RESEARCH ARTICLE

DOI: 10.5935/0946-5448.2024.S1.14

International Tinnitus Journal. 2024;S1:80-86

Epidemiological study of dermatophytes spread in Anbar Governorate

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ABSTRACT

The current study was conducted during the period from October 2022 until March 2023, based on 80 specimens collected from people infected with dermatophytes and clinically diagnosed by dermatologists at Ramadi Teaching Hospital, Fallujah Teaching Hospital, and private outpatient clinics. Information about the patients was enlisted in a questionnaire form. Laboratory studies were conducted for these samples, which included direct microscopic examination using potassium hydroxide (KOH) to detect the presence of dermatophytes initially and then culturing them in Sabouraud Dextrose Agar (SDA) media for diagnosis, relying on the traditional methods. Results obtained from the study showed that there was a significant increase ($P \leq 0.05$) in the infection percentage for males (62.5%), compared to the percentage for females (37.5%).

The study also revealed, through routine examination of direct examination and laboratory culture on SDA medium containing the antibacterial chloramphenicol and the opportunistic antifungal ammonium hydroxide at a concentration of 30% instead of cycloheximide that 68 specimens, at a percentage of 85%, showed a positive result from the laboratory culture, and 12 specimens, or 15%, exhibited a negative result. Direct microscopic examination showed that the positive specimens were 77, at a percentage of 96.25%, and the number of negative specimens was three, at a percentage of 3.75%.

Six types of clinical patterns of Tinea were isolated: Tinea corporis, which had the highest percentage of infection among other clinical cases, reaching 47.5%; Tinea manuum came second and represented 20%.

Keywords: Dermatophytes, Tinea, Disease in humans.

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Paper submitted on February 05, 2024; and Accepted on February 19, 2024

INTRODUCTION

Dermatophytosis is a common disease in humans; even though it is not life-threatening, it becomes a significant public health problem due to its high infection rates and unaesthetic effects damage[1]. Its prevalence has reached about 25% of the world's population. Dermatophytosis diseases are caused by filamentous fungi that cause surface infections and can invade human and animal keratinized tissues (skin, hair, and nails)[2]. Many studies have confirmed that dermatophytes can infect humans of all ages and spread in areas with humid and warm climates, such as Iraq and Iran. They are also widespread in remote and crowded areas[3]. Infection with filamentous dermatophytes can be transmitted from one person to another in several ways. Dermatophytes can be classified into three categories based on their primary host: anthropophilic dermatophytes, which infect humans; zoophilic dermatophytes, which infect animals and can be transmitted to humans through contact with animals; and geophilic dermatophytes, which live in soil and can infect humans through contact with contaminated soil. Dermatophytes can also be transmitted indirectly through the use of personal items such as hairbrushes and the clothes of infected people[4]. Filamentous dermatophytes cause superficial infections called ringworms because they cause infections in the form of ring spots with inflammatory, scaly edges, and redness and blisters can also be observed[5]. Its common name is Tinea, which is used according to its location on the body, such as Ringworm infects body (Tinea corporis), Ringworm infects head (Tinea capitis), Ringworm infects groin (Tinea cruris), Ringworm infects hand (Tinea mnum), Ringworm infects foot (Tinea pedis), Ringworm infects nails (Tinea unguium), and Ringworm infects face (Tinea faciei)[6].

The objective of the study was to conduct an epidemiological investigation of infections with dermatophytes isolated from patients attending Fallujah Teaching Hospital, Ramadi Teaching Hospital, and some private clinics by studying the infection percentage with some ringworms and their prevalence among both genders.

MATERIALS & METHODS

A sample of 80 people with Tinea of both sexes and all ages was collected by taking crusts from the injury area that a dermatologist clinically diagnosed in a dermatology consultant at the Fallujah Teaching Hospital, Ramadi Teaching Hospital, and outpatient clinics for the period from October 2022 to March 2023. A questionnaire form was prepared that included information about the infected person (name, age, gender, residence, sampling date, accompanying symptoms, type of injury, and sampling site). The skin crust samples were taken using a sterilized glass slide after sterilizing the affected area with 70% alcohol, and a part of the skin crusts taken from the infected person was placed directly on a clean glass slide for the direct microscopic examination with KOH at the concentration of 10%. The other part was placed in sterile bags and brought to the Microbiology Laboratory, Department of Biology / College of Education for Pure Sciences/ University of Anbar for examination and cultivation.

Fungal Specimens examination

Specimen Direct Microscopic Examination: Having the specimens transferred to the laboratory, the skin crusts were placed on a sterilized glass slide, and drops of potassium hydroxide at a concentration of 10% were added. Then, they were covered with a slide cover and heated at 30 degrees Celsius to dissolve the keratins. Next, the slide was left for 5-10 minutes, gently pressed, and examined under the microscope using power magnification x10 and then x40[7].

Culturing specimens: The specimens were directly cultured in SDA media containing 250 mg/L of chloramphenicol to prevent bacterial growth, as well as ammonium hydroxide NH_4OH at a concentration of 30% was added to the culture medium instead of cycloheximide to prevent opportunistic fungi[8], where 4-6 drops of NH_4OH at a concentration of 30% were placed in 250 ml of culture media and gently stirred to mix with the solution, then poured into sterilized dishes, after that, the samples were cultured and the dishes were incubated at 25-28°C for at least 1-4 weeks prior to considering negative. The dishes were constantly checked every 2–3 days of incubation for growth observation[9].

Statistical analysis: The results obtained in this study were analyzed relying on the Student (t) test at a significance level of $P \leq 0.05$.

RESULTS & DISCUSSION

The study demonstrates that the percentage of males suffering from skin fungi infection was higher than that of females, as the percentage of males reached 62.5% while the percentage of females reached 37.5%. The reason behind the percentage superiority of dermatophytes infection in males to the percentage of females may be due to the females' reluctance to go to see male doctors due to their shyness and prefer to see female doctors due to religious and social traditions[10]. These percentages were consistent with the study conducted by[11] in Basra, in which the infection percentage of males and females was 52.6% and 47.4%, respectively. They were also consistent with the study conducted by[12], in which the percentage of infected males (56.5%) was superior to that of the females (43.3%); however, the study differed from that conducted[8] at the University of Misrata in Libya as the percentage of females reaching 53.2%, outperforming the percentage of males which reached 46.8%, as well as the study conducted by[13] at the University of Karbala, who found that the percentage of females was 57.14%, and the percentage of males was 42.85%.

During the routine direct examination and examination of crusts cultured on SDA media containing the antibacterial

chloramphenicol and the opportunistic antifungal ammonium hydroxide at a concentration of 30% instead of cyclohexamide, it was found through laboratory culture that 68 specimens out of 80 specimens (85%) were positive and 12 specimens (15%) were negative Figure 1. Through the direct examination, the positive specimens were 77 samples (96.25%), while the negative samples were three samples (3.75%) Figure 2. The number of positive specimens at the direct examination and positive at the culture was 67 (83.75%). The number of specimens that were positive at the direct examination and negative at the laboratory culture was ten samples (12.5%), while the number of specimens that were negative at the direct examination and also negative at the culture was 2 (2.5%), and only one specimen was negative at the direct examination and positive at the laboratory culture, constituting a percentage of 1.25%. These results were also consistent with the study of[14], where 85% of the specimens were positive for laboratory culture, and 15% of them were negative. The results were also consistent with the study of[15] related to dermatophytes; where a percentage of positive specimens were 100% for direct microscopic examination and 66.7% for laboratory culture. Although detecting dermatophytes by direct examination with potassium hydroxide is considered a traditional technique, it is still used globally, achieving straightforward and rapid results for detecting filamentous fungal skin infections[16]. These differences in percentage between studies of dermatophytes may be due to the clinical case type, laboratory conditions, climatic factors, the culture media suitability for fungal growth, the experience of the doctor who diagnosed the disease, and the number of patients seen[17]. The negative result of the direct examination or laboratory culture may be due to the medications and antifungals the patient took that affected the fungi viability and their failure to grow on the culture media, or the sample size may be small, or the not taken correctly from the edges of the infection area but from the center of the infection that has acquired local immunity[18]. Also, keeping the specimens in contaminated or damp containers or bags can damage specimens, resulting in negative culture results[19].

Clinical Patterns of Tinea: Six clinical types of tinea corporis were isolated, with the highest percentage of infection with skin fungi among other clinical cases, recording 47.5%. The higher percentage of tinea corporis than others may be because it can occur in many parts of the body, including the trunk, shoulders, neck, and upper and lower limbs, except for the head, palms, and

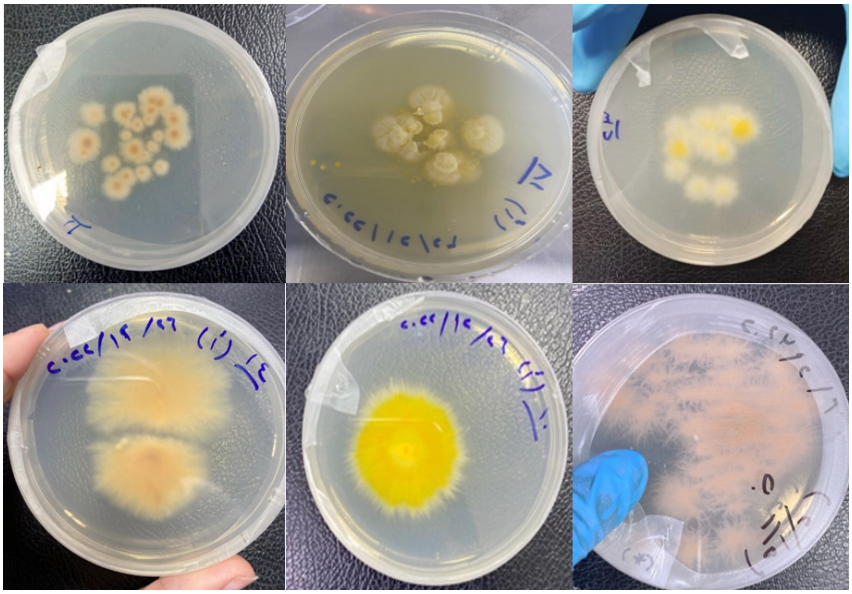

Figure 1: Result of laboratory culturing in Sabouraud Dextrose Agar (SDA) media.

Figure 2: Image of the Fungal Hyphae examined directly under the microscope.

feet. This result was consistent with a study conducted by[20] in Karbala, where the incidence of tinea corporis infection was 43.07%. It was also consistent with a study conducted in India by[21], where the infection percentage of tinea corporis was 50.39%, as well as it was consistent with a study conducted in Babylon, where the infection percentage was 52.89%, which ranked first among other tinea infections[22]. However, the recorded percentage was not consistent with the study conducted by[23], where the first rank for infection with dermatophytes was tinea unguium, reaching 31.57%, nore with the study of[11], in which the percentage of tinea corporis and tinea capitis was equal with a percentage exhibiting 46.62%. Tinea mnum came in second rank, recording 20% which may be due to the frequent use of hands in daily chores and their constant contact with detergents and materials that could irritate the skin. Direct contact with pets can also cause dermatophyte infections. This result was consistent with the study of[24] conducted in Tikrit, where tinea mnum ranked second with a percentage of 22.2% after tinea corporis of 25%, which was at the first rank. Tinea cruris came at the third rank, recording 12.5%, which was consistent with the study of[25] conducted in the center of Maysan Governorate, in which the rate of infection with tinea cruris ranked third, at a percentage of 17.4%. It was also close to what[26] obtained, reporting that the infection percentage with tinea cruris was 12%. The skin infection with ringworm of the foot (Tinea pedis) ranked fourth with a percentage of 8.75%, similar to a study conducted[27], which recorded an infection percentage of 8.1%. In fifth place, tinea capitis came with a percentage of 7.5%, which did not go in line with the studies of[28], which recorded the highest percentage of dermatophyte infections with tinea capitis, which was 48.1%. This variance suggests that tinea capitis is more prevalent in developing and tropical countries, while developed countries have high rates of tinea pedis[29]. The sixth and last rank was for tinea faciei, recording a percentage of 3.75%. This result was consistent with a study conducted in India, where tinea faciei recorded the lowest percentage among other clinical conditions (2.6%)[30].

Relationship between clinical patterns and patients' gender: Results of the current study showed the distribution of clinical cases of dermatophyte infection between males and females, as tinea corporis formed a greater percentage in males, amounting to 31.25%. In comparison, its percentage in females was 16.25%. The highest percentage of infection was tinea cruris, tinea mnum, and tinea pedis were also in males, with percentages reaching 8.75%, 16.25%, and 6.25%, respectively, and their percentages in females were 3.75%, 3.75%, and 2.5%, respectively Table 1. The results demonstrated that the highest infection percentage with dermatophytes in males and females was tinea corporis (31.25% and 16.25%, respectively). Tinea capitis and tinea faciei did not record any infection in the male, while they formed 7.5 and 3.75 % in the female, respectively; on the other hand, the lowest ringworm infection in the female was with tinea pedis by 2.5%, while the tinea capitis and tinea faciei recorded in the female 7.5 and 3.5%, respectively. The cause of males being infected with this ringworm (Tinea corporis) more than the female may be hard physical work, wearing tight clothes, and working under hot and humid environmental conditions, resulting in excessive sweating and hence providing a suitable environment for infection with the fungus. This study was consistent with the epidemiological survey study conducted in 2016 in Japan, where the cases of tinea corporis in males were 423 cases and in females were 241 cases in females[31]. This study was also consistent with[32], as the infection with tinea corporis, tinea cruris, tinea mnum, and tinea were recorded higher in males than in females since the number of cases reached 349, 78, 91, and 69 cases, in males and 232, 0, 61, and 44 cases in females, respectively, with an exception for the tinea faciei as the number in females was 183 cases, which is higher than the number of cases in males, which reached 137 cases. It is also consistent with a study conducted[28] in Ethiopia, recording the incidence of tinea capitis and tinea faciei at percentages of 28.3% and 2.8%, respectively, in females, which were higher than in males, where the percentages recorded 19.8% and 1.6%, respectively.

Distribution of clinical cases according to the patients' residential area: The current study showed that the infection percentage of dermatophytes in rural areas is greater than that in the urban area, as in the countryside, it was 55%, while in the city, it was 45%. The infection percentage with tinea corporis in the countryside was 27.5%, while its percentage in the city was 20%. Regarding the percentage of infection with tinea cruris, it in the city was greater than its rate in the countryside, recording 8.75% and 3.75%, respectively. In contrast, tinea capitis and tinea faciei were only found in the countryside, with percentages of 7.5% and 3.75%, respectively. The percentage of infection with Tinea mnum in the countryside was greater than its rate in the

Table 1. Numbers and percentages of clinical cases according to patient gender.

Clinical cases	Total number (%)	Number of males (%)	Number of females (%)
Tinea corporis	38(47.5%)	25(31.25%)	13(16.25%)
Tinea cruris	10(12.5%)	7(8.75%)	3(3.75%)
Tinea capitis	6(7.5%)	-	6(7.5%)
Tinea faciei	3(3.75%)	-	3(3.75%)
Tinea mnum	16(20%)	13(16.25%)	3(3.75%)
Tinea pedis	7(8.75%)	5(6.25%)	2(2.5%)
Total	80(100%)	50(62.5%)	30(37.5%)

city, with percentages of 12.5% and 7.5%, respectively. All cases of tinea pedis infection were found in the city, with a percentage of 8.75% Table 2. The widespread spread of skin fungi within rural areas may be due to low standards of living and poor health conditions, and the cause behind infection with dermatophytes of ringworm in such areas is due to zoophilic and geophilic fungi. The infection can occur as a result of the transmission of dermatophytes through interaction between people and animals through contact with soil contaminated with skin crusts and hair falling from animals, as well as through soil containing spores of dermatophytes[33,34]. This result was consistent with the study conducted by[35] in Baghdad, which recorded the infection percentage in rural areas (66.8%) higher than that in urban areas, which amounted to 33.91%; nevertheless, it was not consistent with a study in Dhi Qar, which showed that the infection percentage with dermatophytes is more prevalent among people who live in urban areas (64.71%) than people who live in rural areas (35.29%)[2]. It also contradicted the study of[36], who recorded the highest infection percentage in urban areas, which amounted to 86.36%, while in rural areas, it was only 13.64%.

Distribution of clinical cases according to age categories: Dermatophytes can infect all age categories, young and old. However, the study showed that the most common age infected was 21-30 years of age, with a percentage of 35%, followed by the age of 11-20 years old, where the infection percentage was 17.5%. We notice in Table 3 that the age categories 1-10, 31-40, and 41-50 years were equal in the dermatophytes infection percentage of 10%, and in the category 51-60 years was 11.25%. The categories most susceptible to ringworm of tinea corporis, tinea cruris, and tinea mnum were people whose ages ranged from 21-30 years, with percentages of 21.5%, 8.75%, and 11.25%, respectively. The category most susceptible to tinea capitis infection was 1-10 years old, recording 3.75%, whereas the highest incidence of tinea faciei was in the 41-50 year category with a percentage of 2.5%, and for the tinea pedis, the highest percentage was 2.5%, recorded by the two categories 11-20 and 41-50 years old.

The results of this study were consistent with the study of[37], which recorded the highest dermatophytes infection percentage (36.98%) in people aged 21-30, and the group aged older than 60 years was the least age category infected with dermatophytes, at a percentage of 6.25%. In a study by[38], it was reported that the higher percentage of infection with dermatophytes among young people than in old ones is due to physical activity and continuous and increased sweating in the young category. He[39] mentioned that the lack of infection in people older than 60 may be due to the thickness of the skin layer and the increase in sebaceous secretions. This result was consistent with the study[40], which recorded that the highest number of tinea corporis cases was in the 21-30 and 31-40-year-old categories, reaching 6 cases for each and the highest number of cases of tinea cruris amounted to 8 cases in the 21-30 age categories. The results of this study also agreed with those of[41], who recorded the highest tinea capitis percentage was in patients under the age of 14 years, reaching 36.16%, which is due to the lack of natural protective fatty acids secreted in the children scalp.

Distribution of clinical cases according to the presence or absence of chronic diseases: The study showed that chronic diseases did not affect infection with dermatophytes since the infection percentage in people who suffer from chronic diseases such as hypertension, diabetes, and others was 15%. In comparison, it was 68% in the absence of chronic diseases. The infection percentage with all ringworms in the absence of the

Table 2. Distribution of clinical cases according to the patients' residential area.

No	Clinical case	Countryside (%)	City (%)	Total number (%)
1	Tinea corporis	(%27.5) 22	(%20) 16	(%47.5) 38
2	Tinea cruris	(%3.75) 3	(%8.75) 7	(%12.5) 10
3	Tinea capitis	(%7.5) 6	-	(%7.5) 6
4	Tinea faciei	(%3.75) 3	-	(%3.75) 3
5	Tinea mnum	(%12.5) 10	(%7.5) 6	(%20) 16
6	Tinea pedis	-	(%8.75) 7	(%8.75) 7
	Total	(%55) 44	(%45) 36	(%100) 80

Table 3. Distribution of clinical cases according to patients' age.

No	clinical case	10-1 (%)	20-11 (%)	30-21 (%)	40-31 (%)	50-41 (%)	60-51 (%)	<60 %	Total number (%)
1	Tinea corporis	-	(11.25)9	(12.5)10	(3.75)3	(7.5)6	(8.75)7	(3.75)3	(47.5)38
2	Tinea cruris	(1.25)1	-	(8.75)7	(2.5)2	-	-	-	(12.5)10
3	Tinea capitis	(3.75)3	(2.5)2	-	(1.25)1	-	-	-	(7.5)6
4	Tinea faciei	-	-	(1.25)1	-	(2.5)2	-	-	(3.75)3
5	Tinea mnum	(3.75)3	(1.25)1	(11.25)9	(1.25)1			(2.5)2	(20)16
6	Tinea pedis	(1.25)1	(2.5)2	(1.25)1	(1.25)1	-	(2.5)2	-	(8.75)7
	Total	(10) 8	14 (17.5)	(35) 28	(10) 8	(10) 8	(11.25)9	(6.25)5	(100) 80

Table 4. Distribution of clinical cases according to the presence or absence of chronic diseases.

No	Clinical case	Chronic diseases presence (%)	Chronic diseases absence (%)	Total number (%)
1	Tinea corporis	**(%7.5) 6**	**(%40) 32**	**(%47.5) 38**
2	Tinea cruris	**(%2.5) 2**	**(%10) 8**	**(%12.5) 10**
3	Tinea capitis	**-**	**(%7.59) 6**	**(%7.5) 6**
4	Tinea faciei	**(%1.25) 1**	**(%2.5) 2**	**(%3.75) 3**
5	Tinea mnum	**(%3.75) 3**	**(%16.25) 13**	**(%20) 16**
6	Tinea pedis	**-**	**(%8.75) 7**	**(%8.75) 7**
	Total	**(%15) 12**	**(%85) 68**	**(%100) 80**

chronic disease was higher than its percentage in the presence of the chronic disease and the percentages of tinea corporis, tinea cruris, tinea capitis, tinea faciei, tinea mnum, and tinea pedis when there were no chronic diseases were 40%, 10%, 7.59%, 2.5%, 16.25%, and 8.75%, respectively. In contrast, their percentages in the presence of chronic diseases were 7.5%, 2.5%, 0%, 1.25%, 3.75%, and 0%, respectively Table 4. Tinea corporis had the highest infection incidence in people who suffer from chronic diseases, including diabetes and hypertension, at a percentage of 7.5%. This result was consistent with a study[42], which recorded an infection percentage of 14.65% with dermatophytes for people suffering from chronic diseases, which indicates that dermatophytes can infect all healthy patients' people who suffer from weak immunity.

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