A Clinico - Mycological Profile of Dermatophytosis at a Tertiary Care Hospital in Bihar

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ABSTRACT

Skin infections are common diseases in developing countries. Skin infections due to Dermatophytes has become a significant health problem affecting children, adolescents and adults of which Dermatophytosis are of particular concern in the tropics. The availability of scanty data on the prevalence and some associated epidemiological factors of Dermatophytosis in the Bihar state in northern India prompted us to take up the present study which utilizes conventional methods for isolation and identifications of Dermatophyte species from superficial mycosis in human patients. The Study was conducted from January 2013 to December 2014 one year at Nalanda Medical College Patna. All samples in the form of skin, hair and nails were collected randomly in batches under aseptically condition from both out patients & in-patients. KOH mount was done for direct exam. Culture was done on Sabouraud’s dextrose agar with antibiotics and cycloheximide and on dermatophyte test medium. Lactophenol cotton blue mounts and urease test were performed for species identification A total of 200 clinical samples were collected from patients at NMCH in which 110 out of 200 cases (55%) were positive by direct microscopy & 85 (29.29%) were positive by culture. The commonest age group involved was 21 – 40 years. Males 93 [88%] were more prone to dermatophytoses than females 13 [12%] in total positive case. Tinea cruris was the most common clinical presentation and Trichophyton rubrum was the most common fungal pathogen isolated from clinical samples. This study analyzed the Dermatophytic infections are prevalent throughout the world—due to a lack of education or resources for diagnosis and became a larger threat. In the present study Dermatophytosis is a major problem in North Indian territorial & prevalence of dermatophytic infection is alarming in both rural as well as urban community in Patna, Bihar.

Introduction

Skin infections are common disease in developing countries. Skin infections due to Dermatophytes has become a significant health problem affecting children, adolescents and adults therefore Dermatophytosis are of particular concern in the tropics. A fungal infection is usually resulting of the presence of some types of keratinophilic fungi on the skin. These fungi grow frequently on those areas of the skin that are warm, dark and moist. Dermatophytes are parasitic fungi that infect
the skin and cause infections of the skin, hair and nails because of their ability to obtain nutrients from keratinized material. These organisms colonize the keratin tissues and in response to their metabolic byproducts, host experiences inflammatory reactions. The organisms belong to 3 genera, Trchphyton, Epidermophyton and Microsporum. Dermatophytes may be grouped into 3 categories based on host preference and natural habitat. Anthropophilic species predominantly infect humans, Geophilic species are soil based and may infect both humans and animals, and Zoophilic species generally infect non-human mammals. Tinea infections are among the most common dermatologic conditions throughout the world. To avoid the misdiagnosis, identification of Dermatophyte infections requires both a fungal culture on Sabouraud's agar media and direct microscopy mycological examination from clinical samples (skin scrapings, hair & nails). The tinea infections are prevalent globally but they are common in tropics and may reach epidemic proportions in geographical areas with higher humidity, overpopulation and poor hygienic living conditions hot and humid climate of India makes Dermatophytosis a very common superficial fungal infection.

The climatic conditions of our country is favorable for maintenance of dermatophyte infections situated within the tropical and subtropical belts of the world. India has remarkably varied topography which favours fungal growth. Cases of superficial mycoses in the country were first reported from upper Assam by Dr. Powell in 1900. Since then, the prevalence of dermatophytes has been reported from different states of the country.

A study of dermatophytosis in a population is important as it may reflect the climatic condition, customs, hygienic and socio-economic status of the people and Dermatophytosis & other cutaneous fungal infections, tends to be a chronic, are disfiguring and is associated with social stigma. Therefore The present study was carried out to evaluate the prevalence rate of Dermatophytic infection and to assess the clinic epidemiological profile of dermatophytic infection to identify the species of fungi and to compare the clinical diagnosis with potassium hydroxide (KOH) smear positivity and culture positivity.

**Materials and Methods**

The present study was conducted at Department of Microbiology Nalanda Medical College Patna Bihar from January 2013 to December 2014. All samples in the form of skin, hair and nails were collected randomly in batches under aseptically condition from both out patients & in-patients with the inclusion criteria include.

All clinical samples were collected from the patients attending hospital setting where the study was conducted and Only skin scrapings, nails, Hair roots & Pus were subjected for this study and Exclusion Criteria was Patients on antifungal therapy & Samples containing commensal fungal and bacterial elements.

Performa must be filling during the collection of sample to obtain information on duration of the lesion, clinical picture, prior therapy as well as demographic data such as age, sex and duration of illness etc.

**Examinations by Direct KOH Mount**

Hair follicles, scrapings of skin and nails was treated with 10 to 20 % KOH for 10 minutes, mounted on a glass slide and examined under microscope for the presence
of fungi under low power of magnification. The positive samples were taken for the isolation of the dermatophyte species on Sabouraud’s Dextrose Agar (SDA, Himedia).

**Isolation of Dermatophytes**

The samples were mainly culture on the Sabouraud’s Dextrose Agar (SDA, Himedia) containing Cyclohexamide (0.05%) and chloromphenicol (0.004%) under sterile conditions. The two slopes were incubated one at 25°C & other one at 37°C for four weeks and monitored for the growth. Dermatophytic growth was picked up with L- shaped inoculating needle and streaked on SDA slants.

The colonies on the slants were examined for their morphology, texture and pigmentation (obverse and reverse) etc. The confirmation was done on the basis of colony characteristics appeared in appropriate culture medium microscopic examination of the stained preparations (as described below).

**Dermatophyte Test Medium**

At 25°C, it was used to isolate and distinguish dermatophytes from the fungal or bacterial contaminants found in cutaneous lesions. The dermatophytes turned the medium red by raising the pH through metabolic activity while most fungi and bacteria do not.

**Biochemical Test**

The trichophyton species is subjected to the urease test. This is performed on Christensen’s medium. Trichophyton, mentagrophytes hydrolysedurea give positive results, while *Trichophyton rubrum* give negative result.

**Identification by Microscopy**

Colony of each isolate was stained with Lactophenol Cotton Blue (LCB) mount and observed under low (10× lens) as well as high power (40× lens) of light microscope. The identification was based on features such as organization of hyphae (pencil shaped, spiral, pyriform, septations etc.), microconidia and macroconidia (tear shaped, drop like, spherical, in bunches, abundance or rare etc.), or any using cromogenic Dermatophyte Hi media (Commercially available) and urease test which is positive in some species of dermatophytes. [5]

**Statistical Analysis**

The statistical analysis is done using appropriate formula and p value was calculated.

**Result and Discussion**

A total of 200 clinically suspected cases of cutaneous mycotic infections were examined for the presence of dermatophytes. Out of 200 clinical samples mostly obtained from skin 137 then Hair 43 & least from nail 20 by which 110 were KOH positive by direct microscopy & 85 were culture positive clinical isolates identified. (Table 1)

**Prevalence of Dermatophytoises on the Basis of Age and Sex**

In present study, males are infected more than females with a ratio of 2.1 &. In total clinical cases 97 males and 13 females were found. None of them had any systemic disease. we observed that dermatophyte infections were more common in the age group of 21-40 years 56 (70%), followed by 11 – 20 yrs, 41 – 50 yrs, > 50 yrs, 0 – 10 yrs, in oder of 43 (61.40%), 07 (38.8%),
2 (16.6%) and 02 (10.0%) respectively. (Table 1).

**Microscopy and Culture**

Out of 200 clinical cases, diagnosis was confirmed by microscopic examination (KOH) in 110 (55%) cases and causative agents were isolated in 85 (42.5%) culture positive cases. A total of 58 (29%) cases were positive on direct examination as well as on culture. 37 (18.5%) cases were positive on direct microscopy but negative on culture, 12 (6%) cases were negative by direct microscopy but yield growth on culture also. 50 (25.35%) cases were negative by both techniques. (Table 3)

In the present study, out of 110 KOH positive clinical types, *Tinea cruris* was the most common clinical presentation with 40/110 (36.4%) followed by *Tinea corporis* 29/110 (26.4%), *Tinea unguium* 13/110 (12%), *Tinea capitis* 12/110 (11%), *Tinea pedis* 8/110 (7.3%) and *tinea mannum & tinea barbae* 3/110 (2.8%) both were equally find out respectively. *Tinea faciae* was found least with 02/85 (1.8%) occurrence. We observed that close contacts, overcrowding in family and low personal hygiene were primary regions for the development of the dermatophytose. (Figure 1)

Among the 200 samples taken, only 85 (42.5%) were culture positive and subjected for further investigation. Microscopical and macroscopical observation suggested the high prevalence of clinical type *Tinea cruris* was the most common clinical presentation with 33/85 (38.8%) followed by *Tinea corporis* 23/85 (27%), *Tinea unguium* 10/85 (11.7%), *Tinea capitis* 07/85 (8.2%), *Tinea pedis* 5/85 (5.8%) and *Tinea mannum* 3/85 (3.5%) find out respectively. *Tinea faciae & Tinea barbae* were both equally found least with 2/85 (2.3%) prevalent. (Figure 2)

**Clinical and Mycological Correlation**

The most common dermatophyte was the *Trichophyton rubrum* 53/85 (62.3%) followed by *Trichophyton mentagrophytes* 12/85 (14.1%), *Epidermophyton floccosum* 06/85 (7.05), yeast like *Candida* 4/85 (4.7%), *Trichophyton violaceum* and *Trichophyton schoenleinii* 3/85 (3.5%) each followed as *Trichophyton tonsurans* 2/85 (2.3%) and *Microsporum gypseum* 1/85 (1.8%) cases were identified from culture positive isolates. Clinical and mycological correlation is shown in Table 2.

Dermatophytose are superficial infections of keratinised tissue, the skin, hair and nails, caused by dermatophytes. The prevalence of dermatophytosis is determined by environmental conditions, personal hygiene and individuals’ susceptibility. The variation in clinical presentation is related to the species of the fungus, size of the inoculum, the involved sites, and the immune status of the host [5]. The higher incidence of superficial mycoses is seen in month of July to September due to rainy season & humid atmosphere which is also correlating well with other studies. [5, 6]

In the present study, males (73%) were more frequently affected than females (27%). Male to female ratio was 7.4:1. This finding is well correlated with studies done by P. V. Doddaman, G. Kumaran and M. Jeya respectively [6, 7]. Study showed that males were predominantly affected than females. The higher incidence in males could be due to greater physical activity and increased sweating & other factors like environmental conditions such as hot and humid weather.

In our study, adult age group of 21-40 years 56 (70%) is most commonly affected followed by adolescent age group of 11 – 20 yrs, 41 – 50 yrs, > 50 yrs, 0 – 10 yrs, in
order of 43 (61.40%), 07 (38.8%), 02 (16.6%) and 02 (10.0%) respectively. This finding is well correlated with studies done by Aruna Aggarwal, Grover WCS, Parul, M Misra respectively. Higher frequency in adults confirm to the predominant age group which is physically active outdoors.\[10,11,12\]

**Table 1** Analysis of Different Clinical Samples

| Site     | No. of samples | KOH Positive samples | Culture positive samples |
|----------|----------------|----------------------|-------------------------|
| Skin     | 125            | 72                   | 60                      |
| Hair     | 47             | 21                   | 16                      |
| Nail     | 28             | 17                   | 9                       |
| Total    | 200            | 110                  | 85                      |
| Total Percentage | % | 55                   | 42.5                    |

**Table 2** Prevalence of Dermatophytoses on the Basis of Age and Sex

| S.No. | Age  | No. of patient(s) | Males + Ve case | Females + Ve Case | Total + Ve Case | %   |
|-------|------|-------------------|-----------------|-------------------|----------------|-----|
| 1     | 1-10 | 20                | 01              | 01                | 02             | 10.0|
| 2     | 11-20| 70                | 37              | 06                | 43             | 61.4|
| 3     | 21-40| 80                | 50              | 06                | 56             | 70.0|
| 4     | 41-50| 18                | 06              | 01                | 07             | 38.8|
| 5     | >50  | 12                | 01              | 01                | 02             | 16.6|
| Total |      | 200               | 97              | 13                | 110            | 55.0|

**Table 3** Distribution of Clinical Types on the Basis of Microscopy and Culture

| Fungal Agents | KOH + ve | Culture + ve | KOH +ve culture +ve | KOH +ve culture -ve | KOH -ve culture +ve | KOH -ve culture -ve | Total (N = 200) |
|---------------|----------|--------------|----------------------|---------------------|---------------------|---------------------|-----------------|
| T. corporis   | 29       | 23           | 16                   | 12                  | 3                   | 11                  | 43              |
| T. capitis    | 12       | 7            | 5                    | 0                   | 0                   | 4                   | 21              |
| T. mamum      | 03       | 3            | 2                    | 2                   | 1                   | 2                   | 13              |
| T. unguium    | 13       | 10           | 07                   | 04                  | 2                   | 6                   | 25              |
| T. pedis      | 08       | 5            | 4                    | 3                   | 1                   | 7                   | 17              |
| T. cruris     | 40       | 33           | 21                   | 15                  | 5                   | 17                  | 62              |
| T. barbae     | 03       | 2            | 2                    | 1                   | 0                   | 2                   | 12              |
| T. faciae     | 02       | 2            | 1                    | 0                   | 0                   | 1                   | 7               |
| Total + Ve (N=110) | 110  | 85           | 58                   | 37                  | 12                  | 50                  | 200             |
| Percentage %  | 55       | 42.5         | 29                   | 18.5                | 6                   | 25                  | 100             |
Table 4: Clinical and Mycological Correlation is shown in Table 2

| Fungal pathogen | T. rubrum | T. tonsurans | T. violecutum | T. schoeleini | T. mentagrophytes | E. floccosum | M. gypseum | Candida /others | Total |
|-----------------|-----------|--------------|--------------|--------------|-----------------|-------------|-----------|----------------|-------|
| T. corporis     | 14        | 1            | 1            | 1            | 3               | 2           | 0         | 1              | 23    |
| T. capitis      | 4         | 0            | 0            | 1            | 1               | 1           | 0         | 0              | 07    |
| T. mannium      | 2         | 0            | 0            | 0            | 1               | 0           | 0         | 0              | 03    |
| T. unguinum     | 5         | 0            | 0            | 1            | 2               | 1           | 0         | 1              | 10    |
| T. pedis        | 3         | 0            | 1            | 0            | 1               | 0           | 0         | 0              | 05    |
| T. cruris       | 23        | 1            | 2            | 0            | 3               | 1           | 1         | 2              | 33    |
| T. barbae       | 1         | 0            | 0            | 0            | 0               | 0           | 1         | 0              | 02    |
| T. faciae       | 1         | 0            | 0            | 0            | 1               | 0           | 0         | 0              | 02    |
| Mixed           | 0         | 0            | 0            | 0            | 0               | 0           | 0         | 0              | 00    |
| Total           | 53        | 02           | 04           | 03           | 12              | 06          | 01        | 04             | 85    |
| %               | 62.3      | 2.3          | 4.7          | 3.5          | 14.1            | 7.05        | 1.8       | 4.7            | 100   |

Fig:1 Direct Microscopy (KOH) Positive Clinical Types

Fig:2 Culture Positive Clinical Types
Out of 200 clinical cases, diagnosis was confirmed by microscopic examination (KOH) in 110 (55%) cases and casual agents were isolated in 85 (42.5%) culture positive cases. A total of 58 (29%) cases were positive on direct examination as well as on culture. 37 (18.5%) cases were positive on direct microscopy but negative on culture, 12 (6%) cases were negative by direct microscopy but yield growth on culture also 50 (25.35%) cases were negative by both techniques. which is comparable with the study of G.Kumaran and M.Jeya, Doddamani et al (2013). KOH positive and culture negative could be due to non-viability of fungal elements in some cases.\cite{8,9} 

The commonest clinical type seen in our study is T. cruris (38.8%) followed by T. corporis (27%), Tinea unguium (11.7%), Tinea capitis (8.2%), Tinea pedis (5.8%) and tinea mannum (3.5%) find out respectively. Tinea faciae& tinea barbae were both equally found least with (2.3%) prevalent. Difference in the incidence of clinical types were observed in different studies and Our results are in good agreement with the earlier studies conducted in Gujarat & MP.\cite{13,15}

The most common dermatophyte isolated was Trichophyton rubrum 53/85 (62.3%) followed by Trichophyton mentagrophytes 12/85 (14.1%), Epidermophyton floccosum 06/85 (7.05), yeast like Candida and Trichophyton violaceum 4/85 (4.7%) each, Trichophyton schoenleinii 3/85 (3.5%) followed as Trichophyton tonsurans 2/85 (2.3%) and Microsporum gypseum 1/85 (1.8%) cases were identified from culture positive isolates. These results are mostly comparable with the study of Anup Kainthola & Puneet Gaur et al and KAK Surendran, Ramesh M Bhat et al (2014).\cite{12,13,14}

High prevalence of Trichophyton rubrum and Trichophyton mentagrophytes followed by Epidermophyton floccosum and Trichophyton verrucosum was found with males (48.5%) being more prone to the dermatophytoses. Our study reports that species of Trichophyton genus was responsible of majority of the infection which is also supported by reports of other investigators. In this study our findings provide further evidence for the existence of the strong correlation between occurrence of dermatophytoses and living standards. Our results are in good agreement with the earlier studies It could be explained on the basis of different climatic conditions and geographic distribution \cite{12,13,14}.

Trichophyton rubrum was the commonest isolate in cases of Tinea cruris, Tinea corporis, Tinea mannum and Tinea pedis, It is due to better adaptation, more virulence and easy colonization on hard keratin with T. violaceum emerging as the common etiological agent in Tinea capitis. Tinea unguium caused by the anthropophilic species, T. mentagrophytes and E. floccosum. Because of their adaptability to a wide range of environmental conditions and their intimate association with humans, these fungi occur wherever human dwell.\cite{17}

In conclusion, Dermatophytosis is a public health problem affecting children, adolescents and adults even in our area. The present study indicates the widespread occurrence and dissemination of dermatophytopses in Bihar and suggests for the establishment of healthcare units and upgrading the beliefs of rural population about dermatophytes infection and its consequences. The present study shows that tinea cruris is the most common clinical type of dermatophytosis and Trichophyton rubrum is the most common isolate in this part of North India. Our study supports the
belief that age group 21-40 years is considered as highly active group & Males were more frequently affected than females and there are greater chances of interaction between them leading to dissemination of fungal infections (dermatophytosis) more rapidly in the community. It was well observed during the study that level of knowledge about dermatophytosis or other fungal infections was below average in the local dwellers and hence further worsened the situation in this part of India. Hence, an abrupt intervention is needed in the form of upgrading their knowledge, precautions and need for urgency of treatment.

Dermatophytosis diagnosis based on the clinical manifestations confirmed by the KOH examination and gold standard culture is best strategy for the diagnosis and correct treatment of the patient. Even if culture facility not available, KOH examination should be performed on routine basis. In the present study 55% clinical samples were positive for dermatophytosis which indicates a higher prevalence rate in east Bihar because most of the patients were from a lower socio-economic strata and came from over-crowded living conditions, which could have aggravated the infectiousness of these dermatophytic fungi.

This study analyzed the Dermatophytic infections are prevalent throughout the India—due to a lack of education or resources for diagnosis and became a larger threat. In the present study Dermatophytosis is a major problem in North India territorial & prevalence of dermatophytic infection is alarming in both rural as well as urban community in Patna, Bihar.

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