

## Detection of dermatophytes in clinically normal extra-crural sites in patients with tinea cruris

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### ABSTRACT

**Introduction:** Although dermatophytes are not part of the normal human skin flora, it has been postulated that carriage of dermatophytes in clinically normal sites such as toe webs, scrotum and other areas may serve as reservoirs for the recurrence of infection.

**Aim:** This study was designed to identify the presence of pathogenic fungi in possible carriage sites in patients with tinea cruris. This is to verify the possibility that dermatophytes in clinically normal sites may act as sources for the spread, chronicity and/or recurrence of tinea cruris.

**Patients and methods:** Fifty males with clinically suspected tinea cruris were included. All have apparently healthy four extra-crural sites including scrotum, thigh, natal cleft and toe web spaces. Every patient was subjected to careful history taking, thorough clinical and dermatological examination and mycological study. Duplicate sets of skin scrapings were collected from the crural lesion. Other duplicate sets were collected from the other clinically normal sites. These scrapings were examined by direct microscopy and culture. One set of scrapings was mounted in a drop of KOH/DMSO solution and examined microscopically for the presence of fungal elements. The second set was inoculated on Sabouraud's dextrose agar to identify the causative dermatophyte.

**Results:** Among the 50 patients, direct KOH mount was positive in 38/50 (76%) while mycological culture showed positive results in 23/50 (46%) of patients. We detected dermatophytes not only in the lesion but also in the clinically normal sites. *T. rubrum* was the most common organism isolated from the lesion (crural area) in 15 patients (30%) followed by *T. verrucosum* in 5 patients (10%), *E. floccosum* in 2 patients (4%) and *T. violaceum* in 1 patient (2%). On the other hand, from the extracrural sites, we isolated *T. rubrum* from the scrotum in 7 (14%), the thigh in 1 (2%) and the natal cleft in 1 patient (2%). *T. verrucosum* was isolated from the scrotum in 5 (10%), the thigh in 2 (4%) and the toe web in 1 patient (2%).

**Conclusion:** The current study demonstrated the presence of dermatophytes in clinically normal sites in patients with tinea cruris which might be the cause of spread, chronicity and/or recurrence of infection.

**KEYWORDS:** Tinea cruris, dermatophytes, KOH mount, fungal culture

### INTRODUCTION

Dermatophytosis is common in tropical countries and may reach epidemic proportions in areas with high rate of humidity and in over population with poor hygienic conditions.<sup>1</sup> Although various species of dermatophytes produce clinically characteristic lesions, a single species may produce variety of lesions depending upon site of infection<sup>2</sup>. Tinea cruris may be caused by any of the

dermatophytes making up the genera *Trichophyton* (*T*), *Microsporum* (*M*) and *Epidermophyton* (*E*).<sup>3</sup> The causative organism can invade both the stratum corneum and the terminal hair of the affected areas.<sup>4</sup> The lesion of tinea cruris extends from the groin down the thighs and backward on the perineum or about the anus; the scrotum and labia majora are generally excluded.<sup>5</sup> Tinea pedis may be accompanied by dermatophyte infec-

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tion of other parts of the body including groin, hands or nails.<sup>6</sup>

Once infected, scales may be transmitted through direct contact between individuals, or indirectly through contact with objects that carry the infected scales.<sup>7</sup> This transfer of infection is thought to occur through arthroconidia that are shed by the infected host in skin scales.<sup>8</sup> Autoinfection by other dermatophytes elsewhere in the body, especially the foot to the groin, may also be a method of contracting a tinea infection.<sup>9</sup>

Because of the broad range of differential diagnosis of tinea cruris infections, it is important to perform a mycologic examination, consisting of a 10%-20% KOH preparation, from skin scrapings, and a fungal culture on Sabouraud's dextrose agar (SDA). Examination of the infected scales from the leading edge of the lesion may reveal septate hyphae coursing through the squamas.<sup>10</sup> Cultures incubated at room temperature should grow the causative organism within 2-4 weeks.<sup>11</sup>

The aim of this work was to identify the presence of dermatophytes in possible carriage sites (thighs, scrotum, natal cleft and toe webs between fourth and fifth toes) in patients with tinea cruris. This is to verify the possibility that dermatophytes in clinically normal sites may act as sources for the spread, chronicity and/or recurrence of tinea cruris.

## PATIENTS AND METHODS

Fifty male patients clinically suspected to suffering from tinea cruris were included in this study and selected after full history taking, general and local examination. Their ages ranged from 13- 52 years with a mean age of 29 years. Patients with any affection of the extra-crural sites (scotum, thigh, natal cleft and toe web spaces) as well as

patients that have received systemic antifungal treatment in the last four weeks and/or topical antifungal medications in the last two weeks prior the study were excluded.

For every patient, duplicate sets of skin scrapings were collected from the crural lesion as well as from the other clinically normal sites. The selected areas were cleaned with alcohol then skin scrapings were collected using sterilized instruments starting from the four clinically normal sites then from the lesion to minimize cross-contamination of scales from the different sites. These scrapings were examined by direct microscopy and culture.

### Direct microscopy

One set of scrapings was examined microscopically for the presence of fungal elements. Specimens were placed on a clean glass slide, and a drop of 10% KOH / 40% dimethyl sulfoxide (DMSO) mixture was added (DMSO increases sensitivity of the preparation and softens keratin more quickly than KOH alone in the absence of heat).<sup>12</sup> A cover slip was applied with gentle pressure to drain away excess solution. The samples were kept for 20 minutes and then examined thoroughly for the presence of filamentous, septate, branched hyphae with or without arthrospores. Cases showing hyphae and/or spores were considered positive. Query cases were repeated for confirmation.

### Cultures on Sabouraud's dextrose agar (SDA)

The second set of scrapings was inoculated onto two types of SDA culture media: One with cycloheximide (to suppress the growth of contaminant fungi) and the other without cycloheximide. Chloramphenicol was added to both culture media (to prevent bacterial overgrowth). The media were

then incubated in a warm, moist environment at 28°C and examined regularly to detect growth of any fungus.

Observation for growth was done periodically for at least 4 weeks after which the media were reported as positive or negative. The fungi were identified by noting their growth rate, colonial morphology, and microscopic structures. Colonial morphology includes color, size, texture, and topography of the colony. The microscopic structures of fungi usually provide definitive identification. Microscopic features that were looked for are the type, size, shape and arrangement of spores and the size and color of hyphae.

## RESULTS

### KOH results

Thirty eight (76%) out of the fifty patients showed microscopically positive KOH results obtained from the crural lesions characterized by the presence of long tubular branched septated structures (hyphae) with or without arthroconidia (Table 1, Fig. 1).

**Table 1** Results of KOH mount.

KOH mount (no. 50)				
Positive cases		Negative cases		KOH sensitivity
No.	%	No.	%	
38/50	76%	12/50	24%	76%



**Fig. 1** KOH test: long tubular branched septated structures (hyphae) with arthroconidia (KOH mount x 200).

### Culture results

Twenty three (46%) out of the 50 patients showed mycologically positive cultures obtained from the crural lesions. On the other hand, 12 (24%), 3 (6%), 1 (2%) and 1 case (2%) showed positive cultures obtained from the scrotum, thigh, natal cleft and toe webs respectively (Table 2).

#### *From the lesion (crural area)*

*T. rubrum* was isolated in 15 patients (30%) followed by *T. verrucosum* in 5 patients (10%), *E. floccosum* in 2 patients (4%) and *T. violaceum* in 1 patient (2%) (Table 2, Fig. 2a and b, Fig. 3, Fig. 4, Fig. 5, Fig. 6a and b, Fig. 7a and b).

#### *From the extracrural sites*

From the scrotum, *T. rubrum* was isolated in 7 patients (14%) and *T. verrucosum* in 5 patients (10%). From the thigh, *T. verrucosum* was isolated in 2 patients (4%) and *T. rubrum* in 1 patient (2%). From the natal clefts, *T. rubrum* was detected in 1 patient (2%) while in toe webs; *T. verrucosum* was detected in 1 patient (2%) (Table 2, Fig. 2a and b, Fig. 3, Fig. 4, Fig. 5).

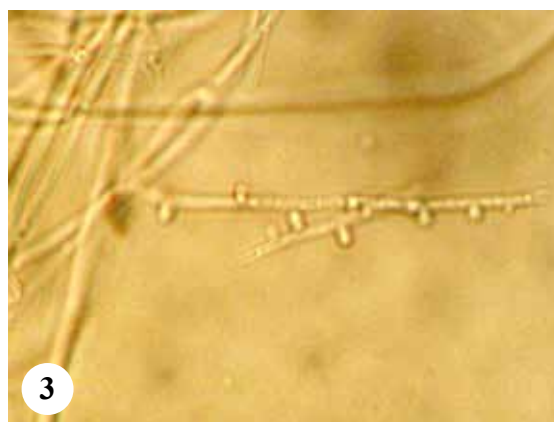
In other words, *T. rubrum* represents the most common dermatophyte as it was isolated from the lesion in 30% of the patients; while in clinically normal sites, it was isolated from the scrotum in 14% and from the thigh in 2% of patients. *T. verrucosum* was isolated from the lesion in 10% of patients and, in clinically normal sites, from scrotum in 10%, thigh in 4% and toe web in 2% of patients. On the other hand, *E. floccosum* was isolated from the lesion in 4% of patients and *T. violaceum* in 2% of patients. Both couldn't be detected in clinically normal sites.

**Table 2** Results of fungal culture

Fungal culture (no. 50)						
Positive cases		Negative cases			Culture sensitivity	
No.	%	No.	%		46%	
23/50	46%	27/50	54%			
Isolated dermatophytes						
From the lesion			Other sites			
Isolated fungi	No.	%	Site	Isolated fungi	No.	%
<i>T. rubrum</i>	15	30%	Scrotum	<i>T. rubrum</i>	7	14%
				<i>T. verrucosum</i>	5	10%
<i>T. verrucosum</i>	5	10%	Thigh	<i>T. verrucosum</i>	2	4%
<i>T. floccosum</i>	2	4%		<i>T. rubrum</i>	1	2%
<i>T. violaceum</i>	1	2%	Natal clefts	<i>T. rubrum</i>	1	2%
			Toe webs	<i>T. verrucosum</i>	1	2%



**Fig. 2a** Macroscopic morphology of *T. rubrum* - surface: colonies are flat to slightly raised, white to cream, suede-like to downy.



**Fig. 3** Microscopic morphology of *T. rubrum* (downy type): Typical slender clavate microconidia resting directly on the hyphae with absence of macroconidia.



**Fig. 2b** Macroscopic morphology of *T. rubrum* - reverse: yellow-brown pigment.



**Fig. 4** Macroscopic morphology of *T. verrucosum*: colonies are small, button-or-disk-shaped, golden-yellow, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth.



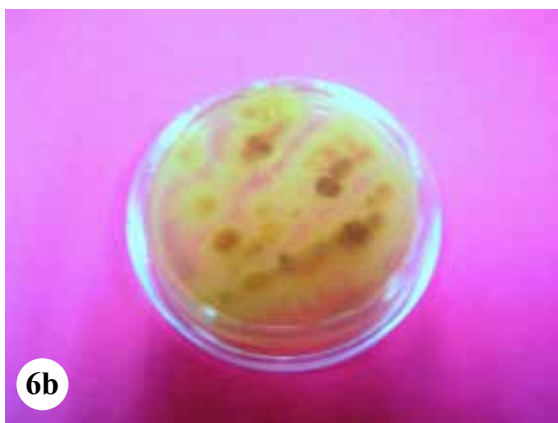
**Fig. 5** Microscopic morphology of *T. verrucosum*: broad irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The tips of some hyphae are broad and club-shaped.



**Fig. 7a & b** Microscopic morphology of *E. floccosum*: characteristic smooth, thin-walled macroconidia, which are often produced in clusters growing directly from the hyphae.



**Fig. 6a** Macroscopic morphology of *E. floccosum* - surface: colonies are greenish-brown to khaki coloured with a suede-like surface, raised and folded in the centre, with a flat periphery and submerged fringe of growth with white pleomorphic tufts of mycelium.



**Fig. 6b** Macroscopic morphology of *E. floccosum* - reverse: deep yellowish-brown pigment.

## DISCUSSION

It has been postulated that carriage of dermatophytes in toe web spaces, the scrotum and satellite areas may serve as reservoirs for the recurrence of infection; though the infection in these areas may not be clinically evident.<sup>13</sup> However, Pau *et al.*<sup>14</sup> reported that mycological exams in patients without clinical signs were always negative for dermatophytes.

To declare this discrepancy, we planned this work and we were able to isolate variety of dermatophytes from the crural lesions as well as from the extracural sites. Isolated dermatophytes from the crural lesions included *T. rubrum* in 15 patients (30%), *T. verrucosum* in 5 patients (10%), *E. floccosum* in 2 patients (4%) and *T. violaceum* in 1

patient (2%). From the extracutaneous sites, we isolated *T. rubrum* from the scrotum in 7 (14%), from the thigh in 1 (2%) and from the natal cleft in 1 patient (2%). *T. verrucosum* was isolated from the scrotum in 5 (10%), from the thigh in 2 (4%) and from the toe webs in 1 patient (2%). The results of this study are in accordance with the results obtained by Chakrabarti et al<sup>15</sup> who detected dermatophytes in scrapings from cutaneous lesions as well as from clinically normal sites including the thighs, scrotum, natal cleft and the web spaces between the 4th and 5th toes.

In this study, the *T. rubrum* was the predominant etiological agent isolated in tinea cruris. This coincides with the findings of most of the earlier works with variable percentages.<sup>2,16,17,18</sup> In extracutaneous sites, *T. rubrum* was the most predominant organism too; presenting in 18% in our study versus 28% in Chakrabarti et al<sup>15</sup> study.

In a study carried out by Silva-Tavarez et al<sup>19</sup> *T. rubrum* was the prevalent dermatophyte in 90% of tinea cruris cases, followed by *T. tonsurans* (6%) and *T. mentagrophytes* (4%). However, in contrast to the later study, beside *T. rubrum* we isolated *T. verrucosum*, *T. violaceum* and *E. floccosum*. Also, in 35 isolates of tinea cruris, Kumar et al<sup>2</sup> isolated *T. rubrum* in 26 cases (74.28%) *T. mentagrophyte* in 4 (11.43%), *E. floccosum* in 4 (11.43%) and *M. audouinii* in 1 case (2.85%). Our results are more similar with Singh and Beena<sup>16</sup> who could isolate *T. rubrum*, *T. mentagrophytes*, *T. violaceum* and *E. floccosum*. The observed differences between these results could be explained by the different study conditions eg, number of patients, geographical and environmental factors as well as socioeconomic standards.

This work and majorities of studies on dermatophytosis world-wide revealed that *T. rubrum* was

the main dermatophyte isolated from ringworm lesions (except tinea capitis). This may be attributed to the fact that *T. rubrum* lesions are more apt to become chronic and (being anthropophilic) non-inflammatory, a reason that may delay in seeking medical help and increasing the chances of fungal transmission. On the other side, infection by zoophilic fungi as *T. verrucosum* and *T. mentagrophytes* often is associated with an acute inflammatory clinical presentation.

The second most frequently isolated fungus in this study was *T. verrucosum*. To our knowledge, this is the 1st report documenting that *T. verrucosum* is the second most frequently isolated fungus in tinea cruris lesion (5 patients). Moreover, it is the second most frequently isolated fungus from the apparently healthy scrotum (5 patients). Also it was isolated from the thigh (2 patients) and may be the only reported isolated fungus from the apparently healthy web space (1 patient). The increased isolation of the zoophilic *T. verrucosum* from these sites may be due to increased exposure to the natural animal reservoirs and closeness of animal to human contact as most patients came from residential outskirts.

*T. rubrum* was the most common organism isolated (62.02 %) followed by *E. floccosum* (25.14%) by El-Mazny et al<sup>20</sup> whereas Chakrabarti et al<sup>15</sup> isolated *T. rubrum* from 32/60 (53%) and *E. floccosum* from 4/60 (6.6%) patients. Contrary, only few works reported that *E. floccosum* was the most frequently isolated fungus in tinea cruris. Sadri et al<sup>21</sup> concluded that *E. floccosum* was the most frequently isolated fungus in their cases. Fleming<sup>22</sup> reported it as the infecting agent in 90 out of 159 cultures from patients, while *T. rubrum* was isolated in 48 cases. Also, Shahindokht et al,<sup>23</sup> mentioned that *E. floccosum* remains the most



prevalent fungal pathogen in dermatophytosis and increased incidence of this species was observed in tinea cruris. This difference of dermatophytosis aetiology may be related to variations in climate conditions and natural reservoirs.

Although the scrotum is typically spared<sup>5,24</sup> an important finding in this study is that the largest isolate from extra-lesional sites was present in the scrotum (24%). This is in agreement with Chakrabarti *et al.*<sup>15</sup> who isolated the dermatophytes from the scrotum in 20% of cases. This might be explained by the direct contact of these sites to the lesion.

Direct microscopy with KOH mount from the crural lesions was positive either for hyphae or arthroconidia or both in 38 (76%) patients; these results are similar to Chakrabarti *et al.*<sup>15</sup> in which 46 (77%) patients were positive for hyphae or arthroconidia.

In this work, KOH mount has higher sensitivity compared to that of mycological culture [38/50 (76%) versus 23/50 (46%) respectively]. This result is comparable with many other works as in Singh and Beena<sup>16</sup> study who revealed that 157 cases (60.38%) were positive for fungus on direct microscopy while 116 (44.62%) were culture positive. Among 100 cases of dermatophytosis, Suman and Singaracharya<sup>25</sup> showed that 59 cases were positive by direct microscopy and 56 cases were positive by culture. Abdo *et al.*<sup>26</sup> declared that direct microscopy was positive in 30/35 (85.7%) while mycological culture showed positive results in 21/35 (60%) of patients. Also, in agreement with this study, Shenoy *et al.*<sup>27</sup> proved that KOH mount and mycological culture showed positive results in 53% and 35% of patients respectively. In addition, out of 174 specimens, Ali *et al.*<sup>28</sup> revealed that 65.5% had fungal elements

on KOH mount while 50% were culture positive. Although these results document that mycological culture was less sensitive than KOH mount, the later is unable to identify and isolate the causative dermatophytes as does fungal culture.

The host's immune response against dermatophyte infection basically depends on the host's defense against metabolites of the fungi, virulence of the infecting strain or species and anatomical site of the infection.<sup>29</sup> Local cutaneous factors appear to be very important in determining whether or not infection will occur after an exposure to a dermatophyte.<sup>30</sup> The warm and moist conditions were thought to be related to the high incidence of dermatophytosis in combat troops in the swampy areas of Vietnam.<sup>31</sup> Also, occlusion over the site appears to enhance susceptibility to experimental dermatophyte infections in humans and other animals.<sup>31,32</sup> Occlusion has been postulated to increase hydration of the underlying skin and emission from the skin of carbon dioxide which could favor dermatophyte growth.<sup>33</sup> The fungus/host interaction, which includes fungus species, host species, immune response capacity and response modulation by the organism, will exert influence on the degree of inflammatory reaction, which will define the clinical presentation and duration of the lesion.<sup>34</sup>

Dermatophytes are known to be not true commensals. Their carriage in clinically normal sites such as thighs, scrotum, may be attributed to a humid environment, warm weather, wet and/or restrictive clothing or obesity causing constant apposition of skin folds including apposition of the scrotum and thigh. The raised question "how dermatophytes are present in these extra-lesional sites without causing pathology?", however, needs more effort. Is there may be variations in regional skin immu-

nity?; is the density of fungus is low so that not permitting it to colonize and harm a host? or is this just a latency (incubation) period after which the fungus will exert its pathogenicity?. All these questions need further research to be declared.

## CONCLUSION

In this work, we were able to isolate variety of dermatophytes from tinea cruris lesions as well as from apparently normal extracutaneous sites. Their existence in clinically normal sites might be the cause of spread, chronicity and/or recurrence of such infections. This finding must be kept in mind when treating such lesions. Thus, topical antifungal agents may be applied also to the potential carriage sites to prevent recurrence. In addition, it may be necessary to give systemic antifungals in chronic and/or recurrent infections.

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