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Survey of Scalp Dermatophyte Carriage in a Day Care Center in Turkey

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Abstract The prevalence of tinea capitis and the symptom-free colonisation of the scalp with dermatophytes were examined in 502 mentally retarded participants who attended day care centers in the Tarsus district, Mersin, Turkey. Between December 2006 and May 2007, a screening study was conducted in three centers on a total of 316 (62.9%) male and 186 (37.1%) female participants aged 12 ± 6.2 years. The examinations were carried out in parallel with the hairbrush, toothbrush, and cotton swab methods by inoculation onto Sabouraud glucose agar. No participant was diagnosed with tinea capitis; however, we detected three carriers, all of whom were boys aged 2–16 years. The total prevalence of carrier state was 0.6%. Of three boys, *T. tonsurans* was seen in two cases (66.7%), and in one case a zoophilic variant of *T. mentagrophytes* (33.3%) was isolated. The diagnosis was made via the hairbrush method in all three carriers. We also did a screening study on ten households of the three asymptomatic carriers. *T. mentagrophytes* also was isolated in a 5-year-old sister of the boy with *T. mentagrophytes* colonisation. All the carriers were followed-up without any antimycotic treatment. In two of the participants, the carrier state persisted at the 13th and 17th week follow-ups, and mycological clearance was documented at the 20th and 24th week for these individuals. The third case and the household's culture were found negative at the 7- and 12-week follow-ups. Despite poor hygienic conditions and the participants' difficulties in performing basic hygiene practices, asymptomatic carriage was found to be surprisingly less prevalent among the mentally retarded individuals.

Keywords Asymptomatic carriage · Dermatophyte · Diagnosis

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Introduction

Tinea capitis is a typical illness of the scalp caused by dermatophyte fungi with diverse clinical presentations. It occurs in all age groups, but predominantly in prepubertal children [1, 2]. The problems caused by tinea capitis are its contagiosity and the long duration of the treatment, even with modern antimycotics [1]. The asymptomatic carrier is defined as an individual without signs or symptoms of tinea capitis, but who has dermatophyte-positive scalp culture. Asymptomatic carriage is not an infrequent status in

dermatophytes' epidemiology depending upon human, animal, and environmental colonisation. However, for the most part, clinicians and mycologists have been discussing the significance of this parameter only for the last 2 decades. Hence, little is known about the epidemiology, diagnostic techniques, ways of spreading, and management strategies of asymptomatic carriage (AC) [2].

Anthropophilic and zoophilic dermatophytes as well as geophilic species are related with AC with considerable geographic differences [1, 2]. Anthropophilic dermatophytes, i.e., *T. tonsurans* [3–6], *T. violaceum* [7, 8], *M. audouinii* [9], *M. ferrugineum* [10], and *M. rivalieri* [11], have generally been associated with high rates of AC. This has been attributed to a relative lack of host response; hence, these fungi were thought to be good candidates for being AC [12]. In contrast, zoophilic organisms usually presenting with a symptomatic inflammatory response are less likely to lead to AC [4, 10, 13]. However, more recently, *M. canis* [14, 15] and *T. mentagrophytes* [15, 16] were reported as the predominant species for AC. Geophilic species, *M. nanum* [14], *T. terrestre* [17], *T. ajelloi* [5], and *M. gypseum* [5, 16], were also reported to be associated with AC, albeit at a decreased level. Today, the diagnosis of the carrier state is only possible by fungal culture by using the hairbrush [4, 5, 7, 9–11, 16], toothbrush [8, 16], cotton swab [15, 16], scalpel blade [3], carpet disc [13], or gauze [6] method.

This study aimed to (1) determine the prevalence of scalp carriage and related fungi and (2) discuss the efficacy of diagnostic techniques among mentally retarded participants who attended day care centers in the Tarsus district, Mersin province, Turkey.

Material and Methods

The Tarsus district, with a population of 318,553, is located in the Çukurova region by the Mediterranean coast at latitude 36.55°N and longitude 33.54°E. The climate is typical of the Mediterranean region, with very hot summers, and chilly and damp winters. The climate is cool (9.5°C) and rainy (132.2 kg/m^2/month) in winter, and hot (32.7°C) and dry (1.6 kg/m^2/month) in the summer. The relative humidity is high (57.9–76.9%) for most of the year.

Participants

The study was conducted in three day care centers in the district of Tarsus, Mersin province, Turkey. All parents of the 535 participants of the three day care centers were informed of this study, and 502 (93.8%) of them agreed to participate in it. In this investigation, the participants were all day cases, and none of them were on any kind of antimycotic treatment in the last 3 months. The families were of lower socioeconomic status, and the physical conditions of the centers were below average. The study protocol was reviewed and approved by the Faculty of Medicine's Ethics Committee of the University of Çukurova, and written consent was obtained from each participant's parents or legal counselor. The age of the participants ranged between 1 and 42 years with a mean of 12 ± 6.2; 316 (62.9%) of the participants were male, and the remaining 186 (37.1%) were female.

Sample Collection

Each participant's scalp was examined for broken hairs and/or alopecia, scaling, and crusting. However, scalp samples were taken from all children irrespective of the clinical symptoms. The scalp samples were taken by three methods in the following order: (1) by gently brushing each side of the scalp four times vigorously with a plastic hairbrush [14], (2) by using a plastic toothbrush rubbing [8], and (3) by rotating a cotton swab on the scalp [15]. The hairbrushes and toothbrushes were commercially provided as no-name products from a local market and were also used in our previous study [16]. The cotton swab procedure was carried out after the instrument had been dipped in sterile 0.1% Tween 80. Each method sampled the same area among the four quadrants of the scalp. The surface areas of the hairbrush, toothbrush, and cotton swab were 38 cm^2, 3 cm^2, and 2 cm^2, respectively. The hairbrush consisted of 167 plastic prongs, was circular in shape, and of a size to fit in a Petri dish.

Household Members

Further extensive examinations were conducted with the children having a dermatophyte colonisation of the scalp in domestic surroundings. Once the carriers

were identified, household members and inanimate objects at the dwelling of the cases were analysed in terms of dermatophyte fungi. Samples were taken from inanimate objects, i.e., 11 pillowcases, 6 blankets, 1 sheet, 1 sofa, 4 towels, and 3 combs, with the cotton swab method. The ten household members (five adults and five children) of the three carriers, aged between 5 and 43 years, with a mean of 25.5 ± 15.1, comprising four (40%) males and six (60%) females, were questioned and inspected for dandruff, scalp scaling, or recent hair loss, as well as the presence of any dermatophyte infection. In addition, scalp samples of the households were collected by the three methods described above. None of the households had been on any kind of antifungal treatment in the last 3 months. Follow-up of three carriers and their households had been done at the 19th and 24th weeks when they were available.

Fungal Culture

Brushing the scalp with any plastic brush-based technique builds up static electricity on the brushes, which attracts particulate material (including fungal propagules) onto the prongs of the brushes. This material is then dislodged when the brushes are inoculated onto the agar surface. Sabouraud glucose agar (SGA; Acumedia, Baltimore, MD) plates containing 100 μg/ml cycloheximide (Sigma, Steinheim, Germany), 100 μg/ml chloramphenicol (Fluka, China), and 50 μg/ml gentamicin (Sigma) were used as a study medium. Each hairbrush was stabbed onto the medium, creating 167 inoculations corresponding to the 167 prongs of the hairbrush. The toothbrush method was also streaked over the study medium. The cotton swab was inoculated onto the study medium by rotating the swab head while streaking the surface of the medium. All plates were transferred to the Mycology Laboratory at the Faculty of Medicine, University of Çukurova. The cultures were incubated at 25°C in air and were examined after 7, 14, and 21 days for evidence of growth.

Spore Load

Colonies were counted on each plate for the hairbrush method, and a total colony count (equivalent to number of spores retrieved) was obtained for each participant. A spore load system was assigned as follows: light for 1–5 colonies, moderate for 6–10 colonies, and heavy for >10 colonies [16].

Identification of Dermatophyte Species

Any dermatophyte isolates were subcultured on SGA and potato dextrose agar (Merck, Darmstadt, Germany) in Petri dishes. These species were identified by colony morphology and microscopic examination with lactophenol cotton blue preparation. To confirm the identities of *T. tonsurans* and *T. mentagrophytes* strains, in vitro hair perforation test, urease activity in Christensen's urea broth, and growth in bromcresol purple-milk solids-glucose agar (Himedia, Mumbai, India) testings were also performed [18].

Organisms

The following reference strains were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands: *T. asteroides* CBS 424.63, *T. interdigitale* 428.63, *T. langeronii* CBS 764.84, *T. mentagrophytes* CBS 318.56, *T. mentagrophytes var. mentagrophytes* CBS 110.65, and *T. quinckeanum* CBS 572.75.

Statistical Analysis

Statistical analysis of the mean, median, and standardised deviation was done using the statistical package SPSS version 10.0.

Results

Participants

Clinical symptoms were not recognisable in any of the cases, but 3 (0.6%) out of 502 participants were detected as carriers. While two of the carriers (who are in the same day care center, but in different classes and services) were positive for *T. tonsurans,* one was positive for *T. mentagrophytes.* In these three cases, diagnoses were made via only the hairbrush method, and the spore loads were determined as light for two and moderate for one (case 2). First follow-ups of these three cases were performed in the 12th, 13th, and 17th weeks; two of the cases

Table 1 The mycological findings and the follow-up results of the scalp dermatophyte carriers

Case no.	Age (year)/ gender	Diagnosis	Initial screening	First follow-up		Second follow-up	
			Species/method (spore load)	Time (week)	Species/method (spore load)	Time (week)	Species/ method (spore load)
1	16/M	MR	*T. mentagrophytes*/Hairbrush (4)	17	*T. mentagrophytes*/Hairbrush (1)	24	–
2	9/M	MR	*T. tonsurans*/Hairbrush (6)	13	*T. tonsurans*/Cotton swab	20	–
3	2/M	MR + CP	*T. tonsurans*/Hairbrush (3)	12	–	19	–
4	5/F[a]	–	–	–	*T. mentagrophytes*/Cotton swab	7	–

MR mental retardation; *CP* cerebral palsy; *M* male; *F* female; – culture negative

[a] Household of Case 1

remained culture-positive (one via the hairbrush and one with the cotton swab method). For these two cases, the second follow-ups were performed at the 20th and 24th weeks, where all cultures were sterile (Table 1).

Household Members

In this study, the 5-year-old sister of case 1 (16/M) was also found to be positive for the zoophilic variant of *T. mentagrophytes* via the cotton swab method. However, fungal cultures were found be free of dermatophyte fungi at the 7th week after the first determination (Table 1). On the other hand, all environmental sites were negative for dermatophyte fungi. Case 1 and his sister had had animal contact, e.g., cows and cats on a farm where they spent their summer holidays.

Discussion

A relatively frequent occurrence of asymptomatic dermatophyte scalp carriage depending upon severable variables has been reported in the literature [3–11, 13–17]. More recently, in two studies among primary school children that were performed by our group in Adana province (the fifth biggest province of Turkey, near the Tarsus district), the prevalence of carrier state was found to be 0.1% [15] and 1.3% [16], respectively. In the present study, the symptomless colonisation of the scalp with dermatophytes was found in 0.6% of the examined persons and would have demonstrated no difference in the two patient populations, mentally challenged students (e.g., mental retardation and/or cerebral palsy) compared to normal students who lived in the same area of Turkey. In addition, the overlapping of AC and STC prevalence in our first study (0.1% vs. 0.1%) [15], the latter study (1.6% vs. 0%) [16], and this present investigation (0.6% vs. 0%) revealed that AC also may have been detected in a population without any symptomatic tinea capitis (STC) cases in the community. Hence, the results of this study suggest that mental disability is not a predisposing factor for day care attendees in terms of AC. Moreover, in this study, we could not demonstrate where the 'carrier state' originated from, close contact or poor hygiene, because of the limited number of cases.

In this study, a total of four carriers were detected, three from the day care centers and one from the households. The related fungi were identified as *T. tonsurans* (50%) and zoophilic *T. mentagrophytes* (50%). When we look at these studies together, we identify *T. mentagrophytes* as an emerging fungi related with carrier state in and around Adana province. Presumably the zoophilic fungi were acquired from an animal; however, we were not able to determine the source of *T. mentagrophytes,* i.e., cats or dogs. On the other hand, human-to-human transmission of the zoophilic species is also possible and should not be underestimated. In addition, we could not detect the source of anthropophilic *T. tonsurans*.

In this investigation, there was no obvious transmission of fungi and following infection. This is interesting since the children were accommodated in a day care center and had close contact with each

other. This could be related to the 'spore load system,' which could give a logical explanation for the results in this study. Indeed, up to a maximum of ten colonies per affected child is a quantity that probably lies below the critical infectious dose. The Concise Oxford Dictionary (9th edition, 1998) defines 'carrier' as "a person or animal that may transmit a disease or a hereditary characteristic without suffering from or displaying it, and an illness transmission is not described." Therefore, the following question about terminology arises: Is the word 'carrier' correct for the situation described here?

Asymptomatic scalp carriage has also been demonstrated in a child living with index cases of STC. The household setting is the most common place for close prolonged contact where sharing of combs, brushes, hats, clothing, furniture, toys, and bed linen occurs [19]. Consistent with this picture, AC was detected in a 5-year-old sister of patient 1 (Table 1). Due to the limited number of carriers, we could not define and quantify the risk factors associated with AC.

Akbaba et al. [16] reported that the hairbrush method was significantly more effective in detecting dermatophyte fungi than the toothbrush ($P < 0.01$) and the cotton swab ($P < 0.05$) methods. For laboratory diagnosis, since there is not a single method that is accepted as a gold standard, a combination of methods was implemented by the authors. Therefore, since there is no standard diagnostic technique to detect the carrier state as discussed above, we must assume that the actual prevalence of AC could be higher than the estimated prevalence. In this investigation, we cannot compare the three examination methods, as only three positive results were detected. On the other hand, we could monitor the gradual decrease and disappearance of the spore load only in case 1. We believe close monitoring of spore load may provide valuable information in that it may enable us to predict whether the carrier state will persist or convert to STC or disappear. It is of special interest that colonisation had disappeared in all four children with a proved dermatophyte colonisation by 24 weeks after the first sampling (Table 1). It is important to note that an antimycotic therapy was not carried out on the carriers, and mycological clearance occurred spontaneously.

To the best of our knowledge, Midgley and Clayton [10] were among the first reporting an outbreak of scalp ringworm due to *T. violaceum* in a hospital for the mentally subnormal. Five of the 44 (11.4%) children in one ward had this infection, and several members of the staff had also developed skin lesions. Over 20% of the other children in the ward were found to be carriers of *T. violaceum*, although no infection was apparent on their scalps. The results of the bedding samples showed that *T. violaceum* was isolated from 27%. However, in our study, all the inanimate objects were negative for dermatophyte fungi.

Family physicians have a specific responsibility to provide person-centered and community-oriented health services within the context of the person, the family, the community, and their culture. For this reason, preventive medicine should be an integral part of clinical practice. It is well known that the transmission of tinea capitis is fostered by poor hygiene and overcrowding, and after being shed, affected hairs can harbor viable organisms for more than 1 year [20]. In terms of AC and STC, we must emphasise prevention through individual education of the families about personal hygiene.

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