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

Cut Hazlianda, Kamaliah Muis, Isma Lubis

Universitas Sumatera Utara, Fakultas Kedokteran, Dermatology and Venereology, Medan, Indonesia

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
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 | 100.0|

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| NC/G             | Negative | T. mentagrophytes|
| 2      | Negative| NCG              | Positive | T. rubrum       |
| 3      | Negative| NCG              | Negative | T. mentagrophytes|
| 4      | Positive| T. rubrum        | Negative | T. rubrum       |
| 5      | Negative| NC/G/Paeckimyces | Negative | T. mentagrophytes|
| 6      | Negative| NC/G/Aspergillus flavus | Positive | T. mentagrophytes|
| 7      | Positive| T. rubrum        | Positive | T. rubrum       |
| 8      | Positive| T. vitreum       | Positive | O               |
| 9      | Negative| NC/G/Clausoponum | Negative | -               |
| 10     | Positive| M. malienni      | Negative | -               |
| 11     | Negative| NCG              | Negative | -               |
| 12     | Positive| T. tonsurdi      | Negative | -               |
| 13     | Negative| NC/G/Aspergillus flavus | Positive | E. floccosum |
| 14     | Positive| M. malienni      | Positive | O               |
| 15     | Negative| NC/G/Aspergillus flavus | Negative | -               |
| 16     | Negative| NC/G/Aspergillus | Negative | T. mentagrophytes|
| 17     | Negative| NC/G/Aspergillus nigler | Negative | -               |
| 18     | Negative| NC/G/Aspergillus flavus | Negative | -               |
| 19     | Positive| T. rubrum        | Positive | O               |
| 20     | Negative| NC/G/Aspergillus | Positive | T. vencosus      |
| 21     | Positive| T. tonsuran      | Positive | T. tonsuran     |
| 22     | Positive| T. erinaceae     | Negative | -               |
| 23     | Positive| T. tonsuran      | Positive | O               |
| 24     | Positive| T. rubrum        | Positive | T. rubrum       |
| 25     | Negative| NC/G/Aspergillus flavus | Negative | -               |
| 26     | Positive| T. rubrum        | Negative | -               |
| 27     | Positive| T. rubrum        | Positive | T. rubrum       |
| 28     | Negative| NC/G/Paeckimyces | Positive | T. vencosus     |
| 29     | Positive| T. rubrum        | Positive | T. rubrum       |
| 30     | Positive| T. rubrum        | Positive | O               |
| 31     | Positive| T. Schoentieni   | Positive | O               |

NGC: 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. rivalens      | 2  | 6.9|
| T. erinacei      | 1  | 3.2|
| T. rubrum        | 8  | 25.8|
| T. schoenleini   | 1  | 3.2|
| T. tonsurian     | 3  | 9.7|
| T. violaceum     | 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          | n  | %  |
|------------------|----|----|
| E. floccosum     | 1  | 3.2|
| T. mentagrophytes| 2  | 6.5|
| T. rubrum        | 5  | 16.1|
| T. tonsurian     | 1  | 3.2|
| T. verrucosum    | 2  | 6.5|
| O                | 14 | 45.2|
| NCG              | 6  | 19.4|
| Total            | 31 | 100.0|

*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 |
|------------------|---------|----------|
| M. rivalens      | 2       | 6.5      |
| T. erinacei      | 1       | 3.2      |
| T. rubrum        | 8       | 25.8     |
| T. schoenleini   | 1       | 3.2      |
| T. tonsurian     | 3       | 9.7      |
| T. violaceum     | 1       | 3.2      |
| E. floccosum     | 1       | 3.2      |
| T. mentagrophytes| -       | 2        |
| T. verrucosum    | -       | 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      |
| Negative         | -       | 14       |
| O                | 6       | 19.4     |
| Total            | 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].

References

1. Garg J, Tilak R, Garg A, Prakash P, Gulati AK, Nath G. Rapid detection of dermatophytes from skin and hair. BMC Res Notes. 2009;2:1-6. https://doi.org/10.1186/1756-0500-2-6 PMid:19374765 PMCid:PMC2678142

2. Hay RJ, Moore J. Mycology. In: Burn T, Breathnach S, Cox N, Griffiths C, editor. Rook’s Text Book of Dermatology. 7th Ed. United State: Black-Well; 2004.1425-7.

3. Schieke SM, Garg A. Superficial fungal infection. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff Krauss, editor. Fitzpatrick's Dermatology In General Medicine. 8th Ed. New York: McGraw-Hill Companies Inc.; 2012:2277-97.

4. Hainer BL. Dermatophyte infections. American Family Physician. 2003;67(1):101-8. PMid:12537173

5. Nasution MA. Mikologi dan mikologi kandungan infeksi yang umum di Indonesia. Jakarta: PT Medical Multimedia Indonesia; 2005:30.

6. Hajar S. Penyebab infeksi jamur dermatofitosis di rumah sakit umum daerah dr. Pirngadi Medan [Thesis]. Medan: Universitas Sumatera Utara, 2005.

7. Lakshmipathy DT, Kannabiran K. Overview on dermatomycosis: pathogenesis and treatment. Natural Science. 2010;2(7):726-31. https://doi.org/10.4236/ns.2010.27090

8. Daili ESS, Menaldi SL, Wisnu IM. Penyakit Kulit yang Umum di Indonesia. Jakarta Pusat: PT Medical Multimedia Indonesia; 2005:30.

9. Hajar S. Penyebab infeksi jamur dermatofitosis di rumah sakit umum daerah dr. Pirngadi Medan [Thesis]. Medan: Universitas Sumatera Utara, 2005.

10. Wiederkehr M, Schwartz RA. Tinea cruris. Available at: http://emedicine.medscape.com/article/1091806-overview#showall. Accessed on 28 March 2013.

11. Lakshmipathy DT, Kannabiran K. Review on dermatomycosis: pathogenesis and treatment. Natural Science. 2010;2(7):726-31. https://doi.org/10.4236/ns.2010.27090

12. Wiederkehr M, Schwartz RA. Tinea cruris. Available at: http://emedicine.medscape.com/article/1091806-overview#showall. Accessed on 28 March 2013.

13. Hajar S. Penyebab infeksi jamur dermatofitosis di rumah sakit umum daerah dr. Pirngadi Medan [Thesis]. Medan: Universitas Sumatera Utara, 2005.

14. Blikes Spektrum klinis dan faktor predisposisi dermatofitosis di beberapa Puskesmas wilayah kota Medan [Thesis]. Medan: Universitas Sumatera Utara, 2005.

15. Liu D, Coloe S, Baird R, Pedersen J. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol. 2000;49:493-7. https://doi.org/10.1099/0022-1317-49-6-493 PMid:10847201

16. Alexander CL, Shankland GS, Carman W, Williams C. Introduction of a dermatophyte polymerase chain reaction assay to the diagnostic mycology service in Scotland. Br J Dermatol. 2011;164: 966-72. https://doi.org/10.1111/j.1365-2133.2010.10186.x PMid:21166858

17. Gutzmer R, Mommert S, Küttler U, Werfel T, Kapp A. Rapid identification and differentiation of fungal DNA in dermatological specimens by LightCycler PCR. J Med Microbiol. 2004;53:1207-14. https://doi.org/10.1099/jmm.0.45779-0 PMid:15585499

18. Handoyono D, Rudiretna A. Prinsip umum dan pelaksanaan polymerase chain reaction (PCR). Unitas. 2001;3(1):17-29.

19. Aryani A, Kusumawaty D. Prinsip-prinsip polymerase chain reaction (PCR) dan aplikasinya. Kursus Singkat Isolasi dan Amplifikasi DNA, 2007:71-4.

20. Mirzahoseini H, Omidinia E, Shams-Ghahfarokhi M, Sadeghi G, Razzaghi-Abyaneh M. Application of PCR-RFLP to rapid identification of the main pathogenic dermatophytes from clinical specimens. Iran J Publ Health. 2009;38(1):18-24.

21. Elavarashi E, Kindo AJ, Kalyani J. Optimization of PCR-RFLP directly from the skin and nails in cases of dermatophytosis, targeting the ITS and the 18S ribosomal DNA regions. JCDR. 2013:1-6.

22. Gupta AK, Chaudhry M, Elewski B. Tinea corporis, tinea cruris, tinea nigra, and piedra. Dermatol Clin. 2003;21:395-400. https://doi.org/10.1016/S0733-8635(03)00031-7

23. Hainer BL. Dermatophyte infections. American Family Physician. 2003;67(1):101-8. PMid:12537173

24. Patel GA, Wiederkehr M, Schwartz RA. Tinea cruris in children. Pediatric Dermatology. 2009;8:133-7.

25. Fernandes NC, Akkari T, Barreiros MDGC. Dermatophytoes in children: study of 137 cases. Rev Inst Med Trop S Paulo. 2001;43(2):83-5. https://doi.org/10.1590/S0036-46652001000200006 PMid:11340481

26. Andrews MD, Burns M. Common tinea infections in children. American Family Physician. 2008;77(10):1415-20. PMid:18533375

27. Howell N. Jock itch in children. Available at: http://www.livestrong.com/article/525292. Accessed on 9 May 2014.

28. Wisselink GJ, Zanten EV, Koosstra-smid AMD. Trapped in keratin: a comparison of dermatophyte detection in nail, skin and hair samples directly from clinical samples using culture and real-time PCR. J. Microbiol. Methods. 2011:1-5. https://doi.org/10.1016/j.mimet.2011.01.023

29. Girgis SA, El-Fakkar NMZ, Bard H, Shaker OA, Metwalli FE, Bassim HH. Genotypic identification and antifungal susceptibility pattern of dermatophytes isolated from clinical specimens of dermatophytosis in Egyptian patients. Egyptian Dermatology Online Journal. 2006;2(2):1-23.

30. Kim JY, Choe YB, Ahn KJ, Lee YW. Identification of dermatophytes using multiplex polymerase chain reaction. Ann Dermatol. 2011;23(3):304-12. https://doi.org/10.5021/ad.2011.23.3.304 PMid:21909200 PMCID:PMC3162595