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Scalp Dermatophyte Carriage in Pregnant, Pre-, and Postmenopausal Women: A Comparative Study Using the Hairbrush and Cytobrush Methods of Sample Collection

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Abstract Tinea capitis is a dermatophyte infection of the scalp that is most often seen in prepubescent children. In this investigation, we examined the prevalence of tinea capitis and symptom-free colonization of the scalp with dermatophytes in 786 pre- and postmenopausal women aged 12–84 years. Scalp samples were collected from all participants by cytobrush or hairbrush, and cultures were then grown from these samples on Sabouraud glucose agar. No participant was diagnosed with tinea capitis; however, one 43-year-old patient (0.1%) was positive for a "scalp carriage" related to anthropophilic *Trichophyton rubrum*, as detected using a hairbrush. The internal transcribed spacer (ITS) regions of the isolate were sequenced, and the assembled DNA sequences were examined using the basic BLAST (nucleotide–nucleotide) software of the National Center for Biotechnology Information Web database. This patient was followed up without any antimycotic treatment, and after 4 weeks, mycological clearance was documented. In addition, the contacts and environment at home were screened, where all fungal cultures were sterile. To the best of our knowledge, this study is the first report of a "scalp carriage" related to a cosmopolitan fungus, *T. rubrum*.

Keywords Carrier state · Cytobrush · Dermatophyte · Hairbrush · *Trichophyton rubrum*

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Introduction

Tinea capitis is generally observed in children over the age of 6 months and before puberty [1]. The quantity of fungistatic saturated long-chain fatty acids in sebum increases at puberty, and this change is thought to explain the rarity of tinea capitis in adults [2]. However, a reduction in triglycerides in sebum may predispose postmenopausal women to the development of tinea capitis more frequently than other adults [1]. In addition, colonization of the skin with *Malassezia* spp. may interfere with dermatophyte contamination, and a thicker caliber of adult hair may protect against dermatophytic invasion [3].

Tinea capitis in adults generally occurs in patients who are (1) immunosuppressed (e.g., HIV, diabetes, and organ transplants); (2) receiving immunosuppressive drugs [1–3]; (3) experiencing estrogen-level changes, such as during pregnancy and menopause [1, 4]; and (4) exposed to the dermatophytic fungi

from tinea elsewhere on the body or from infected family members, animals, or inanimate objects [5–9]. The clinical picture of tinea capitis in adults varies, mainly depending on the species of dermatophyte that causes the infection and the immune state of the host [10, 11]. In contrast, it was noted that this varying clinical picture is regardless of the microorganism and the host [3]. The microorganism predominantly responsible for tinea capitis in children geographically varies e.g., *Trichophyton tonsurans* in the USA [1] and *Microsporum canis* in Central and Southern Europe [12], as in adults [2, 3, 5–9, 11, 13]. Confirming the diagnosis of tinea capitis is best undertaken with more than one sampling method that includes scraping of the scalp [13], with a hairbrush [9, 14, 15], toothbrush [6–9], moistened cotton swab [9, 16], or cytobrush [17].

Tinea capitis may also present as a minimal infection, termed a "carrier state". An asymptomatic carrier is defined as an individual who is positive for a dermatophyte scalp culture but lacks the signs or symptoms of tinea capitis. Anthropophilic dermatophytes, including *T. tonsurans* and *T. violaceum*, have been generally associated with high rates of asymptomatic carriage [18]. In this investigation, we aimed at (1) identifying the prevalence of symptomatic and asymptomatic scalp ringworm in women who visit a gynecology clinic (2) comparing the hairbrush to the cytobrush method in diagnosing the "carrier state", and (3) examining the household contacts that have positive scalp cultures in Adana, Turkey.

Materials and Methods

Data Collection

A total of 786 women visiting the outpatient clinic of the Department of Gynecology and Obstetrics, Faculty of Medicine, University of Cukurova, between January and June 2010, were included in this investigation. In detail, 303 (38.5%) patients had gynecological disorders, 249 (31.7%) pregnant, 130 (16.5%) postmenopausal, and 104 (13.2%) were with gynecologic cancers. Patient from who dermatophyte was recovered but who lacked clinical symptoms was considered to be asymptomatic carrier. The clinical diagnosis, mycological results, and detailed history of each participant were recorded.

Sample Collection

Each participant's scalp was examined for broken hairs and/or alopecia, scaling, and crusting. Scalp samples were taken from all participants, irrespective of clinical symptoms, by gently brushing each side of the scalp 4 times vigorously with a rotating plastic cytobrush [17] and plastic hairbrush [9, 14, 15]. The cytobrush has plastic bristles that extend parallel to the plastic handle and are attached to a base in a "V" shape. It is rotated 360° longitudinally across the scalp in a single motion and again on the study medium. The hairbrush consisted of 167 plastic prongs, was circular in shape, and of a size that would fit in a Petri dish (Fig. 1) [9, 14, 15]. The study was reviewed and approved by Faculty of Medicine's Ethics Committee of Çukurova University.

Fungal Culture

Clinical specimens were dislodged when the brushes were inoculated on the agar surface. Sabouraud glucose agar (SGA; Acumedia, USA) plates containing 100 μg/ml cycloheximide (Sigma, Germany), 100 μg/ml chloramphenicol (Fluka, China), and 50 μg/ml gentamicin (Sigma) was used as a study medium. Each hairbrush was stabbed onto the study medium, creating 167 inoculation points corresponding to the 167 prongs of the hairbrush. The cytobrush (Medbar, Turkey) was inoculated onto the study medium by rotating the cytobrush head while streaking the surface of the medium. The hairbrush was commercially available as a generic product from a local market; this type of hairbrush was also used in our previous studies [9, 14, 15]. All plates were transferred to the Mycology Laboratory at the Faculty of Medicine, University of Çukurova. The cultures were incubated at 25°C on the bench and were examined after 7, 14, and 21 days for evidence of growth [9, 14, 15].

Spore Load

Colonies were counted on each plate of the hairbrush method, and a total colony count (equivalent to the number of spores retrieved) was obtained for each participant. A spore load system was assigned as follows: light, 1–5 colonies; moderate, 6–10 colonies; and heavy, for >10 colonies [9, 14, 15] per plate.

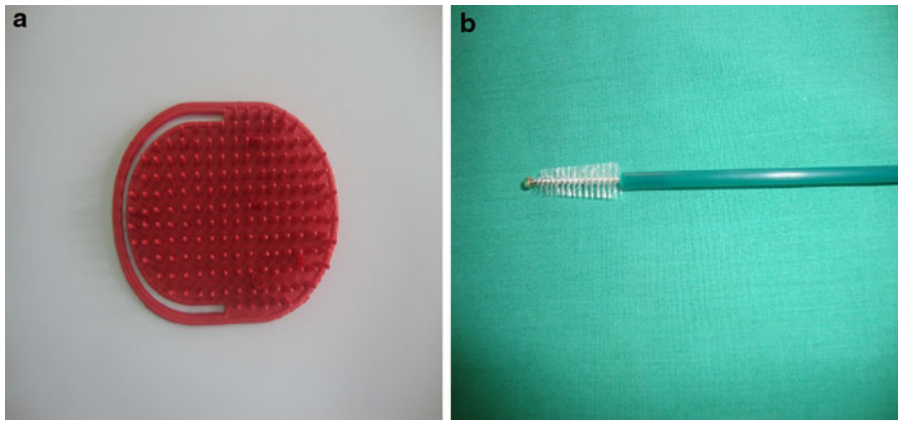

Fig. 1 Sabouraud agar plates inoculated with the samples from scalp, (**a**) hairbrush and (**b**) cytobrush methods

Household Members

Further extensive examinations were conducted at the patient's household. Once the carrier was identified, the household members, the father (aged 50 years), the girl (aged 20 years), and the boy (aged 15 years) were tested for dermatophytic fungi. Samples were also collected from a total of 12 inanimate objects, e.g., the pillowcases ($n = 4$), sheets ($n = 4$), and towels ($n = 4$) used by the each participant.

Fungal DNA Isolation, PCR Amplification, Sequencing, and Analysis of ITS Region

DNA isolation and PCR amplification were performed according to the protocol described by Turin et al. [19]. The rDNA regions spanning the internal transcribed spacer (ITS) 1, 5.8S rRNA, and ITS2 regions were amplified using the universal fungal primers ITS1 and ITS4. The amplified DNA products were sequenced in both directions using PCR primers on an ABI PRISM 3130xl Genetic Analyzer at the DNA sequencing service of Refgen Biotechnologies, Ankara, Turkey. The DNA sequences of the forward and reverse strands were analyzed and aligned with the CAP contig assembly software included in the BioEdit Sequence Alignment Editor 7.0.9.0 software package [20]. The assembled DNA sequences were examined using the basic BLAST (nucleotide–nucleotide) software of the National Center for Biotechnology Information Web database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The DNA sequence of the strain was 100% identical with the 619 nucleotides spanning the 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2, and the 28S rRNA gene (partial) from 28 *T. rubrum* complex strains (21 *T. rubrum*, 5 *T. raubitschekii*, 1 *T. kanei*, and 1 *T. fischeri*).The strain was deposited at the Centraalbureau voor Schimmelcultures (CBS) culture collections, Utrecht, The Netherlands (CBS number: 127447).

Results

The mean age of the participants was 39.4 ± 13.6 years. Clinical symptoms were not recognizable in any of the cases, but only 1 of 786 participants (0.1%) was identified as a "scalp carrier". A 43-year-old woman, gravid 2, parity 3, visited the gynecology clinic with the complaint of irregular uterine bleeding. In medical history, she has had a stage II lung sarcoidosis, for the last 2 years. In addition, she had also no past history of tinea capitis.

In this case, diagnosis was made only using the hairbrush method, and the spore load was determined as heavy, i.e., 17. The first follow-up of the case was performed in the fourth week after diagnosis, and the second follow-up occurred in the sixth week after diagnosis; the fungus had cleared by these follow-ups. The patient exhibited no evidence of tinea at other body sites. In addition, all household members were uninfected with dermatophytic fungi. Also, all screened inanimate objects did not carry any fungal elements.

Discussion

Although tinea capitis rarely diagnosed after puberty, it should be considered in all adults with a patchy,

inflammatory scalp disorder [2]. In 1952, Pipkin [21] reported 1,034 cases of tinea capitis in the Southwest United States with only 4.9% occurring in adults over 20 years of age. In parallel, Romano [4] observed 181 cases of tinea capitis and reported that all of the adult cases (2.8% of the total cases) were postmenopausal women. Cremer et al. [3] described eight adult cases of tinea capitis over a one-year period (accounted for 11% of all cases reported), with 5 of 8 (62.5%) being immunocompromised and 6 of 8 (75%) were women. In adults, women are diagnosed with tinea capitis more frequently than men [3, 4, 13, 21, 22]; however, the female predominance in adult cases has not yet been explained [13].

Household members of infected children are at an increased risk of infection because of the opportunity for close and prolonged contact [23]. Vargo and Cohen [6] found that the prevalence of tinea capitis in family contacts was 3.5 times higher than in a control group. The authors noted that 63% of child contacts were positive for mycology but only 5% of adults tested positive. The prevalence of the "carrier state" was found to be −2.5% [22], 9.4% [9], 11.4% [6], 12% [7], 19.4% [11], 30.4% [5], and 30.6% [8] among adult family members of a child with culture-proven tinea capitis. This result is important because asymptomatic household members may act as reservoirs of infection [6]. Interestingly, these scalp carriers were almost always women [3, 5, 7–9, 11]. In other studies, the incidence rates of adolescent and adults who had clinical signs of tinea capitis were 16.7% [22] and 3–3.8% [6, 22], respectively.

Recently, we authored two separate studies among primary school children that indicated the prevalence of the "carrier state" was 0.1% [16] and 1.3% [9], respectively, in Adana, Turkey. In addition, 0.6% of mentally challenged students showed asymptomatic colonization of the scalp with dermatophytes [14]. The above-mentioned three studies noted that no participant was diagnosed with tinea capitis. More recently, we detected a clonal outbreak of *T. tonsurans* tinea capitis gladiatorum among 14 wrestlers in Adana, Turkey. Even though the number of the cases was small, we demonstrated that the carrier state, specifically scalp carriage, was more common than the tinea capitis superficialis cases (31.1 vs. 17.2%) [15]. Hence, the eradication of asymptomatic carriage seems to be a logical step in controlling the spread of tinea capitis [12].

The traditional standard method of scraping the scalp to collect hair and skin scales also has limitations [17]. A number of different techniques can be used for obtaining clinical specimens, and using more than one approach increases the sensitivity and reduces the chance of failing to isolate the causative fungi [9, 12, 14, 18]. Akbaba et al. [9] reported that the hairbrush method was significantly more effective in detecting dermatophytic fungi than the toothbrush ($P < 0.01$) and the cotton swab ($P < 0.05$) methods. Because there is not a single method that is accepted as the gold standard for the laboratory diagnosis of tinea capitis, we used a combination of methods. Therefore, because there is not a standard diagnostic technique to detect the carrier state [9, 14, 18], we must assume that the actual prevalence of asymptomatic carriage could be higher than the estimated prevalence.

Bonifaz et al. [17] investigated the use of the cytobrush to harvest scale and affected loose hair compared to the standard method of scraping the scalp and collecting hair and cell debris. To diagnose symptomatic tinea capitis in 135 culture-positive cases, the authors achieved isolation rates of 97.7% using the cytobrush compared to 85.1% using the standard method, with a reduced time until positive detection of 8.5 days using the cytobrush compared to 11.2 days using the standard method ($P = 0.025$). The authors also noted that the cytobrush is commercially available in a sterile state and has soft bristles that could be easily used on inflamed scalps without discomfort to the patient [17].

Anthropophilic *T. rubrum*, a cosmopolitan fungus, is the most common agent of tinea glabrosa and tinea unguium; *T. rubrum* rarely causes tinea capitis (<1%) [24–27], specifically, tinea capitis superficialis, i.e., gray patch [24, 28], seborrheic [24, 28], and black-dot types [28, 29] as well as kerion Celsi [28, 30, 31] and scutulum formation [29]. It is producing both endothrix and ectothrix invasion of the hair shaft that does not fluoresce on Wood's light examination [18, 31]. On the other hand, higher prevalence rates of *T. rubrum* recovered from scalp lesions were reported as 8.5% [13], 21.6% [24], and 26.6% [28], respectively. To the best of our knowledge, the occurrence of *T. rubrum* in asymptomatic carriage is also a novel finding of the present investigation. However, the detection rate of *T. rubrum* (0.1%) in this study was lower than that in the other previously reported studies [13, 24, 28]. This reason may be due to: (1) *T. rubrum* can be more related

to tinea capitis superficialis than the "carrier state" [24–29] and (2) this fungus is a quite rare etiologic agent of tinea capitis in Turkey [9, 14–16, 18].

Scalp *T. rubrum* infections have been documented in individuals as young as 4 weeks [32] and as old as 85 years [33]. In addition, simultaneous occurrences of *T. rubrum* and *T. mentagrophytes* [34], *T. violaceum* [31], and *M. canis* [31] have been reported. Interestingly, Ziemer et al. [30] emphasized the possibility of an ongoing asymptomatic carrier state that transforms into acute inflammation after scratching and co-colonization with bacteria. It is important to note that an antimycotic therapy was not carried out in our carrier case and that mycological clearance occurred spontaneously, as mentioned previously [8, 14, 16]. Moreover, in this investigation, we observed that this clearance was noted with a heavy spore load, i.e., 17. The patient's contacts and home environment are the most probable source of this anthropophilic fungus [18]. However, in this investigation, all of the contacts and inanimate objects that were screened were found sterile, and the source of the "carrier state" remains unclear.

In this investigation, our methodology involved a specific type of cytobrush and hairbrush to sample the same areas of the scalp. However, we recovered only one dermatophyte strain, *T. rubrum*, by the hairbrush method. Although *T. rubrum* appears to be a rare cause of tinea capitis, this fungus recovered from an adult "scalp carrier", which might be a potential vector of the fungus to household and community contacts. In conclusion, the efficacy of fungal culture via the hairbrush method is a key approach in diagnosing scalp dermatophyte carriage. This investigation has provided a more complete epidemiological evaluation of scalp ringworm in Adana, Turkey.

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