Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

Dermatophyte Identification In Skin And Nail Samples Using Molecular Method

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ABSTRACT:

Objective- The microscope investigation of fungi is rapid but these diagnostic procedures show lack of sensitivity and specificity with 15% fallacious results and species or genus level identification is not feasible.

Material and Methods- This 18-month prospective study included all dermatophytes that were isolated during that time. Dermatophytes were discovered using standard phenotypic techniques. Therefore, in recent years many molecular methods have been developed which are more specific, precise, rapid and cost effective than conventional phenotypic methods. Samples of skin and nails were analyzed under a microscope using 20% KOH and cultivated on Sabouraud dextrose agar supplemented with DTM Media, cycloheximide, and chloramphenicol. By sequencing the internal transcribed spacer (ITS) r-DNA region, the identity of the fungal isolates was verified.

Results- Out Of the 320 samples 270 (84.37%) samples showed growth of fungi (dermatophytes) after culturing on SDA media. Whereas with AP-PCR, 303 (94.68%) samples were positive for dermatophytes. Seven species of dermatophyte namely *Trichophyton mentagrophytes, Trichophyton rubrum*, *Epidermophyton floccosum, Trichophyton tonsurans, Microsporum gypseum, Trichophyton verrucosum* and *Microsporum canis* identified after culturing on SDA media from these 270 samples were confirmed by AP-PCR also.

Keywords ►Dermatophytes ►Trichophyton► AP-PCR► Molecular methods

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

Introduction

The examination of fungus under a microscope can be completed quickly, but the diagnostic methods lack specificity and sensitivity, yielding 15% false positives, and they are not feasible for identifying species or genera at the species level (1). Thus, important study is required for a precise and quick diagnosis of dermatophytes. The main obstacles to this kind of research could include socioeconomic limitations, other widely occurring health problems, and a lack of qualified experience in the field of medical mycology. As a result, several molecular techniques that outperform traditional phenotypic techniques in terms of specificity, accuracy, speed, and affordability have been developed recently. Pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, polymerase chain reaction amplification using NTS, internal transcribed spacer (ITS) primers, nested-PCR, PCR-restriction fragment length polymorphism (RFLP) analysis, arbitrary primer PCR, qPCR, and multiplex real-time PCR are some of the molecular techniques used to identify dermatophytes. Using agarose gel electrophoresis, the AP-PCR technique produces species-specific banding patterns quickly, easily, and affordably (2). Using molecular typing techniques has shown to be helpful in quickly identifying and detecting dermatophyte species. Molecular typing is required for the identification of fungal isolates up to the genus and species levels, particularly for epidemiological purposes (3). Previous studies using AP-PCR primers for OPAA11, OPAA17, OPD18, and OPU15 revealed the distinction of 20–25 dermatophytes species. (4,5,6) Despite the high frequency of dermatophyte infection, little is known about the pathogenicity mechanism of these causative organisms due to inadequate molecular investigations of this infection. However, the last ten years have seen a revolution in the identification and detection of dermatophytes due to the advent of molecular biology techniques for the study of superficial mycosis (7).

Material and Methods-The department of dermatology, venereology, and leprosy collaborated with the microbiology laboratory unit of Index Medical College Hospital and Research Centre, Indore (M.P.), India, to conduct this prospective observational study. Over a period of eighteen months, the study was conducted. The study included 320 samples in total, 48 nail samples and 272 skin scraping samples, which revealed the presence of dermatophytes in fungal cultures and potassium hydroxide (KOH) mounts. A hair sample was not collected during this period. A comprehensive case history, examination, and other relevant workup were finished and documented on a proforma after the patient gave their

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

informed consent. The skin and nail samples were used to extract the genomic DNA. By examining the band intensity of the samples, agarose gel electrophoresis was used to quantify DNA. The UV spectrophotometer was also used to quantify DNA concentration with accuracy. Four randomly primed PCR primers (OPAA11, OPAA17, OPD18, and OPU15) were employed in the current investigation. (4,5,6) Four primers were used individually for the arbitrary primed PCR amplification of the genomic DNA extracted from each of the skin and nail samples that were obtained. In the current investigation, dermatophytes were identified by amplification of the ITS region using primers ITS1 and ITS4, which were obtained from Chromos Biotech Pvt Ltd., Bangalore. To ascertain Trichophyton mentagrophytes' phylogenetic relationship to other species, the ITS region gene sequence of the dermatophyte species was compared with the non-redundant collection of GenBank sequences using the Basic Local Alignment Search Tool (BLAST).

Result- Of the 320 samples 303 (94.68%) samples were positive for dermatophytes with AP-PCR. In the present study total 272 skin samples were characterized using AP-PCR, whereas 259 samples out of these 272 samples were positive for dermatophytes with AP-PCR. Of the 48 nail samples 44 samples were positive for dermatophytes with AP-PCR. In the present study, characterization of seven dermatophyte species *Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum, Trichophyton tonsurans, Microsporum gypseum, Trichophyton verrucosum* and *Microsporum canis* was done using random AP - PCR primers OPAA11, OPU15, OPAA11 and OPD18. On the basis of ITS sequencing results, *Trichophyton mentagrophytes* was found to be associated with *Trichophyton interdigitale.* All the *Trichophyton mentagrophytes* accessions belonged to *Tinea corporis.* Both *Trichophyton inderdigitale* and *Trichophyton mentagrophyte* were identified from *Tinea cruris. Trichophyton mentagrophytes* accession number MN999935 has shown 52% similarity with accessions KY765898.1 and KY765897.1 of *Trichophyton mentagrophytes* and KY765896.1 of *Trichophyton verrucosum.*

Discussion

Although pathogen culture is still regarded as the gold standard, according to Spiliopoulou et al. (2015), the PCR test has a better sensitivity (85.3%) and specificity (80.6%) than the culture method (64.7%). [8] Lubis et al. (2018) stated that fungal culture is still the gold standard for onychomycosis diagnosis, despite PCR being a better confirmatory diagnostic

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

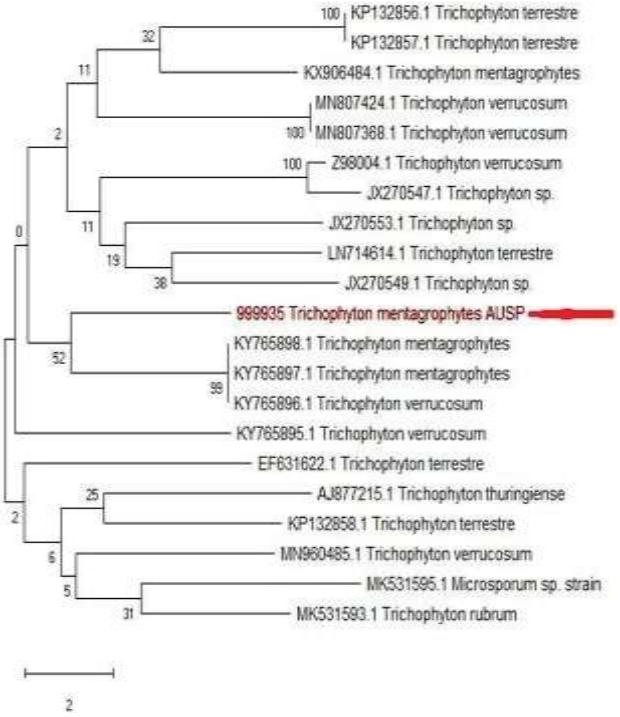
test with 85.71% sensitivity and 28.57% specificity. [9] The positive predictive value was 82.76%, whereas the negative predictive value was 33.33%. The current work used AP-PCR as a molecular diagnostic method to distinguish between distinct dermatophyte species. When compared to nested PCR and real-time PCR, this is more affordable. Zarrin et al. (2017) previously documented the use of the same four random primers—OPAA11, OPU15, OPAA17, and OPD18—for the identification of dermatophyte species in this work. [5] While Girgis et al. (2006) successfully employed only one primer, OPAA17, for differentiating dermatophyte species, the current finding is consistent with their investigation. [2] According to Watanabe and Ishida (2017), their PCR identification method yielded 100% success rate and was a faster way of identification than cultural methods, which had 69.8% success rate for the identification of Trichophyton rubrum and Trihcophyton mentagrophytes species .[10]In order to diagnose onychomycosis, Emam and El-salam (2016) evaluated the use of real-time PCR with nested PCR, KOH microscopy, and culture methods. Contrary to the current study, Trichophyton rubrum and Trichophyton mentagrophytes were the most often isolated fungi. Additionally, it was shown that nested PCR had the lowest sensitivity of 73.3% and real-time PCR had the maximum sensitivity of 93.3%.[1]

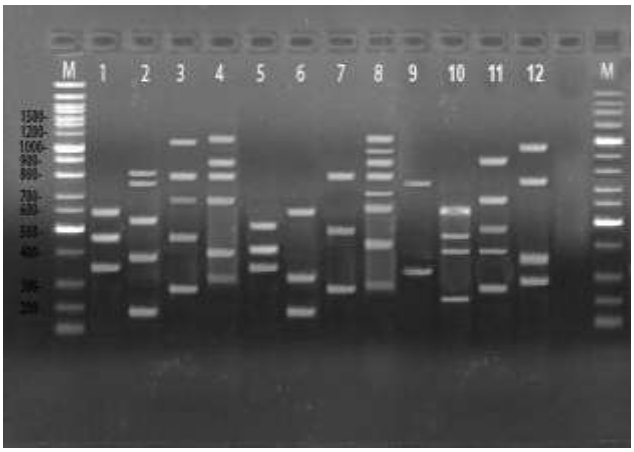
The current results have clearly demonstrated that ITS1 and ITS4 have not been able to do so accurately, despite the claims made by Makimura et al. (1999) and Malinovschi et al. (2009) that the nucleotide sequence of the ITS1 region of pathogenic dermatophytes was useful for identification of dermatophytes at species level and also to understand the phylogenetic relationship within dermatophytes.[11,12] Based on sequencing data, the accessions were identified as Trichophyton mentagrophytes and Trichophyton interdigitale. Accession numbers MN999935, MN999936, MN999937, MN999940, and MN999941 are included for the species Trichophyton mentagrophytes, with query coverage of 52%, 54%, 76%, 40%, and 47%, respectively. Accession numbers MN999938 and MN999939, which had query coverage of 85% and 99%, respectively, have been integrated for Trichophyton interdigitale. Among the participants in this study, 61.56% were from rural areas and frequently resided in unhygienic settings; also, 27.5% had a positive family history of dermatophyte infection, indicating that they got the infection from family members. Trichophyton mentagrophytes, the most often identified species in the study, was further investigated for its evolutionary links with other related species using the ITS1 and ITS4 genomes.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

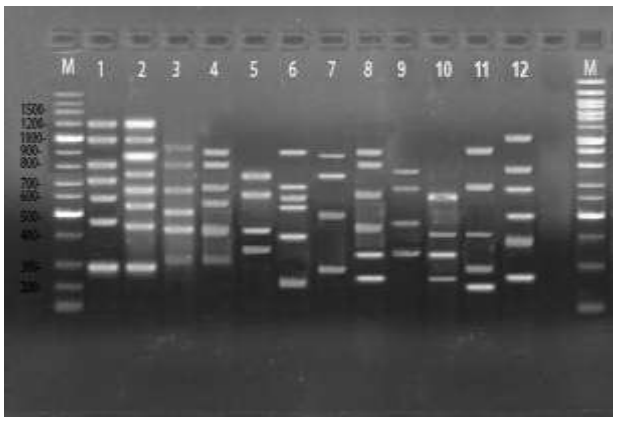
The DNA sequences of Trichophyton mentagrophytes and Trichophyton interdigitale (ITS1 and ITS4) were compared with other comparable sequences obtained from GenBank using the Molecular Evolutionary Genetics Analysis (MEGA X) program. To access their phylogenetic relationships at the species and familial levels, the obtained sequences were separated into separate data sets. This study comprised 320 people with dermatophyte infections; of these, 48 had nail infections and 272 had skin infections. Using SDA culture and microscopic examination, Trichophyton mentagrophytes (40.3% samples) was the most often isolated fungus in the current study. Trichophyton rubrum (30.6% samples), Trichophyton tonsurans (4.6% samples), Epidermophyton floccosum (13.1% samples), Microsporum gypseum (2.5% samples), Trichophyton verrucosum (2.1% samples), and Microsporum canis (1.2% samples) were the next most frequently isolated fungus. The four randomly selected primers (OPAA11, OPU15, OPAA17, and OPD18) were employed to provide a molecular description of the species that the culture method had identified. Twelve, fifteen, fourteen, and sixteen DNA fragments of different sizes were obtained by PCR from the genomic DNA of these seven dermatophyte species using primers OPAA11, OPU15, OPAA17, and OPD18. Despite the fact that the four primers utilized to create the banding pattern distinguished the seven recognized species. According to Ramaraj et al. (2016), Tricophyton rubrum (48.95%) was the most frequently isolated species, followed by Epidermophyton floccosum (0.70%), Microsporum gypseum (1.40%), Trichophyton tonsurans (3.50%), and Trichophyton mentagrophytes (44.75%).[13] Gürcan et al. (2008) and Nagaral et al. (2018) noted a similar finding, stating that Trichophyton rubrum was the most often isolated fungus, followed by Trichophyton mentagrophytes.[14,15]

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

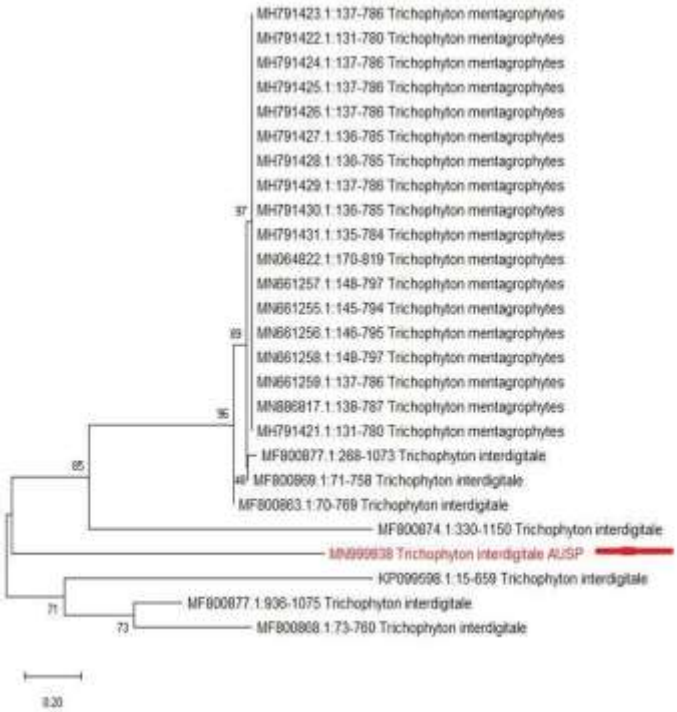


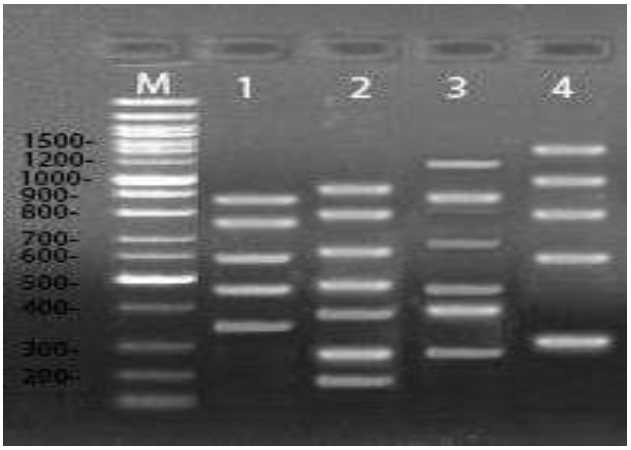


Lanes 1- 4 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 obtained from *Trichophyton rubrum*, respectively. Lanes 5-8 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Trichophyton verrucosum*, respectively. Lanes 9-12 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Trichophyton mentagrophytes*, respectively.



Lanes 1- 4 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Microsporum gypseum*, respectively. Lanes 5-8 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Epidermophyton floccosum*, respectively. Lanes 9-12 show the products of primers OPAA11, OPU15, OPAA17 and OPD18 from *Microsporum canis*,respectively.





Lanes 1- 4 show the products of primers OPAA11, OPU15, OPAA17 andOPD18 from *Trichophyton tonsurans*, respectively

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

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ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 06, 2024

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