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Original Research Article

Mixed dermatophytic infections: a learning perspective and report of ten cases

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ABSTRACT

Background: Isolation of two or more than two pathogenic fungi from the same body site in a patient is considered as a rare entity and very few cases have been reported in literature. These types of infections are called as mixed/ combined fungal infections. Author are enumerating ten cases of superficial mycoses in which two different dermatophytes were grown from the same focus.
Methods: From clinically suspected cases of dermatophytosis, skin and hair samples were collected from the affected sites and examined by standard mycological procedures. Microscopy was done by using 10% KOH wet mount. Culture was put on Sabouraud's dextrose agar with cyclohexamide medium. Growth was identified by lactophenol cotton blue mount.
Results: Mixed dermatophytes were obtained from tinea corporis (five cases), tinea capitis (four cases) and tinea cruris (one case) patients. Fungal combinations from given cases involved two different species of genus *Trichophyton* which were as follows: *T. violaceum+T. tonsurans*, *T. verrucosum+T. tonsurans*, *T. violaceum* (violet) and *T. violaceum* (white), *T. mentagrophytes+T. Violaceum*, *T. rubrum+T. tonsurans*, *T. violaceum+T. rubrum*, *T. rubrum+T. mentagrophytes*, *T. verrucosum+T. mentagrophytes*, *T. mentagrophytes+T. tonsurans*, *Malassezia+T. mentagrophytes*.
Conclusions: Inspite of the frequent occurrence of dermatophytic infections worldwide, reports on mixed dermatophytes are very few. With proper sample collection and proper identification procedures, more cases can be identified and added to the existing literature.

Keywords: Antifungal resistance, Combined fungal infections, KOH mount, Mixed dermatophytes, Trichophyton

INTRODUCTION

Superficial fungal infections of skin, hair and nails caused by dermatophytes are a common health problem all over the world. *Trichophyton*, *Microsporum* and *Epidermophyton* are three types of dermatophytes causing these infections. The infection can be transmitted to humans by anthropophilic (person to person), geophilic (from soil) and zoophilic (from animals) fungus. Proper identification of causative dermatophyte is needed from an epidemiological point of view to determine the source of infection as well as for the choice of the antifungal treatment.[1]

Fungal infections are considered as "mixed or combined" when two or more than two pathogenic fungi are isolated from the same site in the same person. If the two different pathogenic fungi are isolated from different body sites without any causative relation in the same person, infection is said to be "concurrent".

When a fungal infection due to one pathogen is followed by a second pathogenic fungus different from the first then the infection is said to be "consecutive". These terms are confusing and in practice it is quite difficult to tell which type suits best for a particular case.[2]

In case of dermatophytosis, mixed/combined infection can occur either due to simultaneous presence of different genera of dermatophytes (*Trichophyton*, *Microsporum*, *Epidermophyton*) or different species of the same genus.

Inspite of the frequent occurrence of dermatophytic infections, simultaneous presence of two pathogenic dermatophytes in a patient is considered as a rare entity by several authors and few cases are reported in the literature.[3,4] Hereby, author enumerating ten cases of mixed dermatophytosis which are quite exceptional in the literature.

METHODS

Study design and study population includes a hospital-based study was planned in collaboration of microbiology and dermatology departments of the tertiary care hospital to find out the prevalence, clinical and mycological profile of dermatophytosis in patients attending outpatient clinic of skin during a period of one year from March 2015 to February 2016.

During study period 1000 clinically, suspected cases of dermatophytosis were sent to laboratory for mycological examination. In this article, author was enumerating the details of ten cases out of 1000 from which mixed growths of dermatophytes was obtained on culture.

Clinically suspected new cases of dermatophytosis of all age groups and both sexes, who visited dermatology department for the first time irrespective of previous episodes of similar illness and treatment taken in past for the same were enrolled in the study.

Patients of all types of dermatophytic infections like tinea capitis, tinea cruris, tinea corporis, tinea faciei and tinea incognito were included. Patients with chronic systemic illnesses like diabetes mellitus, chronic obstructive pulmonary disease, carcinomas, tuberculosis and HIV were also included.

Patients with other skin diseases which mimic dermatophytosis such as eczematous dermatitis, seborrheic dermatitis, pityriasis rosacea, psoriasis, lupus erythematosus were excluded from the study. The study was approved by the hospital ethical committee under the protocol number IEC-51.

Procedure

The patients were enquired about the age, onset and duration of disease, recurrent episodes, any chronic systemic illness present or not, history of similar illness in other family members or in close contacts, contact with animal or soil, concurrent symptoms like itching present or not and treatment taken in past for same illness.

Contact details of each patient were noted. Samples were collected after explaining procedure and taking consent. Mycological examination taken place where skin scrapings were collected from active margin of the affected body sites by using sterile scalpel. In case of hair infection, affected hairs were plucked by sterile forceps.

Direct microscopic examination was done by 10% potassium hydroxide (KOH) mount and culture was put on Sabouraud's Dextrose Agar (SDA) slants with cyclohexamide. The tubes were incubated at 250ºC for four weeks and monitored for the growth.

Growth on SDA was identified by morphological characteristics, pigment production and by lactophenol cotton blue (LPCB) mount. Additional tests like hair perforation and urease was performed when necessary. Presumptive treatment was prescribed to each patient and was asked to come for follow up examination after one month.

RESULTS

A total of 1000 clinically suspected cases were received in laboratory, out of which 798 (79.8%) showed positive results by either microscopy or by culture or by both. Among 798 positive cases, mixed growth of dermatophytes was observed in ten (1.25%) cases. Out of 10 cases, six were of paediatric age group and four were adults. Seven were male patients and three were female patients (2.3:1). Five cases were of tinea corporis, four were of tinea capitis and tinea cruris was present in one case.

The clinical detail of each patient is enumerated in Table 1. Author found that all mixed growth combinations obtained were involved different species of genus Trichophyton which were as follows: *T. violaceum+T. tonsurans*, *T. verrucosum+T. tonsurans*, *T. violaceum* (violet) and *T. violaceum* (white), *T. mentagrophytes+T. Violaceum*, *T. rubrum+T. tonsurans*, *T. violaceum+T. rubrum*, *T. rubrum+T. mentagrophytes*, *T. verrucosum+T. mentagrophytes*, *T. mentagrophytes+T. tonsurans*, *Malassezia+T. mentagrophytes*. The mycological detail of each patient is enumerated in Table 2. Clinical failure of certain antifungal drugs occurred in three cases, the details of those are described in Table 3. The photographs showing mixed growths are numbered from Figures 1-7.

Table 1: Clinical detail of cases.

Case no.	Age (years)/ Sex	Clinical description
1	6/M	Multiple patches of hair loss over scalp since one year. On examination black dots were seen within the patches. On other body parts scaly, circumscribed, itchy lesions were also present. Patient gave history of local application of steroids. Similar lesions were also present in other siblings. Tinea capitis-grey patch
2	7/F	Single tender, erythematous and indurated swelling, which was studded with pustules and crusting present over left side of the occipital area since four months. Cervical lymphadenopathy was also present. Tinea capitis- kerion
3	5/M	Single annular patch of partial hair loss with erythema and severe itching since six months. Within this patch numerous dull and lustreless broken hairs were seen. Patient gave history of steroid application locally as advised by a private practitioner. Tinea capitis- grey patch
4	40/F	Multiple well-marginated plaques with raised borders and central clearing with scaling associated with marked itching were present on back, upper and lower limbs. Case of recurrent infection since three years. Patient gave history of local application of clotrimazole on and off. Similar lesions were also present in her husband. Tinea corporis
5	60/M	Multiple annular erythematous plaques which are coalescing to form polycyclic plaques associated with marked itching noticed on upper arms, abdomen, back and lower limb since one year. Patient was a known case of diabetes mellitus and gave history of taking oral anti-histaminics. Tinea corporis
6	7/F	Multiple annular cutaneous lesions present on the abdomen and face since two weeks with similar annular, itchy, scaly lesions on the scalp with total loss of hairs. Tinea corporis+ capitis
7	4/M	Multiple erythematous plaques with scaling present on the flexor aspect of right arm since five months. Patient told some ointment was applied on lesions on the day of visit as prescribed by local practitioner previously. Tinea corporis
8	12/M	Single indurated, inflammatory, boggy swelling with partial hair loss on the frontal part of the scalp since three months. Fever and lymphadenopathy were also present. Tinea capitis-kerion
9	26/M	Multiple polycyclic, scaly, itchy lesions present on abdomen and bilateral thigh since two months. History of similar lesions in other family members was given. Tinea cruris
10	22/M	Multiple annular scaly lesions present on bilateral upper arms since one month. Tinea corporis

Table 2: Mycological detail of cases.

Case no.	KOH mount	Culture
1	Hair shaft-endothrix type Cutaneous lesions-long hyaline septate hyphae seen	Hair culture-*T. violaceum*+*T. Tonsurans* Skin culture- *T. violaceum* (Figure 1)
2	Hair shaft- both ectothrix and endothrix	Hair culture-*T.verrucosum*+*T.tonsurans*(Figure 1)
3	Hair shaft-endothrix type	Hair culture-*T. violaceum* (violet)+*T. violaceum* (white) (Figure 2)
4	Long thin hyaline septate branching hyphae	*T. rubrum*+*T. mentagrophytes* (Figure 3)
5	Long thin hyaline septate branching hyphae seen	*T. rubrum*+*T. tonsurans* (Figure 4)
6	Hair shaft-endothrix Skin-Long thin hyaline septate branching hyphae seen	Culture hair- *T. violaceum* Skin scrappings- *T. violaceum*+*T. rubrum* (Figure5)
7	Negative	*T. mentagrophytes* + *T. violaceum* (Figure 5)
8	Ectothrix type	*T. verrucosum* + *T. mentagrophytes* (Figure 5)
9	Long thin hyaline septate branching hyphae seen	*T. mentagrophytes*+ *T. tonsurans* (Figure 6)
10	Long thin hyaline septate branching hyphae seen + spaghetti and meat balls of Malassezia (Figure 7)	*T. mentagrophytes*

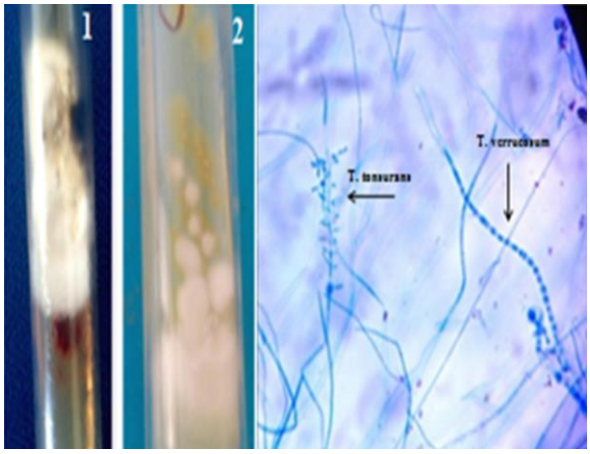

Figure 1: Mixed growths of *T. tonsurans*+*T. violaceum* (case 1), *T. verrucosum*+*T. tonsurans* and microscopic picture of case 2.

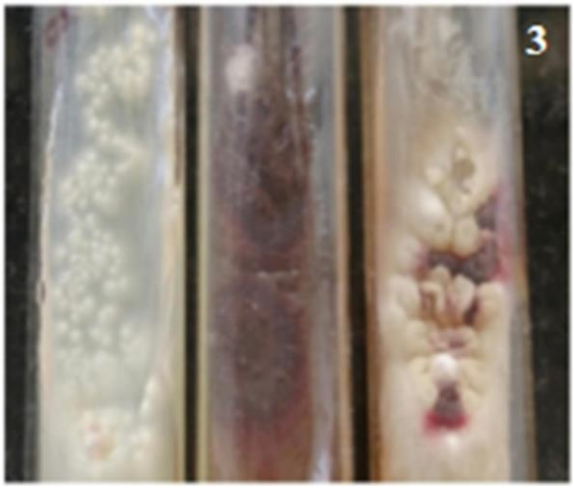

Figure 2: Mixed growth of white+violet *T. violaceum* (extreme right tube) and pure growths after subculture (case 3).

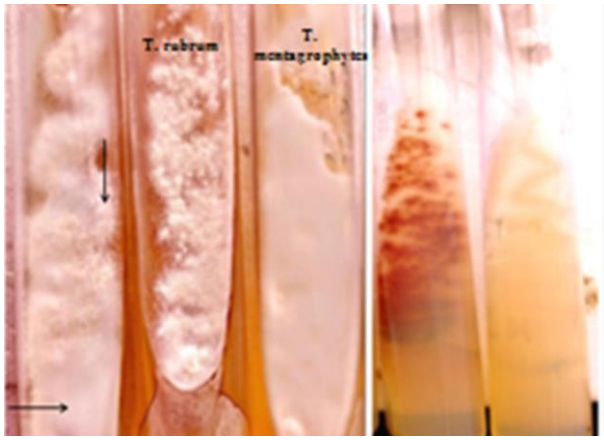

Figure 3: Mixed growth of *T. rubrum*+*T. mentagrophytes* and pure growths after subculture (left to right) (case 4).

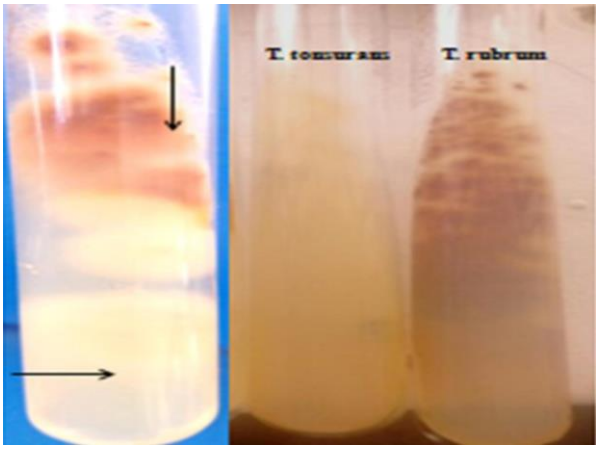

Figure 4: Mixed growth of *T. rubrum*+*T. tonsurans* and pure growths after subculture (case 5).

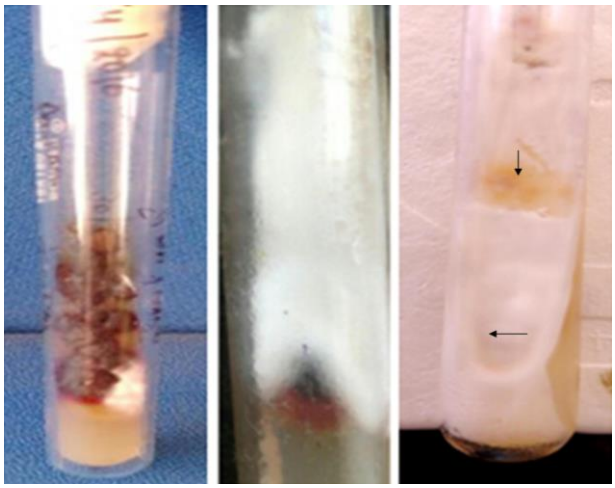

Figure 5: Mixed growth of *T. violaceum*+*T.rubrum* (case 6), *T. mentagrophytes*+*T.violaceum* (case 7), *T. verrucosum*+ *T. mentagrophytes* (case 8).

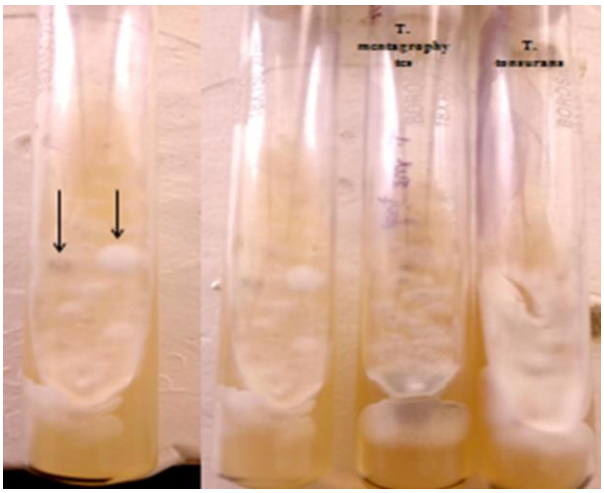

Figure 6: Mixed growth of *T. mentagrophytes*+*T. tonsurans* and pure growths after subculture (case 9).

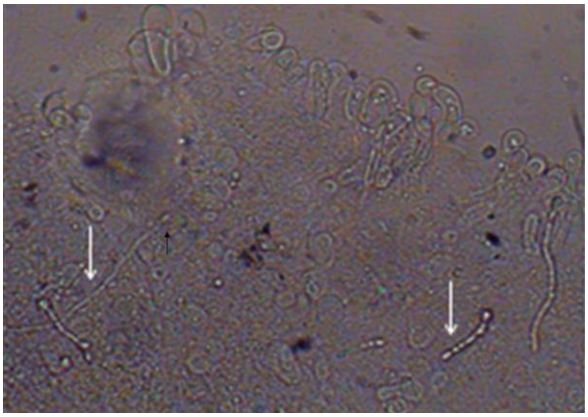

Figure 7: Long septate hyphae+spaghetti and meat balls of Malassezia (case10).

DISCUSSION

The literature on mixed/combined fungus infection is scanty. Mixed growth of dermatophytes was first noted by Aubert from two cases of favus of scalp.[5]

Muskatblit E et al, observed six cases of combined fungus infections over a period of ten years from 1930-1940. Along with these cases, he also described 36 cases of combined or concurrent fungous infections in his article which were already reported in literature at that time, thus making a total of 42 cases.[3] Adding to existing literature, Lewis GM and Hopper ME reported 23 cases of combined, concurrent and consecutive fungal infections among 1200 patients of suspected fungal infections.[2] A statistical study showed that between 1876 and 1950, 91 cases of tinea capitis were diagnosed caused by more than one fungus worldwide.[6] Authors from Montreal noticed double fungal infection in 102 (1%) specimens of skin, hair and nail out of 10057 specimens examined during a period of ten years, from 1963-1973. Out of 102, combined growth of dermatophytes with *Candida* species was found in 88 specimens and combined growth of two different dermatophytes was found in nine specimens (5 skins, 2 nails, 2 scalps).[7]

Few case reports are also found in literature which mentioned combined growth of dermatophytes from tinea patients.[8-12] On the detailed literature search, there are no cases reported between 1973 to 2015. Key findings of this study: author obtained mixed growth of dermatophytes from tinea capitis (4 cases), tinea corporis (5 cases) and tinea cruris (1 case) patients.

Table 3: Treatment details and follow-up result of cases.

Case no.	Treatment prescribed	Follow-up (FU)
1	Oral Griseofulvin -25mg/kg/day with topical ketoconazole shampoo with topical clotrimazole cream on cutaneous lesion for 6weeks.	After 6weeks showed complete clearance with slight hair growth over the patch.
2	Oral Griseofulvin -25mg/kg/day with topical ketoconazole shampoo for 3months.	After 3months of follow-up showed complete clearance.
3	Oral Griseofulvin -25mg/kg/day with topical ketoconazole for 2months.	After 3months of follow-up there was marked improvement.
4	Tablet terbinafine 250mg once a day with terbinafine cream twice a day was given for 4weeks, but patient didn't respond after one month so started capsule itraconazole 100mg BD x 2weeks.	Complete improvement at the end of therapy of itraconazole.
5	Tablet terbinafine 250mg once a day with terbinafine lotion for 4weeks.	At the end of therapy, there was marked improvement.
6	Tablet terbinafine 125mg with topical terbinafine cream and ketoconazole shampoo thrice a week for 4weeks.	At the end of treatment there was a marked improvement.
7	Patient was started on fluconazole 50mg once a week with 1% clotrimazole cream twice a day for 4 weeks. But there was no response after 4weeks then the patient was shifted to tablet terbinafine.	Complete improvement after 4weeks of terbinafine therapy.
8	Capsule Griseofulvin 250mg once a day with ketoconazole shampoo thrice a week for 2months was given. But there was no response then the patient was shifted to tablet terbinafine for 4weeks.	Marked improvement was seen at the end of terbinafine therapy.
9	Patient was started on tablet terbinafine once daily with clotrimazole cream twice daily	Marked improvement with no relapse during 3months of treatment period.
10	Patient was started on tablet terbinafine once daily with clotrimazole cream twice daily.	Marked improvement after 4weeks of therapy.

Table 4: Different combinations of fungi reported in past and of present cases.

Name of study	Combinations of different dermatophytes		
	Scalp	**Skin**	**Skin+Nail**
Weidman et al[14]		*M.furfur+Microsporum minutisimmum*	
Bresciani et al[3]		*T. violaceum+ M. furfur+ Microsporum minutisimmum*	*T. violaceum+ M. audouinii*
Karrenberg et al[13]	*E. inguinale+ E. rubrum* *T. cerebriforme+ T. niveum* *E. inguinale+ T. purpureum* *T. faviforme+ T. cerebriforme+ T. niveum*		
Lane et al[3]		*T. purpureum+ M. albicans*	
Muskatblit et al[3]	*T. violaceum+ T. schoenleinii* *T. schoenleinii+ T. crateriforme* *T. schoenleinii+ M. lanosum* *T. violaceum+ M. lanosum* *T. violaceum+ T. cerebriforme*	*Epidermophyton interdigitale +M.albicans*	*E. rubrum+ M.albicans*
Lewis & Hooper[2]		*T. gypseum+ E. Inguinale* *T. gypseum+ M. furfur* *T. purpureum+T. gypseum +E. Inguinale* *T. purpureum+ M. furfur* *E. Inguinale+ M. furfur* *C.albicans+M.minutisimmum* *T. gypseum+T. purpureum+ M. furfur* *T. purpureum+C. albicans* *M. minutisimmum+M. furfur*	*M.gypseum+T. purpureum* *M. gypseum+ C. albicans* *T.purpureum+ E. inguinale*
Muskatblit et al[8]		*T.interdigitale+T.purpureum*	
Lowenthal et al[9]	*M. audouinii+M. lanosum*		
Tanissa et al[6]	*T. granulosum+T. violaceum*		
Vilanova et al[11]	*T. violaceum+T. tonsurans*		
Dion et al[7]		*T. rubrum+E. Flocossum* *T.rubrum+T. mentagrophytes* *T. rubrum+M. canis* *M. canis+M. audoinnii*	
Rameshwari et al[12]	White+violet T. violaceum		
Present study	*T. violaceum+T. tonsurans* *T. verrucosum+T. tonsurans* White+violet *T. violaceum* *T.verrucosum+T.mentagrophytes*	*T.rubrum+T. mentagrophytes* *T. rubrum+ T. tonsurans* *T. violaceum+T. Rubrum* *T.mentagrophytes+T.violaceum* *T.mentagrophytes+T.tonsurans* *T.mentagrophytes+Malasseza*	

The culture results of these cases were different from previous reports in following ways:

- All ten cases in current report represent true combined fungal infection as mixed growth of dermatophytes was obtained from the same focus. Most of the previous studies have reported mixed dermatophytes from combined, concurrent as well as consecutive fungal infections i.e. from two different body sites. Cases showing true combined dermatophytic infections are very few in numbers. Karrenberg's CL report mentioned mixed growth of *T. cerebriforme* and *T. niveum* from a single focus on scalp of a patient.[13] Weidman F reported mixed growth of M. furfur and M. minutisimmum from erythematous lesions present in axilla of a white man aged 24.[14] Muskatblit E observed mixed growth of *C. albicans* and E. interdigitale from same toe webs in two patients.[3] All nine cases described by authors from Montreal were due to combined dermatophytic infections.[7]

- The combinations of dermatophytes obtained in these cases were- *T. violaceum+T. tonsurans*, *T. verrucosum+T. tonsurans*, *T. violaceum* (violet) and *T.violaceum* (white), *T. mentagrophytes+T. violaceum*, *T. rubrum+T. tonsurans*, *T. violaceum+T. rubrum*, *T. rubrum+T. mentagrophytes*, *T. verrucosum + T. mentagrophytes*, *T. mentagrophytes + T. tonsurans*, *Malassezia + T. mentagrophytes*. All were different and new combinations and were not reported in literature before except combination of *T. violaceum* plus *T. tonsurans*, *T. rubrum + T. mentagrophytes* and combination of white and violet strains of *T. violaceum*.[7,11,12]

- All fungal combinations from these cases involved two different species of genus *Trichophyton*. *Microsporum* and *Epidermophyton* were not isolated from these cases. However, in previous reports all types of mixed growths such as combinations of different genera of dermatophytes, dermatophyte plus *Candida* spp., dermatophyte plus M. furfur as well as combinations of different species of the same genus of dermatophyte were described (Table 4).

- Along with mixed growths author also noticed some uncommon culture findings. In the two cases of tinea corporis *T. violaceum* was isolated with *T. mentagrophytes* in one and with *T. rubrum* in another. *T. violaceum* is a common cause of tinea capitis but it is less commonly reported as a causative agent of tinea corporis. Studies conducted in various parts of the country showed *T. rubrum* and *T. mentagrophytes* as the commonest agents of tinea corporis.[15-18] However, some case reports are on record which showed isolation of *T. violaceum* from *T. corporis* patients.[19,20] White variants of T. *violaceum* are also less commonly reported in past studies. Few cases of tinea capitis caused by white *T. violaceum* are sporadically reported from African, Asian and European countries.[21,22] Author noticed clinical resistance to antifungals in three cases. Two cases were of tinea corporis, one patient was not responding to terbinafine and another patient was not responding to fluconazole. Third case was of inflammatory tinea capitis and was not responding to griseofulvin. The antifungal resistance in dermatophytes is increased over the last few years and many authors have reported in vitro as well as clinical resistance in superficial mycosis patients.[23-25] However, author didn't find any evidence in existing literature to show that mixed growth of dermatophytes contributes more to clinical failure of antifungal therapy.

CONCLUSION

The simultaneous presence of two dermatophytes at the same body site is not a new entity. Cases have been known since 1876. Inspite of this, limited literature is available. So, whether combined dermatophytic infections are rare as indicated by sparse case reports mentioned in literature so far or there is underreporting of such cases? author think that already existing reports are not an exact state of affairs. There seems to be a possibility that by conducting more studies, many more such cases can be uncovered in future. In this manner, light might also be thrown on a possible causal relation between resistance to treatment and double infection.

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