ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Scalp hair fungal infection in Miraj-Sangali with reference to antifungal susceptibility testing of dermatophytes.

Vijay Mane[1,2], Shivdas Mali[1], SA Patil[1], Nilima Patil[1], AD Urhekar[2] and [3]Ajit KG

[1]Department of Microbiology, Government Medical College & Hospital Miraj-Sangali, Maharashtra, India
[2] Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai-410206
[3] Department of Biotechnology, RCOE, Mumbai

ABSTRACT

In India the superficial cutaneous mycotic infection is quite common because of the favorable climatic conditions such as high temperature and humidity. Various reports have appeared on dermatophytoses from the different parts of India, in all studies emphasis has been laid on dermatophytes along with scanty references to other pathogenic fungi. In this study patients with *Tinea capitis* infections were screened by clinical points of view through dermatologists and isolation, confirmatory test, and antifungal susceptibility tests were done as per the standard microbiological procedures. *Tinea capitis* is caused by species of Trichophyton and Microsporum. The Systemic study of dermatophytes began 150 years ago when Robert Remak described the mycelial nature of clinical disease 'favus' type of dermatophytic infection of scalp. During the last 40 years, studies of mycotic infections in human and animals have increased significantly. Nearly every medical speciality now includes mycotic diseases in its scientific programmes and publications. Fungal infection of scalp and hair is superficial cutaneous mycotic infection. It presents with a variety of clinical picture and more or less depends upon type of dermatophyte involved. Fungal infection of scalp and hair has worldwide distribution. The present study suggests that every patient of scalp hair infection should be properly studied for mycological examination and should be treated accordingly. This study revealed that Ketaconazole, fluconazole, and griseofulvin were the most ideal antifungal drugs for the treatment of *Tinea capitis* fungal infection

Keywords: Dermatophytosis, Dermatophyte, agar dilution method, *Tinea capitis* and antifungal agents,

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Depending on many factors, there exists a wide range of variation of immunity to the dermatophytes. Absolute immunity to infection is rare. A person enjoys a relative or incidental immunity because of anatomical and physiological factors. Thus, girls with long hair and adults with increased sebum or a change in the molecular structure of fatty acid chains are less likely to acquire Microsporum infection. Conversely, boys with short hair and children in general through lack of sebum are more vulnerable to Microsporosis. *M. audouinii and T. rubrum* infection occurs with a minimum of demonstrable resistance on the part of the host, with these infections, as with others there is a tendency to chronicity and efforts to cure by medical methods are frustrating. In contrast, infection caused by *M. canis. M. gypseum or T. mentagrophytes* characteristically induce inflammatory reaction, indicating considerable host resistance, and clinically the outlook for cure within a reasonable period [1-4]. After recovery from fungal infection caused by the later dermatophytes, there usually exist a resistant state in which reinfection is less likely than before. Such immunity is usually not permanent and may afford protection only for a few weeks or at most for few months. Marcussen and others submitted a evidence stating that circulating antibodies are detected by passive transfer tests. So one must conclude that such mechanism have no place in body defense against dermatophytes. The experience about immunity to fungal disease is limited as compared to bacterial, viral or parasitic infection. A wide range of immunity to dermatophytes exists depending upon many factors. The infection depends upon the exposure to sufficient inoculum size, size of the organism and general resistance of host. Even then a symptomatic or sub clinical infection with a particular fungal pathogen may provide immunity. Immunological and non immunological mechanisms are involved in defense against fungi causing ringworm infection [5-7]. It was reported that following the first experimental Trichophyton infection, Guinea pigs were resistant to subsequent infections and the fungus is difficult to demonstrate. The positive reaction depends to some degree on antigen selected for testing but there is uniformity in the antigen preparation. As a result the acquired immunity crosses specification (i.e. infection with *T. mentagraphytes* produces immunity also to *T. rubrum* and other dermatophytes). Type IV delayed hypersensitivity response plays a major immunological defense mechanism in dermatophytes infection. The keratinization produces delayed types of hypersensitivity reaction. The monopeptide are immunologically active while the glycopeptides are not [8-10]. Absolute immunity to infection by dermatophytes is rare. Evidence is submitted by Marcussen and others (1937) of circulating antibodies detectable by passive transfer tests. This mechanism do not play significant role in defense against infection by dermatophytes .Ayres and Anderson in 1934 reported that circulating fungistatic antibodies can be demonstrated by nothing but the inhibition of growth of the fungus on Sabouroud's slants containing 8% of patient serum. Sera from patients without dermatophytes were not inhibitory. A fresh normal human serum also shows fungistatic activity which is present at birth and remains active for some years [11-17]. Host develops IgM, IgG, IgE and IgA but it has been accepted that IgE plays a role in the suppression of cell medicated immunity. The development of CMI, which is correlated with delayed type hypersensitivity, is usually exclusion of dermatophytes elements from the stratum corneum. Certain serum factors inhibit the growth of the dermatophytes; unsaturated transferrin is one of the factors its mode of action appears

to be independent of iron binding capacity. Dermatophytes also activate complement but not as much as yeast zymogen [18-22]. When *T. rubrum* or another dermatophyte tries to involve into the viable epidermis from the stratum corneum, complement may be activated and inhibit the fungal growth. Normal epidermal turnover may then push the organism back into the stratum corneum. Activation of compliment may also cause an influx of PMN leucocytes which even more potentially inhibits or kills *T. rubrum*. An alpha 2 macroglobulin keratinase inhibitor has also been identified in stratum and may modify the growth of the organism. The patients developed a wide spread granulomatous dermatophytic infection in which titer of serum inhibition factor was found to be very low. Immunological cross-reactivity between glycoprotein isolated from *T. mentagrophyte* and human isoantigenA has been demonstrated. Total IgE level in serum was correlated to different dermatophytes species and it is observed that patient with serum IgE level >100 Kμ/ml had a tendency to increased frequency of dermatophytes infections when compared with patients with a serum IgE level <100Kμ/ml. Normal intact dry epidermis is resistant to dermatophytes infection [23-27]. The important factor is moisture in many anthropophilic infections. Dermatophyte produces catalases which act as defense against the myeloperoxidase system of killing. It was found that mannan from cell wall inhibit the immune response. Mannan isolated from *T. rubrum* and *M. canis* were compared for their immunoinhibitory activity. *T. rubrum* cause chronic, relatively non inflamed infections, where as *M. canis* characteristically produce highly inflamed infection including kerion. Human hair contain saturated fatty acid, mainly 9, 7, 11 and 13 carbon chains abstracted from adult hair which are found to be inhibitory to dermatophytic fungi. *T. capitis* caused by *M. audouinii* is known to clear spontaneously at puberty when there was a change in serum composition in the form of higher concentration of these inhibitory fatty acids. Prevention and spontaneous cure of T. capitis is known after the use of undecenoic acid ointment. The skin of an adult Negro person seems to be relatively less susceptible to dermatophytes infection than the Caucasian people showed common antigens to be shared by dermatophytes and saprophytic fungi found in mans environment. The antibodies can be demonstrated by agar gel diffusion, aemagglutination and haemagglutination inhibition tests. It was observed by the absorption tests that the human dermatophytic antibodies were completely removed by the antigens of saprophytic fungi. Thus it may suggest that the so called dermatophytic antibodies found in human serum was the result of stimulation by either dermatophytes of saprophytic antigens. This could explain the titers of dermatophytes antibody found in children and adult. After the study of most investigators it is believed that normal limb of immune system has a minor role in development of acquired resistance to dermatophyte infections. Infections produce precipitating hemagglutination and complement fixing antibodies. But these antibodies are not species specific and cross react with other dermatophytes and saprophytic fungi including the air born moulds. The immune response to dermatophytic infection has also been studied in experimental animals like guinea pigs, rabbits, rats and calves. It is found that CMI is the defense mechanism to antigen of infecting dermatophytes. In guinea pigs with experimental *T. mentagraphytes* infections, maximal erythema occurs in the infected skin. The delayed hypersensitivity reaction has been showed by increase in the rate of desquamation of stratum corneum [28-32].

ISSN: 0975-8585

MATERIALS AND METHODS

About 3000 cases of fungal infection were clinically screened and among them 20 cases were found as Tinea capitis infection. After proper & aseptically sample collection, samples were used for culture; identification was done as per the standard protocol. A total 20 cases of *Tinea capitis* were included in this study .The antifungal drug such as Ketaconazole, Fluconazole and Griseofulvin were used by agar dilution method. Out of 20 cases of *Tinea capitis* cases four isolates were grown on Sabouraud's Dextrose agar. MIC was determine as the lowest concentration of the antifungal drug preventing growth of visible colonies on drug containing slants and compare with visible growth of drug free control tubes of *Aspergillus niger* NCIM 1165.

RESULTS AND DISCUSSION

Clinical presentation of *Tinea capitis* were done on basis of clinical types, which showed grey patch 9 (45%) &5(25%) in males and females cases respectively. Kerion showed 2(10%) & 2 (10%) in males and female case while black dot was showed only in 2 (10%) male cases.

I) Age and Sex distribution:

The predominant Age group was 0-10 age, it consist of 9 (35%) cases, consist of 7 &2 males and females respectively. Age group 11-20 showed 6 (30%) cases which comprised 5 & 1 Males & Female while age group above 21 were showed 5 cases, comprised 1 male and 5 females. Total 13 (65%) cases were males in all the age groups while 7 (35%) cases were females in all age groups (Table 1/2).

Table 1:- Age and Sex Distribution

Age	Patients		Total	Percentage
	Male	Female		
0-10	7	2	9	35
11-20	5	1	6	30
21-above	1	4	5	25
Total	13(65%)	7(35%)	20	100

The age group most affected was 0-10 years (35%).

Table 2:- Distribution of cases according to clinical types of *Tinea capitis*

Sr.No	Clinical types	Males	Females	Percentage
a)	Grey patch	9(45%)	5(25%)	(14)70
b)	Kerion	2(10%)	2(10%)	(4)20
c)	Favus	-	-	-
d)	Black dots	2(10%)	-	(2)10
Total		13(65%)	7(35%)	(20)100

The most common clinical type was grey patch (70%).

ISSN: 0975-8585

II) KOH positive and culture positivity or negative:

KOH positive & growth on culture media cases were 2 (10%) while 9(45%) cases were showed KOH positivity and no growth on medium (Table 3).

Table 3:- KOH and culture study

KOH examination	Culture examination	
	Positive	Negative
Positive	2(10%)	9(45%)
Negative	1(5%)	8(40%)
Total	3(15%)	17(85%)

In the present study majority of the cases (45%) were KOH positive but culture negative.

III) KOH Negativity and culture positivity or negative:-

KOH negative & growth on culture media case was found 1 (5%) and 17 (85%) cases were showed KOH negativity and no growth on culture media (Table 3/4).This isolates belongs to two genera and four species, Out of four isolates two were *Trichophyton. violaceum* and other two were *Trichophyton tonsulans* and *Microsporum gypsum* each respectively. Incidence of dermatophytes was 0.66 % found in this present study.

Antifungal Susceptibility was done with *Trichophyton violaceum* by using three drugs. It showed MIC 1µg/ml and 0.5 µg/ml and 1µg/ml to Ketaconazole, Griseofulvin and Fluconazole respectively (Table 5-9) and Figure 1 / 2.

Table 4:- Distribution of cases according to clinical types

Dermatophytes isolated	Clinical types of Tinea capitis		
	Grey patch	Kerion	Black dots
T. violaceum	1	1	-
T. tonsurans	1	-	-
M. gypseum		1	-
Total	2	2	-

The most common dermatophyte isolated from the cases was *T.violaceum*.

Table 5:- Incidence of species

Species Isolated	No.
T. violaceum	2(10%)
T. tosurans	1(5%)
M. gypseum	1(5%)
Total	4(20%)

The maximum number of isolates was *T.violaceum* (10%)

ISSN: 0975-8585

Table 6:- Incidence of *T.capitis* with other series

Series	Total cases	Cases of T.capitis	Percentage
Mankodi series	600	30	5.0
Desai et al	467	43	9.2
Gupta series	620	20	3.2
Kalra series	454	14	3.1
S.A.Patil series*	150	1	0.67
Present series	**3000**	**20**	**0.66**

*(Unpublished data at Miraj in 1982)

Table 7:- *In Vitro* susceptibility of dermatophytes to Ketoconazole

Isolates	No	0.0001 µg/ml	0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	0.5 µg/ml	1 µg/ml	2.5 µg/ml	5 µg/ml	10 µg/ml	100 µg/ml
T. violaceum	1	+	+	+	+	+					

T.violaceum- **MIC: 1µg/ml**

Table 8:- *In Vitro* susceptibility of dermatophytes to Griseofulvin

Isolates	No	0.0001 µg/ml	0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	0.5 µg/ml	1 µg/ml	2.5 µg/ml	5 µg/ml	10 µg/m	100 µg/ml
T. violaceum	1	+	+	+	+						

T.violaceum-**MIC:0.5µg/ml**

Table 9:- *In Vitro* susceptibility of dermatophytes to Fluconazole

Isolates	No	0.0001 µg/ml	0.001 µg/ml	0.01 µg/ml	0.1 µg/ml	0.5 µg/ml	1 µg/ml	2.5 µg/ml	5 µg/ml	10 µg/m	100 µg/ml
T. violaceum	1	+	+	+	+						

T.violaceum-**MIC:0.5µg/ml**

ISSN: 0975-8585

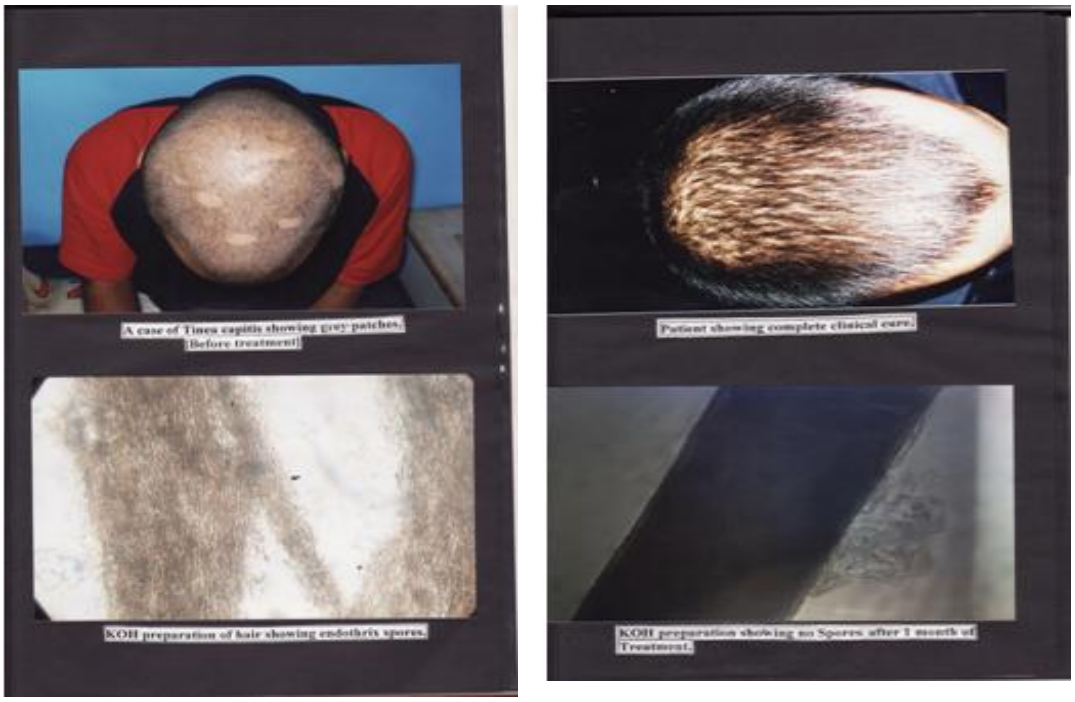

Figure 1/2:- The photograph shows before and after treatment of drugs in treatment of *Tinea capitis* fungal infection.

CONCLUSIONS

The present study suggests that every patient of scalp hair infection should be properly studied for mycological examination and should be treated accordingly. This study revealed that Ketaconazole (1µg/ml), fluconazole (0.5µg/ml) and griseofulvin (0.5µg/ml) were the most ideal antifungal drugs for the treatment of Tinea capitis fungal infection.

REFERENCES

[1] Mohrenschlager M, Seidl H.P, Ring, Abeck D: Am J Clin Dermatol 2005;6(4): 203-13.
[2] Moraes M.S, Godoy-Martinez P, Alchorne M.M: Mycopathologia 2006;(2): 91-5.
[3] Emmons CW: IJCP 1992, 3; 20-23.
[4] Else Svejgaard: Acta dermatology, Venerology (Stock) suppl. 121, 19-26. 1986
[5] Rattan A: Indian J Medical Microbiol 1999; 17; 125-128.
[6] Moore G. S, Jadow D. M: Publication Company. Virginia 1979. Page 113.
[7] P.Nagabhushanam, D.Tirumalarao. Indian J Dermatol Venerol 1972;38:2.
[8] Pushpa Talwar, Hanjan B. S, S. Subramanian: Indian J Dermatol Venerol Leprol 1980; 44(4):209-215.
[9] Tanaka S, Summer Bell RC. J Med Vet Mycol 1992;30(suppl. 1): 29-39.
[10] Dermis. J Am Dermatol 1994;31:2.
[11] Nath P Agarwal AK. Indian J Dermatol Venerol 1971;36:182.
[12] Ziemer A, Kohi K.Mycoses 2005;48(1):76-9.

[13] Silverberg NB. J Am Acad Dermatol 2002;46(2suppl): 120-4.
[14] Arenas R, Toussant s, Isa-Isar: Kerion. Int J Dermatol 2006;45(3): 215-9.
[15] Kondo M, Nakano N, Shiraki Y, Hiruma M. J Dermatol 2006;33 (3): 165-8.
[16] Asayaya SO, Kamar KK. East African Med J 2001;78(10):531-5.
[17] PH Sanderson, JC Slopper. J Dermatol 1953;65:302-372.
[18] Young E, Roth FJR. J Invest Dermatol 1979;72: 46-57.
[19] Cristiansen AH, Svejgard E. Acta Pathol Micro Scand 1976: 84;337- 341.
[20] Reyes AC, Frieelman L. J Invest Dermatol 1996;47:27.
[21] James L, Harris. J Clinical Microbiol 1986;1:460-461.
[22] Henry Earl Jones. J Am Acad Dermatol 1993; 23: 779-81.
[23] Venugopal PV and Venugopal TV. IJDVL (1992) 58: 360-371.
[24] Hussain H. J Dermatol Treat 1995; 6: 167-9.
[25] Jones TC. J Dermatol 1995; 132: 683-9.
[26] Baurdrez Rasselet F. J Dermatol 1996; 135: 1011-12.
[27] S Ranganathan. Indian J Dermatol Venerol Leprol 1995; 61: 16-8.
[28] Sanchita Karmakar, G Kalla. Indian J Dermatol Venerol Leprol 1995; 61: 200-203
[29] Ashok Kumar Khare. Indian J Dermatol Venerol Leprol 1985; 51: 328-333.
[30] Nagabhushnam P, Singh N. Indian J Dermatol Venerol Leprol 1972. 38. 56-87.
[31] A Kamalam, AA Thambiah. IJMR 1979;70: 403-406.
[32] Amin AG. IJDVL 1973;19: 22.