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Investigation of interleukin-8 from patients infected *Trichophyton rubrum*

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Abstract---The most prevalent form of chronic dermatophytoses causing pathogenic fungus in humans is *Trichophyton rubrum*. Keratinocytes, the main epidermal cells, are crucial in local immune interactions because they represent the first line of defense against microbial assaults, skin In response to varied stimuli, keratinocytes express a wide range of receptors and create host defense peptides, cytokines, and chemokines, this immunological study's objective was to use an ELISA to determine the function of inflammatory interleukins (IL-8) in *Trichophyton rubrum* patients. The study included 30 patients with verified *Trichophyton rubrum* and 10 individuals as a control group. Age, gender, the date of the surgery, the patient's occupation, their address, and the findings were all collected for every infected patient, the ELISA (Linked Immunosorbent Assay) technique was used to measure immune cells' cellular kinetics for secreting interleukin-8 were calculated And there was a significant increase in the level of significance for persons infected with interleukin-8 ($p > 0.05$), compared to control persons.

Keywords---*Trichophyton rubrum*, interleukin-8, Investigation, dermatophytoses.

1. Introduction

Dermatophytosis is a contagious skin condition that affects both humans and animals. According to(1), dermatophytes are classified into three groups based on how they interact with their environment: zoophilic, geophilic, and anthropophilic fungi, dermatophytoses is a more accurate name for a group of filamentous fungi that cause diseases like ringworm and tinea, the most frequent cause of fungal infections, affecting millions of individuals annually, is dermatophytes, there are three main genera that produce diseases that vary in location, severity, and

development: *Trichophyton*, *Microsporium*, and *Epidermatophyton* (2), it is also known as a keratinolytic fungus since some of it may infect tissues inside the body while others can breakdown keratin outside of a live body (in vitro) the body (3).

On the other hand, *T. rubrum* infection is uncommon and primarily affects people with impaired immune systems, treatment failure could be brought on by re-infection or poor adherence. *T. rubrum* infections can be tenacious, challenging to treat, and recur frequently after antifungal therapy is discontinued(4), in individuals with severe superficial dermatomycosis, cellular immune responses (CMI) against the causative culprit have been linked to infection clearance. However, in dermatophytes patients, macrophages (macrophages and neutrophils) are unable to deal with *T. rubrum*, the only obvious cause of pathogenesis; as a result, these patients are unable to display a CMI response(5), local tissue macrophages are likely assisting phagocytic cells in swallowing and/or limiting fungal development, producing an acute inflammatory response, and transferring fungal antigens to T cells, which can phagocytose fungal cells, present antigens, and release cytokines, chemokines, and other molecules. Innate immunity is required for the establishment of adaptive cell-mediated immune responses that limit the spread of mycolic infections and illness. Substances that cause an inflammatory response and control infection (6)(5), this study was conducted, which the aim to:

- 1 Isolation of the human skin disease-causing fungus *T. rubrum*
- 2 Research on the immunological response in *T. rubrum* dermatoses patients to an enzyme-linked immunosorbent assay (ELISA) test.

2. Materials and Methods

Patients who visited the medical clinic between October 2021 and February 2022 provided a total of (118) samples, using a sterilized sharp blade, samples of skin were scraped from the edge of the infected area, the damaged hair was cut for analysis, and the nails were retrieved from a sharp blade with fragments of strange shape and color, the study also included direct diagnosis and transplantation on plates. Transplantation of samples: Samples were added right away to the SDA medium, which also included cyclohexamide and antichloramphenicol(7), Planting samples on the potato dextrose agar (PDA) medium allowed researchers to identify *T. rubrum*(8).

Blood sample collection

Blood samples were taken from 50 healthy individuals and 80 of the same dermatophytes patients. 5mL of venous blood was collected using sterile medical syringes, deposited into a gel tube, and centrifuged for ten minutes at 4000 rpm to extract the serum, the serum was then split into sterile eppendorf tubes and stored at a temperature of -20°C until the immunological tests were finished.

ELISA Assay for *T. rubrum* (Protocol Procedure):

Buffer Wash 15 ml of the washing solution and 375 ml of distilled water should be added to the washer. Standards 2000 pg/ml of buffer should be used to dilute the sample in a standard vial.

Then, we create 7 tubes with 0.3 ml of sample dilution in each. buffer we add 0.3 ml of the standard, which has a concentration of 2000 pg/ml, to the first tube of the seven tubes and thoroughly mix it, we then add 0.3 ml of this first tube to the second tube of the seven tubes and thoroughly mix it, Finally, we take from tube 2 and add the tube whose concentration reached 500 pg/ml to the third tube, and for the seventh tube, we don't do anything with it until it's still blank, we combine 100 microliters of serum with 100 microliters of sample dilution buffer to produce a 1:2 dilution. Making a working solution for biotin-labeled antibodies, the amount of biotin-labeled antibody solution required for 40 samples, 8 standards, a black sample, and 36 samples is then determined, according to calculations made using patient finances, each well requires 100 liters of water, thus 4 ml are required, with an additional 4.5 ml added as a precaution, in other words, since the dilution ratio is 1:100, we dilute the samples and build the pallet by adding 4455 microns of the antibody dilution ratio to the 45 microns of the biotin detecting antibody.

Add 50 l of tomb substrate to each well after that, and then incubate for 10 to 20 minutes at 37 °C. Then fill each well with 50 l of L-stop solution. After one minute, shake quietly and start reading at 45(9).

***Trichophyton rubrum* is separated using psychological tests**

- * Baby hair should be used for the "hair perforation test," which involves placing it on a glass slide with a drop of lacto phenol and examining it under a microscope to observe how the fungus is attacking the hair (3).
- * Depending on the technique, the urease test was performed to assess the capacity of fungi to produce urea enzyme (1).
- * PCR assay-based diagnostic procedure (3).

Results and Discussion

Fungi were present. *T.rubrum* 30 produced positive samples, with 14 isolates from the skin, 7 from the nails, and 9 from the hair, accounting for less than half of the cutaneous skin fungus. Fungi were recognized using the following technique, a blood sample was taken from each person infected with the fungus *T.rubrum*, and the blood was separated from the serum with centrifuge from between October 2021 and February 2022, fungi produce large conidial with long, smooth, long-stemmed, thin-walled, and helical walls, as well as tiny round cones along the hypha in grape clusters. *Trichophyton* species complicated, which is comparable to the behavior of species in this species and the different genotypes may seem remarkably similar, is a problem with genetically diagnosing *Trichophyton* spp (3).

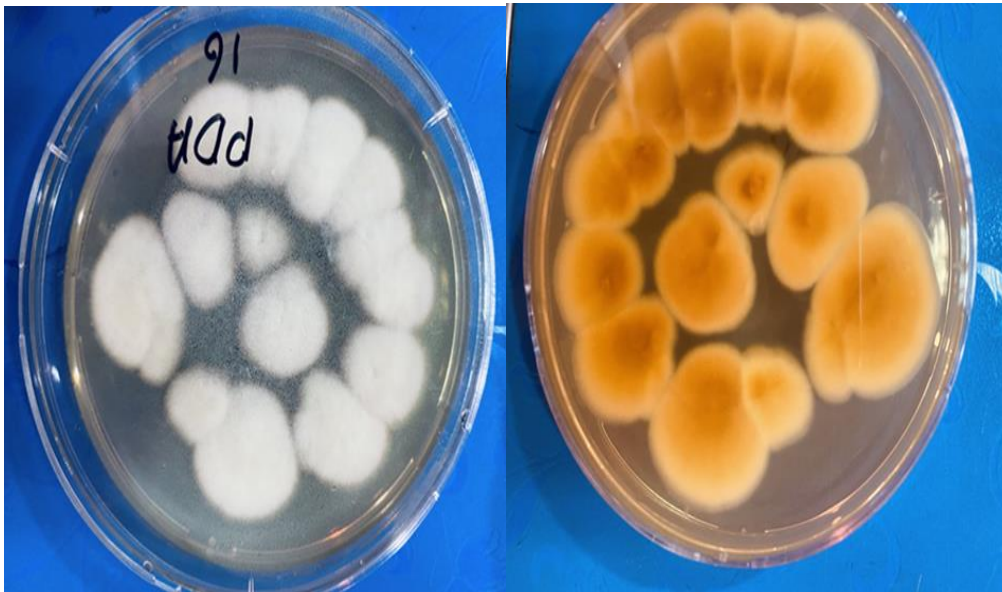


Figure 1. Shows the form of a *Trichophyton rubrum* colonies on P.D.A.C.C medium

The huge conidia were quite small and came in a variety of shapes, including pencil-shaped and cigar-shaped conidia, Microscopy revealed a significant number of small conidia that were either sitting or gripping a small bump on the fungal strings in a reciprocal fashion (Teardrop-shaped) or peg-shaped, as shown in the following figure (2), this result agree with(3).

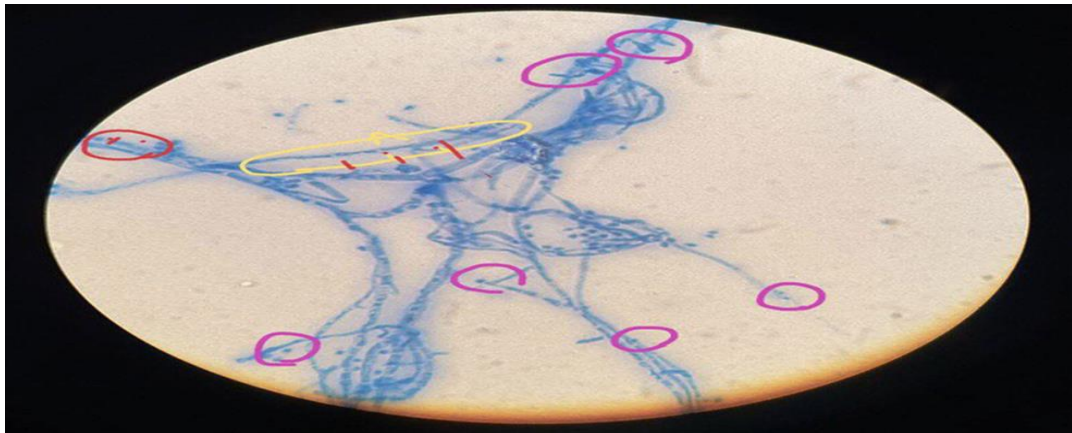
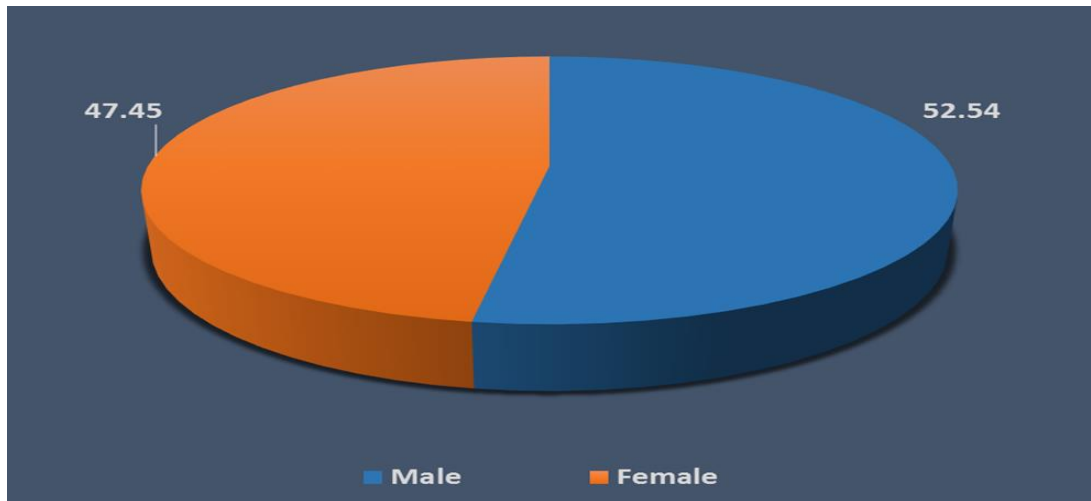


Figure (2) Pink markings Microconidia. It's more than the yellow macroconidia under *T.rubrum* slides

Dermatophytes Infection and gender

Studies have been done on individuals ranging in age from 5 months to 70 years, the infection rates of males and females with dermatophytes were discovered to be converging. It appeared that the male infection rate in the Al-Hussein teaching

hospital was 62 samples and 52.54 percent, while the female infection rate was 56 cases and 47.46 percent. These ratios revealed significant differences in infection between genera, as shown in Figure (3).



* significant differences on $P \leq 0.05$.

Figure (3) Dermatophytes Infection and gender.

This conclusion was in agreement with the findings of other researchers in Iraq, such as (10), and outside of Iraq, in South Africa (11). They discovered that men are more prone to contracting dermatophytes infections than women are. The divergence in findings between this study and others in Baquba (12), and outside of Iraq (13), might be linked to healthy habits and personal cleanliness, which is one of the most important factors in dermato disease in humans. A little increase in the incidence rate among males is due to the nature of their occupation, which offers a favorable environment for infection because the majority of them work low-level, heavy jobs, several other studies, including the findings of, concur, with it, as do the findings of the current study (13) (12).

Studied Samples IL-8 in Patients with *T.rubrum*.

Compared to the control group, where the highest value was in the infected patients with a concentration of 110 pg/ml, the current results in Figure (3) showed a significant increase in the concentration of interleukin-8 in the group of patients infected with the fungus *T.rubrum*. This result is consistent with the results (14).

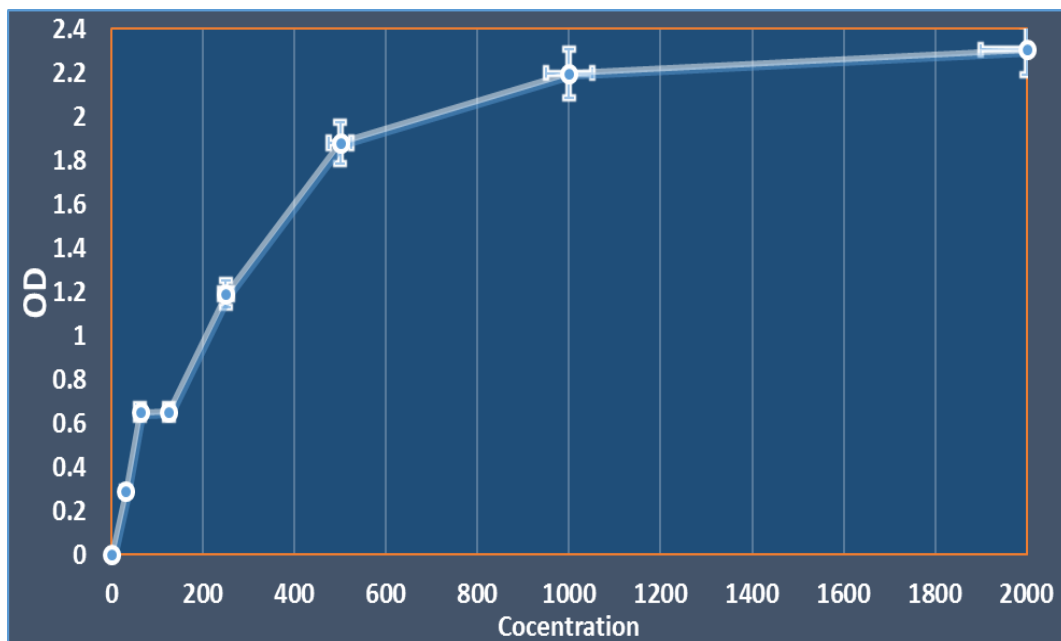


Fig (4) shows the distribution of the mean IL-8 level in controls and Patients infected with the fungus *T.rubrum* with a probability.

Table (5) shows the distribution of the mean IL-23 level in controls and patients with a probability less than 0.05.

Groups	Mean (%) \pm SD	Total
Control	10 (27.78%) \pm 1.336	36
Patients	26 (72.22%) \pm 9.241	
X^2	3.625	
P Value	0.00001	

*Error bars represents standard deviation on significant level 0.05

Neutrophils produce the protein L-8, also referred to as neutrophil chemotactic factor. It causes IL-8 stimulates chemotaxis, which stimulates phagocytosis. It has been demonstrated that IL-8 is a potent activator of angiogenesis, in target cells, IL-8 induces a variety of physiological processes that are necessary for migration and phagocytosis, including intracellular Ca^{2+} increases, exocytosis (such as the release of histamine), and the respiratory burst, as directs cells to go toward the infection site, especially neutrophils but also other granulocytes. Any cell containing toll-like receptors involved in the innate immune response will release IL-8, which has also been demonstrated to be a signature chemokine of $CR2^+$ naïve T cells, also referred to as recent thymic emigrants, in the keratinized tissues of human and animal skin and hair, dermatophytes are frequently seen. The physical and chemical makeup of the skin serves as a protective barrier

against fungus infections, the cutaneous immune system most likely starts the immune responses that function to stop and eliminate invasive pathogens(15).

Conclusions

- 1 Males were more likely than females to develop dermatophytoses, and patients aged 1 to 10 years had greater infection rates.
- 2 The results showed a significant increase in interleukin-8 levels in *T. rubrum* infected individuals at a meaningful threshold of 0.05, indicating that *T. rubrum* infection mostly affects those who are already immunocompromised (macrophages and neutrophils).

References

1. Pal M. Dermatophytosis in an adult cattle due to *Trichophyton verrucosum*. *Anim Husbandry, Dairy Vet Sci.* 2017;1(1).
2. Sachin K, Seema B. Clinico-mycological profiles of dermatophytosis in Jaipur, India. *African J Microbiol Res.* 2016;10(35):1477–82.
3. Alshibly MK, Al-marshedy AM. Molecular study of virulence factors influencing the pathogenicity of *Trichophyton rubrum*. 2019;(January).
4. de Silva NI, Maharachchikumbura SSN, Thambugala KM, Bhat DJ, Karunarathna SC, Tennakoon DS, et al. Morpho-molecular taxonomic studies reveal a high number of endophytic fungi from *Magnolia candolli* and *M. garrettii* in China and Thailand. *Mycosphere.* 2021;12(1):163–237.
5. Nielsen J, Kofod-Olsen E, Spaun E, Larsen CS, Christiansen M, Mogensen TH. A STAT1-gain-of-function mutation causing Th17 deficiency with chronic mucocutaneous candidiasis, psoriasiform hyperkeratosis and dermatophytosis. *BMJ Case Rep.* 2015;2015:1–6.
6. Campos MRM, Russo M, Gomes E, Almeida SR. Stimulation, inhibition and death of macrophages infected with *Trichophyton rubrum*. *Microbes Infect.* 2006;8(2):372–9.
7. Kannan P, Janaki C, Selvi G. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. *Indian J Med Microbiol [Internet].* 2006;24(3):212–5. Available from: [https://doi.org/10.1016/S0255-0857\(21\)02353-7](https://doi.org/10.1016/S0255-0857(21)02353-7)
8. Xu Y, Hall C, Wolf-Hall C, Manthey F. Fungistatic activity of flaxseed in potato dextrose agar and a fresh noodle system. Vol. 121, *International Journal of Food Microbiology.* 2008. p. 262–7.
9. Huang, X. Z., Liang, P. P., Ma, H., Yi, J. L., Yin, S. C., Chen, Z. R., ... & Chen, J. (2015).
10. Ali, T. H., Ali, N. H., & Mohamed, L. A. (2011). PRODUCTION, PURIFICATION AND SOME PROPERTIES OF EXTRACELLULAR KERATINASE FROM FEATHERS-DEGRADATION BY *ASPERGILLUS ORYZAE* NRRL-447. *Journal of Applied Sciences in Environmental Sanitation*, 6(2).
11. Falahati M, Fateh R, Nasiri A, Zaini F, Fattahi A, Farahyar S. Specific identification and antifungal susceptibility pattern of clinically important dermatophyte species isolated from patients with dermatophytosis in Tehran, Iran. Vol. 13, *Archives of Clinical Infectious Diseases.* 2018.
12. Khazali, H., Parhiskar, A., & Jafari, K. (2005). Effect of serotonin infusions on the mean plasma concentrations of growth hormone, thyroid hormones and

- the amount and constituents of milk in the Sanan goat. *African Journal of Biotechnology*, 4(12).
13. Farag, A. G., Hammam, M. A., Ibrahim, R. A., Mahfouz, R. Z., Elnaidany, N. F., Qutubuddin, M., & Tolba, R. R. (2018). Epidemiology of dermatophyte infections among school children in Menoufia Governorate, Egypt. *Mycoses*, 61(5), 321-325.
 14. Nouh, S. A., Benthami, K., Samy, R. M., & El-Hagg, A. A. (2020). Effect of gamma radiation on the structure and optical properties of polycarbonate-polybutylene terephthalate/silver nanocomposite films. *Chemical Physics Letters*, 741, 137123..
 15. Giri, M. K. W. (2019). Immunological side in overtraining exercise. *International Journal of Health & Medical Sciences*, 2(1), 1-6. <https://doi.org/10.31295/ijhms.v2n1.52>
 16. Hube B, Hay R, Brasch J, Veraldi S, Schaller M. Dermatmycoses and inflammation: The adaptive balance between growth, damage, and survival. *J Mycol Med* [Internet]. 2015;25(1):e44–58. Available from: <http://dx.doi.org/10.1016/j.mycmed.2014.11.002>.
 17. Suryasa, I. W., Rodríguez-Gómez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>