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ORIGINAL ARTICLE

Shifting etiological agents of dermatophytosis: a molecular epidemiological study from Iran

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ABSTRACT

Background and Objectives: Dermatophytosis is a significant worldwide health concern, particularly in tropical and subtropical regions. Tinea unguium (TU) and Tinea capitis (TC) are among the most prevalent clinical manifestations of dermatophytosis caused by several dermatophyte fungi. This study investigated the molecular epidemiology and distribution of dermatophytes causing TU and TC in Tehran, Iran.

Materials and Methods: From March 2023 to March 2024, a clinical mycology center in Tehran received 342 suspected cases of TU and TC. The diagnostic methods included the conventional and molecular methods by sequencing the ITS region of ribosomal DNA.

Results: Overall prevalence of dermatophytosis was 59/342 (17.2%) among suspected patients by direct examination. TU and TC were diagnosed in 31/59 (53%) and 28/59 (47%), respectively. The final prevalence among suspected patients was 43/342 (12.5%) by PCR-sequencing, and TC accounted for the largest group of them, 25/43 (58%). Females represented the largest group of suspected TU cases (204/303, 67%; mean age: 57 years), while males predominated among TC patients (28/39, 74%; mean age: 10 years). PCR-sequencing revealed *Trichophyton tonsurans* was the most common agent of TC, 22/25 (88%), and *Trichophyton indotineae* emerged as a notable cause of TU in 5/18 (28%) of confirmed cases.

Conclusion: In our study, *T. tonsurans* remained the predominant cause of TC, while *T. indotineae* emerged as a significant cause of TU. Agreement between conventional and molecular methods was substantial (κ=0.73, 95% CI: 0.61–0.85), with 81.8% misidentification of the *T. mentagrophytes* complex but complete accuracy for *T. tonsurans* and *Microsporum canis*.

Keywords: Tinea capitis; Tinea unguium; Molecular epidemiology; *Trichophyton indotineae*

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INTRODUCTION

Dermatophytosis, or tinea, is a widespread superficial fungal infection affecting near 20-25% of people globally, particularly in tropical and subtropical climates where humidity is elevated and hygiene conditions are inadequate (1, 2). Among the clinical types, TU and TC are the most significant, affecting nails and scalp, respectively (1). Dermatophytes are keratinophilic fungi traditionally classified into the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* depending on morphological characteristics (3). Recent taxonomic advancements, however, have expanded this classification to seven genera, including *Trichophyton*, *Microsporum*, *Epidermophyton*, *Lophophyton*, *Paraphyton*, *Nannizzia*, and *Arthroderma* (4). Despite the broader taxonomy, the most commonly isolated human pathogens remain *Trichophyton*, *Microsporum*, *Epidermophyton*, and *Nannizzia* (5). Globally, *T. rubrum* and *T. mentagrophytes* are major causes of TU, while *T. tonsurans* and *M. canis* are commonly associated with TC (6-8). The worldwide prevalence rate for TU stands at approximately 5.5%, with considerable variation by region (9). The prevalence of TC varies widely across regions, ranging from less than 1% to over 80%, depending on geographic, climatic, and socioeconomic factors (10). In Iran, the historically predominant dermatophytes consist of *T. interdigitale*, *E. floccosum*, *T. mentagrophytes*, and *T. violaceum* (11-14). In recent years, *T. Indotineae*, a novel species of the *T. mentagrophytes* complex (ITS genotype VIII), poses a major global health threat due to its prevalence and resistance to terbinafine and other antifungals (5, 15). Reports from Iran have documented a growing prevalence of *T. indotineae*, particularly in skin infections showing resistance to terbinafine (16). This shift in etiological patterns may be influenced by multiple factors, including geographic, ecological, and socioeconomic variables (2, 17). Although numerous studies have examined dermatophyte epidemiology in Iran, many have relied solely on morphological identification methods, which lack precision in differentiating closely related species (12, 18). The use of molecular techniques has significantly enhanced species-level identification and epidemiological accuracy (19). Therefore, the objective of this study was to determine the current molecular epidemiology and species distribution of dermatophytes causing TU and TC in patients at a mycology referral center in Tehran, Iran.

MATERIALS AND METHODS

Specimen collection and processing. From March 2023 to March 2024, 59 positive cases in direct microscopy (nail, n=31; and scalp, n=28) were diagnosed in 342 referred patients with suspected TU and TC, to the clinical mycology laboratory at Tehran University of Medical Sciences, Tehran, Iran. Inclusion criteria comprised patients with clinical manifestations of TU or TC who presented to the mycology laboratory within one year. Exclusion criteria included patients with incomplete clinical records, recent antifungal treatment within the past four weeks, or negative findings in both direct examination and culture. Of the 59 direct positive microscopy samples, 43 (73%) were positive in culture, and 16/59 (27%) despite microscopic confirmation remained culture-negative. Patient demographic and clinical characteristics, including age, sex, predisposing factors, and antifungal treatments, were collected. Nail and scalp scraping were collected from the mentioned patients. Fungal elements were examined microscopically by KOH based on the presence of septate, branching hyaline hyphae in skin and nail samples, and invasion patterns in hair shafts, and inoculated on Sabouraud Dextrose Agar (SDA) (BD Diagnostics, Franklin Lakes, NJ, USA). A 4-week incubation period at 28°C was applied for all cultures. Colonies were macroscopically identified based on texture, color, pigmentation, and growth rate. Microscopic features such as size, shape, distribution, and arrangement of both microconidia and macroconidia, along with hyphal characteristics, were diagnosed with lactophenol cotton blue (20). Ethics Code of this study was: IR.TUMS.SPH.REC.1402.116 (Tehran University of Medical Sciences).

Morphological and molecular identification. Morphological features of slide cultures were observed with an optical microscope (Olympus BX41, Olympus Corporation, Japan). All isolates morphologically identified to species levels (20) dermatophytes were subjected to further molecular analysis.

For molecular identification, all strains isolated from the culture were specified up to the genus level by sequencing of ribosomal DNA internal transcribed spacer (ITS) regions (16). Briefly, a com-

mercial DNA extraction kit (DNA EXTRACTION Kit DNP, Sinacolon, Iran), which relies on silica column binding for DNA purification, was utilized according to the manufacturer's instructions. Primers V9G (5′-TTACGTCCCTGCCCTTTGTA-3′) and LS266 (5′-GCATTCCCAAACAACTCGACTC-3′) were used to amplify the ITS regions (21, 22). All purified PCR products were subjected to BigDye Terminator v3.1Cycle Sequencing Kit (Applied Biosystems, USA) using primer ITS1, and Sanger sequencing (Core Facilities Laboratory, Isfahan, Iran). The sequences were edited in Geneious software (http://www.geneious.com), and further analyzed with the basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) on NCBI, and they were identified to species level based on comparison with reference ITS sequences in GenBank.

Statistical analysis. Data were analyzed utilizing IBM SPSS Statistics version 26.0. Categorical variables were presented as frequencies (%) and compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables (e.g., age) were implied as geometric mean (GM) or median with interquartile range (IQR) and analyzed using the Mann–Whitney U test. A p-value <0.05 was considered statistically significant. The agreement between diagnostic methods was assessed by Cohen's Kappa coefficient (κ).

RESULTS

Between March 2023 and March 2024, 59 positive cases in direct microscopy (nail, n=31; and scalp, n=28) were diagnosed in 342 referred patients with suspected TU and TC, to the clinical mycology laboratory at Tehran University of Medical Sciences, Tehran, Iran. Direct microscopy and culture were positive in 43 out of 59 cases (73%), while 27% (16/59) remained culture-negative ($p = 0.032$). Furthermore, antifungal therapy prior to sampling was reported in 8 patients (13.6%), and 7 of them had negative culture results ($p < 0.01$). TU accounted for 52.5% (31/59) of cases, predominantly affecting women (67%, $p < 0.001$, $\chi^2=15.2$) with a median age of 57 years (IQR: 50-68). In contrast, TC represented 47.5% (28/59), showing male predominance (74%, $p < 0.001$) and a younger median age of 10 years (IQR: 7-13) ($p < 0.001$, Mann-Whitney U=85). Among TU cases, toenail involvement was the most frequent (64.5%), followed by fingernail (32.3%) and combined infections (3.2%). Regarding hair invasion, the most frequent clinical form of TC was endothrix 22/28, followed by endo-ecto 3/28, ectothrix 2/28, and favus 1/28. In one favus case, the culture negativity was also associated with previous antifungal use. Based on direct microscopic examination, the frequencies of dermatophytosis, which depend on age and gender, are presented in Table 1. Of the 59 patients with confirmed direct examination, 17 individuals (28.8%) had underlying diseases. The most commonly reported conditions were diabetes mellitus, cancer, and long-term corticosteroid use. Morphological and microscopic analysis of the 43 isolates identified *T. tonsurans* as the predominant agent in 27 cases (63%), followed by *T. mentagrophytes* (n=11, 25%), and *M. canis* (n=5, 12%). Among 43 isolates that underwent ITS-based molecular identification, *T. tonsurans* predominated (n=27, 63%), followed by *T. indotineae* (n=6, 14%), *M. canis* (n=5, 12%), *T. mentagrophytes* (n=2, 5%), *T. interdigital* (n=1), *T. rubrum* (n=1), and *T. vanbreuseghemii* (n=1). Morphological identification showed 100% concordance with molecular methods for *T. tonsurans* and *M. canis*, but *T. indotineae*, *T. interdigitale*, *T. vanbreuseghemii*, and *T. rubrum* isolates were morphologically misidentified as *T. mentagrophytes*. The outcomes of species identification for the type of clinical presentation are detailed in Table 2. Agreement between conventional and molecular methods was substantial (κ=0.73, 95% CI: 0.61–0.85), with 81.8% misidentification of the *T. mentagrophytes* complex but complete accuracy for *T. tonsurans* and *M. canis.*

DISCUSSION

In this study, we utilized ITS-rDNA as the gold standard region to identify dermatophytes (19) at the species-level. Notably, while most prior studies in Iran rely on conventional mycological methods, our approach enhanced the accuracy of species identification by incorporating molecular tools. Some studies have indicated dermatophytosis incidence among Iranian patients suspected of TU ranging from 5 to 44% in Tehran and 1 to 28% in other provinces. In this study, the TU frequency rate was 10.2%, This finding aligns with some prior Iranian epidemiological reports (23-25). Based on our results, the frequency of TC was 71.8 % in children, aligning with

Table 1. Demographic and diagnostic characteristics of dermatophytosis cases with statistical comparisons

Clinical Form	n (%)	Median Age (IQR)	Female n (%)	Microscopy Positive n (%)	Culture Positive n (%)	p-value (age) *	p-value (gender) **
Tinea un-guium	31 (52.5%)	57 (50-68)	16 (51.6%)	31 (100%)	18 (58.1%)	<0.001	0.003
Tinea capitis	28 (47.5%)	10 (7-13)	5 (17.9%)	28 (100%)	25 (89.3%)	<0.001	0.003

*Mann-Whitney U test, **Chi-square test

Table 2. Species identification by DNA sequencing of the ITS regions.

Sample ID	Morphological Identification	Clinical Site	Sequence Identity (%)	Molecular Identification	GenBank Accession Number
2	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053852
3	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053824
4	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053825
5	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053826
6	*T. tonsurans*	Scalp	99.85%	*T. tonsurans*	Pv053827
7	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053828
8	*T. tonsurans*	Scalp	99.85%	*T. tonsurans*	Pv053829
9	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053830
10	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053831
11	*T. tonsurans*	Scalp	99.84%	*T. tonsurans*	Pv053832
12	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053849
13	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053833
14	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053834
15	*T. tonsurans*	Scalp	99.64%	*T. tonsurans*	Pv053835
16	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053836
17	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053851
18	*T. tonsurans*	Scalp	99.81%	*T. tonsurans*	Pv053837
19	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053838
20	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053839
21	*T. tonsurans*	Scalp	99.69%	*T. tonsurans*	Pv053840
22	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053853
32	*T. tonsurans*	Scalp	100%	*T. tonsurans*	Pv053842
12a	*M. canis*	Scalp	100%	*M. canis*	Pv052815
317	*T. mentagrophytes*	Scalp	100%	*T. men-tagrophytes*	Pv059277
1128	*T. mentagrophytes*	Scalp	100%	*T. indotineae*	Pv059282
34	*M. canis*	Toe nail	100%	*M. canis*	Pv053843
262	*M. canis*	Toe nail	100%	*M. canis*	Pv052813
301	*M. canis*	Toe nail	100%	*M. canis*	Pv052814
1158	*M. canis*	Toe nail	99.13%	*M. canis*	Pv053848
461	*T. mentagrophytes*	Toe nail	100%	*T. men-tagrophytes*	Pv059278
76	*T. mentagrophytes*	Toe nail	99.32%	*T. van-breuseghemii*	Pv053850
185	*T. mentagrophytes*	Toe nail	100%	*T. indotineae*	Pv059275
220	*T. mentagrophytes*	Toe nail	99.22%	*T. interdigital*	Pv059276
37	*T. tonsurans*	Toe nail	100%	*T. tonsurans*	Pv053844
38	*T. tonsurans*	Toe nail	100%	*T. tonsurans*	Pv053845
1072	*T. mentagrophytes*	Toe nail	100%	*T. indotineae*	Pv059280
614	*T. mentagrophytes*	Finger nail	99.60%	*T. indotineae*	Pv077899

Table 2. Continuing...

1012	*T. mentagrophytes*	Finger nail	100%	*T. indotineae*	Pv059279
1118	*T. mentagrophytes*	Finger nail	99.87%	*T. indotineae*	Pv059281
261	*T. mentagrophytes*	Finger nail	99.31%	*T. rubrum*	Pv077898
914	*T. tonsurans*	Finger nail	100%	*T. tonsurans*	Pv053847
30	*T. tonsurans*	Finger nail	100%	*T. tonsurans*	Pv053841
40	*T. tonsurans*	Finger nail	100%	*T. tonsurans*	Pv053846

epidemiological profiles in Iran and globally (26). The discrepancies in these results can arise from the sample size, regional and ecological conditions, as well as methodological variations. According to our findings, TU was rare in children and predominantly affected adults, with the highest frequency in those over 50 years, which is consistent with the global trends showing that the prevalence of TU rises with age (27-29).

Unlike studies reporting higher TU prevalence in men (30), our findings showed a female predominance, possibly due to greater healthcare-seeking behavior among women. However, several studies indicated equal participation of both genders (31). Gender predominance discrepancies between geographical locations might be attributed to changes in culture, climatic conditions, and socioeconomic levels.

According to age distribution, TC primarily affects prepubertal children. Studies from Iran, China, Bosnia, and Poland found that the most common age group for TC is < 19 years old (32-35). According to Gharaghani et al. analysis of dermatophytosis in Iran during the previous 60 years, TC affected people between the ages of 1 and 19 more often (96.8%) (37). Similarly, prepubertal children aged 10 years on average were most impacted by TC infection in the current study. *T. tonsurans* and *T. indotineae* were equally represented among TU cases (n=5 each). Overall, *T. tonsurans* accounted for the highest number of isolates across all clinical forms, followed by *T. indotineae* (n=6). Consistent with our findings, *T. indotineae* was one of the causative agents of TU in studies in Iran, Italy, and France (16, 36, 37). Contrary to our finding, *T. mentagrophytes* and *T. rubrum* were reported as the predominant agents of TU (38-40). Since the 2000s, the pattern of TC fungal agents has steadily altered, with a notable increase in the prevalence rate of *T. mentagrophytes*, *T. tonsurans*, and low-prevalence or emerging species like *T. indotineae*, as well as a notable decrease in the incidence rate of *T. schoenleinii* (35). The leading TC agent in France, the United Kingdom, and North and Central America is *T. tonsurans* (25, 41). Whereas *M. canis*, a zoophile, is the most common species in Europe, Australia, South American, and the Mediterranean regions (41, 42). Our data and recent molecular surveys indicate that *T. tonsurans* has increasingly replaced *M. canis* as the dominant dermatophyte in Iran. Separately, the anthropophilic species *T. violaceum*, once prevalent in the region, now appears to be on the verge of local extinction (12, 31, 43-46). Accurate identification of dermatophytosis and its causative agents is crucial for tracking changes in frequency and establishing a reference framework for epidemiological analysis. In developing countries, clinical examination, direct mycological microscopy , and conventional culture are the standard diagnostic methods for dermatophytosis (18). *T. interdigitale* was historically thought to be a variety of *T. mentagrophytes* due to their near resemblance; however, molecular analysis in 2017 revealed that *T. interdigitale* is distinct from *T. mentagrophytes* (5). In the present study, *T. interdigitale* and *T. vanbreuseghemii* strains isolated from TU were identified using molecular analysis, which had been mistakenly identified by the traditional method as *T. mentagrophytes*. Microscopic features of *T. indotineae* are similar to *T. mentagrophytes* and are indistinguishable from it. *T. indotineae* was recently described by Kano et al., and terbinafine resistance was shown in some strains isolated from six patients in this study: one TC case and five TU cases. Previously identified as *T. mentagrophytes* rDNA-ITS genotype VIII, *Trichophyton indotineae* is a novel species within the *T. mentagrophytes* complex that has quickly replaced *T. rubrum* as the dominant dermatophyte and is reportedly on the rise in Iran and other countries across the world (5, 36). Although our study identified *T. indotineae*, we did not perform antifungal susceptibility testing. Including such data would strengthen clinical relevance.

CONCLUSION

In this study, *T. tonsurans* remained the predominant cause of TC, while *T. indotineae* emerged as a significant cause of TU, matching *T. tonsurans* in prevalence. Agreement between conventional and molecular methods was substantial (κ=0.73, 95% CI: 0.61–0.85), with 81.8% misidentification of the *T. mentagrophytes* complex but complete accuracy for *T. tonsurans* and *M. canis.* Thus, molecular methods should be used with strains identified as *T. mentagrophytes*, but may not be necessary for those identified as *T. tonsurans* and *M. canis.*

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