STUDY OF CLINICAL AND MYCOLOGICAL PATTERNS OF DERMATOPHYTOSIS AND SENSITIVITY PATTERN OF DERMATOPHYTES IN NEPAL

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ABSTRACT

One of the most common reasons for outpatient visits to the dermatology departments are superficial fungal infections. There has been an emergence of chronic and recalcitrant superficial fungal infections not responding to conventional antifungal agents in our parts of the world. This has become a significant public health issue where patients spend their money first on over-the-counter topical Fixed Drug Combination (FDC) creams containing steroid and then on visiting numerous physicians and dermatologists due to the recalcitrant nature of infection. One of the ways of overcoming this problem would be to identify the causative agents and determine their sensitivity patterns to the common antifungal agents. This study, therefore, aims to counter this significant public health problem by providing information on identification of these dermatophytes and their sensitivity patterns to the routinely used antifungal agents. The results of this descriptive cross-sectional study done at Dermatology Department of Nepal Medical College in Kathmandu will provide a base for formulating new guidelines on the management of these conditions. The patients diagnosed to have dermatophytosis were registered by dermatologist. Skin scraping was taken from the rim of the skin lesion and further processed for potassium hydroxide (KOH) smear examination, culture and sensitivity test. Out of total 274 patients 150 were male and 124 were female. Most of the patients belonged to the age group 20 to 40 years. Tinea corporis was the commonest followed by tinea unguim, tinea pedis and cruris. KOH smear was positive in 60.94% and culture was positive in 47.4%. Trichophyton spp. was commonest (35.40%) followed by Microsporum spp. (7.67%) among the isolates. In Trichopyton spp. the mean minimum inhibitory concentration (MIC) of itraconazole, ketoconazole, terbinafine, griseofulvin and fluconazole was 0.25, 0.26, 0.32, 2.28 and 5.957µg/ml, respectively. In Microsporum spp., MIC of itraconazole, ketoconazole, terbinafine, griseofulvin and fluconazole was 0.41, 0.9, 0.05, 0.4 and 25.7µg/ml, respectively. Trichophyton spp. is the commonest causative agent of dermatophytosis in our part of the world. MIC of commonly used antifungal drugs like itraconazole and terbinafine are low which signifies that these fungi are still susceptible to these drugs. However, the MIC of fluconazole is high compared to other antifungal drugs.

KEYWORDS

Dermatophytosis, clinical pattern, antifungal sensitivity, Nepal

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INTRODUCTION

Dermatophytoses are superficial fungal infections of keratinized tissue caused by members of the genera *Trichophyton*, *Microsporum* or *Epidermophyton.*[1] These infections are a worldwide problem and their incidence has increased in the last two decades constituting a major category of cases attending the dermatology out-patient clinics.[2] They also constitute one of ten most common reasons for out-patient consultations in Nepal with a frequency of 4.4 to 5.7% of out-patient visits.[3,4]

There has been a surge of chronic and recalcitrant dermatophytosis reported in India in the last few years.[5,6] One of the reasons attributed to the surge of these infections are overuse of easily available over the counter, inexpensive fixed drug combination (FDC) topical agents (steroid, anti-fungal and anti-bacterial).[7] Steroid abuse is also a common problem seen in Nepal.[8,9] As a result of this, the textbook recommended dosage and duration of antifungal treatment for dermatophytosis have been rendered inadequate.[6] One of the ways to counter this would be to support the clinical diagnosis by identification of the etiological agent with the aid of culture and initiate treatment based on the results of the sensitivity patterns.[10] Therefore, this study aims to provide information on determination of the clinical profile of dermatophytosis, etiological agent as well as susceptibility of the agents to the currently available antifungal drugs. With the information gained from this study, newer guidelines can be formulated for the management of these superficial fungal infections.

MATERIALS AND METHODS

Study design: A descriptive cross-sectional study was carried out at the Department of Dermatology and Department of Microbiology of Nepal Medical College Teaching Hospital (NMCTH), Gokarneshwor-8, Kathmandu, Nepal for a duration of two years (2019 - 2021). Patients of all ages and sex clinically diagnosed as dermatophytosis by two registered dermatologists were included in the study

Sample collection: Samples consisting of epidermal scales were scraped from the rim of lesions using a sterile scalpel blade following cleaning of the affected sites with 70% v/v isopropyl alcohol. The scrapings were collected on a piece of sterile brown paper and transported to the laboratory within 2 hours for microscopy and culture of dermatophytes.

Sample processing: Potassium hydroxide (KOH) smear examination: Specimen collected for microscopy was mixed with 10% KOH over a glass slide and covered with coverslips. The wet smear after digestion of keratin was carefully examined under low (X10) and high (X40) power objective for the presence of hyphae and/or arthroconidia.

Culture of dermatophytes: Specimens collected for culture were inoculated into Sabouraud dextrose agar with antibiotics, cycloheximide or dermatophytes test medium (DTM). The plates were incubated at 28°C for up to 4 weeks and examined at 2 to 3 days intervals for fungal growth. Identification of dermatophytes was done by studying colony characteristics, microscopic morphology, slide culture, hair perforation test and urease test according to standard microbiological methods.

Antifungal susceptibility testing: Minimum inhibitory concentration (MIC) of all antifungal agents tested for dermatophytes were calculated after performing antifungal susceptibility testing by broth micro-dilution method using CLSI document M38-A2 guidelines.[11]

Preparation of inoculum: Isolated dermatophytes were grown on potato dextrose agar (PDA) for conidia formation. After the appearance of the sufficient growth, the fungal colonies were covered with 5 ml of sterile saline (0.9%), and the suspension was made by gently probing the surface with the tip of a sterile Pasteur pipette. The resulting suspended mixture was withdrawn and filtered in a sterile tube having a gauze piece filter to remove hyphae. The filtered suspension with conidia in the test tube was mixed with a vortex mixer for 15 seconds. Concentration of conidia in the suspension was calculated by counting on cell counting haemocytometer (Neubauer chamber). Cell suspension was then diluted or concentrated accordingly to get standard inoculum size of 1-3x 10^3 cfu/ml.

Preparation of antifungal drug dilutions: Stock solution of all antifungal agents to be tested was prepared by dissolving them in their respective solvents (ketoconazole, terbinafine, griseofulvin, itraconazole in dimethyl sulfoxide and fluconazole in sterile distilled water). From the stock solution, with the serial two-fold dilution, antifungal agents of different concentration were prepared. Final concentrations ranged from 0.125 to 64 µg/mL for fluconazole, 0.03 to 16 µg/mL for ketoconazole, itraconazole and terbinafine, and 0.03 to 8 µg/mL for griseofulvin.

Test procedure: The tests were performed in polysterene microtitre plates with flat bottom wells. Each well was inoculated with 100 µl of the standard inoculums of the conidial suspension prepared in RPMI-1640 medium and 100 µl of diluted drugs were added correspondingly to each well. The plates were incubated at 28°C for 7 days for growth of the fungi. Growth and sterility control wells were also maintained for each assay and all the tests were performed in duplicate. The highest dilution of the drug, which inhibited ninety percent of the fungal growth, was taken as the MIC. *Trichophyton mentagrophytes* ATCC MYA-4439 was used as the control.

RESULTS

Among the 274 cases, 150 (54.74%) were males and 124 (45.25%) were females with M:F of 1.2:1. The majority of the patients (51.09%) were from the age group of 20-40 years (Table 1).

Table 1: Age wise distribution of patients

Age (years)	n of patients
<21	43
21-40	140
41-60	73
>60	18

Regarding the clinical variant, the commonest variant was tinea corporis found in 71 cases (25.91%). Tinea faciei, on the other hand was the least common variant found in 4 cases only (1.46%) as shown in Table 2.

Table 2: Distribution of cases according to the clinical variant of dermatophytosis

Tinea type	n of patients
Tinea corporis	71
Tinea unguium	55
Tinea pedis	48
Tinea cruris	45
Tinea capitis	26
Tinea manuum	13
Tinea incognito	12
Tinea faciei	4

Table 4: Types of fungal isolates (n=130)

Fungal type	n
Trichophyton spp	97
Microsporum spp.	21
Epidermophyton spp.	5
Candida albicans	4
Non-albicans *Candida*	2
Aspergillus spp.	1

The mycological study showed the KOH positivity in 167 (60.94%) cases with culture positivity in 130 cases (47.40%). Both KOH and culture positivity was detected in 109 cases (39.78%). However, 58 cases (21.16%) had positive KOH with negative culture report. Interestingly, around 21 cases (7.66%) had negative KOH but positive culture report (Table 3).

Of the total 130 fungal isolates, the *Trichophyton* spp. (74.60%) were amongst the commonest followed by *Microsporum* spp. (16.15%), *Epidermophyton* spp. (3.84%), *Candida albicans* (3.07%), non-albicans *Candida* (1.53%) and *Aspergillus* spp. (0.76%) (Table 4).

Among total fungal isolates (n=130), 40 isolates (*Trichophyton* spp.: 35 and *Microsporum* spp.: 5) were selected by convenience sampling method and further subjected for antifungal susceptibility testing by broth dilution method. Regarding antifungal sensitivity test, the MIC range along with their mean (µg/ml) of five different antifungals tested against *Trichophyton* spp. and *Microsporum* spp. are shown in Table 5. The *Epidermophyton* spp. was excluded from the susceptibility testing as they do not produce microconidia and due to the limited budget and time frame susceptibility testing for all isolates were not performed.

This shows that the MIC range of fluconazole was highest against both *Trichophyton* spp. and *Microsporum* spp. whereas itraconazole was found to have lowest MIC against *Trichophyton* spp. (0.25 µg/ml) and terbinafine had the lowest MIC against *Microsporum* spp. (0.05).

Table 3: Distribution of cases according to KOH mount and culture report

Finding pattern	KOH +ve Culture +ve	KOH +ve Culture -ve	KOH -ve Culture +ve	KOH -ve Culture -ve
Number	109	58	21	86
%	39.78 %	21.16 %	7.66 %	31.40 %

Table 5: Antifungal susceptibility test results

Fungal isolates	Antifungal drugs (µg/ml)	0.0156	0.0312	0.0625	0.125	0.25	0.5	1	2	4	8	16	32
Trichophyton spp. (n=35)	Terbinafine	10 (28.6%)	5(14.3%)	-	5 (14.3%)	5(14.3%)	2 (5.7%)	8(22.3%)	-	-	-	-	-
	Fluconazole	-	-	-	3(8.6%)	5(14.3%)	1(2.8%)	2(5.7%)	6(17.1%)	2(5.7%)	7(20.0%)	8(22.3%)	1(2.8%)
	Ketoconazole	-	4(11.4%)	5(14.3%)	8(22.3%)	6(17.1%)	12(34.3%)	-	-	-	-	-	-
	Itraconazole	3(8.6%)	-	8(22.3%)	-	15(42.8%)	9(25.7%)	-	-	-	-	-	-
	Griseofulvin	-	-	-	10(28.6%	8(22.3%)	9(25.7%)	-	2(5.7%)	1(2.8%)	4(11.4%	-	1(2.8%)
Microsporum spp. (n=5)	Terbinafine	-	4(80.0%)	-	1(20.0%)	-	-	-	-	-	-	-	-
	Fluconazole	-	-	-	-	-	1(20.0%)	-	-	-	-	-	4(80.0%)
	Ketoconazole	-	-	-	-	-	1(20.0%	4(80.0%	-	-	-	-	-
	Itraconazole	-	-	1(20.0%)	-	-	4(80.0%)	-	-	-	-	-	-
	Griseofulvin	-	-	-	-	2(40.0%)	3(60.0%)	-	-	-	-	-	-

Table 6: MIC range and mean MIC of isolates against various antifungals			
Isolates	**Antifungals**	**MIC range (µg/ml)**	**Mean (µg/ml)**
Trichophyton spp.	Terbinafine	0.0156-1.0	0.32
	Fluconazole	0.125-32.0	**5.95**
	Ketoconazole	0.0312-0.5	0.26
	Itraconazole	0.0156-0.5	**0.25**
	Griseofulvin	0.125-32.0	2.28
Microsporum spp.	Terbinafine	0.0312-0.125	**0.05**
	Fluconazole	0.5-32.0	**25.7**
	Ketoconazole	0.5-1.0	0.9
	Itraconazole	0.0625-0.5	0.41
	Griseofulvin	0.25-0.5	0.4

DISCUSSION

Dermatophytosis is a superficial infection of the skin caused by a group of fungi known as dermatophytes.[1] The dermatophytes may originate from the soil (geophlic species), from animals (zoophilic species) or may be restricted to the human skin (anthropophilic species).[1] In this study, we analyzed the clinic-mycological profile of 274 clinically diagnosed cases of dermatophytosis.

Regarding the age wise distribution of dermatophytosis, maximum number of cases was from the age group 20-40 years (51.09%). A similar finding has been reported by Singh *et al*[12] of which the most common age group affected was 21 to 30 years (27.8%). Likewise, Janardhan *et al*[13] and Das *et al*[14] found the commonest age group affected to be 31-40 years (32%) and 20-30 years (24%), respectively. The increased frequency of dermatophytosis in this age group could be due to the increased physical activity of the people as this group comprises the most active age group.

Among 274 patients in our study, 54.74% were males and 45.25% were females with a male to female ratio of 1.2:1. A similar kind of male preponderance has been shown by Mathur *et al*[15] where 56.50% were males and 43.30% were females with a male to female ratio of 1.3:1. The study by Noronha *et al*[16] also found that males (62.0%) were more commonly affected than females (38.0%) with a male to female ratio of 1.63:1. This higher incidence in males could be due to the increased indulgement of males in outdoor activities making them more prone to fungal infections.

In the present study, the most common clinical variant of dermatophytosis found was tinea corporis (25.91%) followed by tinea unguium (20.07%), tinea pedis (17.51%), tinea cruris (16.42%), tinea capitis (9.48%), tinea manuum (4.74%), tinea incognito (4.37%) and tinea faciei (1.45). Comparing this with other studies conducted in Nepal, the study by Mathur *et al*,[15] Agarwalla *et al*,[17] Poudyal *et al*,[18] and Khadka *et al*[19] also found tinea corporis as the commonest variant accounting for 47%, 43%, 31.20%, and 25%, respectively. Internationally studies conducted by Kumar *et al*,[1] Doddamani *et al*,[10] and Poluri *et al*[20] have also reported similar finding of tinea corporis being the commonest variant.

Regarding mycological studies, our study reported KOH positivity in 60.94%. Similar positivity rate (58.18%) has been reported by Poluri *et al*.[20] The study by Pradhan *et al*[21] showed that 65.3% cases were positive for fungal elements on direct microscopic examination. Likewise, Mathur *et al*[22] showed direct microscopy positivity rate as 64.8%.

Overall culture positivity in our study was 47.40% which is lower than the positivity rate of direct microscopy. Similar findings with lower culture positivity rate than direct microscopy has been reported in several studies including the ones by Kumar *et al*,[1] Mathur *et al*[15] and Poluri *et al*[20] with positivity rate of 42.40%, 56.36% and 62%, respectively. However, studies by Khadka *et al*,[19] Grover *at al*[23] and Sen *et al*[24] have found that culture positivity rate was higher than the direct microscopy positivity rate. In our study culture positivity was more in KOH positive cases (109 cases) as compared to KOH negative cases (13 cases) in accordance with the study by Poluri *et al*[20] which indicates that KOH examination is a good screening test for dermatophytosis. Not only that, cases with KOH negative but culture positive reports highlights the significance of both direct

microscopy and culture for definitive diagnosis of dermatophytosis.[25]

In this study, out of 130 total fungal isolates, the most common isolate detected was *Trichophyton* spp. (74.60%) followed by *Microsporum* spp. (16.15%) and *Epidermophyton* spp. (3.84%). Similar studies with *Trichophyton* spp. as the commonest isolate has been reported by Noronha *et al*[16] (*T. mentagrophytes* – 48.30%, *T. rubrum* – 38.30%), Agarwalla *et al*[17] (*T. rubrum* – 45.74%, *T. mentagrophytes* – 26.60%) and Munir *et al*[25] (*T. rubrum* – 52.94%, *T. mentagrophytes* –29.42%).

According to the fungal culture and sensitivity test report of our study, fluconazole was found to have the highest MIC (0.125-32.0, mean: 5.95µg/ml) against *Trichophyton* spp. whereas itraconazole had the least MIC (0.0156-0.5, mean: 0.25µg/ml). Likewise, for *Microsporum* spp., fluconazole had the highest mean MIC (25.70 µg/ml) whereas terbinafine had the lowest mean MIC (0.05µg/ml). Our result indicates high MIC for fluconazole might be due to its frequent use against fungal infections in our set up and hence is showing less effectivity compared to past and hence itraconazole and terbinafine could be the antifungals of choice against dermatophytosis. Besides, another important result seen in our study is the low MIC of griseofulvin with *Microsporum* spp. Griseofulvin has fallen out of dermatologists list of favored medication for treatment of dermatophytosis other than tinea capitis. But this sensitivity pattern shows that it still could be considered a much needed alternative in therapeutic armamentarium for dermatophytosis.

As per the study by Girish *et al*,[26] the MIC (mean, µg/ml) obtained ranged from 0.06 to 0.5 µg/ml (0.121) for terbinafine, 0.06 to 4 (0.62) for itraconazole, 0.06 to 4 µg/ml (0.857) for ketoconazole and 0.5 to 8 µg/ml (2.151) for griseofulvin; thus highlighting the efficacy of terbinafine amongst all antifungals. Likewise study by Indira *et al*[27] has also reported the MIC range for terbinafine to be lowest (0.001 to 0.64 µg/ml) followed by ketoconazole (0.01-3.84 µg/ml), itraconazole (0.32-5.12 µg/ml) whereas griseofulvin and fluconazole showed a highest MIC range (0.32-5.12 µg/ml). The study done by Fernandez *et al*[28] displayed the lowest MIC of eberconazole (0.11 µg/ml) while those of cotrimazole, miconazole, and ketoconazole were 0.22, 0.43, and 0.72 µg/ml, respectively.

The first study related to fungal culture and sensitivity in Nepal by Mathur *et al*[22] showed that dermatophytes had the highest sensitivity to miconazole (48.2%), followed by itraconazole (27.7%), clotrimazole (12%), with surprisingly no sensitivity to ketoconazole and fluconazole. Similarly, as per the study by Pradhan *et al*,[21] dermatophytes had the highest susceptibility to itraconazole (75.2%), followed by clotrimazole (74.8%). However, in the contrary, there was a high resistance (91.6%) to fluconazole. Further, according to study reports of Singh *et al*,[29] terbinafine was found to have the highest activity followed by ravuconazole, posaconazole, ciclopirox, itraconazole, griseofulvin and fluconazole by broth microdilution assay against dermatophytes showing these antifungals as attractive newer options.

This study has highlighted that *Trichophyton* spp. is the commonest organism causing dermatophytic infections in our setting. In addition, it has reflected the importance of fungal culture and sensitivity as the drugs like fluconazole has shown high MIC indicating that fungi are developing less susceptibility to it, however, low MIC against itraconazole and terbinafine indicates dermatophytes are still susceptible to these drugs. Similar kind of studies with detail studies of fungal species are advocated to do in future so that the problems related to the resistance (sensitivity pattern) and recurrence of dermatophytosis will no more be a burning issue as it's been these days.

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