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Original Research Article

Study of onychomycosis

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ABSTRACT

Background: Onychomycosis is one of the most common fungal nail infections caused by Dermatophytes, Non-Dermatophytic Molds (NDM) and Yeast. Though it is not life-threatening, can cause pain, discomfort, and disfigurement. It decreases the nail growth rate. Objectives: This study was carried out to document the clinico-mycological pattern and antifungal susceptibility pattern of onychomycosis.
Methods: The study group included 130 consecutive patients with suspected fungal nail infections, attending Dermatology outpatient department of King George Hospital, Visakhapatnam, Andhra Pradesh during November 2012 to August 2014. The nail clippings of the patients were collected and subjected to KOH mounts for direct microscopy and fungal culture and antifungal susceptibility tests.
Results: Onychomycosis was common among males (66.92%) than females (33.08%) with highest incidence was in age group 31-40 years (41.37%). Finger or toenails were exclusively involved in 32.18% and 55.18% patients respectively while these were involved concurrently in the rest of the 12.65% patients. Distal and lateral subungual onychomycosis seen in 64.36% of the patients was the most common clinical type. KOH and culture positivity were recorded in 56.92% and 48.46% cases respectively. Dermatophytes (50.58%) were predominant isolate followed by NDM (27.58%) and yeast (21.84%). Clotrimazole and ketoconazole were most effective antifungals against dermatophytes. For NDM, itraconazole, nystatin and amphotericin B and for yeast fluconazole and itraconazole were effective.
Conclusions: The present study gives an insight about the aetiological agents causing onychomycosis and their anti-fungal susceptibility pattern in this region. Thus, it can help in taking adequate control measures to prevent it.

Keywords: Clotrimazole, Dermatophytes, Ketoconazole, Onychomycosis

INTRODUCTION

Onychomycosis comprises all fungal infections affecting the nail apparatus, i.e., nail matrix, nail plate, cuticle, mesenchymal tissue and nail folds.[1] It accounts for up to 50% of nail disorders and 30% of all superficial fungal infections of the skin.[1,2] Nail changes in onychomycosis can occur in various forms ie. onychodystrophy, onycholysis, subungual hyperkeratosis, discoloration (melanonychia or leuconychia), or thickening of nail-plate.[3] Many affected nails become discolored, possibly resulting from the mixture of organisms present and from the opacity of the amorphous keratin itself.[4]

Onychomycosis, though not life-threatening, can cause pain, discomfort, and disfigurement.[5] Onychomycosis is

worldwide in distribution. Prevalence rates range from 2%-3% in temperate climates to 12% in tropical climates. Prevalence rates in children are 30 times less than in adults, ranging from 0%-0.2%.[6] Approximately 30% of patients with tinea elsewhere also have nail infection. Risk factors include psoriasis, diabetes mellitus and immunosuppression (HIV, cancer, rheumatological disorders), poor peripheral circulation, repeated nail trauma.[7,8] Onychomycosis is one of the early manifestations of HIV infection. Multiple nail involvement, isolation of both common and rare species and resistance to treatment are the characteristics of onychomycosis in HIV.[9]

Most studies have shown a higher prevalence among males and those who involve in sport activity. The prevalence of onychomycosis increases with age and reaches nearly 20% in patients over 60 yrs.[10] The prevalence is low among children compared with adults due to reduced exposure to infected environments (communal showers, public changing rooms and saprophytic fungi), faster linear nail growth, less cumulative trauma, due to smaller and thinner nail surface and lower prevalence of tinea pedis.[11] Patients with asthma, urticaria and angioedema are more likely to have onychomycosis, this could be explained by an allergic reaction to the fungus that causes the atopic diseases. Patients with a history of other fungal infections also have a higher risk of onychomycosis. Toenails are about seven times more frequently affected than fingernails due to three times slower growth rate.[12] Toenail infections was commoner in males and fingernail infections in females.[13]

The clinical presentations of onychomycosis are Distal Lateral Subungual Onychomycosis (DLSO), Proximal Subungual Onychomycosis (PSO), White Superficial Onychomycosis (WSO), Endonyx Onychomycosis (EOM), Candida Onychomycosis (CO) and Total Dystrophic Onychomycosis (TDO).

The etiological agents of onychomycosis differ from place to place and even within the same place differ with the passage of time. Dermatophytes are the most frequently implicated causative agents in onychomycosis (approximately 90% in toenail and 50% in fingernail). Non-Dermatophyte Molds (NDM) mainly affect toenails and occasionally fingernails. NDM accounts for 1.5-6% of all onychomycosis and it mainly affects older persons, who are over 60 yrs of age and patients with keratin abnormalities.[14] Onychomycosis caused by NDM is becoming increasingly prevalent even in healthy people. This apparent emergence might be an artifact of improved diagnostic techniques or increased awareness that these fungi are potential etiologic agents.[15] Yeasts which were previously regarded as contaminants, are now increasingly recognized as pathogens in fingernail infections.[16] Candida albicans accounts for 70% of cases, while C. parapsilosis C. tropicalis and C. kruzei account for the remaining. It is seen commonly in women because of the practice of frequent submerging of their hands in water.

Laboratory diagnosis includes direct microscopy (KOH preparation) combined with culture of nail clippings, which still remains the gold standard. If neither microscopy nor culture yields a diagnosis, histological analysis using Periodic Acid-Schiff (PAS) staining will help to determine whether pathogen is a fungus. It is the current gold standard test, as it not only proves the presence of fungus within the nail plate, but also gives a valuable clue to the level of invasion and its arrangement.[17] It is the most sensitive diagnostic test.[18] Subungual hyperkeratosis contains more fungal elements than the nail plate itself. Though rarely used DNA-based methods are effective for identifying mixed infections and quantification of fungal load.

Objectives of the study were to study risk factors and clinical presentation of onychomycosis in and around Visakhapatnam, to isolate and identify the etiological agents in patients with onychomycosis, to speciate the identified etiological agent, to study the antifungal susceptibility pattern of isolated fungi.

METHODS

The present study was carried out in the dept. of microbiology, Andhra Medical college, Visakhapatnam, India. Study group included 130 patients with suspected fungal nail infections attending the outpatient Department of Dermatology at King George Hospital, Visakhapatnam, India, during the period of November 2012 to August 2014.

Inclusion criteria

- Nail abnormalities like thickening of nail, discoloration, disfigurement, split, subungual hyperkeratosis.

Exclusion criteria

- Patients who received antifungals (topical/systemic) within past one month were excluded from the study.

After informed consent, detailed history of the patient was recorded. The affected nails were cleaned with 70% alcohol to remove any potential contaminants. Depending on the thickness of the nail plate and the amount of subungual hyperkeratotic material, either a nail clipper, or sterile scalpel blade was used to collect nail clippings. In case of paronychia, i.e. where yeast infection was suspected, exudate was expressed from the paronychial folds by probing with a flat excavator and on a pre-moistened swab, the material was collected. Collected material was placed in pre-sterilized Whatman no.1 filter paper envelope and put in sterile Petri dish for transportation.

The nail samples were submerged in 20% Potassium hydroxide (20% KOH) solution in a sterile container and left overnight for dissolution. Later, the softened material was placed on a sterile glass slide, coverslip was placed, examined under the microscope for the presence of any fungal elements (Figure 1).

After the direct microscopic examination, irrespective of demonstration of fungal elements, the samples were inoculated in two sets of Sabouraud Dextrose Agar (SDA), one supplemented with actidione (cycloheximide, 0.05 mg/mL) and another without it. The inoculated SDA bottles were incubated at 250C in Biological Oxygen Demand (BOD) incubator. The other set was kept at room temperature. The cultures were examined daily for first week and thereafter twice weekly for next three weeks and discarded if no growth was found at the end of 4 weeks. Standard mycological tests were done to identify the isolate.

The fungal isolates were identified as dermatophytes or filamentous fungi or yeast by their macroscopic and microscopic features.

Diagnosis of non-dermatophytic onychomycosis was made based on the following criteria:

- Nail abnormalities consistent with the diagnosis
- Positive KOH preparation with presence of hyphae in the nail keratin
- Failure to isolate a dermatophyte in culture and
- Growth of the same mold in at least two consecutive nail samples.

Macroscopic examination included,

- Rate of growth
- Colony characteristics

  Colour of growth (e.g., black, green, white, yellow)
  Consistency (e.g., cottony, fluffy, granular, suede like)
  Topography (e.g., flat, folded, rugose)
  Colony on reverse for significant pigment production (e.g., wine red, yellow, reddish brown, yellowish brown).

The dermatophytes were identified by tease mount technique stained with Lacto Phenol Cotton Blue (LPCB), growth on Dermatophyte Test Medium (DTM), and for filamentous fungi, tease mount technique with LPCB and slide culture technique when required were employed. Yeast like isolates were identified by Gram's staining, Germ tube test, and growth on CHROM agar Candida medium.

For presumptive identification of dermatophytes, Dermatophyte Test Medium (DTM), (Figure 2), was used. Most of dermatophytes species produced red colour within 3 to 6 days due to liberation of alkaline metabolites. If there was no change up to two weeks, the medium was discarded.

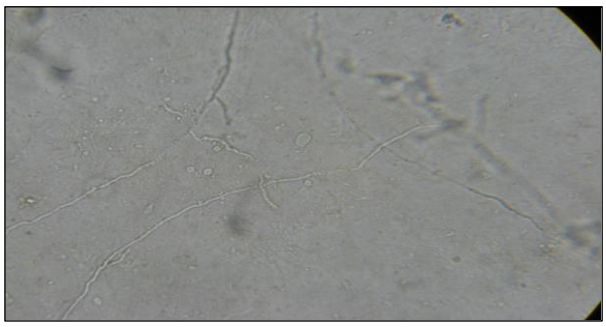

Figure 1: KOH mount showing fungal hyphae.

Figure 2: Dermatophyte test medium showing colour change.

Identification of isolates

Trichophyton spp.

T. rubrum colonies were white to fluffy, occasionally powdery to granular with diffuse wine-red colored pigmentation on reverse. Microscopically showed pyriform shaped microcondia borne along the sides of hyphae and Macrocondia, usually rare, which were pencil shaped. A negative urease test further confirmed.

T. mentagrophytes colonies were creamy tan, powdery to granular, flat surface with buff colour. Microscopically abundant spherical microconidia were seen in grape like clusters and macroconidia were thin walled and cigar shaped. Sometimes spiral hyphae were seen. They were identified by urease test positive.

T. tonsurans colonies were white, tan to yellow, suede-like to powdery, wrinkled with heaped or sunken centre with reverse, tan colored. Microscopically, numerous microconidia some, clavate or elongate, variable in size and shape borne singly along hyphae and macroconidia are rare, cylindrical with curved tips were seen. Some balloon forms of macroconidia were also seen.

T. verrucosum were very slow growing, glabrous to velvety white colonies with tendency to skin into agar surface. Microscopically, conidia were absent, sometimes macroconidia when present had characteristic rat-tail appearance and microconidia were large and teardrop shaped. Many *chlamydoconidia* were seen in chains, best at 370C.

T. schoenleinii colonies were glabrous, waxy, heaped, cerebriform, grey to tan colored colonies with absent microconidia and characteristic antler-like hyphae (favic chandeliers) and chlamydospores were frequently seen.

Epidermophyton floccosum

Colonies grew rapidly within 3 to 5 days initially gray white then developed distinct characteristic khaki-green pigment when matured. Yellow white streamers of hyphae were seen radiating from the center of the colony to periphery.

The surface became granular on further maturity as conidia were produced and microconidia were never produced. Macroconidia were usually produced in profusion and were typically club shaped, have three to five cells and thin, smooth walled. They often clustered in groups of three to four. *Chlamydoconidia* were typically present, particularly in older cultures.

Aspergillus spp.

Identified by rapidly growing filamentous fungi exhibiting various colors due to production of dense pigmented spores, and microscopically by chains of oval to spherical conidia borne from the tips of one or two rows of sterigmata arranged radially over the surface of the swollen tips of conidiophores called vesicle. *Aspergillus niger* colonies showed wooly, white to yellow at first and then turns to dark brown or jet black, granular surface due to dense proliferation of black spores. Microscopically it showed hyaline septate hyphae with conidiophores of variable length, biseriate phialades, covered entire vesicle and conidia were jet black covering the entire vesicle and obscuring them.

Aspergillus fumigatus colonies were velvety or powdery at first, turning to smoky green. Reverse was white to tan. Conidiphores were smooth, uniseriate phialides, covered upper half vesicle, parallel to the axis of stalk. Conidia were borne in chains from tips of sterigmata. *Aspergillus flavus* colonies were yellow to green and reverse golden to red brown. Conidiophores were pitted and spiny, biseriate or uniseriate, phialides covering entire vesicle.

Curvularia spp.

Rapidly growing, floccose, brown with black reverse. Hyphae were dematiaceous, septate and conidia were golden brown, multicell and curved with a central swollen cell giving a boomerang appearance.

Alternaria spp.

Dark grey brown and microscopically the conidia were muriform (multicelled with both transverse and longitudinal septa) and elongated with rounded broad end of one attached to pointed beak like end of adjacent conidia in chain formation.

Penicillium spp.

Rapidly growing blue green with rugosities, and microscopically there was brush like branching of conidiophores bearing long chains of small, spherical conidia borne on flask shaped blunt sterigmata.

Scopulariopsis spp.

Fast growing, initially appeared white, later became light brown and powdery in appearance. Truncate conidia were formed in chains on well- developed conidiogenous cells which were produced either singly or in penicillate arrangement.

Acremonium spp.

Rapidly growing, became white to gray in colour. Small septate hyphae that produced single, tube like phialides, which gave rise to clusters of elliptical, single celled conidia contained in a gelatinous cluster.

Rhizopus spp.

Fast growing, white to light grey initially which became darker as sporangia matured. The hyphae were broad, non-septate, with brown root-like short branches i.e. rhizoids arised adjacent to nodal position. Sporangia were at the tip of sporangiophore contained oval to cylindrical sporangiospores.

Candida spp.

Identified by cream colored, smooth and pasty colonies on SDA. Gram stain showed Gram positive budding yeast cells. It was identified as albicans by positive germ tube test (Figure 3). Speciation was done using Hi CHROM *Candida* differential medium (Figure 4).

Selective and differential chromogenic medium for identification of various Candida species.

Colors of colonies at 37°C for 48 hrs:

C.albicans Green
C.glabrata Pink to Purple
C.kruzei Pink
C.parapsilosis Cream to pale pink
C.tropicalis Violet

Antifungal susceptibility testing was done by disk diffusion method as per CLSI guidelines.

Preparation of Inoculum and Agar well diffusion Method-Preparation of Inoculum and media on which the disks were placed, and incubation period was different for Dermatophytes, *Candida* and non-dermatophyte filamentous fungi.

For dermatophytes, 21 days old grown culture of dermatophytes were scraped with sterile scalpel and dissolved in sterile saline solution to make different dilutions (1/2). One of diluted suspensions was used as inoculum which had absorbance of 0.600 at 450 nm determined spectroscopically (Electronics India). Antifungal screening was carried out using the agar well diffusion assay (CLSI, M-27A, M28A)16. 20 ml of sterilized Sabouraud dextrose agar medium was poured into a 15 cm Petri dishes in triplicates for each fungi. 20 µl of inoculum suspension of dermatophytes was distributed evenly over the surface and antifungal disks were placed respectively and plates kept in BOD at 27°C for 10 to 15 days and zones of diameter were noted by the help of zone scale reader (Hi Media) (Figure 5).

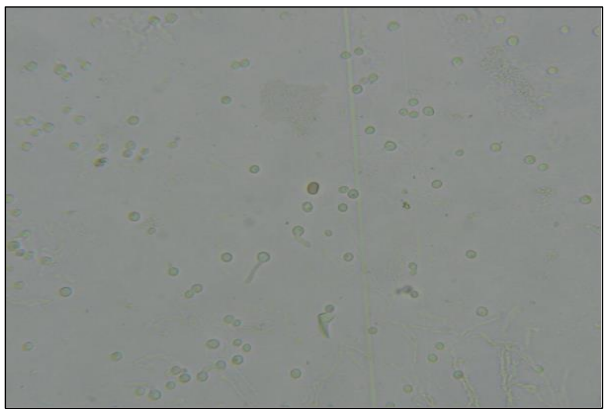

Figure 3: Germ tube test in *Candida albicans*.

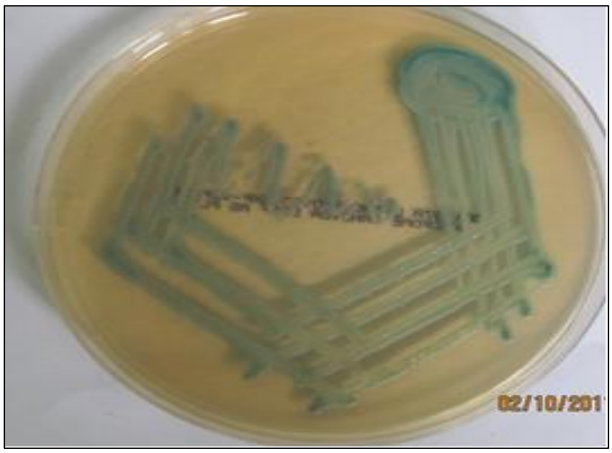

Figure 4: CHROM agar showing colonies of *Candida albicans*.

For Candida, it recommends the use of Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye medium. Inoculum was prepared from the yeast grown on SDA for 24 hrs, adjusted to match the turbidity of 0.5 Mc Farlands standard in the spectrophotometer. Sterile applicator swab was moistened in that cell suspension and used to inoculate the surface of Mueller-Hinton agar. Antifungal disks were placed and incubated in BOD for 24 hrs and observed for zones of inhibition (CLSI, M44A). (Figure 6).

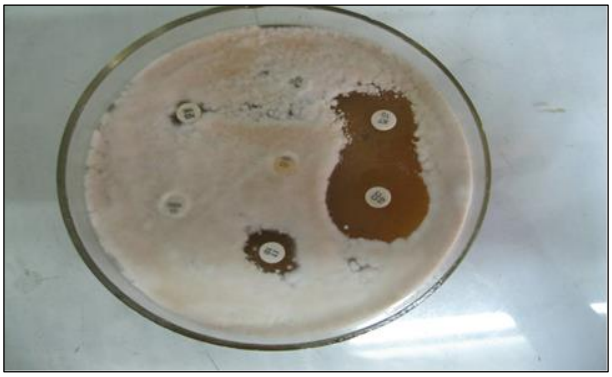

Figure 5: Antifungal susceptibility testing for dermatophytes.

Figure 6: Antifungal susceptibility testing for *Candida spp.*

For NDM, the fungi were sub cultured on potato dextrose agar one week before to testing and the inoculum suspensions were prepared to optical densities ranged from 0.09 to 0.11. The agar plates were inoculated with inoculum suspension with sterile swab and antifungal disks (HiMedia) were placed (CLSI, M51A) and incubated for 48 hours and observed for zones of inhibition (Figure 7).

Antifungal disks used were

Amphotericin B - 20 µg,
Itraconazole - 10 µg,
Fluconazole -10 µg,
Clotrimazole -10 µg,

Nystatin -100 units /disc
Ketoconazole - 10 µg

For Azoles sensitivity zones were

Susceptible - when ≥ 17 mm diameter,
Intermediate - in between 14 mm-16 mm diameter,
Resistant - when ≤ 13 mm diameter

For Amphotericin B sensitivity zones were

Susceptible - when ≥ 15 mm diameter,
Intermediate - in between 13 mm-14 mm diameter,
Resistant - when ≤ 12 mm.

For Nystatin

Susceptible – ≥ when 15 mm diameter,
Intermediate - in between 10 mm -14 mm diameter,
Resistant - when no zone.

RESULTS

Out of 130 patients with fungal nail infections, 87(66.92%) showed positive for fungal growth and 43(33.08%) were culture negative Table 1. Highest number of patients were from low income group 67(51.54%) followed by middle income group 54(41.53%). Least number of cases were from high income group 9(6.93%) Table 2.

Most of the patients were from urban area 74(56.92%) than rural area 56(43.08%) Table 3. The peak incidence of fungal nail infections was seen during July- September 2013(16.92%). Distal and lateral subungual onychomycosis was the common clinical variety prevalent in 56(64.36%) cases, followed by *Candida onychomycosis* 17(19.55%).

Table 1: Culture results of the study group.

Type of isolates	No. (N=130)	%
No. of cases showing fungal growth	87	66.92
No. of cases showing no growth	43	33.08
Total	130	100

Table 2: Distribution of study group based on socio-economic status.

Income group	No. (N=130)	%
Low income	67	51.54
Middle income	54	41.53
High income	9	6.93
Total	130	100

Majority of patients belonged to age group 31-40 years (41.37%). Least number of patients were of age group <20 years (2.29%). Elderly patients above 61 years comprised of 5.75% of total positive cases. Male predominance was seen in the present study. Out of total 87 males, 63 cases (72.42%) were culture positive. Total female comprised of 43, of which 24(27.58%) were culture positive.

Fungal infections elsewhere in the body (tinea infections) was the major predisposing factor seen in 23(26.46%), followed by history of trauma in 19(21.86%) patients. Household works were seen in 17(19.55%) and drug usage (immunosuppressant, steroids etc.,) for long duration accounted for 6.89% cases. HIV infected were 9(10.34%) and history of diabetes mellitus was seen in 11(12.65%), associated with psoriasis in 2(2.29%) (Table 4).

Table 3: Distribution of study group in relation to area of residence.

Area	No. (N=130)	%
Rural	56	43.08
Urban	74	56.92
Total	130	100

Table 4: Correlation with risk factors in culture positives.

Factors associated	No. (N=87)	%
Trauma	19	21.86
Diabetes mellitus	11	12.65
Hiv	9	10.34
Household works	17	19.55
Drug usage (long duration)	6	6.89
Other fungal infections elsewhere (hair, skin)	23	26.46
Psoriasis	2	2.29
Total	87	100

Fingernails were involved in 28(32.18%) cases, toenails in 48(55.18%) and both fingernail and toenail were involved in 11(12.65%) patients. In males, toenail 41(65.07%) involvement was predominantly seen and in females it was fingernails 14(58.83%) (Table 5).

Disfigurement and discoloration were seen in 100% patients, subungual hyperkeratosis in 70.11% patients, pain in 11.49% and paronychia in 9.19% patients. Among 87 males, agricultural labourers were 43(49.43%) followed by office workers 21(24.14%) and daily wage workers 20(22.98%). Among 43 females, high incidence of onychomycosis was seen in household works 20 (46.52%) (Table 6).

KOH mounts were positive in 74(56.92%) patients, among which culture were positive in 63(48.46%) cases and culture negative in 11(8.46%). KOH mount were negative in 56(43.08%), among which culture positive were 24(18.46%) patients and culture negative in 32(24.62%) (Table 7).

Table 5: Sex wise distribution of site of nail infection in culture positive cases.

	Male (%)	Female (%)	No. (N=87)	%
Fingernail	14 (22.24)	14(58.83)	28	32.18
Toenail	41(65.07)	7(29.16)	48	55.18
Both fingernail and toenail	8(12.69)	3(12.51)	11	12.65
Total	63(100)	24(100)	87	100

Table 6: Sex wise distribution of study group in relation to occupation (N=130).

Occupation	Male (N=87)		Female (N=43)	
	No.	%	No.	%
Agricultural laborers	43	49.43	14	32.55
Office workers	21	24.14	1	2.32
Daily wage laborers	20	22.98	6	13.95
Household workers	-	-	20	46.52
Students	3	3.45	2	4.66
Total	87	100	43	100

Table 7: Correlation between KOH mount and culture of study group.

	Culture positive		Culture negative		Total	
	No.	%	No.	%	No.	%
Koh mount positive	63	48.46	11	8.46	74	56.92
Koh mount negative	24	18.46	32	24.62	56	43.08
Total	87	66.93	43	33.08	130	100

Out of 87 culture positive, predominant isolates were dermatophytes 44(50.58%), followed by Non-dermatophyte molds 24(27.58%). Yeast accounted for 19(21.84%) in this present study (Table 8).

Out of 44 dermatophytic isolates obtained, Trichophyton species (Figure 8, Figure 9) constituted 39(88.64%) and Epidermophyton species were 5(11.36%) (Figure 10). Among Trichophyton species, *T. rubrum* was the most predominant isolate 24(54.54%) (Table 9).

Out of 24 NDM, *Aspergillus niger* accounted 5(20.83%), *Aspergillus flavus* and *Aspergillus fumigatus* 2(8.33%) each, *Scopulariopsis spp.* 4 (16.67%) , *Penicillium spp.* 3 (12.51%), *Alternaria spp.* and *Acremonium spp.* 2(8.33%) each, *Curvularia spp.* 1(4.16%) and *Rhizopus spp.* in 2(8.33%) (Table 10).

Among *Candida spp.*, *C. albicans* 11(57.89%) accounted for maximum number, others *C. kruzei* 4(21.07%), *C. parapsilosis* 2(10.52%) and *C. tropicalis* 2(10.52%) (Table 11).

Table 8: Group wise distribution of isolates from culture positive patients.

Isolate	No. (N= 87)	%
Dermatophytes	44	50.58
Non dermatophytic molds	24	27.58
Yeast	19	21.84
Total	87	100

Table 9: Distribution of dermatophyte isolated from culture positive cases.

		No. (N=44)	%
Trichophyton species	*T. Rubrum*	24	54.54
	T. Mentagrophytes	9	20.45
	T. Tonsurans	2	4.55
	T. Schoenleinii	2	4.55
	T. Verrucosum	2	4.55
Epidermophyton spp.	*E. Floccosum*	5	11.36
Total		44	100

Table 10: Distribution of non-dermatophyte molds isolated in culture positive cases.

	NO. (N=24)	%
Aspergillus niger	5	20.83
Aspergillus flavus	2	8.33
Aspergillus fumigatus	2	8.33
Scopulariopsis spp.	4	16.67
Alternaria spp.	2	8.33
Curvularia spp.	1	4.16
Acremonium spp.	2	8.33
Pencillium spp.	3	12.51
Rhizopus spp.	2	8.33
Mixed	1	4.16
Total	24	100

Figure 8: Growth on SDA of Trichophyton rubrum.

Figure 9: Growth on SDA of *Trichophyton mentagrophytes*.

Figure 10: Growth on SDA of *Epidermophyton floccosum*.

Antifungal susceptibility testing: In the present study susceptible as well as intermediate sensitive were taken as sensitive. Most of the trichophyton species were 100% sensitive to Ketoconazole and Clotrimazole.

NDM were 100% sensitive to Itraconazole, Nystatin and Amphotericin B. *Candida spp.* were 100% sensitive to Itraconazole, Fluconazole.

Table 11: Distribution of *Candida* species isolated in culture positive cases.

	No. (N=19)	%
C. Albicans	11	57.89
C. Kruzei	4	21.07
C. Parapsilosis	2	10.52
C. Tropicalis	2	10.52
Total	19	100

Table 12: Comparison of KOH wet mount and culture positivity with various studies.

Author	Area	Total KOH positive (%)	Total culture positive (%)	KOH positive and culture positive (%)	KOH negative and culture positive (%)	KOH positive and culture negative (%)	KOH negative and culture negative (%)
D.vijaya et al.	Bangalore	30	40	24	16	6	54
R kaur et al.	Delhi	45.5	60.2	32.8	27.4	12.7	27.1
P veer et al.	Aurangabad	81.8	48.8	48.1	5.6	38.6	12.5
N k das et al.	Kolkata	63.64	95.45	30.58	18.82	2.35	48.23
R. Lone et al.	Kashmir	56	40	36	4	20	40
P. Gelotar et al	Gujarat	17.78	37.78	17.78	20	0	62.22
Present study	Visakhapatnam	56.92	66.92	48.46	18.46	8.46	24.62

DISCUSSION

Onychomycosis is a chronic infection of the nails, nowadays considered as a serious problem for public health, in view of its high occurrence in worldwide population. In this study, higher incidence was seen in urban population (56.92%), which coincided with M Gupta et al, R Kaur et al. A higher incidence in the urban population may be due to their more cosmetic and health consciousness, better accessibility to health services and wearing of closed footwear. Onychomycosis was found to be common in the age group 31-40 years (31.53%). R kaur et al, estimated mean age of 34.96 years to be commonly affected with onychomycosis. This increased incidence in the younger population could be because they are more often exposed to occupation-related trauma, predisposing them to onychomycosis. They may also be cosmetic conscious than the older age group. Higher incidence was seen in males (66.92%) than females (33.08%) in the present study. P veer et al, estimated the ratio of m: f being 1.8: 1. Higher incidence in males may be because they are more exposed to outdoors with greater physical activity and are more prone to trauma. Use of occlusive footwear may be a contributory factor.

Toenail onychomycosis (55.18%) was more frequent than fingernail involvement (32.18%). This correlated with M

Gupta et al, L Adhikari et al.[19] This may be due to wearing of closed footwear for longer duration and trauma. Greater toenail was frequently affected than the rest other toes in this study. This is due to greater size of big toenail predisposing to increased trauma.[20] Fingernail onychomycosis had a greater impact on quality of life compared to toenail onychomycosis.[21]

Most common clinical pattern in the present study was Distal and Lateral Subungual Onychomycosis (DLSO) variety (64.36%), which coincided with R Lone et al. (64.44%).[22] Total dystrophic onychomycosis was the most common clinical type in HIV infection. Concurrent fungal infections elsewhere (tinea) was the highest risk factor (26.46%), followed by trauma (21.86%). This correlated with study conducted by B Sigurgeirsson et al, they found tinea infections were strongly associated with onychomycosis with odds ratio 4.26.

Peak incidence was seen during July-September (16.92%). This represents the monsoon season, the hot, humid atmosphere, offers ideal climate for growth of fungi. This correlated with the study of S Sarma et al.[23]

Among males, agricultural laborers (49.43%) were at high risk followed by office workers (24.14%). This correlated with S Sarma et al, and M Gupta et al. This may be due to increased perspiration, a greater risk of occupation related trauma and exposure to soil saprophytes. Office workers were more prone due to the habit of wearing closed and occlusive footwear for prolonged duration.

Females with household works (46.52%) were at high risk. This correlated with M Gupta et al, and D Vijaya et al. This may be due to the fact that, household work involving hands and feet constantly in water-soaked state, appears to be an important predisposing factor in homemakers. Soaps, detergents can also cause damage to the nails.

Direct microscopy using KOH preparation plays an important role in diagnosing fungal infections, however culture gives a a definitive diagnosis. In this study, 24 of the culture positive samples showed no fungal elements on direct KOH mount. This could be because, the fungus could have been in an inactive sporulating phase difficult to be seen by microscopy, but able to grow in appropriate media.[24] Of the culture negative cases, 11 showed fungal elements on KOH mount, but failed to grow in culture. This could be due to non-viability of the fungi prior to inoculation. This highlights the importance of both KOH preparation and culture in diagnosis. The difference in these rates among different studies may be due to factors involved in the collection, transport and inoculation of specimens, culture conditions, severity, type and stage of disease.[25] and stage of disease 25.Comparision of KOH wet mount and culture positivity with various studies (Table 12).

In the present study, dermatophytes (50.58%) were the predominant isolates, which correlated with N K Das et al, (50%) and R Kaur et al, (48.89%). Among NDM, the predominant isolate was Aspergillus species. This observation coincided with Sung Min Hwang et al.[26]

In the present study, Clotrimazole and Ketoconazole were the most effective antifungals against dermatophytes for non-dermatophyte molds, the effective antifungal agents were Itraconazole, Nystatin and Amphotericin B and for *Candida* species Fluconazole and Itraconazole were effective. This coincided with Jyothi Padmaja et al.[27] Meta-analysis done by N A Trivedi et al, concluded that though both itraconazole and terbinafine are well tolerated and highly effective drugs, continuous terbinafine is more effective than intermittent itraconazole at achieving mycological cure of toenail onychomycosis.[28]

CONCLUSION

In the present study, 66.92% of samples were positive for fungal growth and 33.08% were culture negative. Low socio-economic group (51.54%) were more affected. Onychomycosis was more common in urban areas (56.92%). The peak incidence of fungal nail infections was seen during July- September 2013 (16.92%). Highest incidence was seen in the age group 31-40 years (41.37%) followed by 41-50 years (21.85%). Toenail involvement was commonly seen (55.18%). Toenails were mostly involved in males (65.07%) and in females mostly fingernails (58.83%). DLSO was the common clinical variety prevalent in 64.36% cases. Fungal infections elsewhere in the body (tinea infections) was the major predisposing factor seen in 23(26.46%) patients. Onychomycosis was more common in males and among males, agricultural laborer (49.43%) were commonly affected. Among female's household works (46.52%) was commonly affected.

KOH mounts were positive in 56.92%, among which culture were positive in 48.46% cases. Predominant isolates obtained were dermatophytes (50.58%), followed by Non-dermatophyte molds (27.58%) and yeast (21.84%). Clotrimazole and Ketoconazole were the most effective antifungals against dermatophytes. For non-dermatophyte molds, Itraconazole, Nystatin and Amphotericin B and for *candida spp*. Fluconazole and Itraconazole were effective.

This study emphasized the need for microbiological confirmation for diagnosis and treatment for onychomycosis as antifungal resistance is an emerging condition. This study gives an insight about the etiological agents causing onychomycosis and their anti-fungal susceptibility pattern in this region. Thus, it can help in taking adequate control measures to prevent it.

Recommendations

Health education: The health education system needs to improve knowledge about onychomycosis among the

people by means of improving educational tools, preferably based on audiovisual techniques.

Provision of better facilities: Provision of better facilities in health care set up and spreading awareness about the advantages of better and early diagnosis could be a motivating factor.

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