ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-6 | Issue-6 | Nov-Dec -2024 | **DOI: https://doi.org/10.36346/sarjbab.2024.v06i06.001**

Original Research Article

Identification of Some Fungi Causing Skin Infection and its Relationship with Some Blood Test of Tinea Patients in Tikrit City

Mokhalad Sabah Khalaf Al-Doori[1*]

[1]Ministry of Education, Salah al-Din Education Directorate, Al-Dour Education Department Iraq

***Corresponding Author:** Mokhalad Sabah Khalaf Al-Doori
Ministry of Education, Salah al-Din Education Directorate, Al-Dour Education Department Iraq

Article History
Received: 26.09.2024
Accepted: 01.11.2024
Published: 05.11.2024

Abstract: Dermatophytes infection is a common problem worldwide and frequent in Iraq. Several reports and articles were published on prevalence, distribution, causes and treatment of dermatophytosis. The current study included the collection of 100 clinical samples (70 skin peels, 20 hair samples and 10 nail samples), as well as 50 blood samples from patients with dermatophytes and skin consultants in Tikrit City and 20 healthy blood samples for the period from November 2023 to March 2024. The results showed that the most common dermatophytes isolated was *Microsporum canis* and *T.rubrum* 10 (20 %) isolates from 50 total , followed by *M.gypseum*, 6(12%) and 5(10%) *T.mentagrophytes* and *T.verrucosum* and 4(8 %) isolates *E. floccosum* and *T.erinacei* and finally appeared 3(6 %) isolate *T.schoenleinii* and *T.concentricum*. The results of the statistical analysis showed non significant difference P <0.05 in the total number of white blood cells and monocyte (5.15 ± 2.636, 0.89±0.51) in patients with dermatophytes compared with the group Control mean± SD (5.73±1.821, ± 0.69 ± 1.591) respectively. Lymphocytes significant decreased (1.82 ± 1.301) in patients with dermatophytes compared to control group (4.60 ± 1.35) P≤0.01. Furthermore granlocytes increased (2.30±1.591) in patients with dermatophytes compared to control group (0.33± 0.403) P<0.01. The study concluded that perhaps the reduction of some of the body's WBCs parameters has an important role in the incidence of fungal infection in people infected with them when compared to healthy people.

Keywords: Dermatophytes, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton flocosum*. hematological parameters.

INTRODUCTION

Dermatophytes are a collection of closely related filamentous fungus that are keratinophilic and keratinolytic. They are generally taxonomically classified under the Trichophyton genus, and less frequently under the Microsporum or Epidermophyton genera [1]. A category of closely related fungus known as dermatophytes have the capacity to inhabit tissues that have been keratinised. These organisms are classified based on the characteristics they exhibit when cultivated on an appropriate agar medium. The group of morphologically and physiologically related moulds known as dermatophytes are responsible for causing clearly characterised illnesses in vertebrates. Onset of dermatophytoses has risen in recent years, especially in patients with weakened immune systems [2]. Fungal infections primarily target the skin, nails, and hair, where keratin serves as the primary structural protein, resulting in a diverse range of symptoms. Dermatophytes are a group of fungi that specifically penetrate keratinised tissues such as hair, skin, nails, and mucous membranes. The classification of dermatologophytes as anthropophilic, zoophilic, or geophilic is based on their typical habitat being either on humans, animals, or in the soil [4]. Initially, tinea progresses by the fungus attaching to the skin, where it undergoes germination and infiltrates the skin barrier. Subsequently, it produces and secretes Keratinases and other essential enzymes to disrupt the outermost layer of the skin [5]. Upon colonisation of the skin, dermatophytes induce keratinocytes to synthesise many cytokines that facilitate the inflammatory response and the buildup of leukocytes, mainly neutrophils, in affected tissues [6]. Enhancing cellular immunity, the Th1 and Th17 cells make a substantial contribution to the control ofcutaneous infection. Th17 cells both promote the entry of Neutrophils into the injury site and stimulate the activation of epithelial cells to produce chemicals such as Chemotactic and antibacterial peptides. Various strategies may be employed by the host

to eliminate fungal infections. These methods involve the recruitment of neutrophils and macrophages into cutaneous lesions through inflammation mediated by Th1 cells [7-9].

MATERIALS AND METHODS

Sample Collection

Clinical samples from ringworm patients of all ages and sexes were taken. Clinically diagnosed by Tikrit City Dermatology Consultant specialists from January to June 2024. It had 100 clinical samples (70 skin peels, 20 hair, 10 nails). Distribution and recording of patient information included gender, age, other diseases, area of infection, type of ringworm (and place of residence and presence of animals), number of family members, and physiological condition.

An ethyl alcohol concentration of 70% is employed for reducing bacterial contamination. Sterile blind blades were used to collect small scrapings from the borders of the ulcers, sterile flat forceps were used to get hair samples, and infected nails with subungual debris were sterilised with a scalpel, separated obliquely, and kept in SDACC into three partitions-. portion was injected directly into medium-containing tubes in sterile paper bags and portion was kept in nylon bags. The samples were taken to the Faculty of Science graduate studies laboratory, where the inoculation tubes were cultured under Petri dishes at 29°C. The remaining samples were refrigerated until dish preparation, ˚ 29-27 m. Maintain the same temperature and check for fungal growth everyday. Use microscopic and culture investigations to identify the disease-causing fungus.

Direct microscopic examination

After taking pathological samples (skin scales, nails, and hair), a drop of 15% potassium hydroxide solution was added to a glass slide, covered with a 40x10 cover, and left for five minutes until the keratin dissolved. The fungal structures were then examined microscopically under magnification.

Culturing of specimens

One part of the pathological samples (skin scales, nails and hair) was cultured on plates containing Saproid medium supplemented with Cycloheximide (Actidione) to inhibit the growth of saprophytic fungi and Chloramphenicol to inhibit the growth of bacteria for the purpose of isolating dermatophytes. The other part was cultured on plates containing Saproid dextrose agar medium supplemented with Chloramphenicol only for the purpose of isolating saprophytic fungi and yeasts causing dermatomycosis. Then the plates were incubated at a temperature of 27±2°C for 2-4 weeks and were continuously examined to observe the appearance of fungal growth [10].

Blood samples.

Collection of blood samples (50) blood samples were collected from the same patients with skin fungi and (20) blood samples from healthy subjects, where 5 ml of EDTA were withdrawn from venous blood using sterile medical syringes and 2 ml of it was placed in anticoagulant tubes. Emerald was transferred to the laboratory for blood cell count using a Tube device.

Statistical Analysis: The results were statistically analyzed using the Independent-Samples T-test, version 22.0 for the year (2009), using the P=test at a value of 0.05.

RESULTS

A total from 100 clinical samples with clinical diagnosed dermatophytes, only 60(**75%**) were positive culture for skin infection of origin 70 samples, while 13(**16.25%**) and 7(**8.75%**) positive culture for hair and nails respectively. As shown in Table (1).

Table 1: Total number of skin, hair and nail peel samples examined during the study

Samples (total)	Number of culture positive
Skin peels 70	60(75%)
Hair 20	13(16.25%)
Nails 10	7(8.75%)
100(100%)	80(100%)
P-value	<0.05

This study showed increase incidence of dermatophytes in male than female. Skin fungi were 35(58.3%) in male, while 25(41.7%) in female. Furthermore hair and nail fungi also increase in male than female that were 35(58.3%), 25(41.7%) in hair fungi respectively, 10(76.9%), 3(23.1%) in nail fungi, at p-value < 0.05. As shown in Table (2).

Table 2: Distribution dermatophytes according to sex

Samples (total)	Male	Female
Skin peels 60	35 (58.3%)	25 (41.7%)
Hair 13	10 (76.9%)	3 (23.1%)
Nails 7	5 (71.4%)	2 (28.6%)
80 (100%)	50 (62.5%)	30 (37.5%)
P-value	0.02	

The results showed that the most dermatophyte type isolated during the study were ***M.canis and T.rubrum*** that were **10(20%)** positive isolates, followed by 6(12 %) *M.gypseum*, 5(10 %) *T.mentagrophytes*, 4(8%) *T.verrucosum*, 4(8%) *E. floccosum*, 4(8 %) *T.erinacei* and finally appeared 3(6%) *T.schoenleinii* and *T.concentricum*. As shown in Table (3).

Table 3: Frequency of isolated dermatophyte species relative to the clinical patterns

Dermatophyte type	Total
M.canis	10(20 %)
T.rubrum	10(20 %)
T.mentagrophytes	5(10 %)
E. floccosum	4(8 %)
T.verrucosum	5(10 %)
M.gypseum	6(12 %)
T.schoenleinii	3(6 %)
T.erinacei	4(8 %)
T.concentricum	3(6 %)
Total	**50(100%)**

M.canis appeared elevated above the surface of the medium, with radial grooves, yellowish-white in color, and cottony, with aerial hyphae protruding upwards, as shown in Figure (1). Microscopic examination showed the presence of large conidia in abundance, with thick walls, and the absence of small conidia.

T.rubrum appeared white and cream color as shown in the Figure (2) and had a granular, downy shape, and from the base they were dark yellow in color, creamy in texture (smooth). Microscopic examination showed the presence of divided hyphae and the presence of large, club-shaped conidia divided by several septa ranging in number from (5-8) septa and small ones in large numbers and tear-shaped.

B- Microscopic image of the fungus under (40x magnification).

T.mentagrophyte Colonies of this fungus appeared growing on SDA medium after seven days of incubation and were characterized by being flat, white in color, domed in the center, and granular in texture, as shown in Figure (3). As for the bottom of the plate, they were yellowish-brown to reddish-brown in color. Microscopic examination showed the presence of a small number of large conidia that were club-shaped and rounded at the end and contained a number of septa. As for the small conidia, they were spherical and arranged in clusters along the fungal hyphae. The colony of this fungus is characterized by its cottony shape that turns into Clayton granular, and the colonies contain spiral-shaped fungal hyphae.

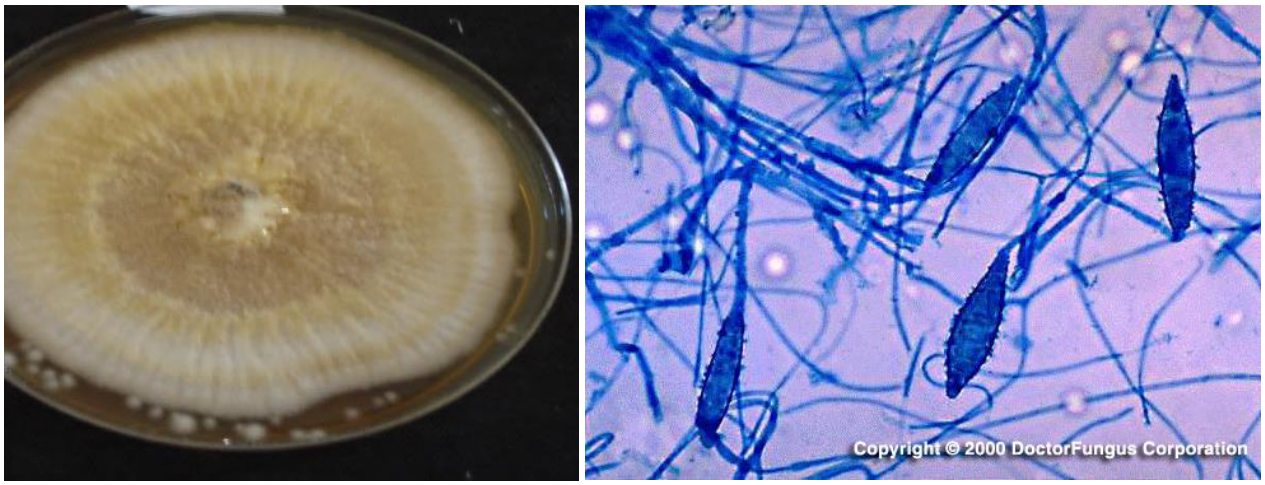

Fig. (1): *A- M.canis* at 27°C growing on medium SDA,B- Microscopic image of a fungus under (40x magnification)

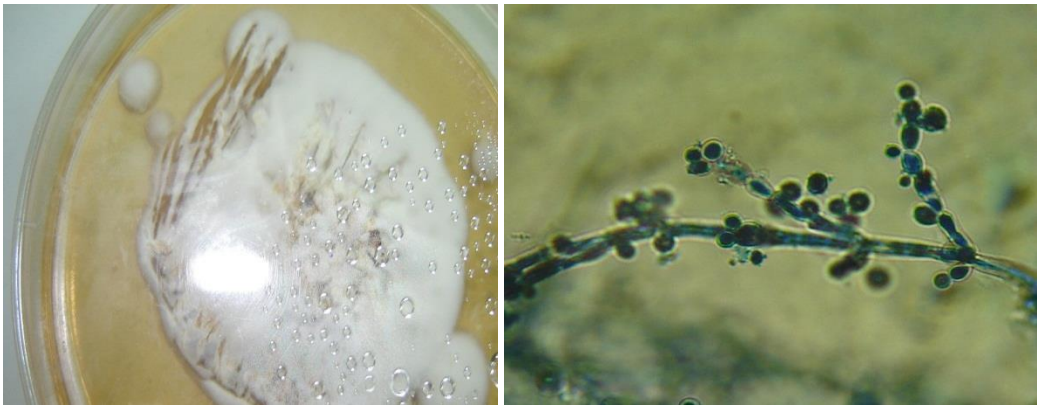

Figure (2): A- External appearance of the fungus T. rubrum incubated at 28°C

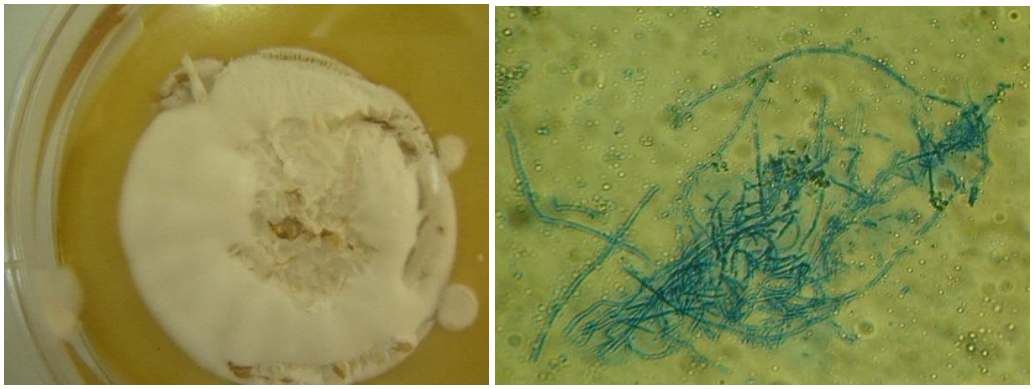

Figure (3): A- External appearance of the fungus T.mentagrophytes incubated at 28°C, B- Microscopic image of the fungus under (40x magnification)

E. floccosum characterized by being flat, white, and thin, and with the progression of growth they become velvety with a central fold and a cream color. The edge of the colony is ciliated, and from the base it appears yellow to reddish brown, as shown in Figure (4). Microscopic examination showed the presence of large conidia that are few, smooth, and thin-walled, and the number of cells is (3-4) with a change in size and shape. As for the small conidia, they are in the form of bundles connected to each other, slightly serrated and extended, or they are spherical and single-celled, carried on a conidial carrier, which is in the form of dense clusters.

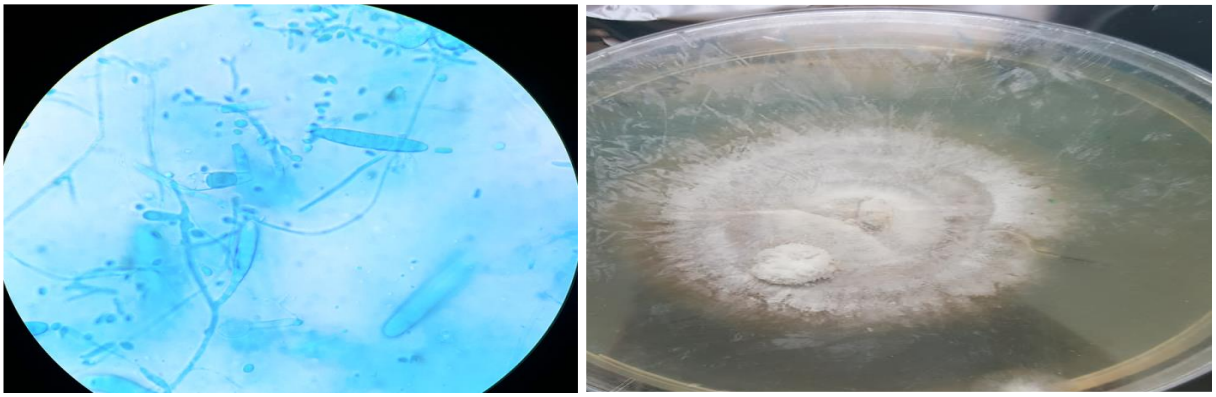

Figure (4): A- External appearance of the fungus E. floccosum incubated at 28°C, B- Microscopic image of the fungus under (40x)>

Blood Test

Complete Blood Cell (CBC)

The results of the statistical analysis showed non significant difference P <0.05 in the total number of white blood cells and monocyte (5.15 ± 2.636, **0.89**±0.51) in patients with dermatophytes compared with the control mean± SD (5.73±1.821, ± 0.69 ± 1.591) respectively. Lymphocytes significant decreased (1.82 ± 1.301) in patients with dermatophytes compared to control group (4.60 ± 1.35) P≤0.01. Furthermore granlocytes increased (2.30±1.591) in patients with dermatophytes compared to control group (0.33± 0.403) P<0.01. As shown in Table (4).

Table 4: The percentage of white blood cells in blood samples taken from infected people

Test	Groups	No.	Mean	SD	p-value
Total WBC (mm^3)	Control	10	5.73	1.821±	0.37
	Patients	50	5.15	2.636±	
LYM%	Control	10	4.60	1.35±	0.01
	Patients	50	1.82	1.301±	
GRA%	Control	10	0.33	0.403 ±	0.01
	Patients	50	2.30	1.591 ±	
MON%	Control	10	0.69	1.591 ±	0.21
	Patients	50	0.89	0.51 ±	

DISCUSSION

Dermatophytosis is a major public health problem in tropical and subtropical countries like Iraq, yet remains unresolved. The incidence is increasing due to wide spread use of corticosteroids and antifungal agents without appropriate microbiological investigations [11]. Therefore, it is essential that good laboratory methods are available for the rapid and precise identification of the dermatophytes involved in order to apply appropriate treatment and preventive measures [12].

The results of this study showed that the isolated dermatophytes belong to the three fungal genera Trichophyton, Microsporum, and Epidermatophyton. This result is consistent with [13], and does not agree with the result of [14], who isolated only the genus Trichophyton. This study showed ***T.rubrum*** and ***M.canis*** the most common fungal speices were isolated that agree with [15]. T. rubrum is more virulent to cause infection because it generates a low immune response with large numbers of mycelia and the pathogen possesses some lipid-degrading enzymes including phospholipase and its ability to produce lipid-degrading toxins, all of which work with the fungus to suppress the host's cellular immunity [16]. It is followed by the fungal species T. mentagrophytes with (17) isolates and a percentage of (25.4%), which is a zoophilic fungus and causes ringworm of the nails, body and feet and is responsible for 10% of the total number of fungal skin infections [17].

The result of this study showed increase incidence in male than female, that agree with study done by [18] in Iraq. While disagree with [19, 20] that demonstrate increase incidence in female than male. The high rate of infection in males may be due to the nature of their work, which provides an environment suitable for infection, as most of them are workers and owners of difficult professions with a low level of health, which causes the transmission and occurrence of infection, or it may be due to the size of the sample studied and based on the patients who visited, as the number of male visitors was more than females because most females prefer to go to outpatient clinics, in addition to the customs and traditions of the society from which the sample was taken, as most females are reluctant to give the sample. However, the results of the current study are consistent with the results of many studies, including the study [21].

The statistical analysis of the results revealed no statistically significant disparity in the overall count of WBC between patients with cutaneous fungus and the control group. A modest reduction in the overall count of WBC may be attributed to the migration WBC to the specific location of skin fungus-induced damage in the layers of the outermost layer of the skin [5]. The chemical affinity of Chemotaxins is attributed to the enzymatic actions of skin fungus, which aid in the examination of the corneal layer of the skin and the extent of damage [4]. This study observed a notable reduction in lymphocytes and a substantial rise in granocytes, however non significant differences in monocyte. Neutrophils play a crucial role in the initial defence against cutaneous fungus by activating the immune response and releasing essential interleukins and immunological molecules like IL-1β, TNFα and IL-8 [22,23]. This study demonstrated that inflammation induced by Th1 triggers the recruitment of WBC, namely neutrophils and macrophages, to the affected skin caused by fungal infection [24]. The increase in granulocytes (neutrophils) and, to a lesser extent, monocytes, at the expense of other types of WBC, particularly lymphocytes, may result in a decrease in skin lesions [25].

CONCLUSION

Males were more susceptible to Skin peels, Hair and Nails compared to female. Aswell as *Microsporum canis* and *T.rubrum* are the most fugus isolated from patients. While the results of the current study showed an increase in the percentage of granulocytes and monocytes and a decrease in the percentage of cells, which indicates that skin infections.

REFERENCES

1. Aditya K, G., Jennifer E, R., Melody, C., & Elizabeth A, C. (2005). Dermatophytosis: the management of fungal infections. *SKINmed: Dermatology for the Clinician*, *4*(5), 305-310.
2. Weinstein, A., & Berman, B. (2002). Topical treatment of common superficial tinea infections. *American family physician*, *65*(10), 2095-2103.

3. Hasan, A. M. (2015). Isolation of dermatophytes species from patients with different types of leukemia in Baghdad Governorate. *Iraqi Journal of Biotechnology*, *14*(2), 122-126.
4. Davies, R. R., & Zaini, F. (1984). Enzymic activities of Trichophyton rubrum and the chemotaxis of polymorphonuclear leucocytes. *Sabouraudia: Journal of Medical and Veterinary Mycology*, *22*(3), 235-241.
5. Brasch, J. (2009). Current knowledge of host response in human tinea. *Mycoses*, *52*(4), 304-312.
6. Martinez-Rossi, N. M., Peres, N. T., & Rossi, A. (2017). Pathogenesis of dermatophytosis: sensing the host tissue. *Mycopathologia*, *182*(1), 215-227. doi:10.1007/s11046-016-0057-9
7. Hau, C. S., Tada, Y., Kanda, N., & Watanabe, S. (2015). Immunoresponses in dermatomycoses. *The Journal of Dermatology*, *42*(3), 236-244. doi:10.1111/1346- 8138.12718
8. Heinen, M. P., Cambier, L., Fievez, L., & Mignon, B. (2017). Are Th17 cells playing a role in immunity to dermatophytosis?. *Mycopathologia*, *182*, 251-261. doi:10.1007/s11046-016-0093-5
9. Mignon, B., Tabart, J., Baldo, A., Mathy, A., Losson, B., & Vermout, S. (2008). Immunization and dermatophytes. *Current opinion in infectious diseases*, *21*(2), 134-140. doi:10.1097/ QCO.0b013e3282f55de6
10. Tilton, R.C. (1992). Fungi In: Clinical Laboratory medicine, by Tilton, R.C.; Balows, A.; Hohnadel, D.C. and Reiss, R.F., Mosby, PP. 727-762.
11. Anupama, A. (2017). Isolation and Identification of Dermatophytes from Clinical Samples—One Year Study. *Int. J. Curr. Microbiol. App. Sci*, *6*(11), 1276-1281.
12. Shalaby, M. F. M., El-Din, A. N., & El-Hamd, M. A. (2016). Isolation, identification, and in vitro antifungal susceptibility testing of dermatophytes from clinical samples at Sohag University Hospital in Egypt. *Electronic physician*, *8*(6), 2557.
13. AL-Mansour & Wafaa, Khalaf, Abood (2018). Isolation and Identification of Bateria and Fungi Caused Dermal Infection and Inhibitory Effect of some Medicinal plants., University of Samarra-college of education-department of biology, Thesis M.Sc Microbiology.
14. Cai, C. G., Lou, B. G., & Zheng, X. D. (2008). Keratinase production and keratin degradation by a mutant strain of Bacillus subtilis. *Journal of Zhejiang University Science B*, *9*(1), 60-67.
15. Hindy, N., & Abiess, A. A. (2019). Isolation and identification of dermatophytes causing Dermatophytosis in Hilla city, Iraq. *Indian Journal of Public Health Research & Development*, *10*(10), 2225-2230.
16. Al-Azawi, T. H. M. (2013). *Epidemiological and Molecular Study of Some Keratinophilic Fungi in Swimming Pools* (Doctoral dissertation, Council of the College of Science for Women, University of Baghdad).
17. Zhang, X., Wang, Y., Chi, W., Shi, Y., Chen, S., Lin, D., & Jin, Y. (2014). Metalloprotease genes of Trichophyton mentagrophytes are important for pathogenicity. *Medical mycology*, *52*(1), 36-45.
18. Mohammed, S. J., Noaimi, A. A., Sharquie, K. E., Karhoot, J. M., Jebur, M. S., Abood, J. R., & Al-Hamadani, A. (2015). A survey of dermatophytes isolated from Iraqi patients in Baghdad City. *Al-Qadisiyah Medical Journal*, *11*(19), 10-15.
19. Bander, K. I. (2012). Epidimological study of dermatophytes infection in Samarraa city. *Tikrit Journal of Pure Science*, *17*(1).
20. Al-Hmadani, A. H., Al-Dhalimi, M. A., & Alrufae, M. M. A. (2014). Epidemiologic study of dermatophytosis in Al-Najaf government. *Al-Kufa University Journal for Biology*, *6*(1).
21. Gnat, S., Łagowski, D., Nowakiewicz, A., & Dyląg, M. (2021). A global view on fungal infections in humans and animals: infections caused by dimorphic fungi and dermatophytoses. *Journal of Applied Microbiology*, *131*(6), 2688-2704.
22. Cambier, L., Mathy, A., Baldo, A., Bagut, E. T., Tabart, J., Antoine, N., & Mignon, B. (2013). Feline polymorphonuclear neutrophils produce pro-inflammatory cytokines following exposure to Microsporum canis. *Veterinary microbiology*, *162*(2-4), 800-805.
23. Wojcicka-Lorenowicz, K., Kostro, K., Lisiecka, U., & Gąsiorek, B. (2018). Phagocytic activity and oxygen metabolism of peripheral blood granulocytes from rabbits experimentally infected with. *Journal of Veterinary Research*, *62*(1), 43-48.
24. Vermout, S., Tabart, J., Baldo, A., Mathy, A., Losson, B., & Mignon, B. (2008). Pathogenesis of dermatophytosis. *Mycopathologia*, *166*, 267-275.
25. Waldman, A., Segal, R., Berdicevsky, I., & Gilhar, A. (2010). CD4+ and CD8+ T cells mediated direct cytotoxic effect against Trichophyton rubrum and Trichophyton mentagrophytes. *International journal of dermatology*, *49*(2), 149-157.