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A Comparative Study of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and Fungal Culture for the Evaluation of Fungal Species in Patients with *Tinea Cruris*

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Abstract

BACKGROUND: Tinea cruris is the second most common dermatophytosis in the world and the most common in Indonesia. The conventional laboratory tests for dermatophyte infection are slow and less specific. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is a PCR method with the addition of enzyme after amplification, therefore enabling for more specific results.

AIM: This study aimed to find whether the PCR-RFLP test could yield the same fungal species result as a fungal culture.

METHODS: The specimens were skin scrapings from thirty-one patients suspected *tinea cruris*. The tools and materials that were used were Sabaroud's dextrose agar media, primer ITS 1 and ITS 4 and MvaI.

RESULTS: The equation percentage of the test result species between PCR-RFLP and fungal culture was 50% of 12 subjects whose the test results were both positive from the fungal culture and PCR-RFLP. The percentage of the test result with fungal culture the fungal species were found, but in the PCR-RFLP test which the fungal species was not found, the percentage was 50% of 12 subjects which the test results were both positive as fungi from the culture and PCR-RFLP test.

CONCLUSIONS: The species from PCR-RFLP examination was the same with the fungal culture.

Introduction

Dermatophytes are a group of keratinophilic fungi that can grow on humans' and animals' keratinous tissues such as skin, hair, and nails causing dermatophytosis [1-4]. *Tinea kruris* is a dermatophytosis that may be found on groins, genitals, pubic area, perineal and perianal skins. It's the second most common dermatophytosis globally and also the most common in Indonesia [3, 5-9]. A study by Hajar (1999), found *tinea kruris* as the most common dermatophytosis in Pirngadi General Hospital, Medan [10]. Other studies by Bilkes, 2005 and Nasution, 2005 also found *tinea kruris* as the most common dermatophytosis in several Puskesmas (community health centre) at 40% of all dermatophytosis cases [11, 12].

The conventional laboratory tests for dermatophyte infection are direct microscopic examination with 10% potassium hydroxide (KOH) and fungal culture [1, 3, 13]. These procedures are rather slow. Thus, a faster diagnostic method is needed. Dermatophytes identification can be made in a fast and specific manner by using nucleic acid amplification technology [13, 14]. Molecular techniques such as the polymerase chain reaction (PCR) method has a high sensitivity and specificity rate and can be used to diagnose myriads of microorganism including pathogenic fungi [1, 14, 15].

PCR is an in vitro method for synthesising and amplifying dermatophyte deoxyribonucleic acid (DNA) [16, 17]. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) may produce an even more specific outcome by adding post-amplification enzymes [18]. A study by

Elavarashi, et al. 2013 suggests using Internal Transcribed Spacer (ITS) primer, MvaI and DdeI enzyme for the PCR-RFLP to have a satisfactory outcome [19]. Species identification, however, can be different from the PCR and culture. A study by Irime, et al. 2011, found different species between the real-time PCR and the culture on four samples [20].

Species identification becomes important as a study by Paramata, et al. in Makassar, found 28% dermatophytosis agents on glabrous skins to be itraconazole-resistant [21].

This study aimed to find whether the PCR-RFLP test could yield the same fungal species result as a fungal culture.

Methods

This is a descriptive study using a cross-sectional design. Samples were collected starting in September 2013 in the mycology division of dermatovenereology outpatient unit of RSUP. H. Adam Malik, Medan. Fungal culture and PCR-RFLP were done in the integrated laboratory of Sumatera Utara University, Faculty of Medicine.

The specimens were skin scrapings from thirty-one patients suspected of having *tinea cruris* from history and dermatological examination. The tools and materials that were used were Sabaroud's dextrose agar media (the gold standard for fungal species identification) added with Cycloheximide (0.5 g/l) and Chloramphenicol (0.05 g/l), thermocycler (applied biosystem Verity 96-type well thermal cycler, Singapore), DNA extraction kit (Promega), PCR kit (Promega), ITS 1 primer (forward) and ITS 4 (reverse) (1st Base), and MvaI restriction enzyme (Fermentas). Preheat (94^{o}C, 10 minutes); denaturation (94^{o}C, 1 minutes); annealing (58^{o}C, 1 minute); extension (72^{o}C, 1 minute (35 cycles)) and final extension (72^{o}C, 7 minute) were done using the thermocycler. This study uses the same basepair used in previous studies by Elavarashi E. and Mirzahoseini H., et al. [19]. No positive controls are being used.

The data from the basic information, history, dermatological examination and specimens were then collected. The scraped specimens were then separated into different envelopes and divided into two groups. The first group were being used for fungal culture and another for the PCR-RFLP. The results were then presented in frequency distribution tables and then analysed in a descriptive manner using other literature as comparisons.

Results

Skin scrapings from 31 subjects were collected in this study.

Table 1: Subject characteristics based on gender

Gender	n	%
Male	16	51.6
Female	15	48.4
Total	31	100.0

According to Table 1, out of 31 subjects, fifteen were identified as female (48.4%), and sixteen were identified as male (51.6%).

Table 2: Subject characteristics based on age groups

Age (y.o.)	n	%
12-21	12	38.7
22-31	3	9.7
32-41	2	6.5
42-51	4	12.9
52-61	5	16.1
62-71	3	9.7
72-81	2	6.5
Total	31	100

According to Table 2, most subjects were part of 12-21 years old group at 38.7% of all subjects.

Table 3: Skin scraping analysis using culture and PCR-RFLP

Sample	Culture	Species detected	PCR-RFLP	PCR-RFLP species
1	Negative	NCG	Negative	-
2	Negative	NCG	Positive	*T. mentagrophytes*
3	Negative	NCG	Negative	-
4	Positive	*T. rubrum*	Positive	*T. rubrum*
5	Negative	NCG/*Paecilomyces*	Negative	-
6	Negative	NCG/*Aspergillus flavus*	Positive	*T. mentagrophytes*
7	Positive	*T. rubrum*	Positive	*T. rubrum*
8	Positive	*T. violaseum*	Positive	O
9	Negative	NCG/*Cladosporium*	Negative	-
10	Positive	*M. rivalieri*	Negative	-
11	Negative	NCG	Negative	-
12	Positive	*T. tonsured*	Negative	-
13	Negative	NCG/*Aspergillus flavus*	Positive	*E. floccosum*
14	Positive	*M. rivalieri*	Positive	O
15	Negative	NCG/*Aspergillus flavus*	Negative	-
16	Negative	NCG/*Aspergillus fumigatus*	Negative	-
17	Negative	NCG/*Aspegillus niger*	Negative	-
18	Negative	NCG/*Aspergillus flavus*	Negative	-
19	Positive	*T. rubrum*	Positive	O
20	Negative	NCG/*Aspergillus fumigatus*	Positive	*T. verrucosum*
21	Positive	*T. tonsuran*	Positive	*T. tonsuran*
22	Positive	*T. ericinae*	Negative	-
23	Positive	*T. tonsuran*	Positive	O
24	Positive	*T. rubrum*	Positive	*T. rubrum*
25	Negative	NCG/*Aspergillus flavus*	Negative	-
26	Positive	*T. rubrum*	Negative	-
27	Positive	*T. rubrum*	Positive	*T. rubrum*
28	Negative	NCG/*Paecilomyces*	Positive	*T. verrucosum*
29	Positive	*T. rubrum*	Positive	*T. rubrum*
30	Positive	*T. rubrum*	Positive	O
31	Positive	*T. schoenleinii*	Positive	O

NCG: no culture growth; o: not detected.

According to Table 3, 16 positive subjects were found on the culture, and 17 positive subjects were found using PCR-RFLP.

According to Table 4, *T. rubrum* is the most common species found in the culture out of all subjects at eight subjects (25.8% of all subjects).

Table 4: Fungal species distribution based on culture result

Species	n	%
M. rivalieri	2	6.5
T. ericinae	1	3.2
T. rubrum	8	25.8
T. schoenleinii	1	3.2
T. tonsuran	3	9.7
T. violaseum	1	3.2
NCG	4	12.9
NCG/*Aspergillus niger*	1	3.2
NCG/*Aspergillus flavus*	5	16.1
NCG/*Aspergillus fumigatus*	2	6.5
NCG/*Cladosporium*	1	3.2
NCG/*Paecilomyces*	2	6.5
Total	31	100.0

NCG: no culture growth.

According to Table 5, *T. rubrum* is the most common species found using PCR-RFLP out of 31 subjects in five subjects (16.1% out of all subjects). It could be concluded from the cultures and PCR-RFLP that *T. rubrum* was the most common fungal species found.

Table 5: Fungal species distribution based on PCR-RFLP

Species Jamur	n	%
E. floccosum	1	3.2
T. mentagrophytes	2	6.5
T. rubrum	5	16.1
T. tonsuran	1	3.2
T. verrucosum	2	6.5
-	14	45.2
O	6	19.4
Total	31	100.0

o: species not detected.

According to Table 6, out of 31 subjects, twelve (38.71%) were found positive both in the culture and PCR-RFLP. Four (12.90%) were found positive on the culture and negative on the PCR-RFLP. Five (16.13%) were found positive on the PCR-RFLP and negative on the culture. Out of twelve subjects that were found positive both on the culture and PCR-RFLP, six (50%) yield the same species and six (50%) were found on the culture but not found on the PCR-RFLP.

Table 6: Fungal species distribution based on fungal culture and PCR-RFLP

Species	Culture		PCR-RFLP	
	n	%	n	%
M. rivalieri	2	6.5	-	-
T. ericinae	1	3.2	-	-
T. rubrum	8	25.8	5	16.1
T. schoenleinii	1	3.2		
T. tonsuran	3	9.7	1	3.2
T. violaseum	1	3.2		
E. floccosum	-	-	1	3.2
T. mentagrophytes	-	-	2	6.5
T. verrucosum	-	-	2	6.5
NCG	4	12.9	-	-
NCG/*Aspegillus niger*	1	3.2	-	-
NCG/*Aspergillus flavus*	5	16.1	-	-
NCG/*Aspergillus fumigatus*	2	6.5	-	-
NCG/*Cladosporium*	1	3.2	-	-
NCG/*Paecilomyces*	2	6.5	-	-
Negative	-	-	14	45.2
O			6	19.4
Total	31	100.0	31	100.0

NCG: no culture growth; o: not detected.

Discussion

There were more male subjects to female in this study. Hajar, 1999 also found more male subjects to female in his study which is around 26.67% [10]. Gupta, et al. 2003 and Daili, et al. 2005 conclude that *tinea cruris* affect more male to female [8, 22]. It's suggested that the preference was caused because scrotal areas on males make a warm and humid condition [4]. Menswear also tends to have more coverings for women, and this also contributes to the humid condition [23].

Patel, et al. 2009 and Fernandes, et al. 2001 found higher incidences of *tinea cruris* in young-adult and adolescent males [24, 25]. Andrews, et al. 2008 also found *tinea cruris* are mostly seen in young-adult males [26] In the current study, *tinea cruris* is mostly seen on the 12- 21 years old age group (38.7%). According to Patel, et al. 2009, increase of obesity cases are seen among children and adolescents, and this may contribute to the rising number of *tinea cruris* cases in those age groups [24]. Children who are using tight shirts or underwear may sweat profusely or causing immune disorder thus rising the risk of contracting *tinea cruris* [27].

This study found *T. rubrum* as the most common fungal species found from the fungal culture and PCR-RFLP. Hajar, 1999 and Nasution, 2005 found *T. rubrum* and *T. mentagrophytes* as the most common aetiology for *tinea cruris* [10, 12]. Schieke et al., 2012 and Wiederkehr, et al found *T. rubrum* dan *E. floccosum* as the most common causative agent of *tinea cruris* followed by *T. mentagrophytes* and *T. verrucosum* [3, 6].

Out of twelve subjects that were found positive by using culture and also PCR-RFLP, six (50%) belong to the same species and on another six (50%) fungal species were found on the culture but the PCR-RFLP yield otherwise result. The thinness of the base pair from the PCR may contribute to the result by causing the splicing enzyme used unable to detect the base pair. A study by Irime, et al., 2011, found difference between the identification done using culture and the real time PCR on four samples. Two samples were identified as *T. rubrum* using the culture but identified as *T. interdigitale* using the PCR. Two other samples were identified as *T. Interdigitale* using the culture but identified as *T. rubrum* using the PCR [20]. A study by Wissenlik, et al., 2011 were using real-time PCR for dermatophytes identification. Four different samples yield different species result between using the fungal culture and the real-time PCR [28]. Another study by Girgis, et al., 2006 found seven samples that yield different species result between using the fungal culture and the real-time PCR [29].

Species identification becomes necessary to plan the therapy since *Epidermophyton* and *Trichophyton* were sensitive to terbinafine but *Microsporum* is less sensitive. Thus, a clear and concise way to identify the species is integral in order to be able to give a correct treatment, so that it may

speed up the patients' recovery [30].

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