JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, September 2010, Vol. 55, No. 5
doi: 10.1111/j.1556-4029.2010.01409.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

PATHOLOGY AND BIOLOGY

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Trichophyton Mentagrophytes Perforates Hair of Adult Corpses in the Gaseous Period*

ABSTRACT: Despite the substantial literature on mycology, there are still limited reports of the interaction between fungi and human hosts in the postmortem period. Thus, the main goal of this study was to investigate the *in vitro* perforation test using *Trichophyton mentagrophytes* on hair from adult corpses in the postmortem period (gaseous period). The protocol was carried out with positive (prepubescent children's hair) and negative controls (healthy adult hair) as well. One strain of *Trichophyton rubrum* was also used as a negative perforation control. Perforations were found in all the hair samples from corpses and prepubescent children after 12–14 days exposure to *T. mentagrophytes* and were absent in the hair samples of healthy adults. Furthermore, hair perforation was not observed with *T. rubrum*. Our preliminary findings suggest the use of *T. mentagrophytes* as a potential marker of the death interval in forensic science.

KEYWORDS: forensic science, forensic mycology, *Trichophyton mentagrophytes, in vitro* hair perforation, gaseous period, markers of time of death

Advances in forensic medicine and the use of biological markers have improved the postmortem analysis of corpses, which is exemplified by the recent findings versus the estimation of the time of death based only on temperature at different body sites (1,2). Thus, there is a need for wider application of bio- and thanatochemical analyses to estimate precisely the postmortem period. The use of sophisticated models (3) appears to be efficient in estimating time of death, and its combination with biomarkers, e.g., flies (4), may improve forensic techniques. Many other agents may be candidates for estimating the time of death.

The fungus *Trichophyton mentagrophytes* is commonly isolated for laboratory diagnosis, such as in the *in vitro* hair perforation test (5). This test, commonly performed in many mycology laboratories, uses the head hair of blond prepubescent children as a positive control. In this age group, the hair is generally fine and soft, with no medulla, which facilitates verification of the capacity of *T. mentagrophytes* to perforate the hair, which is not observed in the hair from healthy adults because of its higher resistance and the influence of hormonal and immunological factors.

Based on the gradual absence of immunological factors after death, we considered the possibility of using *T. mentagrophytes* to estimate the postmortem period by a hair perforation test. Thus, the main goal of this study was to investigate the *in vitro* perforation test using *T. mentagrophytes* on hair from adult corpses in the postmortem period (gaseous period).

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*Supported by CNPq—Conselho Nacional de Desenvolvimento Científico e Tecnológico and by FUNCAP—Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico Process: 9053/08.

Received 23 Sept. 2008; and in revised form 29 June 2009; accepted 2 July 2009.

Materials and Methods

Ethical Aspects

The present study was approved by the Research Ethics Committee of the State University of Ceará (approval under number 064969333-9).

Hair Samples

Scalp hair samples were collected from three different groups for the hair perforation test as follows: (i) hair from corpses (n = 12; victims ranging in age from 18 to 35 years old), (ii) hair from healthy adults (n = 12; with ages ranging from 18 to 35 years old; negative control), and (iii) hair from blond prepubescent children (n = 4; positive control). The hair samples were collected with forceps and a sterile scalpel. None of the living subjects reported recent use of antifungal medicines or similar scalp treatments. The corpses were all from violent death victims, without report of clinical infection caused by fungal microorganisms, and were selected in the gaseous period (between 3 and 5 days after death).

Selections of Strains of T. mentagrophytes *and* T. rubrum

Four strains of *T. mentagrophytes* and one strain of *T. rubrum* (used as a negative control), belonging to the collection of the Specialized Medical Mycology Center (Department of Pathology and

Forensic Medicine of Federal University of Ceará, Brazil), were used in this study.

Exposure to T. mentagrophytes *and to* T. rubrum

Each strain was grown in a small Petri dish (7 cm diameter × 15 mm height) containing unamended agar (Bacto™ Agar; Becton Dickinson Microbiology Systems, Cockeysville, MD). Hair samples were placed on the agar surface with the appropriate fungal strain, according to the previously described protocol for each strain (6). The dishes were then incubated at 25–28°C for up to 30 days. Dishes were evaluated daily; and for each analysis, a part of the hair sample was removed together with the fungi for staining with lactophenol cotton blue (0.05%). Samples were analyzed by light microscopy at 100× and 400× magnification. The test was considered positive when perforation was observed in at least half the thickness of the hair (6).

Results

There were perforations noted in the hair from all the corpses and positive controls (hair from children) exposed to *T. mentagrophytes.* This positive result occurred between days 12 and 14 in children, and 13 and 14 in corpses, after exposure to *T. mentagrophytes* (Table 1).

The microscopic characteristics of the positive samples were noted as the presence of a perforated area in the marrow and/or cortex of the hair penetrating at least half of the hair structure (Fig. 1). The hair samples from the 12 healthy adults (negative control) did not show positive perforation during the 30 days of observation, in any combination of samples and fungal strains. Further, *T. rubrum* was not associated with hair perforation in any case.

TABLE 1—In vitro *hair perforation by* Trichophyton mentagrophytes.

Hair Origin	Strain	Perforation Day (+)	Microscopic Characteristic
Corpses			
1	CEMM 03-3-018	13th	Perforation of the cortex
2	CEMM 03-3-018	14th	Perforation of the cortex
3	CEMM 03-3-018	13th	Perforation of the cortex
4	CEMM 01-4-194	14th	Perforation of the cortex
5	CEMM 01-4-194	14th	Perforation of the marrow
6	CEMM 01-4-194	13th	Perforation of the cortex
7	CEMM 01-5-038	13th	Perforation of the cortex
8	CEMM 01-5-038	13th	Perforation of the cortex
9	CEMM 01-5-038	13th	Perforation of the cortex
10	CEMM 01-1-015	14th	Perforation of the cortex
11	CEMM 01-1-015	14th	Perforation of the cortex
12	CEMM 01-1-015	14th	Perforation of the cortex
Child			
1	CEMM 03-3-018	12th	Perforation of the cortex
2	CEMM 01-4-194	14th	Perforation of the cortex
3	CEMM 01-5-038	13th	Perforation of the marrow
4	CEMM 01-1-015	14th	Perforation of the cortex
Healthy adults			
1	CEMM 03-3-018	–	–
2	CEMM 03-3-018	–	–
3	CEMM 03-3-018	–	–
4	CEMM 01-4-194	–	–
5	CEMM 01-4-194	–	–
6	CEMM 01-4-194	–	–
7	CEMM 01-5-038	–	–
8	CEMM 01-5-038	–	–
9	CEMM 01-5-038	–	–
10	CEMM 01-1-015	–	–
11	CEMM 01-1-015	–	–
12	CEMM 01-1-015	–	–

Discussion

The *in vitro* hair perforation test, initially described by George and Ajello (7), is based on the ability of *T. mentagrophytes* to perforate hair. The standard test is effective and simple to perform, and it is commonly used on blond children's hair because of their low natural resistance and the absence of saturated fatty acid chains, which allow perforation.

Although the defense barriers of humans cease to function after death, this does not happen instantaneously (8). It occurs as part of a natural process of decomposition that involves cell death and the proliferation of microorganisms during phases after death, which are generally divided into four periods: greenish discoloration period (from 24 to 36 h), gaseous period (in days), deterioration period (in months), and skeletonization period (in years) (9). Once started, the various phenomena of cadaver degeneration occur continuously. Besides the hormonal influence during life, the morphology of the hair also undergoes changes after death (10). The cuticle, which during life works as a protective barrier, degenerates after death, allowing attack by microorganisms such as bacteria and fungi.

Despite the substantial literature on mycology, there are still limited reports of the interaction between fungi and human hosts in the postmortem period (11). We have observed the action of hair-perforating enzymes of *T. mentagrophytes* on the scalp hair of adult corpses in comparison with the hair from the healthy adult. The perforation occurs between the 13th and 14th day of incubation, i.e., the gaseous period of adult cadaver decomposition. This indicates that the decay of immunological factors occurs during the gaseous postmortem period, besides the natural after-death degradation of the hair.

In summary, our results open perspectives for an effective characterization of the decay of barriers in the fungus–host interaction after death. Further studies determining the potential of fungal

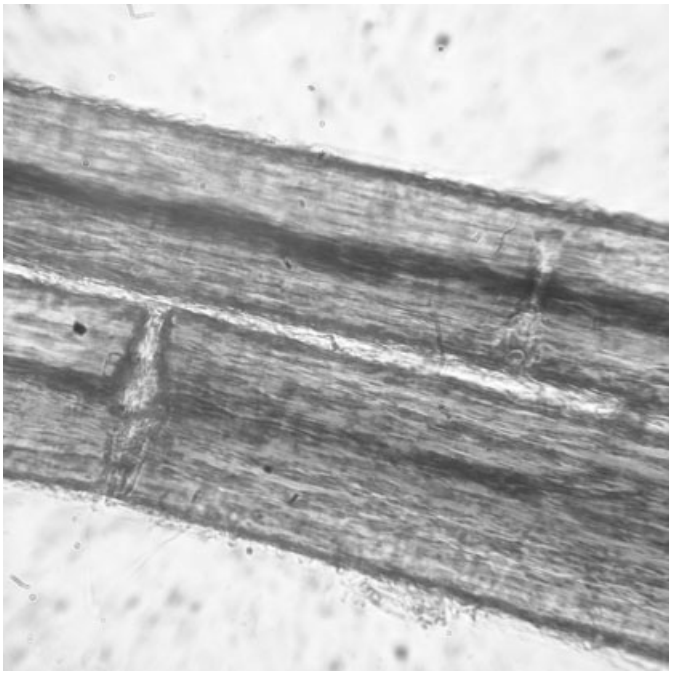

FIG. 1—*The microscopic characteristics of a positive sample with the presence of a perforated area in hair.*

species like *T. mentagrophytes* for use as a marker of the time of death could be interesting for forensic science.

Acknowledgments

This work was supported by the National Research Council (CNPq) and by the Ceará State Foundation for Development of Science and Technology (FUNCAP), Process 9053/08.

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