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Original Article

Study of Dermatophytosis prevalence in Al-Nassiriyah city- Iraq

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ABSTRACT

The present study aimed to investigate the prevalence of some dermatophytes species from patients with dermatophytosis in Thi-Qar Province during the period from August 2014 to January 2015. One hundred eighty samples were examined by 10 % KOH and cultured on sabouraud's dextrose agar with cyclohexamide and chloramphenicol to identify the dermatophytes species. The results showed that out of the 180 cases of dermatophytes, only 94 (52.2 %) cases were positive by both direct KOH examination and culture, whereas false negative results was recorded in 16 (8.9 %) of specimens. Tinea corporis represented the highest fungal infection among patients which represent 47 cases with a percentage of (42.73 %), followed by tinea capitis 19 cases with a percentage of (17.27%), whereas tinea faciei recorded the lowest fungal infection with 4 cases and a percentage of (3.63 %). High fungal infections were reported in females with a percentage of 67.27% in comparison to 32.73 % in males. Age group of 21-30 years recorded the highest percentage of infections among the age groups with 31 cases and a percentage of 28.18%, while age group up to 51 years showed the lowest number of infection with 4 cases and a percentage of 3.63 %. Urban areas recorded the highest percentage of infection with 86.36 % in comparison with 13.64 % in rural areas. The results showed that dermatophytes species which are isolated from patients in the present study included; *Microsporum canis* with a percentage of 40.91 %, followed by *Trichophyton tonsurans* with (32.73 %), *Trichophyton verrucosum* (15.45%), *Microsporum gypseum* 8.18%, and finally *Microsporum fulvum* with a percentage of 2.73 %. This present study considered the first study in Thi-Qar province that isolate and record *M. fulvum as* causative agent of dermatophytosis.

Key words: Dermatophytes - *Microsporum* – *Trichophyton* - Tinea.

INTRODUCTION

Dermatophytes as keratinophilic fungi are able to infect keratinous tissues of skin (the stratum corneum layer), hair, and nail in humans via their keratinase enzymes. They also degrade claws, feathers, hooves, horns, wools in animals [10,40].
Dermatophyte fungi belonging to three genera (*Trichophyton*, *Microsporum*, and *Epidermophyton*). *Trichophyton* and *Microsporum* genera are the most numerous and diverse, there are over 40 species belonging to these two taxonomic groups. Epidermophyton genus has only one representative – Epidermophyton floccosum species [28]. Dermatophytes are characterized by high affinity to keratin-containing tissues, what make them responsible for superficial mycoses of skin, nails and hair [29]. Dermatophytes can be divided into two states on the basis of stages in the life cycle, the anamorphic and the teleomorphic states. The anamorph is the state where asexual reproduction occurs, the teleomorph, is the sexually reproductive (perfect) state [15]. Tinea is a mycosis caused by dermatophytes; filamentous fungi that invade the keratinized tissues, including the corneous layer of the skin, nails, and hair [43]. Dermatophytosis occur most only on dead keratin at the top layer of the skin, hair and nails, thus dermatophytes are restricted to hair, nails, and superficial skin [50], and they do not infect mucosal surfaces because they require keratin for growth [25]. Reaction to a dermatophytes infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors [18]. Clinical manifestations of dermatophytosis vary depending on the site of infestation and the type of strain; therefore, accurate identification of the strain is

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crucial in order to facilitate rapid treatment and to prevent spread of the disease [23]. The present study aimed to investigate the prevalence and diagnosing the common agents of dermatophytosis in Al-Nassiriyah city - Iraq.

MATERIALS AND METHODS

Collection of specimens: One hundred eighty clinical specimens including hair fragments, nails, swaps and skin scrapping were collected from Dermatology Department of Al-Hussein Teaching Hospital in Thi-Qar Province. The collected specimens were kept in sterile and clean paper bags and marked with the information for each. The scarping skin was collected using sterilized slide after wiping the affected area with 70% ethanol. The infected hairs were removed by a sterile forceps [37].

Direct examination: Direct microscopic examination of skin and hair specimens was performed after digestion in 10 % potassium hydroxide (KOH), while nails specimens were digested using 20 % KOH. A portion of each sample was placed on a sterile slide and a few drops of KOH solution were added, and heated gently (warming gently over the burner was done to speed up the process) for about 5-10 minutes, after that the slide was let to be cooled and the cover slip was put and examined under the low (10X) and high (40X) microscope lens [34]. Lactophenol cotton blue stains must be used to enhance the contrast between fungus and skin. This method aids visualizing hyphae and confirmation of the diagnosis of dermatophytosis infection [42].

Isolation of dermatophytes species: All specimens (irrespective of the negative or positive microscopic examination results) were cultured on Sabouraud's dextrose agar (SDA) supplemented with 500 mg/L cyclohexamide to inhibit the growth of non-dermatophytes molds and 250 mg/L chloramphenicol to reduce contamination with fast growth bacteria. Plates were inoculated with skin scraping, nails, hair fragments and swaps. Cultures were incubated at 28±1°C for 14-21 days. Petri dishes were examined daily after 7 to14 days.

Identification of dermatophytes isolates: In order to study the characteristics of isolated fungi and classified them accurately, glass slide were prepared by taking a part of fungal growth using a sterile needle and put it in a drop of lactophenol cotton blue placed on a glass slide and then sealed by a cover slide, then examined by light microscope under (40X). Identification of the growth was performed depends on the following criteria which is morphological features of colony growth that were including (color, reverse of the colony and pigments that produced). Other criteria was microscopical and macroscopical characteristics which include microconidia and macroconidia: size, shape, arrangement, and hyphal structures [14, 31, 47].

Other diagnostic tests for Dermatophytes:

1. Urease test: This test was used for the detection of urease enzyme which is produced by some dermatophytes. Test tubes containing the medium inoculated with fungal isolates and incubated at 28°C for seven days. Positive result detected as a change of the medium color from yellow to pink [13, 36].

2. Rice Grains medium: It was used to induce sporulation and for differentiation of *Microsporum canis* which grow well and heavily from *Microsporum audouinii* which failed to grow [36].

3. Hair perforation test: This test was performed by taking several non-infected hairs from a child's head and divided them into several parts; 1 cm long and then placed in a glass screw tubes and sterilized by autoclave for 10 minutes and then 25 ml of distilled water containing 3-5 drops of 10 % yeast extract was added to the tubes, then the hair was inoculated by taking a part of fungal colony and incubated for 10-14 days [36].

RESULTS AND DISCUSSION

Examination of the specimens: The specimens were examined by direct examination (KOH solution) and culture. Only 110 specimens with a percentage of 61.1 % yielded growth of dermatophytes after culturing on SDA, and considered as positive specimens which were used in the morphological and molecular identification. The results showed that 94 (52.22 %) specimens were positive in both direct examination and culture, while 16 (8.89%) specimens were negative for direct examination and positive in culture and 43 (23.89 %) specimens had shown positive in direct examination and negative in culture, while 27 cases with a percentage of 15 % were negative in both direct examination and culture (Table 1). This was in relatively agreement with [8, 22, 38] whom found that direct KOH examination was positive in 77 %, 58 % and 80 %, respectively. Whereas this study revealed that direct microscopic examination showed false negative results in 16(8.9%) of specimens, this is relatively in agreement with [24,49] whom mentioned that direct microscopic examination showed false negative results up to 13% and 5.3% respectively, while these results disagree with [2,52] whom mentioned that direct microscopic examination

showed false negative results up to 50%. Also the data of the present study revealed that the KOH test have false positive results in 43(23.9%) cases while culture showed negative results after five weeks of incubation at 28˚C although the direct KOH examination was positive. The negative results of direct KOH examination may be associated to an inadequate amount and preparation of sample, skills of observer, a non-suitable temperature and storage in wet containers result in growth of saprophytic fungi which lead to contamination of the sample. Negative results of culture may be due to that most cases have topical antifungal medications before sampling, or may be the sampling was not from the active border of the lesion which invaded by fungi, also some cases showed mixed infection or contamination by saprophytic fungi.

Table (1): Numbers and percentages of diagnostic specimens by direct examination and culture method.

Clinical cases examination	No. (%)
Positive in both direct examination and culture	94 (52.22%)
Negative in direct examination and Positive in culture	16 (8.89%)
Positive in direct examination and Negative in culture	43 (23.89%)
Negative in both direct examination and culture	27 (15%)
Total number	180 (100%)

Clinical types of dermatophytosis: Tinea corporis was the main infection that recorded in 47 (42.73 %) of patients followed by tinea capitis 19 (17.27 %), tinea pedis were 12 (10.91 %) cases, tinea unguium 12 (10.91 %), tinea cruris 10 (9.1 %), tinea manuum 6 (5.45 %), and tinea faciei 4 (3.63 %) (Figure 1). These results were in agreement with [1, 3, 4] whom found that tinea capitis and tinea corporis were possibly the most prevalent clinical types in Iraq. In other countries such as Yemen [35] and Libya [17] demonstrated that tinea corporis accounts the majority of cases followed by tinea capitis. In Poland [32] found that T. corporis and T. capitis were the most dermatophytes infections. While in India, T. corporis was the most common among dermatophyte infections [30]. But in Iran, tinea cruris and tinea pedis recorded as the most common dermatophytosis [46]. The increased prevalence of tinea corporis and tinea capitis may be due to overcrowded and large sizes of families and sharing of clothes and towels and other tools such as combs and shavings tools.

Isolation of Dermatophytes: Five dermatophytes species were identified in specimens of patients. The present study showed that the main causative of dermatophytes infections was *Microsporum canis* with 45 (40.91 %) cases followed by *Trichophyton tonsurans* 36 (32.73 %) cases, *Trichophyton verrucosum* 17(15.45 %) cases, whereas *Microsporum gypseum* and *Microsporum fulvum* recorded the lowest isolated species with 9 (8.18 %) and 3 (2.73 %) cases respectively. The results agreed with [2,16,54] who mentioned that *M. canis* was the main causative agent of dermatophytes infection. While the present study disagreed with [49] who found that *Trichophyton rubrum* was the most frequently isolate, also present results disagreed with [5,26,51] who mentioned that *Trichophyton mentagrophytes* was the most common isolate from the clinical samples. The higher incidence with *M. canis* may be explained to the direct or indirect contact with domestic animals such as cattle because *M. canis* is a zoophilic fungus and cause many ringworm infections. (Table 2).

Sources of Dermatophytes infection: Dermatophytes species were classified into three main ecological groups which include: Zoophilic, Anthropophilic and Geophilic fungi. The results observed that the main source of infection was Zoophilic fungi and represented 62 cases with a percentage of (56.36 %), followed by Anthropophilic fungi with 36 cases and a percentage of (32.73 %). On the other hand, Geophilic fungi recorded the lowest infection source with 12 (10.91 %) cases. These results agreed with [26,44] who found that most causative agents of dermatophytosis were zoophilic fungi. While our results disagreed with [3] who mentioned that anthropophilic fungi causes the most dermatophytosis infection. The differences may be due to occupation, environment, climate and dermatophytes species that vary from area to another [41]. Also the increased incidence of infection with zoophilic fungi may explain as a result of upbringing and contact with animals especially cattle. (Figure 2).

Dermatophytes infections and residency of the patients: The impact of residence on the distribution of the patients was assessed, the present study showed that there was a statistically significant association to the residency of the patients and dermatophytes infection, where urban areas have been strongly affected by dermatophytes infections with 95 (86.36 %) patients in comparison to15 (13.64 %) patients from rural areas (Table 3). The same results was recorded in

other studies as the infection of people living in urban areas were much more than rural area patients [9,20,21]. While these results disagreed with [3,5,26] who mentioned that the rural area patients were higher than urban area patients. It can be explained to the low levels of education and sharing personal belongings such as combs, clothes, towels and shaving tools, which common between family members in low socioeconomic levels; overcrowding of population may leading to contact between patients and healthy, not take care of hygiene and contact with upbringing animals in houses.

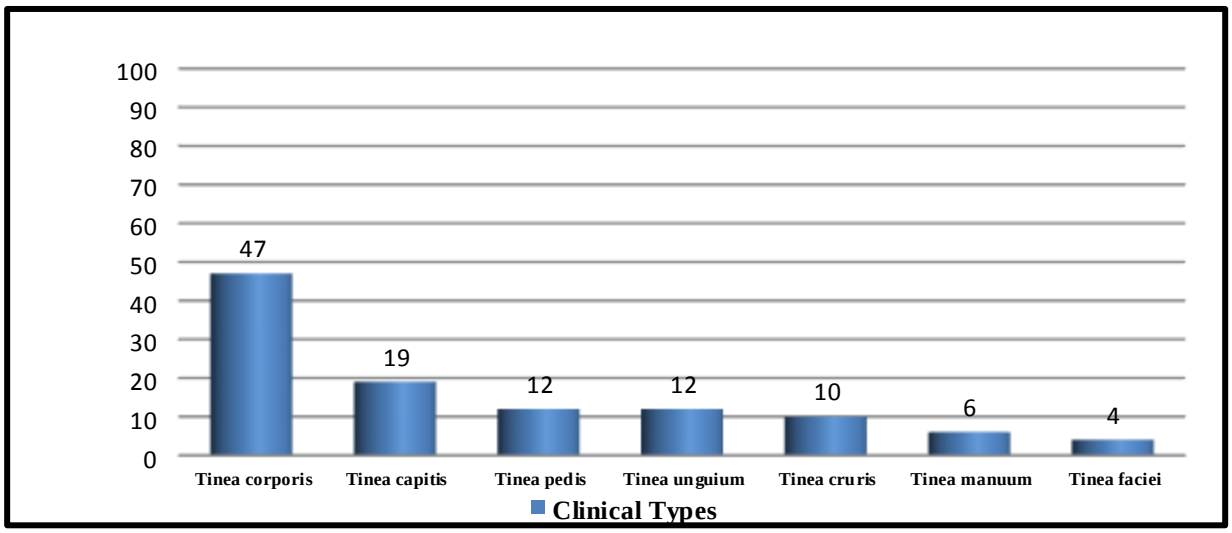

Figure (1): Clinical types of Dermatophytosis and number of cases.

Table (2). Dermatophytes species isolated from different clinical cases of dermatophytes infection.

Fungal species / Clinical cases	*M. canis*	*M. gypseum*	*M. fulvum*	*T. tonsurans*	*T. verrucosum*	Total
Tinea corporis	**21**	**3**	**2**	**13**	**8**	**47**
Tinea capitis	**3**	**2**	**0**	**6**	**8**	**19**
Tinea pedis	**5**	**2**	**1**	**4**	**0**	**12**
Tinea cruris	**3**	**0**	**0**	**7**	**0**	**10**
Tinea unguium	**11**	**1**	**0**	**0**	**0**	**12**
Tinea manuum	**2**	**1**	**0**	**3**	**0**	**6**
Tinea faciei	**0**	**0**	**0**	**3**	**1**	**4**
Total	**45 (40.91%)**	**9 (8.18%)**	**3 (2.73%)**	**36 (32.73%)**	**17 (15.45%)**	**110 (100%)**

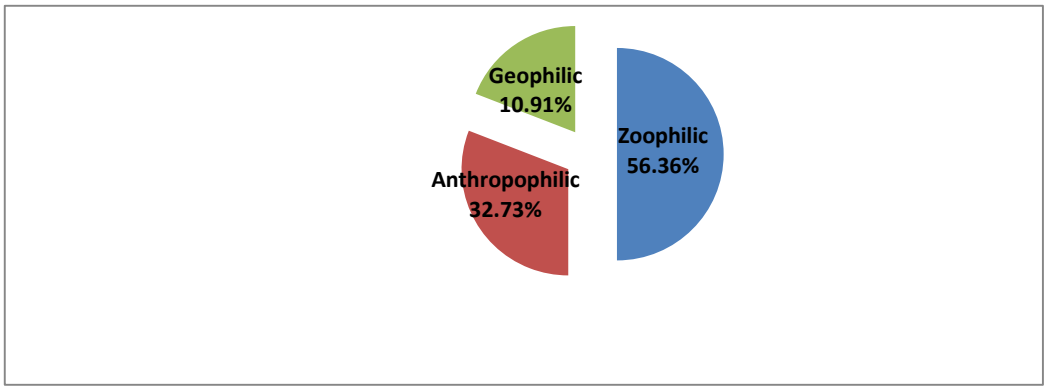

Figure (2) : Types of infection with dermatophytes according to their source.

Table (3). Relationship between dermatophytes infection with residency of patients.

Residency / Clinical cases	Urban	Rural	Total
Tinea corporis	**39 (35.46%)**	**8 (7.27%)**	**47 (42.73%)**
Tinea manuum	**4 (3.64%)**	**2 (1.82%)**	**6 (5.45%)**
Tinea capitis	**17 (15.45%)**	**2 (1.82%)**	**19 (17.27%)**
Tinea cruris	**9 (8.18%)**	**1 (0.91%)**	**10 (9.1%)**
Tinea pedis	**11 (10%)**	**1 (0.91%)**	**12 (10.91%)**
Tinea unguium	**10 (9.09%)**	**2 (1.82%)**	**12 (10.91%)**
Tinea faciei	**4 (3.63%)**	**0 (0%)**	**4 (3.63%)**
Total	**95 (86.36%)**	**15 (13.64%)**	**110 (100%)**

Dermatophytes infection according to gender: The relationship between dermatophytes infection and gender in the present study was shown in table (4). The results indicated that there was a statistically significant association between dermatophytes infection and gender of the patients, whereas the dermatophytes infection was higher in females with 74 (67.27 %) cases, in comparison to males with 36 (32.73 %) cases. Tinea corporis, tinea pedis and tinea unguium recorded higher infection rates in females than males with statistically differences. On the other hand tinea capitis, tinea cruris, tinea faciei displayed higher infections in males than females, while tinea manuum was equal in both females and males (Table 4). These results were in agreement with [7,11,12,53] who found the incidence of dermatophytosis in females were more than males. On the other hand, the results disagreed with [6,19,26,33,39,45,48]. They found that the incidence of dermatophytosis in males was higher than females. The incidence of dermatophytosis in females more than males may explain because of females dealing with water in their home works, so they may be more exposed to humidity which favors by dermatophytes. Other reasons such as life style, physiological differences between male and female and the differences in the social behavior or vocational had an important role in the incidence of dermatophytosis [27].

Table (4). Distribution of patients with dermatophytosis according to gender

Gender / Clinical cases	Male (%)	Female (%)	Total (%)
Tinea corporis	9 (8.18%)	38 (34.55%)	**47 (42.73%)**
Tinea capitis	**12 (10.91%)**	**7 (6.36%)**	**19 (17.27%)**
Tinea manuum	**3 (2.73%)**	**3 (2.72%)**	**6 (5.45%)**
Tinea pedis	**0 (0%)**	**12 (10.91%)**	**12 (10.91%)**
Tinea cruris	**7 (6.36%)**	**3 (2.73%)**	**10 (9.1%)**
Tinea unguium	**2 (1.82%)**	**10 (9.09%)**	**12 (10.91%)**
Tinea faciei	**4 (3.63%)**	**0 (0%)**	**4 (3.63%)**
Total	**36 (32.73%)**	**74 (67.27%)**	**110 (100%)**

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