Polyclonality of *Trichophyton rubrum* Isolates in a Dermatophytosis Patient with Multiple Lesions

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ABSTRACT

We cultured 15 isolates of *Trichophyton rubrum* and one isolate of *Trichophyton mentagrophytes* from an 82-year-old male tinea patient with multiple lesions. To determine whether feet lesions were the source of dermatophytes of other tinea lesions, we extracted total cellular DNA from the *T. rubrum* isolates (13 from feet, two from right waist and buttock). PCR targeting the non-transcribed spacer (NTS) region of ribosomal RNA gene was performed. Molecular polymorphisms were detected by length variation of amplicons.

Four molecular types were found among the 15 isolates. The predominant type, which we previously named Type III, comprised seven isolates cultured from both feet and from left waist and buttock. This was followed by Type VI, five isolates; Type V, two isolates; and Type IV, one isolate. Apart from type III, which was cultured from both feet, isolates were cultured from one foot only. The patient was successfully treated for all types with a six-month course of oral terbinafine and topical luliconazole. The molecular typing supported the notion that tinea pedis was the source of tinea corporis in the patient.

Key words: molecular polymorphisms, *Trichophyton rubrum*, tinea pedis, tinea corporis, multiple lesions

Introduction

Feet are believed to be the portal of human dermatophyte infection, i.e., tinea pedis is the source dermatophyte of tinea found in other sites of the body. There has been no report, however, that directly demonstrates this route. Moreover, our preliminary study revealed differences in molecular types among *Trichophyton rubrum* isolates from tinea pedis, and isolates from tinea on other parts of the body in some patients. In the present study, the molecular types of isolates from an 82-year-old male tinea patient with multiple lesions were determined and compared to verify whether the feet lesions were the source of dermatophytes isolated from tinea lesions in other sites of the patient’s body.

Case report

An 82-year-old Japanese man was referred to us for examination of erythema on the buttocks during treatment for a urinary tract infection. He had, at the minimum, a five-year history of tinea pedis and a one-year history of Alzheimer’s disease. Direct potassium hydroxide examination of scales sampled from margined erythema on the buttocks and hyperkeratosis on bilateral soles, and of clipped nail samples from deformed big toenails (Fig. 1) revealed fungi, and the patient was diagnosed as having tinea corporis and tinea pedis et unguium. Fungal cultures using Mycosel agar slants were positive for dermatophytes, and 16 isolates were identified. Among them, 15 were *T. rubrum* (two from left
waist and buttock, six from right sole, and seven from left sole) and the other was *Trichophyton mentagrophytes* (right heel). The patient was successfully treated with a six-month course of oral terbinafine at 125 mg/day, and topical luliconazole. Total cellular DNA was extracted from the 15 strains of *T. rubrum* using a modified mini-prep method described by Makimura et al. The DNA was used as template DNA in molecular analysis. Intrasppecies typing of *T. rubrum* was determined based on the presence of length polymorphisms in non-transcribed spacer (NTS) of the ribosomal RNA gene using two sets of PCR primers: TrNTSF-2 (5’-ACCGTATTAAGCTAGCGCTGC-3’) and TrNTSR-4 (5’-TGCCACTTCGATTAGGAGGC-3’) for amplification of TRS-1, and TrNTSR-1 (5’-CTCAGTGAACCGTGAGGC-3’) and TrNTSC-1 (5’-CGAGACCAGTGATACATGCG-3’) for amplification of TRS-2. These primer pairs, targeting the highly variable regions in NTS of *T. rubrum*, were previously used to detect 23 molecular types among 101 clinical isolates.

In the present case, four molecular types were detected among the 15 isolates for TRS-1 (Fig. 2). All four were found among isolates cultured from tinea pedis. However, Type IV isolates were cultured from only the left foot and Types V and VI were cultured from only the right foot. Whereas Type III, the most prevalent, was cultured from bilateral feet as well as from right waist and buttock (Fig. 3). No polymorphism among the isolates was observed for TRS-2.

**Discussion**

Feet are the most likely sites to suffer infection with *T. rubrum*. Infection in healthy people usually occurs by acquisition of the fungus from infected individuals, which develops into tinea pedis over a relatively long period. The fungus may then spread from the site initially infected to other parts of the body, including hands, scalp, and trunk. However, no clear evidence of this route has been reported, and analysis using highly sensitive molecular markers is considered necessary for verification.

Several molecular markers have been applied for intraspecies subtyping and strain differentia-
Among them, detection of polymorphisms in NTS\textsuperscript{4} is the most common molecular method for intraspecies typing of \textit{T. rubrum}.

In our preliminary study, we employed the molecular method to type 31 isolates from 12 patients with multiple \textit{T. rubrum} lesions\textsuperscript{1}. Four of the 12 were infected with strains of \textit{T. rubrum} showing different molecular types\textsuperscript{1}, and molecular types from tinea pedis and other parts of tinea in three of these four patients were inconsistent\textsuperscript{10}. Our present case also indicated that multiple molecular types were concomitantly present in the same patient. In this case, one molecular type, namely Type III in our system, was the only type found on bilateral feet, which may indicate the historical origin of the patient’s infection, and was most likely responsible for the widespread tinea. The other strains including Type IV, V, and VI had likely colonized later. Our result is compatible with the hypothesis that tinea pedis is the source of tinea corporis.

Differences in the pathogenesis of these molecular types beyond the feet are unclear. In our preliminary study, we determined the molecular types of 17 strains isolated from tinea lesions at sites other than the feet as: Type I, three strains; Type II, four strains; Type III, three strains; and Type IV, seven strains. Thus, it is difficult to conclude that Type III had higher infectivity than the other types.

The failure to find a relationship among tinea pedis and the tinea found at other sites of the body in our previous study\textsuperscript{1} may have been due to the relatively small number of isolates cultured from the feet.

The presence of multiple types of \textit{T. rubrum} in individual patients has been reported in onychomycosis\textsuperscript{15}. The same group subsequently reported that \textit{T. rubrum} isolated from 10 patients with tinea pedis comprised only one molecular type in each patient\textsuperscript{23}. Our result showing multiple

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig2.png}
\caption{Four molecular types were found among the 15 isolates using NTS region of the ribosomal RNA gene. right waist, buttock, left planta medial border, left (big) toenail, left foot dorsum, left (4th) toe, left heel, left foot medial border, left arch of foot, right planta medial border, right (big) toenail, right foot dorsum, right (1st) toe, right (2nd) toe, right arch of foot, right heel.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Four molecular types were found among 15 isolates in the present case: Type III, seven isolates; Type IV, five isolates; Type V, two isolates, and Type VI, one isolate. All four types were found among isolates from the feet. Type III isolates were cultured from tinea corporis and tinea pedis on both feet.}
\end{figure}
molecular types in one patient contradicts that report. The multiple strains in our case may reflect a long duration of the infection, or may be due to the relatively large number of isolates cultured. Examination of several strains from lesions on each foot is recommended.

Acknowledgments

This study is partially supported by the Research Program on Emerging and Re-emerging infectious diseases from Japan Agency for Medical Research and development, AMED.

Conflict of Interest

Disclosure

In relation to this presentation, I declare that there are no conflict of interest.

References


