological agents of onychomycosis on KOH, Calcofluor white (CFW), KOH treated Nail Clipping with Periodic Acid Schiff (KONCPA) and SDA culture. Objectives: 1) To determine the fungal etiological agents of onychomycosis. 1) To correlate clinical parameters with the mycological findings.
Methods: The study was carried out in department of Microbiology, MGIMS, Sewagram, Wardha. A total of 44 cases with signs of onychomycosis were enrolled in the study which were subjected for microscopic examination by 20% KOH, CFW and KONCPA. Mycological culture was done on Sabouraud's dextrose agar (with and without antibiotics).
Results: On analysis, the positivity by 20% KOH and CFW was 45.5%, 63.4% respectively while by KONCPA it was found to be 25%. In 38.6% fungal cultures revealed growth. At present, the etiological agents were dermatophytes (12.5%), especially Trichophyton rubrum, nondermatophytic isolates (75%) include Aspergillus spp., Penicillium species, Rhizopus and Candida spp. (8.3%). In our study toenails were affected in 84% and distolateral subungual onychomycosis (DLSO) was the commonest clinical presentation.
Conclusion: Along with dermatophytes, NDM and yeasts are also important etiological agents of onychomycosis in our set up.

Keywords: Non-dermatophytic moulds (NDM), Calcoflour white, KOH treated nail clipping (KONCPA)

INTRODUCTION

Onychomycosis (OM) is a common nail problem, accounting for up to half of all nail diseases.[1] Several nail disorders may mimic the onychomycosis clinically. Therefore, a sensitive, quick, and inexpensive test is essential for screening nail specimens for the proper therapeutic management. The etiological spectrum of any superficial mycosis is largely dependent on the microbial flora in the surrounding environment of the individual. It is influenced by the geographic, climatic and occupational factors.

Onychomycosis is an important public health problem because of the increase in immunosuppressive states. Large-scale studies in India are scarce and so the baseline incidence of onychomycosis is not firmly established.[2] The etiological spectrum of any superficial mycosis is largely dependent on the flora in the immediate environment of the individual. It is influenced by the geographic, climatic and occupational factors.

Onychomycosis can be caused by dermatophytes, yeasts and Non-Dermatophytic Moulds (NDM) that are transmitted through infected moist floor areas and less often transmitted via direct personal contact.[3] Non-dermatophytic moulds are accepted as uncommon or secondary pathogens in onychomycosis in already damaged nails by trauma, ischemia or disease, especially by dermatophyte infection and frequently seen in immunocompromised, poor peripheral circulation or temperate climates.[4-6] Dermatophytes, especially Trichophyton rubrum, are the most frequently implicated causative agent in onychomycosis. Previously these infections were regarded as contaminants, yeasts are now increasingly recognized as pathogens in finger nail infections, as are some moulds.[7]

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).[8-10] The prevalence of onychomycosis varies depending on age, sex, regional differences, cultural habits, migration, seasonal conditions, immune status of the host, living and hygienic conditions.[11] It is therefore essential to obtain epidemiological data for different regions to enable strategic planning for control and prevention.

A high rate of false-negative dermatophyte detection is observed when common laboratory methods are used. These methods include microscopic observation of potassium hydroxide-digested nail clippings and culture methods using agar-based media supplemented with cycloheximide, chloramphenicol and gentamicin to isolate dermatophytes. Microscopic detection methods that use calcofluor white staining or periodic acid- Schiff staining may also be substituted for and have previously been reported to be more sensitive than potassium hydroxide-digested nail clippings. DNA protocol is an alternative method for detecting trichophyton infections. When this protocol is used, the presence of T. rubrum DNA is directly detected. However, the viability of the dermatophyte is not addressed and further methods need to be developed for the detection of viable T. rubrum directly from nail samples.[12]

Aim

To determine the utility of different microscopic methods and culture for diagnosing onychomycosis.

Objectives

1. To determine the fungal etiological agents of onychomycosis.
2. To study the clinical pattern of onychomycosis
3. To correlate the mycological findings with the clinical diagnosis of onychomycosis.

METHODS

This cross-sectional study was carried out in the department of microbiology of Central India during the period of five months. Study was approved from the Institutional ethical committee. 44 Clinically suspected patients of onychomycosis attending to the dermatology clinic of tertiary care rural hospital of Central India.

Inclusion criteria

All patients with a clinical diagnosis of onychomycosis and giving consent were included in the study. Detailed history of patients along with clinical pattern and location of disease were taken & documented.

Exclusion criteria

The patients giving history of intake of systemic anti-fungal drugs and not giving consent were excluded from the study.

Sample collection and laboratory procedure

A standard basic laboratory methodology which includes the techniques to recognize the causative agents were included i.e. affected nails were cleaned with 80% alcohol to remove contaminants. Cleaned nails were clipped short with nail clippers and scrapings were collected from the involved nail bed and from the undersurface of the nail proximal to the cuticle with a no. 15 scalpel blade. Nail clippings and scrapings were subjected to 20% potassium hydroxide (KOH) mount, KOH treated Nail Clipping with Periodic Acid Schiff (KONCPA), Calcofluor white (CFW) and mycological culture. Repeat samples were collected only for confirmation of Non-dermatophytic moulds as pathogen.[13]

A total 44 nail clippings studied were divided into two parts and processed. 1st part was subjected to direct 20% KOH examination, CFW and KONCPA and 2nd part cultured on SDA (with and without Antibiotics). The growth obtained was identified as per standard methods. Fungal culture was considered as gold standard.

Direct microscopy - Potassium hydroxide mounts[12]

Normal or soft nail specimen was placed on a slide, and a drop of 10% KOH was added. A cover slip was applied

with gentle pressure to drain away excess KOH. For brittle nail specimen, it was placed in a test tube and a drop of 20% KOH added. Incubation was done for 2 h or more (in few cases incubation done till 48 h) until softening or digestion of the specimen occurred. Slides were then microscopically evaluated for the presence of branching thread-like hyphal elements or beaded spherical structures (Figure 1).

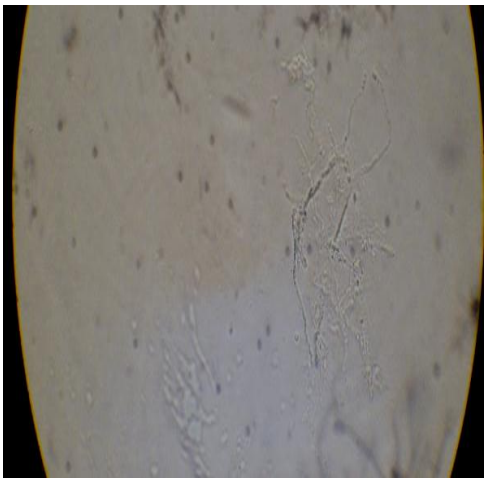

Figure 1: Positive fungal element in Direct KOH mount.

KOH treated nail clipping stained with periodic acid-Schiff (KONCPA)[1]

In this method, specimens were treated with 20% KOH at 56°C for 30 min and washed with isotonic sodium chloride solution. Specimens then centrifuged at 3000 rpm for 5 min and sediment was crushed to form a thin film over the slide. Then, the film was stained with PAS and visualized under microscope.

Observation: Fungi stained bright pink-magenta colour (Figure 2).

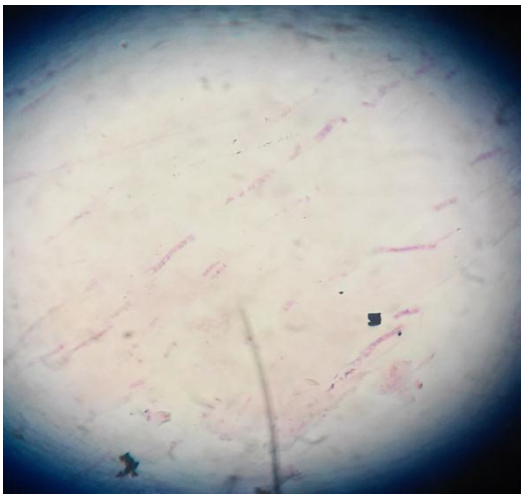

Figure 2: PAS positive stained reddish hyphae within the nail.

Florescent microscopy[1]

In fluorescent microscopy, KOH treated nail material was stained with fluorescent brightener, Calcofluor white (CW), on a glass slide and fungal elements were examined under fluorescent microscope.

Observation: Fungal element when present, fluoresced blue-green under UV light illumination (Figure 3).

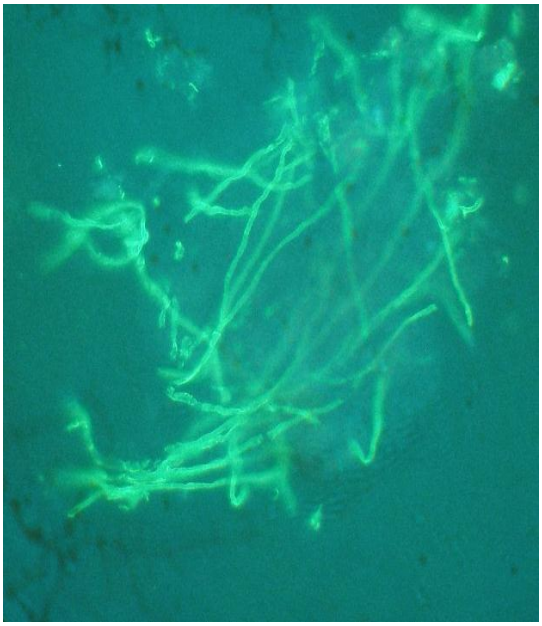

Figure 3: Positive fungal element fluorescing blue-green under UV light.

Mycology culture[13]

Culture was done using

- Sabourauds dextrose agar (SDA) supplemented with cycloheximide (100 µg/ml) and chloramphenicol (50 µg/ml) &
- Sabourauds Dextrose Agar (SDA) without cycloheximide supplement.

These culture tubes were inoculated at 23°C for 4 weeks. The pathogenic microorganisms were identified macroscopically for colony morphology and microscopic examination of lactophenol cottonblue (LPCB) mounts (Figure 4, 5).

Trichophyton rubrum was differentiated from other Trichophyton species by the urease test. Candida albicans was identified by Gram's staining and the germ tube test. Nondermatophytic moulds were isolated by subcultures.

Results obtained were tabulated and statistical evaluation of the results was done.

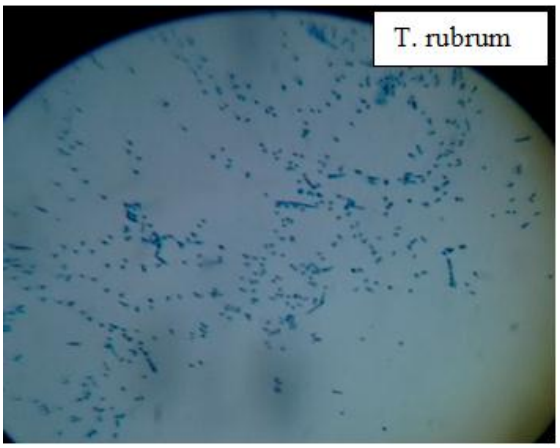

Figure 4: LPCB mounts of mycological culture positive isolates.

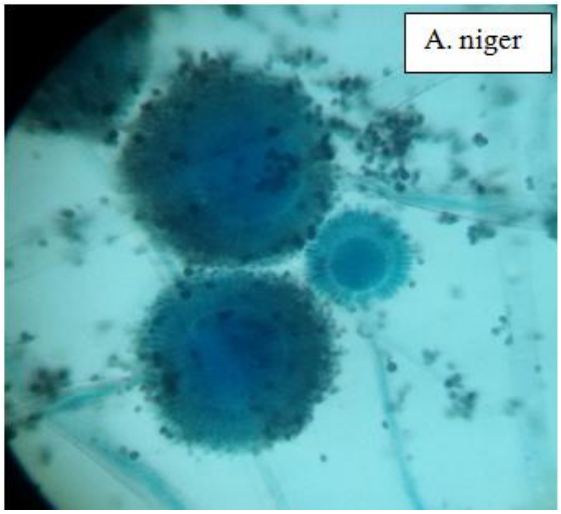

Figure 5: LPCB mounts of mycological culture positive isolates.

RESULTS

From 44 patients with clinically suggestive OM, 19 were men (43.2%) and 25 were women (56.8%) with female to male ratio of 1.32:1. They were belonging to the age-group of 12 to 72 years. Toenail infection constitutes maximum number of cases (84%) followed by fingernail affection in 7 cases (16%). Distal-Lateral Subungual OM (DLSO) was by far the dominant presenting pattern; 30 cases out of 44 (68.1%), while total dystrophic OM were represented by 8(18.2%) cases & white superficial OM are represented by 6 (13.6%) of cases.

Out of 44 patients, direct microscopy with KOH mount, CFW, histopathological examination with PAS staining and mycological culture showed positive results in 20 (45.5%), 28 (63.4%), 11 (25%) and 17 (38.6%) respectively (Table 1).

Culture yielded growth in 24 patients growing dermatophytes in 3 (12.5%), Candida in 2 (8.3%) and Non-Dermatophytic Moulds (NDM) in 18 (75%) patients. Similar significantly high Non-dermatophyte isolation has been reported earlier.[6,14] Aspergillus species were the leading NDM isolates (10) followed by Penicillium species (4), Rhizopus species (4). It is difficult to interpret the role of NDM because the same fungi that can be laboratory contaminants are also occasionally found to be pathogens. Hence it is suggested that if agents other than dermatophytes are isolated, they are probably laboratory contaminants unless KOH or microscopy demonstrates atypical hyphae associated with NDM or if the same organism is repeatedly isolated.[11] Using these criteria, only 11 out of the total 18 non-dermatophytic moulds isolated qualify as the true pathogens. Amongst this 11 NDM 6 were Aspegillus species, 2 were Penicillium species and 3 were Rhizopus species (Table 2).

Table 1: Comparison of positivity by different microscopic & culture methods.

Tests	Positive (Total No. of samples)
KOH	20 (45.5%)
CFW	28 (63.4%)
KONCPA	11 (25%)
Culture	17 (38.6%)

Table 2: Results of mycologic culture.

Fungal agents	Identified fungus as	Number (n=18)
Dermatophytes (n=3)	Trichophyton rubrum	2 (11.1%)
	Trichophyton mentagrophyte	1 (5.5%)
Candida (n=3)	Candida albicans	3 (16.6%)
Non-dermatophytic moulds (NDM)* (n=11)	Aspergillus niger	5 (27.7%)
	Aspergillus terreus	1 (5.5%)
	Penicillium spp.	2 (11.1%)
	Rhizopus spp.	3 (16.6%)

*Positive NDM isolates corrected.

Comparison of different microscopic methods was done. Considering culture as gold standard, the sensitivity and specificity of KOH, CFW and KONCPA were calculated (Table 3). It was found that calcoflour white was found to be having 100% sensitivity while KONCPA having highest specificity of 96.3% (Table 3).

Table 3: Comparison of results by microscopic methods.

	KOH (%)	CFW (%)	KONCPA (%)	M/E+culture (%)
Sensitivity	76.5	100	58.2	100
Specificity	74	59.3	96.3	74.7
PPV	65	60.7	90.9	70.8
NPV	83.3	100	78.8	100

DISCUSSION

The common cosmetic problem in today's era is dystrophic nails. Dystrophic nails may be due to onychomycosis and should be differentiated from other acquired and congenital conditions.[15] To our knowledge, this is the first comprehensive study of onychomycosis performed in people living in this rural and urban areas of central India.

Direct demonstration of fungal elements with KOH mount and the isolation of fungus by culture are the routinely done by laboratory methods. The method of obtaining nail clippings and the size of specimen are also important factors while considering these tests. Reported sensitivity of fungal culture for identifying dermatophytes varies from 25% to 80% with an approximately 30% false negative results with culture and KOH studies.[16] These high false-negative results are unacceptable; hence there is need for a test with higher sensitivity that also gives an early diagnosis. DNA-based techniques are currently being used to identify a number of pathogens and may soon be used regularly in the diagnosis of onychomycosis.[17] These tests are likely to be more expensive and may not be available in all centers dealing with nail infections.

In the literature, there are reports on histopathologic examination with PAS staining (HP/PAS) of nail clips as a highly reliable diagnostic tool for onychomycosis.[16,18-21] Periodic Acid-Schiff (PAS) stain demonstrates the presence of certain polysaccharides present in the walls of fungal hyphae. The study was conducted, in order to know whether the probability & convenience of pathogen identification significantly differs by HP/PAS from that by KOH mount and mycologic culture.

In the present study, direct microscopy was positive more than culture. 63.4% samples were positive by direct examination by CFW, 45.5% by KOH, 25% by KONCPA and 38.6% by 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.[22-24] In our study, 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.[25] 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.[26]

In the present study, we compared KOH mount, CFW versus PAS stained nail clipping to refine all the methods in the diagnosis of OM. Considering culture as gold standard, only 20 cases out of 44 (76.5% sensitivity) proved to be KOH positive, 28 (100% sensitivity) proved to be CFW positive while 11 (58.2% sensitivity) of KONCPA stained samples were positive. Variable results obtained with these procedures are reported in the literatures. Agreeing with our results, Kanga et al.[27] showed that 77/130 (59.2%) patients were positive with KOH while 49/130 (37.6%) proved positive with culture. The positive rates of KOH preparation, KONCPA, fluorescent microscopy, and culture were 66.1%, 61.3%, 79.0%, and 75.8%, respectively in a study of Yadav et al.[28] which correlates well with our study. Also Yadav et al reported the sensitivities of KOH (68%), CFW (89.4%) and KONCPA (65.9%) in the diagnosis of OM correlates well with our study. On the other hand, Machler et al.[29] found that 100% of patients got positive results with both PAS staining and KOH mounting, Our results are on the other side of the results obtained by Weinberg et, al11 and Lawry et al. Weinberg et al.[18] investigated 105 patients with KOH preparation, culture and PAS staining. They reported sensitivities of 92% for PAS, 80% for KOH mount and 59% for culture. Lawry et al.,[21] found PAS to be 85% sensitive while sensitivity for KOH was 53%. Supporting these results, other authors reported superior PAS positivity versus KOH. Liu et al.[30] reported positive PAS in 26/43 (61%) versus 19/43 (44%) for KOH. Reisberger et al.[31] reported 182/387 (47%) versus 156/387 (40%).

Though there are many literatures depicting the high sensitivity of KONCPA, Singh and Lavanya[32] highlighted certain fallacies of PAS staining in this context. They judged it is not an invaluable test in the diagnosis of OM owing to its ineffectiveness in identifying the causative pathogen, which would aid in advocation of correct treatment. Also, false positivity may occur with other inflammatory nail dermatoses as they may be indistinguishable histologically as also clinically.

KOH mount is a simple, rapid & inexpensive test to perform which requires minimum infrastructure but some amount of experience to interpret the smears. CFW method is rapid and easy to perform. Fluorescent brighteners like CW specifically bind to cellulose and chitin, which are the major components in the cell wall of fungi. The dye then fluoresces as it is exposed to UV radiation on fluorescent microscopy. This helps in easy visualization of fungal elements. But keeping a fluorescent microscope is an expensive means.

Our study revealed a mycological culture positivity of 38.6% which include dermatophytes & yeasts 16.7% each while non-dermatophytes were 61.1%. Trichophyton rubrum (11.1%) & Trichophyton mentagrophytes (5.5%) were the dermatophytic isolates while all the yeasts so isolated were Candida albicans (16.6%). Isolation of NDM in our study is higher than that seen in other studies.[33,34] This could be because of frequent exposure to soil saprophytes in our study. The most common of these isolates was the Aspergillus spp. (27.7%) followed by Rhizopus spp. (16.6%) & Penicillium spp. (11.1%). These NDM isolates are

considered to be the true pathogens as are the repeat isolates and also showed positive microscopic findings.

In our study, amongst the 44 clinically suspected patients of OM, 19 were men (43.2 %) and 25 were women (56.8%) with female to male ratio of 1.32:1. This female preponderance was considered to be due to increased participation of female in domestic wet work and also attributed to their field work. Also there are many reports had shown a greater susceptibility of females to this infection.[35,36]

In the present study, Distal-Lateral Subungual OM (DLSO) was by far the dominant presenting pattern; 30 cases out of 44 (68.1%), while total dystrophic OM were represented by 8(18.2%) cases & white superficial OM are represented by 6(13.6%) of cases as was found in many other studies.[14,24]

CONCLUSION

The most common pattern of onychomycosis noted was DLSO; however TDO & WSO were not uncommon. The toenails were most commonly affected compared to fingernails. Among the toenails, the great toe was most commonly affected. In the study out of 44 studied cases, 63.4% were positive by CFW, 45.5% were by direct KOH and by KONCPA 25%. Fungal culture results were positive among 38.6% patients. Non-dermatophytic moulds were the most common organisms isolated, which stresses the role of non-dermatophytic moulds in causing onychomycosis. Dermatophytes & Candida were the other pathogens noted causing onychomycosis. Among the dermatophytes T. rubrum was the most common pathogen. 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 the tests to confirm diagnosis.

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