

Detection of Dermatophytes from Dermatophytosis-Suspected Cases in Iran, Evaluation of Polymerase Chain Reaction-Sequencing Method

Abstract

Background: Dermatophytosis is mostly caused by dermatophytes species, and the diagnosis of disease is very important for early treatment. The aim of this study was to identify the commonly dermatophytes species isolated directly from the clinical samples, using the polymerase chain reaction (PCR) and evaluate both conventional and molecular methods. **Materials and Methods:** This study was performed on 115 clinical samples. Dermatophyte isolates were initially identified by conventional method and confirmed by the sequencing molecular method. In this study, the molecular technique is implemented directly on clinical samples. Statistical analysis of the information was performed by the SPSS software, and the results were statistically analyzed. **Results:** Our findings demonstrated that the most abundant dermatophyte species by PCR-sequencing were *Trichophyton mentagrophytes* (20%), followed by *Trichophyton tonsurans* (10%), *Trichophyton rubrum* (6.7%), *T. interdigital* (6.7%), *Arthroderma otae*, and *Arthroderma vanbreuseghemii*, (3.3%) for each one. **Conclusion:** For medical laboratories, routine procedures are still preferred because of their lower cost, and the results are almost the same as the molecular methods. The sensitivity and specificity values for PCR under our laboratory condition were 60% and 87%, respectively. This study shows that molecular results performed better in nails than other samples, by culture results.

Keywords: *Dermatophytosis, diagnosis, polymerase chain reaction*

Introduction

One of the worldwide health problems is fungal infections which affecting approximately 20%–25% of the world's population.^[1]

These infections are caused mostly by dermatophytes which are a group of keratinophilic fungi that are capable of causing diseases in the skin, hair, and nails of humans and animals.^[1,2] These kind of fungi belong to the oldest groups of micro-organisms which have been known as the agents of human disease.^[3]

Dermatophytes are taxonomically classified into three anamorphic (asexual) genera: *Epidermophyton*, *Microsporum*, and *Trichophyton*. These species are also classified as anthropophilic, geophilic, or zoophilic according to their habitat.^[2,3] These three genera in their classical circumscription is based on the features of macroconidia. Confirmation of dermatophytosis, a superficial fungal infection, relies on diagnostic

test. Conventional approaches for the identification can be made by 1-direct microscopy test of clinical samples with potassium hydroxide (KOH) 10% of clinical samples which is simple, cheap, and rapid-screening method for fungal structures, but it lacks specificity^[2] and 2-Fungal culture which is expensive, time-consuming, and may take up to 4 weeks to obtain the results.^[4] It also requires expert technologist to be able to pointing to correct the identity of dermatophytes by morphology. The conventional method (culture and direct smear) worked well in the diagnosis when fresh isolates were used, but were difficult to maintain and reproduce because of rapid degeneration.^[3] Besides, the culture method is less sensitive than direct microscopy. Among the samples that are positive by direct microscopy, only 40% show positive results by the culture method.^[4] Therefore, alternative methods with sufficient specificity and sensitivity are necessary. In recent years,

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the DNA-based approaches for the detection of fungi have developed for more sensitive and time-saving diagnostics. Some reports describe the polymerase chain reaction (PCR) as a reliable alternative to conventional identification methods, but it has also reported some defects and restrictions.^[4] Molecular-based techniques depend on the detection of genotypic differences in the pathogenic organisms.^[4-6] They are intrinsically more specific and more precise than those based on the phenotypic features. The recent development of molecular technology such as PCR has further increased the sensitivity and fast of nucleic acid-based diagnostic procedures.^[5,7,8]

The aim of our study was evaluated the efficacy of both conventional and PCR-sequencing methods in the diagnosis of dermatophytosis in different clinical samples.

Materials and Methods

Sampling

One hundred and fifteen patients (55 males and 60 females) with dermatophytosis suspected clinically by a dermatologist were enrolled in the study, in Isfahan, Iran, from February 2017 to May 2019. None of the patients had used any antifungal treatment in the previous 3 months. Specimens were divided into three pieces for the following tests: microscopy (KOH 10%), fungal culture, and molecular examination. The investigators evaluating each test were unaware of the results of other examinations.

Ethics

Demographic data were documented for each subject, and this demographic data were collected with the full consent of the patients. It was approved by the ethics review committee of Isfahan University of Medical Sciences. The approval code is IR.MUI.MED.REC.1397.160.

Direct examination

The specimens were transparent with 20% KOH directly on a glass slide to allow for its examination and incubated for about half an hour in a wet plate and were evaluated for about 15 min under a microscope and considered positive if septate mycelium (SM), septate hypha and arthroconidia (SHA), pseudohypha or blastoconidia (PHB) were seen.^[5,9]

Fungal culture

The second part of the specimens was inoculated into Sabouraud dextrose agar medium with 50 mg/L chloramphenicol (SC) (Biolife Italiana Sri, Milan, Italy) and Sabouraud dextrose agar medium containing 500 mg/L cycloheximide along with 50 mg/L chloramphenicol (SCC) (Biolife Italiana Sri, Milan, Italy), and incubated at 25°C. Cultures were examined “periodically” for the fungal growth up to 4 weeks. The specimens were evaluated for colony morphology (growth

rate, appearance, color, and pigmentation) and microscopy characteristics using lactophenol aniline blue.^[4,10]

Molecular identification

The third part of the specimens was verified to the species level using PCR based on internal transcribed spacer regions of rDNA (ITS rDNA).^[11,12]

DNA extraction

The critical point of the present study is extracting the DNAs of fungal elements directly from different cutaneous lesions and identifying the isolates by sequencing method. A small piece of samples was put in a sterile Eppendorf tube. Add 200 µL glass bead (size: 0.5 mm), 200 µL Lysis buffer, and 200 µL phenol chloroform. Specimens were then blended by homogenizer carefully (3 times, 20 sec. 6000Hz). After that, they centrifuge in 5000 rpm. Supernatant was transferred into another sterile tube, and chloroform was added equally and tubes centrifuge in 5000 rpm for 5 min. Again, supernatant was transferred into another sterile tube and pure ethanol was added (2.5 times the volume) and sodium acetate (3M, pH = 5) (0.1 times the volume). They were stored in 20°C for an hour, and tubes were then centrifuged in 10000 rpm. Supernatant was discarded, and 500 µL ethanol (70%) was then added, tubes were centrifuged in 10,000 rpm for 10 min, and the same as prior step, supernatant was discarded. After 10 min, 20 µL distilled water or TE was added.

The PCR with specific oligonucleotide primers was conducted in 25 µL of reaction mixture which made up of 3 µL of genomic DNA, 12.5 µL master mix (red master mix 2X, amplicon, Sina Gene, Iran), 7.5 µL DW, 1 µL ITS-1, and 1 µL ITS-4 (Sina Gene, Iran). The universal fungal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), were used.^[12] The PCR amplification comprised: One cycle of initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C (30 s), 55°C (45 s), and 72°C (45 s), with a final 5-min extension step at 72°C. A tube with no template DNA was used as a negative control.

Detection of amplified products

PCR amplicons were separated by running the products in a 1% (w/v) agarose gel and electrophoresed. A 100 base pair (bp) ladder was used as DNA molecular weight marker in each run. The gels were visualized using the gel documentation system and recorded photographically.

Sequencing

All the amplified products which preliminarily identified by PCR were sequenced by ITS1 primer using an automated DNA sequencer (Bioneer Company, South Korea). The consensus sequences were then compared to all the sequences in NCBI GenBank version. BLAST 2.2.10.

Statistics

Statistical analyses of the information were performed by the SPSS software Version 25 (IBM SPSS Statistics for Windows, IBM Corporation, Armonk, NY). The two-dimensional and multi-dimensional tables in the software were used, and the results were statistically analyzed. The sensitivity and specificity of the methods in the study, as well as the positive and negative predictive values (PPV and NPV) of each method, were calculated.

Results

In the period from February 2017 to May 2019, among 115 suspected cases of dermatophyte infections (31 nail, 81 skin, and 3 hair samples), which originated from Isfahan, Iran, Shafa Medical Mycological Laboratory, 60 patients (52.2%) were female and 55 (47.8%) patients were male. The age range of patients of the study was between 1 and 90 years (mean age: 39 years). Statistical analyses of the information were performed by the SPSS software version 25, and the results were statistically analyzed. The results of the direct smear, culture, and PCR are presented in Table 1 and are further detailed for nail, skin, and hair samples. Of 115 specimens in this study, 32 (27.8%) samples were dermatophyte and 40 (34.8%) were nondermatophyte (ND) held from culture which are detailed in Table 2. From 58 (50.4%) positive cases in direct microscopy, 29 (25.2%) samples were positive in culture which 10 (8.4%) samples were confirmed by sequencing. In 18 (15.6%) specimens, culture and sequencing results were matched to species and genus, and 29 (25.2%) samples were not matched with culture, but nondermatophyte fungi were isolated. From 30 (26.1%) negative samples in direct smear, one (0.9%) sample was dermatophyte in culture. In this study, from 115 suspicious specimens for dermatophytosis, 58 (50.4%) cases were detected by direct smear while from this 58 specimens; only 29 cases were indeed dermatophytosis.

The diagnostic accuracy of the PCR assay was evaluated by calculating the sensitivity, specificity, PPV, and NPV. As direct smear evaluation of the specimens does not identify the genus or species, the sensitivity, specificity, PPV, and NPV of the PCR assay were calculated using the culture-positive samples as the gold standard for

true positives.^[8] The sensitivity, specificity, PPV, and NPV values for PCR were 60%, 87%, 63%, and 85%, respectively. This study shows that PCR results performed better in nails than culture results.

From 115 specimens, 30 (26.1%) samples were positive by PCR, and they were sequenced which 28 (24.3%) cases were matched with culture results. From total 115 specimens, 2 (1.7%) samples were *Malassezia* in direct microscopic which were negative in culture and PCR, and among the 5 (4.3%) specimens which were positive for candidiasis (PHB) in direct microscopy, 2 were positive in PCR, and from these two specimens, one was *Candida* species and one was *Aspergillus* species in culture which both of them were confirmed by sequencing, one was *Cladosporium* species, one was *Candida* species, and one case was negative in culture. From 20 (17.4%) specimens, which were SM in direct microscopic, only 4 (3.5%) cases were positive in PCR that 3 (2.6%) were matched with sequencing and culture and from the specimens with negative PCR, 2 (1.7%) cases were dermatophyte species, 5 (4.3%) *Aspergillus* species, 3 (2.6%) *Candida* species, one (0.9%) *Epicoccum* species, and 5 (4.3%) cases were negative in culture. In total 8 (7%) samples were negative in both direct smear and PCR, but 3 samples were *Aspergillus* species, 4 were *Candida* species, and one specimen was *Rhodotorula* species in culture [Table 3a-c].

According to the sequencing results which were compared to all sequences in NCBI GenBank version. BLAST 2.2.10, the most prevalent dermatophyte species was *Trichophyton mentagrophytes* (20%), followed by *T. tonsurans* (10%), *Trichophyton rubrum* (6.7%), *T. interdigital* (6.7%), *Arthroderma otae*, and *A. vanbreuseghemii* (3.3%) for each one. The most prevalent nondermatophyte fungal species was *Aspergillus flavus* (13.3%), *Candida orthopsilosis*, *Issatchenkia terricola*, *Candida albicans*, *Fusarium proliferatum*, and *A. oryzae* (3.3%) for each one [Figure 1].

Discussion

Conventionally, the diagnosis of cutaneous fungal infection is based on the direct microscopic examination and culture. Microscopy is able to detect fungal hyphae in specimens but cannot identify the exact species, on the other hand, culture will allow the identification of the causative organisms but

Table 1: Combinations of direct smear, dermatophyte culture, and dermatophyte polymerase chain reaction results according to the site of specimens

Samples	Methods						
	Fungal element (DS)		Culture		PCR		
	+	-	+	-	+	-	-
Nail	23 (20)	8 (7)	2 (1.7)	29 (25.2)	6 (5.2)		25 (21.7)
Skin	54 (47)	27 (23.5)	28 (24.3)	53 (46.1)	22 (19.1)		59 (51.3)
Hair	1 (0.9)	2 (1.7)	2 (1.7)	1 (0.9)	2 (1.7)		1 (0.9)
Total	78 (67.9)	37 (32.2)	32 (27.7)	83 (72.2)	30 (26)		85 (73.9)

DS: Direct smear, PCR: Polymerase chain reaction

are rather complex, time-consuming (≥ 2 weeks), and has a high false-negative rate and also require expert personnel and mycological tools. According to the previous studies in Europe, patients with suspicious dermatophytosis

were diagnosed by physicians and dermatologists only based on the clinical features not laboratory findings.^[13] PCR assays have recently been developed to overcome these difficulties.^[6,14,15] Several factors are interfered with dermatophytosis diagnosis such as personnel skills in sampling, laboratory direct microscopic and culture, and also sample volume. Beside, dermatophytes diagnosis procedure is longer than yeasts and saprophytes (sometimes, it takes 4 weeks) due to their fastidious aspects.^[6,16] In this study, we aimed to compare PCR with conventional methods for detecting dermatophytes in clinical lesions from patients were suspected to dermatophytosis. From late 1990, there was a lot of effort to set up a method based on PCR for dermatophytosis diagnosis such as PCR, PCR restriction fragment length polymorphism, and real-time PCR.^[4,6,17,18]

In the past few years, several molecular methods for the detection and identification of dermatophytes from the clinical samples have been developed.^[19] Some of these molecular methods require a culture step, such as oligonucleotide arrays and DNA sequencing.^[18,20,21] In 2007, Bergman *et al.* adapted the PCR-reverse line blot technique, and in 2013, Paugam *et al.* developed the same assay that detects the most prevalent dermatophyte species in the clinical samples.^[4,6] In many molecular methods, dermatophytes DNA isolation directly from the clinical samples has been ignored, and there are isolated from culture so it has challenged the fact that molecular methods are very fast. However, how many molecular procedures (PCR) have been performed immediately after sampling which could diagnose fungal factor's

Table 2: Detail of the dermatophyte and nondermatophyte isolates and negative recovered in culture from the 115 specimens collected from patients included in the survey

Species	Specimens (n=115), n (%)
Dermatophytes	
<i>T. mentagrophytes</i>	14 (12.2)
<i>T. tonsurans</i>	2 (1.7)
<i>T. rubrum</i>	2 (1.7)
<i>T. verrucosum</i>	7 (6.1)
<i>T. interdigitale</i>	2 (1.7)
<i>M. gypseum</i>	1 (0.9)
<i>M. canis</i>	4 (3.5)
Nondermatophytes	
<i>Epicoccum</i> spp.	3 (2.6)
<i>Rhodotorula</i> spp.	2 (1.7)
<i>Aspergillus</i> spp.	17 (14.8)
<i>Candida</i> spp.	16 (13.9)
<i>Fusarium</i> spp.	1 (0.9)
<i>Cladosporium</i> spp.	1 (0.9)
Negatives	43 (37.4)
Total	115 (100)

T. mentagrophytes: *Trichophyton mentagrophytes*, *T. tonsurans*: *Trichophyton tonsurans*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *T. interdigitale*: *Trichophyton interdigitale*, *M. gypseum*: *Microsporium gypseum*, *M. canis*: *Microsporium canis*

Table 3a: Comparison of direct smear, polymerase chain reaction, sequencing and culturing results from clinical specimens suspected dermatophytoses

Direct smear	PCR	Sequencing	Culture	Total (%)
SM	Positive	<i>A. flavus</i>	<i>Aspergillus</i> sp.	1 (0.9)
SM	Positive	<i>F. proliferatum</i>	<i>Fusarium</i> sp.	1 (0.9)
SM	Positive	<i>A. oryzae</i>	<i>Aspergillus</i> sp.	1 (0.9)
SM	Positive	No match	<i>Aspergillus</i> sp.	1 (0.9)
SM	Negative	-	<i>T. mentagrophytes</i>	1 (0.9)
SM	Negative	-	<i>M. canis</i>	1 (0.9)
SM	Negative	-	<i>Epicoccum</i> sp.	1 (0.9)
SM	Negative	-	<i>Aspergillus</i> sp.	5 (4.3)
SM	Negative	-	<i>Candida</i> sp.	3 (2.6)
SM	Negative	-	Negative	5 (4.3)
SHA	Positive	<i>T. rubrum</i>	<i>T. rubrum</i>	2 (1.7)
SHA	Positive	<i>T. mentagrophytes</i>	<i>T. mentagrophytes</i>	5 (4.3)
SHA	Positive	<i>T. mentagrophytes</i>	<i>T. verrucosum</i>	1 (0.9)
SHA	Positive	<i>T. interdigitale</i>	<i>T. verrucosum</i>	1 (0.9)
SHA	Positive	<i>T. interdigitale</i>	<i>T. interdigitale</i>	1 (0.9)
SHA	Positive	<i>T. tonsurans</i>	<i>T. tonsurans</i>	1 (0.9)

SHA: Septate Hyphae and Arthroconidia, SM: Septate Mycelium, PCR: Polymerase chain reaction, *A. flavus*: *Aspergillus flavus*, *F. proliferatum*: *Fusarium proliferatum*, *A. oryzae*: *Aspergillus oryzae*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *T. interdigitale*: *Trichophyton interdigitale*, *M. canis*: *Microsporium canis*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *T. tonsurans*: *Trichophyton tonsurans*

Table 3b: Comparison of direct smear, polymerase chain reaction, sequencing and culturing results from clinical specimens suspected dermatophytoses

Direct smear	PCR	Sequencing	Culture	Total (%)
SHA	Positive	<i>T. tonsurans</i>	<i>T. verrucosum</i>	1 (0.9)
SHA	Positive	<i>A. flavus</i>	<i>Aspergillus</i> sp.	1 (0.9)
SHA	Positive	<i>I. tericola</i>	<i>Candida</i> sp.	1 (0.9)
SHA	Positive	<i>A. vanbreuseghemii</i>	<i>T. interdigitale</i>	1 (0.9)
SHA	Positive	<i>C. albicans</i>	<i>T. mentagrophytes</i>	1 (0.9)
SHA	Positive	No match	<i>T. mentagrophytes</i>	1 (0.9)
SHA	Positive	No match	<i>T. verrucosum</i>	2 (1.7)
SHA	Positive	No match	<i>Epicoccum</i> sp.	1 (0.9)
SHA	Positive	No match	<i>Aspergillus</i> sp.	1 (0.9)
SHA	Positive	<i>A. otae</i>	<i>M. canis</i>	1 (0.9)
SHA	Negative	-	<i>T. mentagrophytes</i>	6 (5.2)
SHA	Negative	-	<i>T. verrucosum</i>	2 (1.7)
SHA	Negative	-	<i>M. gypseum</i>	1 (0.9)
SHA	Negative	-	<i>M. canis</i>	2 (1.7)
SHA	Negative	-	<i>Epicoccum</i> sp.	1 (0.9)
SHA	Negative	-	<i>Rhodotorula</i> sp.	1 (0.9)

SHA: Septate Hyphae and Arthroconidia, *A. flavus*: *Aspergillus flavus*, *F. proliferatum*: *Fusarium proliferatum*, *A. oryzae*: *Aspergillus oryzae*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *T. interdigitale*: *Trichophyton interdigitale*, *M. canis*: *Microsporum canis*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *T. tonsurans*: *Trichophyton tonsurans*, *A. vanbreuseghemii*: *Arthroderma vanbreuseghemii*, *C. albicans*: *Candida albicans*, *I. tericola*: *Issatchenkia terricola*, *M. gypseum*: *Microsporum gypseum*, PCR: Polymerase chain reaction

Table 3c: Comparison of direct smear, Polymerase chain reaction, sequencing and culturing results from clinical specimens suspected dermatophytoses

Direct smear	PCR	Sequencing	Culture	Total (%)
SHA	Negative	-	<i>Aspergillus</i> sp.	3 (2.6)
SHA	Negative	-	<i>Candida</i> sp.	6 (5.2)
SHA	Negative	-	Negative	14 (12.2)
Malassezia	Negative	-	Negative	2 (1.7)
Negative	Positive	<i>T. tonsurans</i>	<i>T. tonsurans</i>	1 (0.9)
Negative	Positive	<i>A. flavus</i>	Negative	1 (0.9)
Negative	Negative	-	<i>Rhodotorula</i> sp.	1 (0.9)
Negative	Negative	-	<i>Aspergillus</i> sp.	3 (2.6)
Negative	Negative	-	<i>Candida</i> sp.	4 (3.5)
Negative	Negative	-	Negative	20 (17.4)
PHB	Positive	<i>A. flavus</i>	<i>Aspergillus</i> sp.	1 (0.9)
PHB	Positive	<i>C. orthopsilosis</i>	<i>Candida</i> sp.	1 (0.9)
PHB	Negative	-	<i>Candida</i> sp.	1 (0.9)
PHB	Negative	-	Negative	1 (0.9)
PHB	Negative	-	<i>Cladosporium</i> sp.	1 (0.9)

PHB: Pseudohyphae or Blastocoonidia, SHA: Septate Hyphae and Arthroconidia, *T. tonsurans*: *Trichophyton tonsurans*, *C. orthopsilosis*: *Candida orthopsilosis*, *A. flavus*: *Aspergillus flavus*, PCR: Polymerase chain reaction

genus and species? It seems that each conventional method (direct microscopic and culture) and molecular method has several weaknesses and strengths, which will discuss based on the method used in this study. First, it is noteworthy that correct sampling method and sufficient sample size are very important in both conventional and molecular methods. In suspected lesions, three classes of microorganisms may be seen in direct smear: (1) SHA for dermatophytosis diagnosis, (2) SM for saprophytes diagnosis, and (3) Pseudohyphae or Blastocoonidia (PHB)

for candidiasis diagnosis. One of the probabilistic errors in the direct microscopic method is differential diagnosis between these three categories. In the next step, it is very important to observe the normal flora and its interpretation. In culture method, several points are notable: Fastidious aspects of dermatophytes (up to 4 weeks), specific culture media which not to be contaminated by environmental saprophytes (Mycosel Agar). Although other nondermatophytes such as *Candida* and *Aspergillus*, growth within 48–72 hours, Mycosel Agar media is not suitable

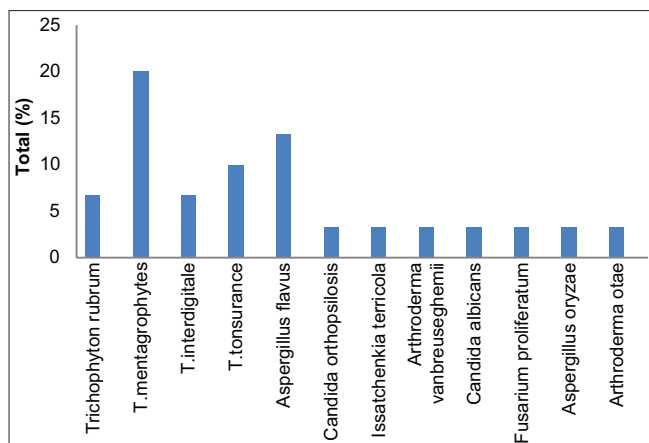


Figure 1: Distribution of different forms of fungi according to sequencing results

for them and cyclohexamide-free media should be used simultaneously. Even if all of the above is followed, some yeast such as *Malassezias* need specific supplementary material in the media and also their optimum temperature is different from dermatophytes. Hence, the inconsistency between culture and direct smear results is due to the above reasons. One of the strengths of the direct microscopic is primary correct diagnosis in nearly half of the cases within 15 to 20 min, and consequently, patient treatment can be done rapidly. Culture is also needed to confirm the diagnosis in <50% of cases. Although culture method is efficient, it is time-consuming and many species do not produce macroconidia or diagnostic elements so, in most cases, dermatophytes may be identified at the genus level.^[1,3,14] Although DNA extraction from the skin and nail sample and other clinical samples is worthwhile, the direct microscopic is superior to the molecular method in terms of material consume and detection speed. Tissue DNA extraction has several problems, for example, false positive and false negative. If a suitable method for organism's DNA extraction from a clinical sample is set up, and the number of samples extracted is high, it will only save time, not the consumables. On the other hand, in simple PCR method, only the presence of fungi can be proven, and quantitative methods such as real-time PCR may help with the diagnosis.^[4,6,19] If the sample is contaminated with normal flora, these methods may have false-positive results. The sequencing method may have a good result, but it is not decisive because only a specific gene or a particular piece of the gene is sequenced, which is not cost-effective and also it is time-consuming.

Conclusion

Dermatophytosis is one of the worldwide health problems. These infections are caused mostly by dermatophytes, so the rapid diagnosis of disease is becoming increasingly important for the early treatment. Conventional approaches for identification include direct smear that lacks specificity and culture which are expensive, time-consuming, and

may take up to 4 weeks to obtain the results and culture method is less sensitive than direct microscopy. In our study, of the samples that is positive by direct microscopy, only 25.2% show positive results by culturing. Hence, alternative method with sufficient specificity and sensitivity seems necessary. The present study provides useful insights on PCR methods for the identification of dermatophytes. It also provides useful information regarding conventional methods in dermatophytes diagnosis.

Our results showed that for medical laboratories, routine procedures are still preferred because of their lower cost. This study shows that molecular results performed better in nails than other samples, by culture results.

Restrictions of the study

This study had several problems, the sample volume which should be adequate to extract the DNA well, also a homogenizer is needed to extract the DNA, which may not be possible for all laboratories in Iran. Sequencing is usually time-consuming and takes time to prepare the results. Moreover, in the molecular method, the sample may contaminate with normal flora and interfere with the results.

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Conflicts of interest

There are no conflicts of interest.

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