

**EPIDEMIOLOGIC, CLINICO-MYCOLOGICAL ASPECTS OF FUNGAL INFECTIONS OF SKIN AND ITS APPENDAGES**Sheikh Munir<sup>1</sup>, Feroze Ganaie<sup>2</sup>, Bilal Kumar<sup>3</sup>, Rituparna Tewari<sup>4</sup>, Syed Badakshaan<sup>5</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: BACKGROUND:** The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. The prevalence of fungal infections seems to vary across the world because of various socioeconomic and cultural factors. **AIM:** This study was undertaken to analyze the epidemiological, clinical and mycological features of fungal infections of skin and its appendages. **SETTINGS AND DESIGN:** Over a period of 1 year (Jan 2010 to Jan 2011) Samples were collected from 402 clinically suspected cases of fungal infections attending outpatient dermatology department of a tertiary care hospital & medical college Kashmir. **MATERIAL AND METHODS:** Clinical samples (Skin, Hair and Nail) from 402 clinically suspected cases were included in the study. All specimens were subjected to KOH examination and culture. Germ tube tests were performed on all growths identified as yeasts. **RESULTS:** 298 (74%) samples showed evidence of fungal elements on direct microscopy, out of which 183 (61.40%) turned out to be positive on culture. The most common dermatophyte isolated from different clinical samples were *Trichophyton rubrum* followed by *Trichophyton mantagrophyte*. **CONCLUSION:** The traditional methods of diagnosing fungal infections are still the best and important tool of diagnosis for the fungal infections in our setting. More studies are required in this part of the country.

**KEYWORDS:** Dermatophytes, fungal infection, appendages, Onychomycosis

**INTRODUCTION:** The cutaneous infection refers to the disease of skin caused by a wide variety of fungi in which the integuments and its appendages are involved. Each pathogenic fungus produces a more or less distinctive clinical pattern so that the causative organism may be suspected. This group includes dermatophytosis, pityriasis versicolor and candidiasis.<sup>1</sup> These fungi have the capability to produce keratinase, which allows them to metabolize and live on human keratin like skin, hair and nail.<sup>2</sup> Fungal infections occur in all climatic zones, affect every strata of the society and are seen in all age groups in humans.

In tropical countries conditions such as climatic, economic (poor hygiene) and social (overcrowding) are known to adversely influence the course of the infections. Dermatophytes have long been classified as anthropophilic, zoophilic and geophilic species on the basis of their primary habitat associations. Zoophilic species tend to produce highly inflammatory reactions and anthropophilic species produce mild but chronic lesions.<sup>3</sup> Although dermatophytosis does not produce mortality, it does cause morbidity and poses a major public health problem, especially in tropical countries like India.<sup>4</sup>

The importance of Onychomycosis is often underestimated; infected nails serve as a chronic reservoir of infection which can give rise to repeated mycotic infections of the skin. Its incidence varies from 0.5% to 5% in the general population in India and the disease rate has increased tremendously in the preceding century, with socio economic, cultural and economic factors

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contributing to the rising trends.<sup>5</sup> There is a long list of fungi which have a tendency to damage the nail, like dermatophytes (50%), yeasts (27%) and molds (23%).<sup>6</sup> Onychomycosis accounts for upto 50% of all nails problems. At least 15% to 20% of persons between 40-60 years of age may have this disease. In addition, immunocompromised persons, diabetics, and athletes are also considered to be at risk.<sup>7</sup>

Certain of the infectious species are geographically restricted and endemic only in particular parts of the world. Other species may be sporadic but have world-wide distribution. The species that are endemic within a population are carried by that population to new places. Troop movements, migration of laborers, emigration, social habits and rapid worldwide travel have all contributed to the changing distribution of infection.<sup>8</sup>

Due to peculiar topographic and climatic variations in Kashmir valley, the prevalence of fungal infections of skin and its appendages is often underestimated. This study was undertaken to investigate the prevalence of fungal infections of skin and its appendages and to study the clinical and mycological features.

### **MATERIALS AND METHODS:**

**STUDY SITE AND STUDY PERIOD:** Samples were collected from 402 clinically suspected cases of fungal infections between Jan 2010 to Jan 2011 attending outpatient dermatology department of a tertiary care hospital & medical college Kashmir.

**CLINICAL EVALUATION AND STUDY DESIGN:** A detailed clinical history was taken from patients including age, sex, duration, occupation, site, extent of infection, type of lesion, and antifungal therapy. Based on the site of involvement, patients were mainly grouped into three groups: Group-I (Patients with infection of Skin), Group-II (Patients with infection of Hair) and Group-III (Patients with Infection of Nail).

**SAMPLE COLLECTION AND PROCESSING:** Clinical specimens like skin scrapings were collected from the active edge of the lesions and roof of the vesicles if any. In tinea capitis, infected lustreless hairs were collected. In tinea unguium, nail scrapings, clippings and sub-ungual debris were collected in small paper envelopes after cleaning the area with 70% alcohol. All specimens were subjected to direct microscopy for fungal elements in 10%/ 20% (for nail) KOH and culture in Sabouraud's Dextrose Agar (SDA) with chloramphenicol and cyclohexamide antibiotics. Both the media were used in duplicate and each replicate were kept at 25°C and 37°C respectively.

All cultures were examined bi-weekly for growth and incubated for four weeks before declaring them negative. The growths were noted for colony characteristics in the form of texture, surface, color, color on the reverse and any diffusible pigment. Tease mount, cellophane tape mount and slide cultures were undertaken for microscopic morphology.<sup>9</sup>

Germ tube tests were performed on all growths identified as yeasts using corn meal with 1% glucose and Sabaraud's agar without antibiotics. The culture studies and identification were done by standard methods.<sup>10, 11</sup>

**ETHICS:** This study was conducted in accordance with applicable laws and regulations. This study was approved by the institutional review board and independent ethics committee.

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**RESULTS:** A total of 402 patients were examined with clinical lesions in the glabrous skin, hair and in the nail. Out of the total 402 samples examined 298 (74%) showed evidence of fungal elements on direct microscopy, out of which 183 (61.40%) turned out to be positive on culture. Ten samples which were culture positive were negative on microscopic examination, making a total of 193 (64.76%) samples culture positive. The age of the patients studied, varied from 2 to 68 years. 80 patients (19.9%) were within 2-40 years of age, 190 (47.24%) within 41-50 years, 132(32.7%) within 51-68 years age group.

The study group comprised 240 males and 162 females. Duration of the disease at the time of presentation varied from one month to three years. 90 (22.3%) patients had complaints of < 15 days duration. 124 (30.84%) had complaints of 1 year, 120 (29%) had complaints of 1<sup>1/2</sup> years, 68 (16.8%) had 2-3 years duration of complaints.

Majority of the patients with dermatophyte infection of skin, hair and nails belonged to low income group (65%) followed by middle income group (23%). Incidence of fungal infections was only (1.8%) in moderately rich group.

The ultimate results of fungal infections of skin and its appendages i.e. hair and nails was analyzed separately based on the age, sex and community distribution (Table-1).

**SKIN (Group-I):** Out of 140 cases of skin infection, 79(56.43%) were males as compared to 61 (43.57%) females. 90 (64.29%) patients belonged to rural area and 50 (35.71%) of patients belonged to urban areas. The most common dermatophyte isolated from 85 culture positive cases of skin infection were *Trichophyton rubrum* (52.94%) followed by *Trichophyton mantagrophyte* (29.42%) (Table-2).

**HAIR (Group-II):** From 108 cases of hair infection, males 63(58.33%) outnumbered the females 45(41.67%). Majority of cases belonged to rural areas 61(56.48%) as compared to urban areas 47(43.52%). Out of the 70 culture positive cases of hair infection, the most common dermatophyte isolated were *Trichophyton rubrum* (44.28%) followed by *Trichophyton mantagrophyte* (32.88%) and *Trichophyton tonsurans* (11.43%) (Table-2).

**NAIL (Group-III):** From the 50 samples of Onchomycosis, males 31 (62%) outnumbered the females 19 (38%). Most of the patients belonged to urban areas 29(58%) as compared to patients from rural areas 21 (42%). Of the 38 culture positive cases of Onchomycosis, the most common dermatophytes isolated in the descending order were *Trichophyton rubrum* (34.21%) followed by *Trichophyton mantagrophyte* (23.69%), *Alternaria* (18.42%), *Aspergillus Niger* (13.15%) and *Candida spp.* (10.53%) (Table-2).

**DISCUSSION:** Fungal infections are the commonest of all infections seen in India. There has been a recent increase as well as the spectrum of causative pathogens associated with these infections. They produce diverse human infections ranging from superficial skin infections to internal organ invasion (systemic disease) hampering person's quality of life. Most superficial and subcutaneous fungal infections are easily diagnosed and readily amenable to treatment.<sup>2</sup>

In relation of that, 12 months long study was carried out at our institute with an outlook to find the clinical pattern of dermatophytosis and species prevalent as per the demographic status in the Kashmir valley. Higher incidence of dermatophytes in males than in females has been reported

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both in India and abroad.<sup>12</sup> In the present study, male 173 (58%) to female 125 (42%) ratio with fungal infection was 1.4:1. In case of skin and hair infection, males 142 (57.25%) outnumbered the females 106 (42.72%) with a ratio of 1.4:1 approximately. Similar high incidence in males has been reported by Madhavi S.<sup>13</sup> Higher incidence in young males could be due to greater physical activity and increased seating.

In case of Onchomycosis, infection was found to be more in men 31(62%) then women 19 (38%) with a ratio of 1.6:1 similar to the observation reported by other workers.<sup>14</sup> The higher incidence of Onchomycosis in males in our study may be because of the result of increased trauma and the greater use of occlusive footwear in males compared to females. Few isolated reports of female preponderance are also present in literature.<sup>15</sup> Lesser incidence of onychomycosis in females as compared to males may be more apparent than real because of underreporting.<sup>14</sup>

In addition, 54% of patients had hobbies such as gardening, pets, and games, cooking and swimming. Increase incidence of fungal infections of skin, hair and nail in these patients can be explained by the domestic activities associated with water, cooking and contact with pets and soil wherein fungal spores reside, Similar observations were found by Jain S. et al in 2000.<sup>16</sup>

The commonest age group affected in our study was 16-30 years (30%) followed by 31-40 years (28.5%), with a peak incidence of infection between 20-30 years. A similar high incidence among the young age group was reported by Gujarat study.<sup>17</sup> In contrast, M mecantini et al,<sup>18</sup> reported a higher incidence among adults over the age of 50 years. Increased participation in physical activity, increased exposure to wet work, shoe wearing habit among this age group and early marriage leading to new house holding responsibilities, could be some of the contributing factors for the increased prevalence in the 16-30 years age group.

In our study, most of the patients with skin and hair infection belonged to rural areas. Out of 248 cases, 151 (61%) cases were from rural areas as compared to urban 97(39.11%). Agriculturists and manual laborers including the farmers forms the majority of cases. The main factor influencing the spread of ringworm is diet, the highest percentage being in areas where the protein deficiency is the greatest. In the present study, probably the same reason was involved for more rural cases, the agriculturists and laborers involve exposure to trauma and direct contact with soil, where fungal spores inhabit and lead to easy chance of infection.

In case of nail infection, majority of cases were from an urban population. Out of 50 cases 29 (58%) were from urban areas in contrast to rural areas 21 (42%). It is possible that hospitals in urban areas attract people from adjoining rural areas where many patients may ignore the asymptomatic nature of the condition. In addition, overcrowding in urban areas, habits of wearing socks and occlusive footwear for long hours may predispose to *T. pedis* and Onchomycosis. Similar findings were observed by other workers.<sup>19</sup>

In the present study, *Trichophyton rubrum* and *Trichophyton mentagrophyte* were the two most common etiological agents isolated from skin and hair infections accounting for 52.24% and 20.5% of infections respectively. This data is close to the reports of Aggarwal et al.<sup>20</sup>

In India the reports published so far unequivocally put *T. rubrum* as the commonest dermatophyte isolated from various lesions followed by *T. mentagrophyte*, which is consistent with our study result.<sup>17</sup> *Trichophyton rubrum* was isolated in all the clinical types of Tinea infections. This fungus has become well adapted to existence in human skin and that tropical climate; crowded living conditions have increased the prevalence of this infection.

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Of the 50 samples of Onychomycosis culture, dermatophytes were grown in 14 (28%), non-dermatophyte moulds (NDM) in 12 (24%), *Candida* in 9(18%), *Alternaria* in 7(14%) and no growth in 8(16%) samples.

Among the dermatophytes, *Trichophyton rubrum* has been reported to be the commonest in fingernail infection as in our study,<sup>21</sup> due to better adaptation, more virulence and easy colonization on hard keratin. Among the NDM, *Aspergillus* spp were the frequent isolate. Same findings were observed by Kaur ravinder et al.<sup>21</sup>

The isolation of *Candida* spp. in our study is in agreement with various studies reporting that *Candida* is almost exclusively found in fingernails in conjunction with candidal paronychia.<sup>14</sup>

The importance of onychomycosis is often underestimated. Although not usually life threatening, onychomycosis can be a source of significant pain and discomfort; it can also pose risk for patients, their families and others in contact with them. Onychomycosis can no longer be considered a simple cosmetic nuisance confined to the nails. It is a significant and important disease which can generate many physical, psychosocial and occupational problems, considerably impairing patient's quality of life.

In the present study, out of 402 cases of suspected fungal infections of skin and appendages, positive evidence under direct microscopy was found in 298(74.1%) cases. A variable incidence of positive cases ranging from 45.4% to 72.4% has been reported by other workers in the country from 2002 to 2012.<sup>1,20</sup> The culture sensitivity in the present study was found to be 193/308 (62.6%). This is in concurrence with the reports of Sanjiv Grover et al study (62.7%).<sup>22</sup> Other studies from India<sup>20,21,23</sup> were able to culture 50.4%, 51.85% and 70.7%, isolates correspondingly.

In the present study, Isolation rate was more by direct microscopy using KOH preparation than culture. Only 183 (61.40%) out of 298 direct smear positive cases, the infectious species were cultured. 10 culture positive samples were negative on microscopic examination. These results highlights the importance of both direct microscopy and culture in definitive diagnosis of fungal infections.

The failure of growth of infectious species in a significant proportion of cases is probably due to use of medicines before the samples are taken from the patients and the use of antifungal agents before the culturing. Another cause may be due to lack of sophisticated methods for isolation of dermatophytes.

**CONCLUSION:** The use of Immunoassays and molecular techniques may increase the sensitivity, speed and accuracy in diagnosis, but the traditional methods of diagnosing fungal infections, such as microscopy and culture are still the best and important tool of diagnosis for the fungal infections in our setting. Limited number of studies are available from this part of the country documenting the prevalence of fungal infections of skin and its appendages.

Thus, large number of studies ought to be conducted to aware the people living here about the pattern of fungal infections, so that effective measures can be taken to control this disease.

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Age groups (Years)	Male %	Female %	Total	Urban %	Rural %	Total
<b>Group-I (Patients with infection of Skin)</b>						
0-15	12	8	20	6	14	20
16-30	18	27	45	25	20	45
31-45	27	13	40	8	32	40
46-65	20	12	32	10	20	30
>65	2	1	3	1	4	5
<b>Total</b>	<b>79(56.43%)</b>	<b>61(43.57%)</b>	<b>140</b>	<b>50(35.71%)</b>	<b>90 (64.29%)</b>	<b>140</b>
<b>Group-II (Patients with infection of Hair)</b>						
0-15	8	4	12	6	9	15
16-30	15	17	32	20	16	36
31-45	13	9	22	12	18	30
46-65	21	13	34	8	12	20
>65	6	2	8	1	6	7
<b>Total</b>	<b>63(58.33%)</b>	<b>45(41.67%)</b>	<b>108</b>	<b>47(43.52%)</b>	<b>61 (56.48%)</b>	<b>108</b>
<b>Group-III (Patients with infection of Nail)</b>						
0-15	6	4	10	3	5	8
16-30	7	5	12	9	9	18
31-45	14	9	23	6	10	16
46-65	2	1	3	2	3	5
>65	2	-	2	1	2	3
<b>Total</b>	<b>31 (62%)</b>	<b>19 (38%)</b>	<b>50</b>	<b>21 (42%)</b>	<b>29 (58%)</b>	<b>50</b>

Table 1: Distribution of patients based on demographic information

SL. No.	Fungal spp.	Group-I	Group-II	Group-III
1	Trichophyton rubrum	45 (52.94%)	31 (44.28%)	13 (34.21%)
2	Trichophyton mentagrophyte	25 (29.42%)	23 (32.86%)	9 (23.69%)
3	Trichophyton tonsurans	7 (8.23%)	8 (11.43%)	-
4	Trichophyton Violaceum	5 (5.88%)	5 (7.14%)	-
5	Trichophyton Schoenleinnii	3 (3.53%)	3 (4.29%)	-
6	Alternaria	-	-	7 (18.42%)
7	Asper. Niger	-	-	5 (13.15%)
8	Candida	-	-	4 (10.53%)
<b>Total</b>		<b>85 (100%)</b>	<b>70 (100%)</b>	<b>38 (100%)</b>

Table 2: Distribution of Fungal species

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