

Genotyping *Tinea Capitis* Fungi based on Barcoding ITS rDNA Marker

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Abstract

The study included isolating and diagnosing of *Tinea capitis* fungi isolated from clinical samples who are visited the outpatient clinics in Babylon / Hilla province. 50 samples were collected 40 clinical samples (hairs and scales), and 10 hair samples as control group. The results showed that: numbers of dermatophytes fungi were identified based on microscopic and Molecular criteria. *T. mentagrophytes*, *T. tonsurans*, *M. audouinii*, *M. canis*. . Also two yeast species were identified: *Candida albicans*, *Candida parapsilosis*.

Key words: *Tinea capitis*, Genotypes, ITS rDNA.

Introduction

Tinea capitis is a scalp skin-attacking disease that attacks hair follicles and shafts, also causes eyebrow and eyelashes infection. The disease is regarded a shallow mycosis or dermatophytosis form. Several synonyms, including scalp ringworm and *tinea tonsurans*, are used¹. The disease possess a health risk due to its ability to change patterns and high prevalence among children after pyoderma². Poor economic and social status and poor hygiene are all associated with infection, in addition there are three factors that help to increase the disease: moving from pets to the house, early identification of suspected pests, incubation period of weeks is infectious but asymptomatic³. The aim of study conduct molecular typing of suspected fungi were associated with *Tinea capitis* samples, and those isolated from patients who are visited the outpatient clinics.

Material and Method

Collection of Specimens:

A 40 hair and scalps samples were collected in this

study from all patients diagnosed with head lesions (outpatient clinics). After that, the infected area was sterilized with 70% alcohol to remove the bacteria. Hair samples were collected using forceps and skin scraps from the scaly edge of the area using a surgical blade. The sample was transferred to the laboratory in sterile filter paper.

Cultivation of Samples:

All clinical samples collected were cultured on SDA agar media that containing chloramphenicol (250mg/l), Streptomycin (150mg/l) and cycloheximide (250mg/l) then all samples incubated at (28-30°C) for two weeks. Then after the result of culture appeared the samples were examined morphologically from where the color of colony, shape, texture and reverse pigment. Then maintained the samples by using a screw-cup slant on SDA and preserved during the study⁴.

All samples that give positive results of *Tinea capitis* purified by taking a pure colony from culture media that obtain a mixed culture.

DNA extraction:

The isolates of yeast and filamentous fungi under interest were subjected to DNA extraction. In brief; a loop full of yeast colonies and tiny portion of mycelia

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were suspended in the lysis buffer supplemented by Promega Yeast Extraction Kit (USA) and following up the extraction steps based on the instruction of this Kit. A dry DNA pellet dissolved in diluted rinse and preserved in - 20 °C until use ⁵⁻⁸.

The genomic DNA was extracted according to Imran and Al.Shukry (2014); The PCR procedure was as previously described by ⁵⁻⁷.The universal primer pair used in this study was ITS5/ITS4 Forward: (5'-GGAAGTAAAAGTCGTAACAAGG-3') and Reverse (5'-TCCTCCGCTTATTGATATGC-3).

Sequencing of Selected PCR Product

A 10 dermatophyte isolates PCR products were sent to Korea's Macrogen Laboratory and obtained the positive sequence information for various species. The sequencing findings were subjected to multiple sequence alignment with reference strains deposits in GenBank for identification.

Results and Discussion

Prevalence of dermatophytes among *Tinea capitis* sufferers:

A total of 50 samples, 40 samples (hairs and scales) were collected from different age and genders of patients with *Tinea capitis* and 10 samples were collected by the same techniques from likely healthy persons (as

control samples), the clinical specimens were collected from private clinics in Babylon province. Identification results based on cultural and microscopic methods showed that 35 (87.5%) of 40 clinical samples were positive cases. The majority of the patients were males 57.14 % (20/ 40) while, 42.85 % (15/40) specimen from females. While only one specimen shown positive out of 10 samples from control group.

The relations between the infection and age for both genders:

It had been registered the number and percentage of infected patients by *Tinea capitis* , depending on gender and age ,table (1) had shown the significant difference between genders and infection with this disease , the number of males (20)(57.14%) and the number of females reached (15)(42.85%) to the consultative of the dermatology .

It was clear from the current study, males infection with *Tinea capitis* was (57.14%) while in females (42.85%) it was found existence of significant differences of the infection in both sexes agreed with results of other researchers outside of Iraq, including Sehagal *et al.*,(1985) in the city of New Delhi, India, Ayaya *et al.*,(2001) in Kenya ,and Ali-Shtayeh,(2002) in Palestine and Morar *et al.*,(2004) in South Africa , Wani *et al.*,(2006) in Kashmir, who found that *Tinea capitis* affects males more than females in their studies.

Table (1) Distribution of age and gender among patients of *Tinea capitis*.

Age/year	Gender				Total Infection	
	Male		Female			
	Number	percentage	Number	percentage	Number	percentage
1 – 10	16	45.71%	14	27.08%	30	85.71%
11 – 20	3	8.57%	0	0%	3	8.57%
21 - 30	1	2.85%	0	4.16%	1	2.85%
31 – 40	0	0%	1	2.85%	1	2.85%
Total	20	57.14%	15	42.85%	35	100%

Phenomenon of wearing veil by female may help in the reduction of the infection and transmission. This result was approached with reached Ali, (1990), who had the highest infection rate among people aged between (5-10) years, and by Abbas (1995), the highest infection rate among people aged 6-9 years.

The Identified fungi:

A total of 6 fungal isolates have currently been identified based on evidence of morphological, microscopic, and molecular analysis. The dermatophytes were: *T. mentagrophytes*, *T. quinckeanum*, *T. tonsurans*, *M. audonii*, *M. persicolor*, and *M. canis*. Also, two *Candida* spp. were *C. albicans*, *C. parapsilosis*.

Table (2): Fungal isolates from hair infected with Tinea capitis.

Fungal isolates	No. of isolate ii i isolates	Percentage of frequency	Males (%)	Females (%)
<i>M. audonii</i>	19	54.28%	10(28.57%)	9(25.71)
<i>M. canis</i>	2	5.71%	2(5.71%)	0(0%)
<i>M. persicolor</i>	9	25.71%	4(11.42%)	5(14.28%)
<i>T. mentagrophytes</i> <i>mentagrophytes</i> <i>mentagrophytes</i> <i>mentagrophytes</i>	1	2.85%	1(2.85%)	0(0%)
<i>T. quinckeanum</i>	1	2.85%	0(0%)	1(2.85%)
<i>T. tonsurans</i>	3	8.57%	3(8.57%)	0(0%)
Total	35	100%	20 (57.14%)	15(42.85%)

Table 2 shows the types of dermatophytes isolated from the human, the most common isolate predominant was *M. audonii* which represent 19 (54.28%) of cases followed by *M. persicolor* 9 (25.71%). It showed the presence of differences between sex and species of the fungi isolated.

Molecular identification

The results genotyping of 12 dermatophytes under interest based on the rDNA region ITS1-5.8S-ITS2, 28S partial sequence shown deferent genotypes. The dermatophytes were: *Microsporum* spp; *Trichophyton* spp. shown in Figure (1).

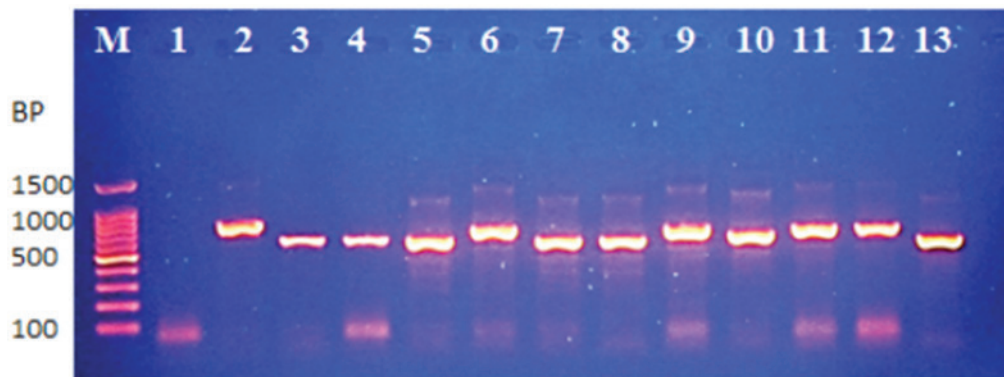


Figure (1): Profile gel electrophoresis of PCR products of 12 dermatophyte isolates of fungi amplified by ITS5/ITS4 primer: Lanes (left to right): isolate of 1- negative control; 2-,*M. audonii* (800 bp and; 3-5 *T. mentagrophytes*(780 bp); 6- *Candida*

parapsilosis (600bp)⁹; 7-9 *T. queckanum* (700 bp); 10-11 - *T. tonsurans* (700 bp); 12 - *M. persicolor* (700 bp), these results according to sequence analysis; 13 - *Candida albicans* (500 bp)⁹; Molecular M (100bp for each step.). 1.6% agarose gel at 70 volt for one hour.

There are high variations in PCR products among of fungi under interest as in used ITS5 / ITS4 as primer pair for amplification of ITS1-5.8S-ITS2, 28S region with primers flanking region, consider good barcoding region due to high variation for another ward the amplification of this region show high polymorphism in PCR product. (*M. audouinii*, *M. canis*, *M. persicolor*, *T. mentagrophytes*, *T. queckanum*, *T. tonsurans*) these range not always require for other specific genetic analysis.⁹

DNA sequence analysis:

About 16 µl of PCR products were sent to Macrogen Laboratory in Korea for sequencing analysis. After the sequence results had been obtained, each sequence alignment with the sent isolate's nitrogen bases sequence. They correspond to reference sequencing samples in the gene bank using NCBI Blast Nucleotide.

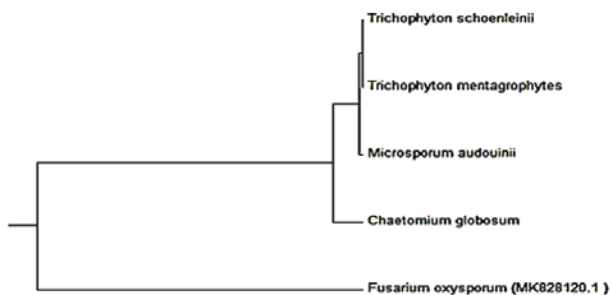


Figure (3): Phylogeny construction for all identified isolates based on sequence charts with one reference strain (marked with the accession number, while our isolates have full names).

Conclusion

Conclusion of this study gave attention that particular study about *Tinea capitis* more producible in their result compared with survey studies of dermatophytes fungi in whole human body.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Health and all experiments were carried out in accordance with

approved guidelines.

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