

A Cross-Sectional, Clinico-Mycolological Research Study of Prevalence, Aetiology, Speciation and Sensitivity of Superficial Fungal Infection in Indian Patients

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Abstract

Introduction: Skin is a mechanically protective layer as well as cosmetically significant anatomical structure. The superficial cutaneous fungal infections involve its outer most covering including appendages like hair and nails. This the most common causes of skin disease in many tropical countries.

Objectives: To study the prevalence, aetiology, speciation and sensitivity of superficial fungal infections.

Materials and Methods: The study was conducted on 100 clinically diagnosed cases of Superficial Fungal Infection attending the Dermatology OPD for a period of 6 months. Detailed history was taken in relation to age, sex, duration of illness, personal history, recurrent infections etc. Clinical examination of lesion included number, types of lesions, scaling, presence of crusts or pustules, scarring, black dot appearances, hair lusture etc. Samples like skin scrapings, hair stubs, nail clippings and pus were collected for KOH study and fungal culture.

Results: Dermatophyte infection mostly *Tinea corporis* is found to be more in male in 20-30 years age group of population. Covered part of the body is affected more and commonly hygiene plays a major part of its occurrence. KOH positivity is found in 68% and culture positivity in 88.2% of the clinically diagnosed cases with *Trichophyton* species as the commonest. Fluconazole and Clotrimazole found to be highly sensitive ; Ketoconazole found to be partially sensitive with 65% .

Conclusion: Dermatophytes were the commonest mycolological isolate with *Trichophyton rubrum* as the common infecting species. Young male preponderance with involvement of covered areas of the body of both skilled and unskilled workers. This can be prevented by a meticulous personal hygiene. Fluconazole and Clotrimazole found to be highly sensitive.

Keywords: Superficial fungal infection; Dermatophytes; Tinea; KOH; Fungal culture; Personal hygiene

Introduction

With the control of most bacterial infections in the developed countries, fungal infections have assumed greater importance [1]. Fungal infections are extremely common in the tropical region and some of them are serious and even fatal. Humans are the normal hosts for this group and transmission may occur by direct contact or indirectly by fomites, [2] however, host factors such as immunologic status and local factors such as trauma, excessive moisture or occlusive clothing may constitute risk factors when combined with exposure to the etiologic fungi. They produce diverse human infections ranging from superficial skin infections to internal organ invasion (systemic disease). These infections usually occur as a result of decrease in natural human defenses (immuno- compromised individuals), or opportunistic heavy exposure to the fungus [3]. Although rarely life threatening, they can have debilitating effects on a person's quality of

life and may in some circumstances spread to other individuals or become invasive. Most superficial and subcutaneous fungal infections are easily diagnosed and readily amenable to treatment [4].

More than 50,000 valid species of fungi exist, but only 100 to 150 species are generally recognized as causes of human disease and approximately 25 species cause the majority of human disease [5].

Fungal infections are generally not communicable in the usual sense from person- to- person transmission. Animal- to- person transfer is also rare, with most such transmissions involving certain dermatophytes. Humans become an accidental host for fungi by inhaling spores or by the introduction of fungal elements into tissue by trauma. Except for disease caused by the dimorphic fungi, humans are relatively resistant to infections caused by the fungi. The major factors responsible for the increase in the number of fungal infections have been alterations in the host, particularly in the immune system.

The infections may occur in patients with debilitating diseases such as progressive infection with the human immunodeficiency virus

(HIV) or diabetes mellitus, or in patients with impaired immunologic function resulting from corticosteroid or antimetabolite chemotherapy. Other common predisposing factors also include long term intravenous cannulation, complex surgical procedures, and antibacterial therapy [5].

From broad perspective, fungal infections may be referred to as either “superficial” or “deep-seated” (or “systemic”). However, this broad categorization no longer serves the needs of clinical practice, as several fungal species once thought to be “superficial” may cause disseminated disease [6]. There is little tissue damage by these fungi and immune response is also of a very low threshold [7].

The superficial fungal infections include some of the most common infectious conditions, such as ringworm or dermatophytosis and pityriasis versicolor, as well as rare disorders including tinea nigra. Their prevalence varies in different parts of the world, but in many tropical countries they are the most common causes of skin disease [8].

Superficial fungal infections (SFIs) affect millions of people worldwide, with an estimated lifetime risk of 10%–20%. [9] The pathogens responsible for SFIs include dermatophytes, yeasts, and molds. Dermatophytes are the most frequently encountered causative

agents of SFIs, leading to tinea infections, which are generally classified according to the body site affected.

The dermatophytes are by far the most significant cutaneous fungi because of their widespread involvement of population at large and their worldwide prevalence. [10] The dermatophytes are molds that can invade the stratum corneum of the skin or other keratinized tissues derived from epidermis, such as hair and nails. They may cause infections(dermatophytoses) at most skin sites, although the feet, groin, scalp, and nails are most commonly affected [8].

Dermatophytosis is defined as “fungal infection of the keratinized tissue of the hair, nail and stratum corneum of the skin” [11]. The disease is caused by fungi belonging to genera Trichophyton, Microsporum and Epidermatophyton [12]. The fungal infections of the skin and its appendages are more common in tropical countries like India due to environmental factors like heat and humidity. The risk factors include socio-economic conditions like overcrowding, poverty and poor personal hygiene [13]. The type and frequency of dermatophytoses may change with time, due to changes in living standards and application of preventive measures like personal hygiene [14].

Age Groups	Tinea	Pityriasis versicolor	Candidiasis
<10 years	1 (1%)	-	-
10- 20 years	16 (16%)	7 (7%)	-
21- 30 years	26 (26%)	6 (6%)	1 (1%)
31- 40 years	10 (10%)	5 (5%)	-
41- 50 years	9 (9%)	4 (4%)	1 (1%)
51- 60 years	9 (9%)	-	2 (2%)
>60 years	3 (3%)	-	-

Table1: Infection type related with age group (Total No. 100).

Malassezia furfur, this lipophilic yeast-like fungus with its natural habitat in stratum corneum of human skin as resident flora causes pityriasis versicolor and has been implicated in pathogenesis of many dermatoses.

The superficial infections also include changes in pigmentation of skin such as tinea nigra. There is formation of nodules along hair shaft as seen in white as well as black piedra.

Sex Group	Tinea	Tinea versicolor	Candidiasis
Male	59 (59%)	15 (15%)	2 (50%)
Female	24 (24%)	7 (7%)	2 (50%)

Table 2: Gender based infection types.

The yeasts are not essentially pathogenic to humans but when the host’s defense mechanism, protective skin barriers or normal flora are altered, colonization, infection and full-fledged disease may be

established. Candida species being most significant of these organisms causes infections of skin and nails in addition to mucous membrane [10]. Superficial fungal infections are usually diagnosed clinically.

Site of present infection	Number
Face	3 (3%)

Palm surfaces	3 (3%)
Groin	19 (19%)
Trunk portion	12 (12%)
Scalp	1 (1%)
Finger or toe nail	12 (12%)
Proximal part of leg or arm	15 (15%)
Genital organ	2 (2%)
Gluteal region	6 (6%)
Axillary region	5 (5%)
Planter surfaces	6 (6%)

Table 3: Site of fungal infection.

The geographic location, cultural background, and population migration patterns significantly affect the characteristics and prevalence of SFIs in particular regions. A significant variation in the pattern of mycotic infection in different countries is clearly evident from studies performed in Algeria, [15] South Africa, [16] Mexico, [17] Italy, [18] Japan, [19] USA, [20] Canada, [21] Brazil, [22] India, [23] and Australia [24]. This heterogeneity in the prevalence of SFIs in different parts of the world has been attributed to factors such as climate (humidity, temperature), lifestyle (e.g., visiting public

swimming pools), involvement in outdoor activities, and the prevalence of underlying diseases (e.g., diabetes rate of 25% in the Saudi population) high incidence of immunocompromised patients as a result of human immunodeficiency virus (HIV) in some African countries. Another factor is the reluctance of patients to seek treatment because of the minor nature of the disease, or because of embarrassment, unless the condition becomes sufficiently serious to affect the quality of life.

Hygeinic practice	Number
Bath per day	
Once	78 (78%)
Twice	14 (14%)
Three times	6 (6%)
Four times	2 (2%)
Regular use of soap	
Yes	56 (56%)
No	44 (44%)
Careful in wiping of wet body parts	
Yes	48 (48%)
No	52 (52%)

Table 4: Hygeinic practice of the infected individuals (Total No. 100).

The identification of the fungal species is epidemiologically important since the source of infection can be traced and its transmission halted [20].

Medical interventions are useful in attending to patients at the individual level. But, due to socio-economic conditions like

overcrowding, poverty and poor personal hygiene the superficial mycoses may contribute to spread in hot and humid areas. Thus, there is an urgent need for public education campaigns and socio-economic interventions at the community level, to overcome the factors like overcrowding and lack of personal hygiene.

Types of work	Number
Skilled labour	11 (11%)
Unskilled labour	33 (33%)
Service	7 (7%)
Business	10 (10%)
Home maker	17 (17%)
Student	22 (22%)

Table 5: Patients type of work (Total No. 100).

The study is undertaken to isolate various fungal agents causing superficial fungal infections, risk factors and sensitivity.

Materials and Methods

The study was conducted on 100 clinically diagnosed cases of Superficial Fungal Infection who attended the Dermatology OPD of Saveetha Medical College & Hospital (SMCH), Kanchipuram,

Thandalam, Taminadu during the period of 6 months. Medical lab investigation was carried out on individuals, who were clinically diagnosed with superficial fungal infection, have been referred to the Mycology unit of Microbiology Department for proper etiological investigation. During the period of study a total number of 100 such individuals were referred to this department for medical attention and they represent the universe of study.

KOH Positive 68 (68%)	Culture Positive 60 (88.2%)
KOH Positive 68 (68%)	Culture Negative 8 (11.7%)
KOH Negative 32 (32%)	Culture Positive 7 (21.81%)
KOH Negative 32 (32%)	Culture Negative 25 (78.12%)

Table 6: Correlation between KOH and Culture.

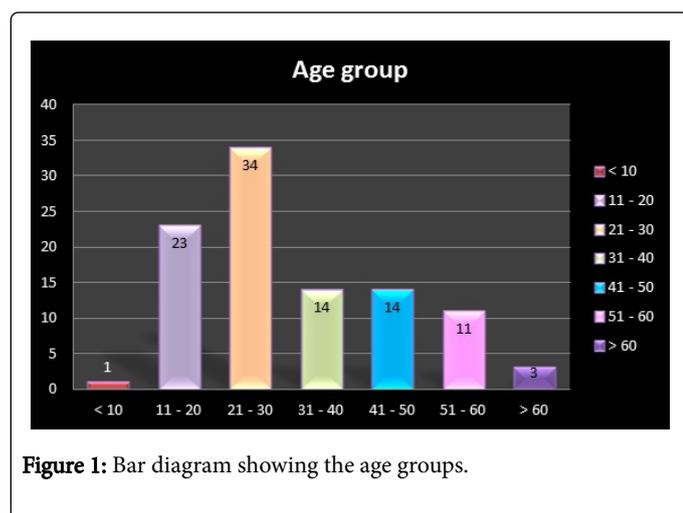


Figure 1: Bar diagram showing the age groups.

A well-tested questionnaire schedule had been designed to collect data on socio-demographic (age, sex, occupational types and family income), hygiene behaviour (source of water, use of soap, wiping of body parts, and sharing of towels) and on infection types.

Logistic regression had been done to find out the risk factors of superficial fungal infection. A number of socio-demographic features (educational level, household income, occupational types, sex, religion) behavioural factors (regular use of soap, sharing towels, frequently

bath/day, source of water) and other related variables (history of previous infection, site of infection, duration of work/day and nature of work) had been used.

Males (69%) were mostly affected, and less affected group were Females (31%) (Figure 2).

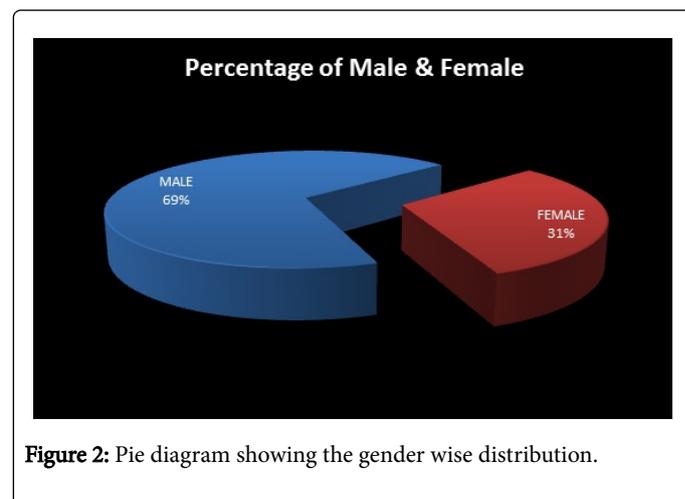


Figure 2: Pie diagram showing the gender wise distribution.

Prior to the collection to the data, the nature of research was explained to the participants and informed consent was taken from them.

Inclusion criteria

Superficial fungal infections including Diabetic patients, Immunocompromised patients.

Exclusion criteria

Deep infections; subcutaneous infections; opportunistic infections; accidental cases and surgical cases

Samples taken for this study were skin scrapings, hair stubs, nail clippings and pus. In the diagnosed dermatophytosis patients any ointments or other local applications present was removed with an alcohol wipe. Using a blunt scalpel, tweezers the lesion was firmly scrapped, particularly at the advancing border. The tops of any fresh vesicles were removed as the fungus is often plentiful in the roof of the vesicle. Other than the pus, the samples were studied by mounting in microscope after treating it with KOH for dissolving the keratin material.

For fungal culture the specimens were inoculated on the following culture media:

SDA

SDA with Chloramphenicol

SDA with Antibiotics and Actidione

SDA with Thiamine

DTM

For fungal staining purpose the Lactophenol Cotton Blue (LPCB) mount is used to study morphological features of fungal isolates.

Antifungal susceptibility testing was done by disk diffusion method using ketoconazole and clotrimazole (10 µg) and Fluconazole (25 µg).

Results

In our study of 100 patients with dermatophytosis the maximum affected age group were in their second decade (34%) followed by 10-20 years (23%) and least affected 3% were sexagenarian and above (Figure 1).

Among the 100 clinically diagnosed cases, Tinea infection (74%) was more than other infections like Pityriasis infection (22%) and less was Candidiasis (4%) (Figure 3).

Study area	Present study, SMCH, Tamilnadu	DD.Belurkar et al,7 Thane, Maharashtra	Huda et al,8 Upper Assam	Patwardhan Dave,4 Aurangabad Maharashtra	and Chowdhary et al,5, Aurangabad Maharashtra	Damie et al,6 ,Ambajogai Maharashtra	Krishnendu Das, et al,10, West Bengal
Predominantly Affected age group	21-30 years	21-40 years	10-30 years	21-30 years	21-30 years	21-30 years	21-30 years
Male:Female ratio	2.2:1	0.6:1	13:7	2:1	9:1	4:1	1:1.03
Tinea corporis	38%	20.19%	27.30%	24.50%	37.84%	23.80%	50.00%
Tinea capitis	1%	19.72%	03.50%	12.00%	15.19%	03.20%	11.60%
Tinea barbae	-	18.78%	-	02.80%	09.39%	-	-
Tinea pedis	08.00%	17.84%	08.30%	17.70%	-	21.60%	6.25%
Tinea unguium	04.00%	14.08%	07.10%	04.00%	-	03.70%	1.78%
Tinea manum	-	09.39%	03.50%	08.50%	-	04.60%	8.03%
Tinea cruris	22.00%	-	-	-	-	-	6.25%
KOH Positivity	68.00%	68.34%	58.33%	37.40%	56.07%	56.88%	69.64%
Culture Positivity	88.2%	71.00%	91.66%	22.81%	46.68%	46.30%	92.30%

Table 7: Comparison with other studies.

Among the 74 Tinea (ring worm) patients, 38 patients were affected with Tinea corporis (38%), followed by 22 patients affected with Tinea cruris (22%), 8 patients with Tinea pedis (8%), Tinea unguium (4%), and least affected clinical manifestations was Tinea capitis (1%), Tinea faciei (1%). However, 22 patients were affected from Pityriasis versicolor. Regarding the Candidiasis cases 2 patients affected with genital candidiasis (2%), and 2 patients are affected with oral candidiasis (2%) (Figure 4).

Tinea infection were commonly found more in the third decade and Pityriasis versicolor in the second decade (Table 1) and on gender based prevalence males were found to be more commonly affected (Table 2).

Mostly affected site of infection was groin region (19%), followed by proximal part of leg and arm, finger and toe nail infection (12%), trunk portion (12%), and less affected area was scalp (1%) (Table 3).

62% of the patients had fungal infection without any previous history of fungal infection, and 38% were infected earlier. Their hygienic practice and mode of work they do were depicted in (Tables 4 & 5).

The samples collected from patients were their fungal skin scrapings, nail clippings, hair stubs and pus (Figure 5). Except pus all other samples were studied by mounting in microscope with KOH. About 68% of the material showed KOH positivity for fungal infection (Figure 6). 62% of the patients had fungal infection without any previous history of fungal infection, and 38% were infected earlier. Their hygienic practice and mode of work they do were depicted in (Tables 4 & 5).

Among the entire KOH positive sample, culture positivity 60 (88.2%), and culture negativity 8 (11.7%). Also 32 KOH negative sample, 7 was culture positive (21.8%) (Table 6).

Among the 73 isolates, most of them were Dermatophytes 48 (65.75%), followed by Malessezia 22 (30.13%), and least were Candida 3 (4.1%) (Figure 7).

Among all the dermatophytes, most common dermatophytes were Trichophyton group 47 (97.9%), followed by Microsporum group 1 (2.08), Epidermophyton was absent in this study (Figure 8).

Most common isolate was *T. rubrum* which accounted for 21 (43.75%). Second one isolate was *T. mentagrophytes* which accounted for 14 (29.16%), *T. violaceum* occupied the third position 6(12.5%). Others like *T. tonsurans* 3 (6.25%), *T. schoenleinii* 1 (2.08%), *T. verrucosum* 1 (2.08%), *T. flavescens* 1 (2.08%). Also Microsporum gypseum which accounted for 1 (2.08%) (Figure 9).

Among three Candida isolates, only one was *Candida albicans* (33.3%) and other two was *C. tropicalis* (66.6%).

Among the isolates 65% of them were shown sensitive to ketoconazole, 100% of the isolates were susceptible to Fluconazole and

clotrimazole. Out of 21 cases of *Trichophyton rubrum* 100% were susceptible to Fluconazole and clotrimazole; and 68% of them only shown sensitivity to ketoconazole.

Discussion

Skin is mechanically protective layer as well as cosmetically significant anatomical structure. The superficial cutaneous fungal infections involve its outer most covering including appendages like hair and nails. The causative fungi colonize only cornified layer of epidermis or supra-follicular portions of hair and do not penetrate into deeper anatomical sites [7].

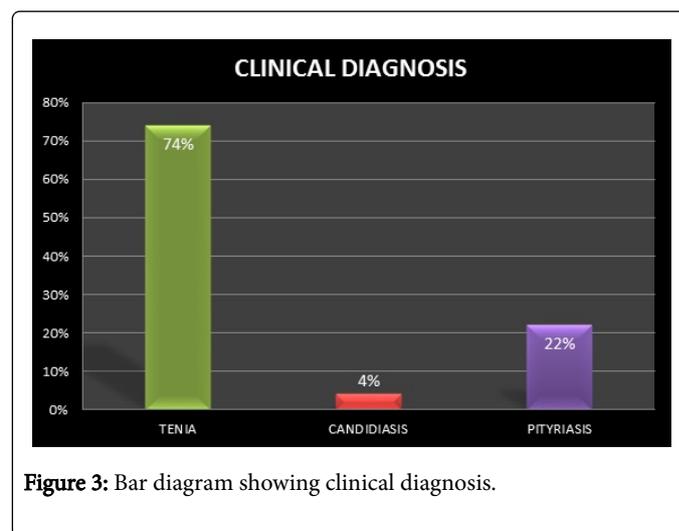


Figure 3: Bar diagram showing clinical diagnosis.

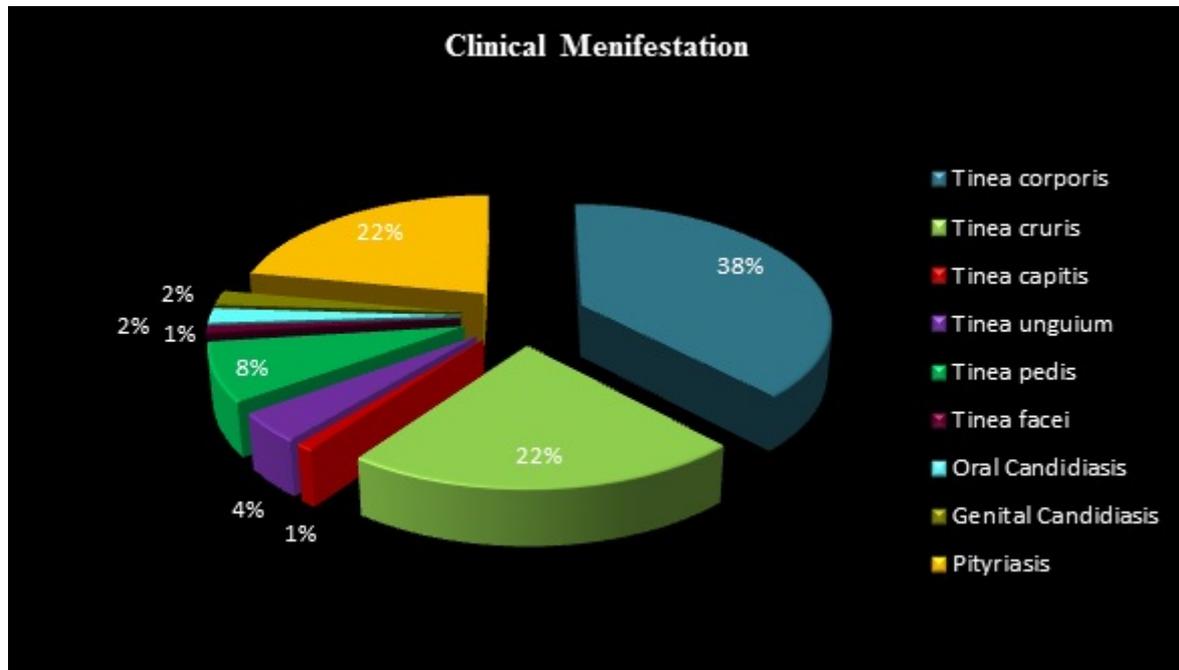


Figure 4: Pie diagram showing clinical manifestations (Total No. 100).

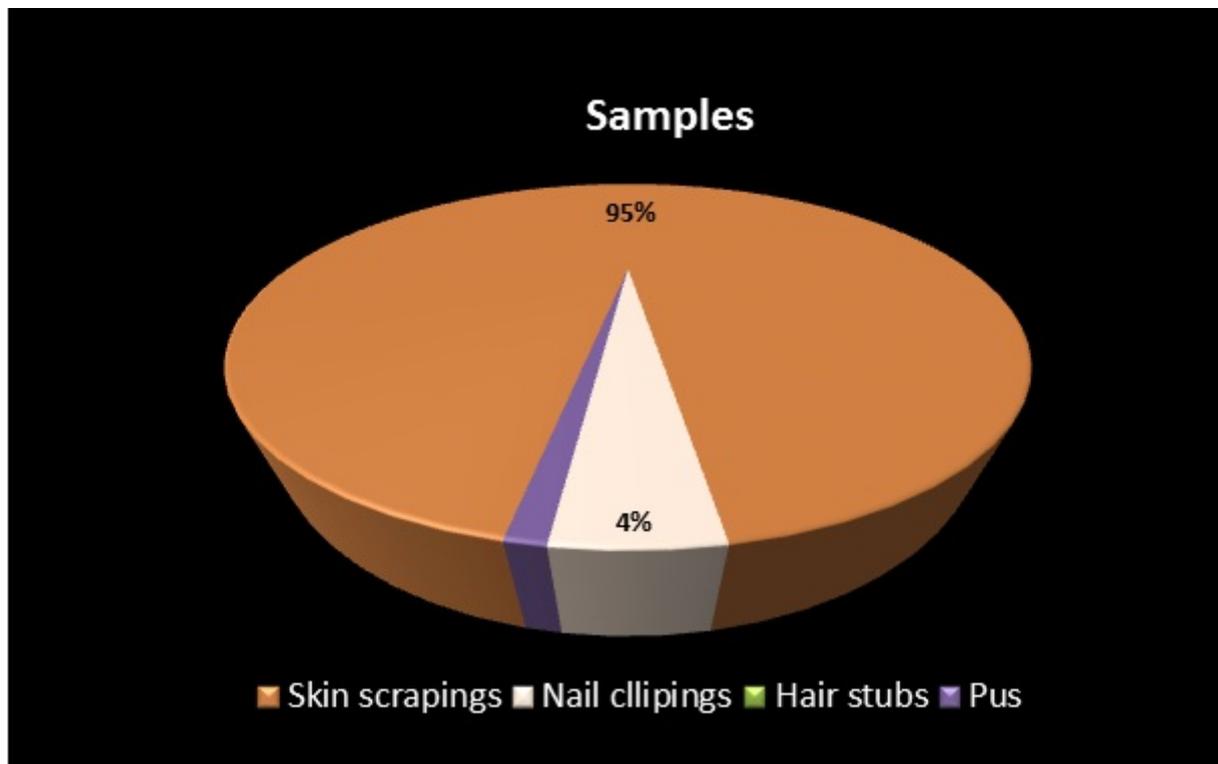


Figure 5: Pie diagram showing type of samples collected (Total No. 100).

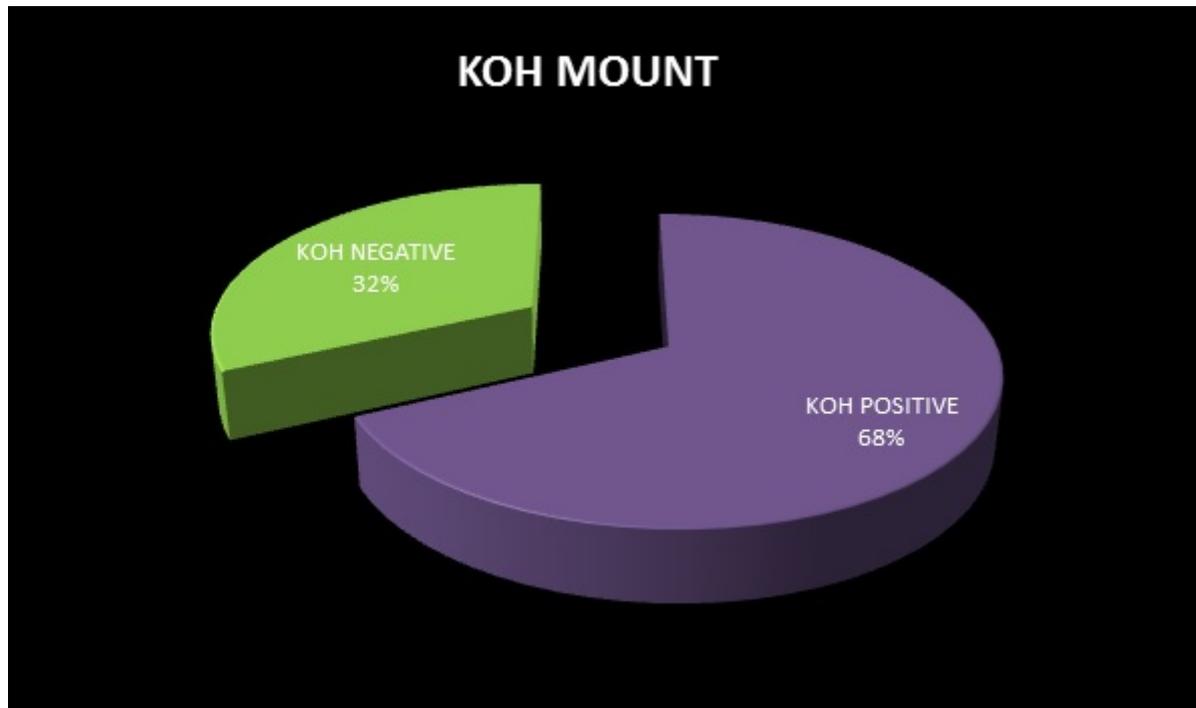


Figure 6: Pie diagram showing the KOH positivity and KOH negativity.

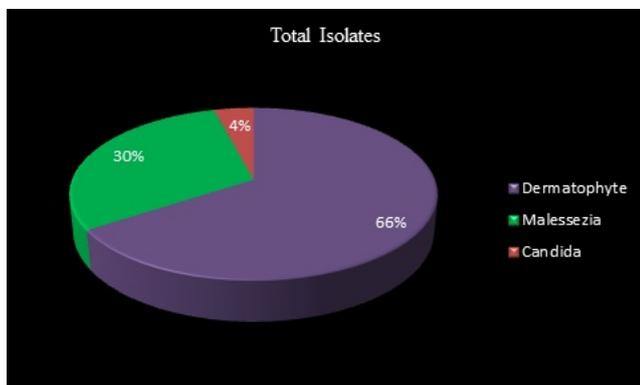


Figure 7: Pie diagram showing total isolates (Total No. 73).

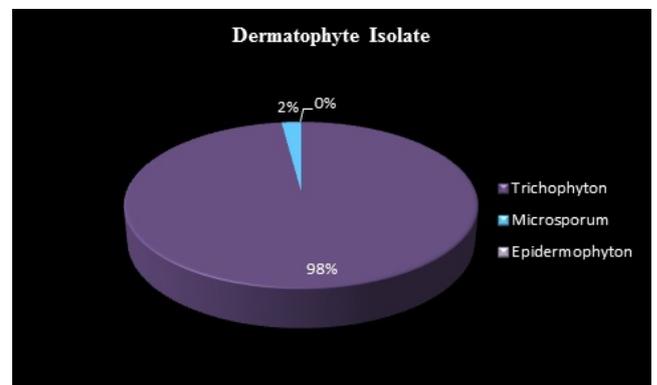


Figure 8: Pie diagram showing dermatophyte isolates (Total No. 48).

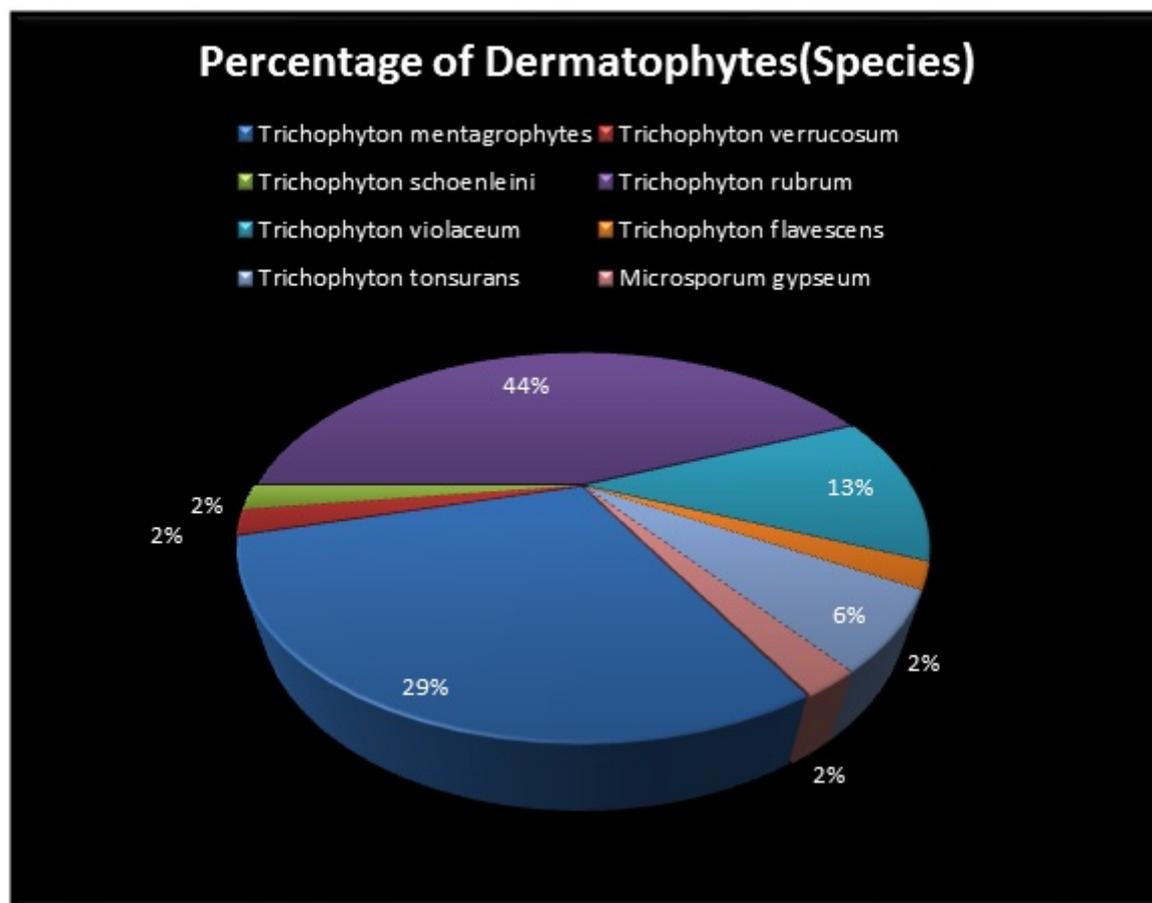


Figure 9: Pie diagram shows distribution of dermatophyte species.

The superficial cutaneous fungal infections (Dermatophytes) is the most common causes of skin disease in many tropical countries [8].

Dermatophytes (name based on the Greek for 'skin plants') are a common label for a group of three types of fungus that commonly causes skin disease in animals and humans [25].

There are three genera of pathogenic dermatophyte fungi

Trichophyton; Microsporum and Epidermophyton

Our study compared with other studies (Table 8) show that the chances of tinea corporis infection can be significantly predicted from age, occupation, previous history of fungal infection and site of the body involved. Dermatophyte infection is found to be more in 20-30 years age group of people. Among the dermatophyte infection Tinea corporis found to be high among the study population. It is found that dermatophytosis infection is more among the home makers, skilled and unskilled workers than the students and other category.

Patients with previous fungal infection found to be less affected than others. The covered parts of the body were commonly affected than the exposed part. Though, our study shows KOH positivity is found in 68% and culture positivity in 88.2% of the clinically diagnosed cases however, in comparison with other studies it is 35% and 37%.

Dermatophytes were the commonest mycological isolate with *Trichophyton rubrum* as the common infecting species followed by mentagrophytes. Regular bathing habits and good personal hygiene were found to be factors that prevent superficial fungal infections. Though decreased sensitivity is found with ketocanazole; Fluconazole and Clotrimazole were found to be highly sensitive.



Figure 10: Macroscopic structure of *T. rubrum* in DTM.

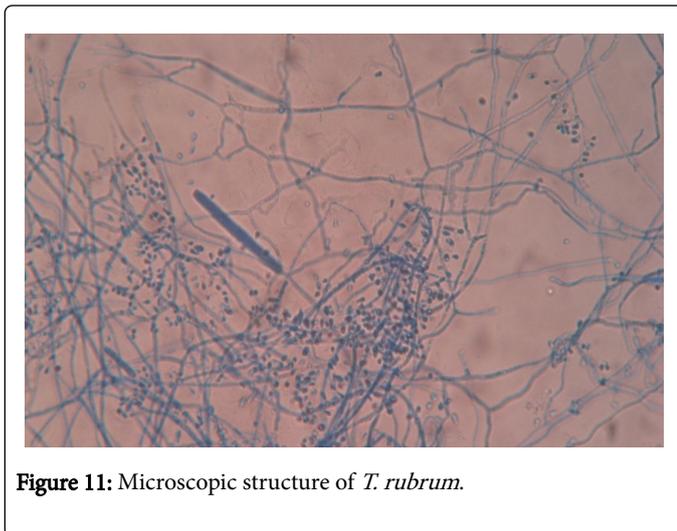


Figure 11: Microscopic structure of *T. rubrum*.

Conclusion

Dermatophyte infections has a young male preponderance with involvement of covered areas of the body common in both skilled and unskilled workers; and dermatophytes were the commonest mycological isolate with Trichophyton taking the lead, among them the commonest species was *Trichophyton rubrum*. This fungal infections can be well prevented by a meticulous personal hygiene. The most sensitive drug for fungal infection is Fluconazole and Clotrimazole.

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