Myco-epidemiologic and genetic study of dermatophytosis and non-dermatophytes in Middle Euphrates, Iraq

Karrema Al-Khafajii

Medical College, Babylon University, Hilla, Babylon, Iraq.

Received 17 December, 2013; Accepted 6 May, 2014

Dermatophytes are the most prevalent infections in human and animals. These infections can cause disease in all age groups and sexes. The objective of this study was to assess the age and sex prevalence, the site of involvement of different kinds of dermatophytes and an evolutionary study of cross sections type in a private clinic patients attended in Middle Euphrates provinces of Iraq (Babylon, Najaf, Dewania and Karbala) in order to prevent and cure these complications. To survey the dermatophytosis, request forms were prepared to evaluate data like age, sex, profession, site of the lesions, the type of lesions and record of contact with animals. Eighty six clinical specimens from the infected sites were collected from 200 patients (125 males and 75 females) and subjected to conventional methods, 16 isolates were diagnosis by molecular assay, and the results were statistically analyzed by SPSS. The results show that 72.7% of the lesions were dry and 27.3% were inflammatory. The dry lesions were mostly (86%) on the head area and inflammatory ones were mostly (54.7%) in the feet area, 21.4% of the patients had previous contact with animals. Tinea corporis was the most prevalent, while Tinea faciei was the least occurring lesion with the highest cultural isolation rate when compared with Trichophyton tonsurans, Trichophyton rubrum, Trichophyton violaceum, Epidermophyton, Trichophyton verrucosum and non dermatophytes. The typing of ITS1-5.8S-ITS2 fragment of rDNA gene for 16 isolated dermatophytes and non-dermatophytes were classified into Epidermophyton floccosum, T. rubrum, Trichophyton mentagrophytes, T. tonsurans and others molds. The observations of molecular analysis were T. rubrum, 800 bp and T. mentagrophytes, 690 bp.

Key words: Myco-epidemiologic, dermatophytosis, diagnosis, microscopy, polymerase chain reaction (PCR), Iraq.

INTRODUCTION

Dermatophytes are keratinophylic fungi that are able to infect keratinized tissues of human leading to infections that are mainly restricted to the corneocyte of skin, hair and nails (Adefemi et al., 2011). They fungi belong to three
anamorphic genera: *Trichophyton*, *Microsporum* and *Epidermophyton*. (Weitzman and Summerbell, 1995; Adeleke et al., 2008). The infection which is caused by these fungi is termed Dermatophytosis and is commonly referred to as ringworm or tinea. These filamentous fungi are usually identified on the basis of clinical features and isolation patterns together with conidial shapes and sometimes with physiological characters such as; hair perforation and urease tests. In some cases, morphological identification can be difficult or uncertain because there is considerable variation among isolates of some species, and it is a time-consuming procedure requiring even 30 day for final isolation and identification of etiologic agent at genus or species level. Likewise, in some instance, the causative dermatophyte fail to produce any obvious reproductive structure in culture (termed sterile mycelia) which makes it impossible for ultimate definitive diagnosis. On the other hand, antifungal drugs are expensive, and they have many side effects in humans and animals. In the last decades, genotyping approaches have proven to be useful for solving problems of dermatophyte taxonomy, as well as enhancing the reliability and speed of dermatophytosis diagnosis (Aho, 1980; Faggi et al., 2001; Jousson et al., 2000; Mochizuki et al., 2003; Jousson et al., 2006; Graser et al., 2006).

Genotyping method offers an alternative way of identifying individual fungal isolates for epidemiological purpose. Genotyping procedure had previously been developed based upon variable rDNA gene cluster of *Trichophyton rubrum* (Jackson et al., 2000). Recently, *Trichophyton mentagrophytes var. interdigitale* has also been shown to possess genetic polymorphisms that map to the rDNA (Mochizuki et al., 2003). By development of PCR technology, a wide variety of molecular techniques such as RAPD-PCR, nested-PCR, PCR-RFLP, PCR-EIA, Real-time PCR and microarray technology were employed as possible alternatives for routine identification of fungi including dermatophytes (De Baere et al., 2010).

Considering the financial and physical costs that dermatophytosis infections impose on the society, urgent actions must be taken to prevent the disease and cure it.

The objective of this survey was to assess the age and sex prevalence, the site of involvement of different kinds of dermatophytes in order to prevent and cure the complication. The ITS1-5.8S-ITS2 fragment of ribosomal DNA gene (rDNA) in the dermatophyte species were used as a reliable marker for species identification.

**MATERIALS AND METHODS**

This survey which is an evaluating study, was carried out on two hundred patients infected with dermatophytosis, their age ranged from 1-70 years (mean 19.8±12.5 SD) they visited and were referred to dermatology private clinic in Hilla (in which patients come from Hilla, Karbala, Dewania and Najaf provinces, all these provinces are called Middle Euphrates). This study was conducted in from March 2012 to March 2013.

The characteristics of the patient such as age, sex, profession, address, the record of contact with animals, the site of the lesion, the type of lesion (dry or inflammatory) were collected in advance forms.

**Hair and skin**

Specimens from the infection sites were collected and subjected to conventional examining by directed (potassium hydroxide) KOH. Microscopic examination and 85 specimens were cultured on primary and selected media. Dermatophytes isolates were identified by their colony characteristics: microscopy, physiologic and biochemical test .16 specimens were genotyped by PCR.

**Extraction of DNA**

Sixteen fungi isolated were used for DNA extraction and PCR assay. DNA of fungal isolates were extracted by picking 1 g of mycelia by using sterile loop and suspending into 300 µl of lysis buffer (10 mM Tris, 1mM EDTA (pH8), 1% SDS, 100 mM NaCl, 300 µl phenol-chloroform (1:1)) shaken for 5 min and centrifuged at 1000 rpm, the supernatant was transferred to new tube and equal volume of chloroform was added, mixed, centrifuged and the supernatant was transferred to new tube. 500 µl of 70% ethanol alcohol was mixed with supernatant and centrifuged at 10000 rpm for 7 min, dry DNA pellet was re-suspended in 75 µl of TE buffer and stored at - 20°C until use (Mousavi et al., 2007).

**PCR assay**

The ITS1-5.8S-ITS2 region of rDNA from various reference strains of dermatophytes and non-dermatophytes) were amplified using the universal fungal primers ITS1 (5'-TCGGTACGGGCAGCGG-3') and ITS4 (5'-TCTCCTGCTTATGATGATGTC-3') used to amplify the entire ITS rDNA region in dermatophytic fungi and non-dermatophytic fungi. The PCR mixture was amplified by thermal cycler PCR System (Labnet, USA) using the following conditions: first denaturation temperature of 95°C for 5 min, followed by 30 cycles including initial denaturation temperature of 95°C for 30 s, annealing temperature of 57°C for 1 min, extensions temperature of 72°C for 1 min, and final extension temperature of 72°C for 7 min. The PCR products were run on 1.2% agarose gel (Bio Basic Canada Inc.) electrophoreses performed at 100 V in TBE buffer. The gel was pre-stained with 0.05% ethidium bromide. The DNA bands were detected by Gel imager scope 21 ultraviolet transilluminator (Korea Company).

The Chi-square test was used to find out if there was any relationship between human contacts with animals and the occurrence of tinea.

**RESULTS AND DISCUSSION**

Two hundred patients were examined, all of them were studied through critical observation, and culture was carried out from 86 patients. From the ages point of view, the patients were at the age group of 1-70 years, the most
involved age group was 11-20 years which were 73 patients (36.5%), these results were consistent with the results of Oyeka and Okoli (2003) and Ameen (2010). From sex point of view, 125 (62.5%) of the patients were males and 75 (37.5%) females. The males showed the most prevalent infections of tinea corporis 78.7%, tinea cruris 77.5%, and tinea pedis 56.3%. In the females, the most prevalent dermatophyte infections were, tinea manuum 49.3% tinea corporis 48%, and tinea cruris 45.3%. Our results were in concordance with the results of Leite et al. (2014) and Havlickova et al. (2008).

From the lesions site of view, patients had skin lesion 30% in trunk, 25% in lower limb, 6%, in upper limb, 3% in the neck, 10% face, 12% head and 14% groin. From lesion type point of view, there were 72.7% dry and 27.3% inflammatory. The dry lesions were mostly on the head area, 86% and inflammatory ones were mostly in the feet area (54.7%). Statistical study of the patients infected with dermatophytes showed that there was a meaningful relation between the type of profession and the lesion sites (Leite et al., 2014). In some specific conditions, tinea corporis in student and housekeepers (p=0.01), tinea capitis in students and children (p=0.03), tinea cruris in drivers and in staff tinea manuum in housekeepers and farmers and tinea pedis in athletes, staff and free professions were more than other cases (p=0.000) (Malinovsch et al., 2009).

The results of culture in 85 patients showed that T. mentagrophytes 28(32.9%) was the most commonly isolated followed by T. tonsurans 16(13.8%) and T. rubrum, 10 (11.7%) (Table 1).

From the residence point of view, 65 (32.5%) patient were in the villages and 135 (67.5%) patients were resident in cities. There was significant relationship (pv=0.000) between human contact with animals and the occurrence of tinea.

According to obtained statistics on quantity of the lesion, 60.8% of patients had one lesion, 31% of them had two lesions, 5.3% had three lesions, 2.9% had four lesions and totally 37.1% of the patients had more than one lesion. Our results are coincidence with results of Adeleke et al. (2008).

The 77 isolated dermatophyte strains were found to belong to six species: E. floccosum (11 isolates) T. mentagrophytes (28 isolates), T. rubrum (10 isolates), T. violaceum (9 isolates), T. tonsurans (16 isolates), T. verrucosum (three isolates) and 3 isolates of non-dermatophytes fungi (Table 1).

### Cultural and microscopic features:

Most of fungal isolates showed standard colonies and microscopic characters especially colony colors and conidial shapes (Figures 1, 2 and 3).

### Genotyping of fungal isolates

Currently, molecular studies are crucial and necessary for identification (Malinovschi et al., 2009). The amplified ITS1-5.8S-ITS2 fragment (including primers) ranked between 380 bp for Geotrichium sp. and 780 bp for E. floccosum in amplicon length (Table 2 and Figure 4). These results agree with results of (Rezaei-Matehkolaei et al., 2012). Shehata et al. (2008) showed in their study the variation in amplicon length of ITS1-5.8S-ITS2 in T. rubrum 800 bp and T. mentagrophytes 690 bp, ribosomal DNA. Some species revealed closely related amplicon length as in lanes 1-4, 7-8, 11, (14 approximately 800 bp). The variation in amplicon length for closely related Trichophyton isolates may arises from isolates source or some isolate are probably not typical strain of T. rubrum or it can be an intermediate strain between T. rubrum and

---

**Table 1.** Concentration, distribution and prevalence, percentage of dermatophyte and non dermatophyte fungi, in terms of the type of the disease in patients that visited private clinic of dermatology (specimens were collected from patients from Hilla, Karbala, Dewania and Najaf province in Iraq).
Figure 1. Colonies of A = *E. floccosum*, B = *T. rubrum*, C = *T. mentagrophyte* on Sabouraud’s Dextrose Agar after 14 days incubation at 37°C.

Figure 2. Conidial shape of A = *E. floccosum*, B = *T. rubrum* on Sabouraud’s dextrose agar after 14 days incubation at 37°C.

Figure 3. Conidial shape of: A- *Acremonium*, B- *Cladophialophora bantiana* on Sabouraud’s dextrose agar 14 day incubation at 37°C.
Figure 4. Gel electrophoresis of PCR products of ITS1-5.8S-ITS2 region of rDNA from various isolates of dermatophyte and non-dermatophyte species were amplified using the universal fungal primers ITS1 and ITS4. Lanes: 1-3, 15-16 = *E. floccosum*; 7, 9, 14 = *T. rubrum*; 6, 11-12 = *T. mentagrophytes*; 5 = *T. tonsurans*; 10 = *Geotrichum*; 13 = *Aspergillus flavus*; N = Negative control; M = molecular marker 100 bp.

Table 2. Amplicon length of entire ITS1-5.8S-ITS2 region of dermatophytic and non dermatophytic fungi. Lane M = molecular marker (100 bp). Lanes 1-16 fungal isolates, N = negative control.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Lane number</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>1</td>
<td>780</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>2</td>
<td>780</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>3</td>
<td>780</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>4</td>
<td>780</td>
</tr>
<tr>
<td><em>T. tonsurans</em></td>
<td>5</td>
<td>680</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>6</td>
<td>690</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>7</td>
<td>800</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>8</td>
<td>780</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>9</td>
<td>800</td>
</tr>
<tr>
<td><em>Geotrichum</em></td>
<td>10</td>
<td>380</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>11</td>
<td>720</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>12</td>
<td>720</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>13</td>
<td>590</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>14</td>
<td>720</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>15</td>
<td>780</td>
</tr>
<tr>
<td><em>E. floccosum</em></td>
<td>16</td>
<td>780</td>
</tr>
<tr>
<td>Negative control</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*T. mentagrophytes*, these results were consistent with the report of Mirzahoseini et al. (2009). *T. tonsurans* is an anthropophilic source that was isolated only from human infections while others were a zoophilic source (Jackson et al., 2000, 2006; Mirzahoseini et al., 2009).

**Conclusion**

With regard to the obtained results, the age, sex, the patient contact record with animal and profession has correlation with the occurrence of different types of tinea. The genotypic differentiation by PCR provides a rapid and practical tool for identification of dermatophytes isolates to the species and strain level within one day which is independent of the culture variations.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.
REFERENCES


