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Original Research Article

Incidence of *Hortaea werneckii* in a tertiary care centre in Maharashtra

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ABSTRACT

Background: *Hortaea werneckii*, a melanized yeast-like fungus is the cause of Tinea nigra, a superficial dermatomycosis. The lesions are differential diagnosis of melanocytic lesions. Tinea nigra mainly affects people in the temperate or subtropical climates with well-demarcated, expanding, hyperchromic plaques on the palms or soles.
Methods: Skin scrapings from various lesions with suspected fungal aetiology were received after clinical assessment and were subjected to laboratory procedures, including direct KOH (10%) analysis, culture on Sabouraud dextrose agar (SDA), and incubation at 25°C and 37°C for 28 days which was further confirmed by lactophenol cotton blue preparation and slide culture.
Results: Out of 295 samples examined, a total number of 15 samples were positive for *Hortaea werneckii* in the time period of 1st January 2023 to 31st December 2023. 7 patients had lesions on both palmar as well as plantar region (46.66%), 3 on palmar and plantar region each (20%), 1 (6.66%) on scalp and 1(6.66%) on face and scalp. The skin scrapings were taken and examined in 10% KOH wet mount which showed positivity of 11(73.33%) samples. The primary isolation of the fungus was done on SDA.
Conclusions: *Hortaea werneckii* is the most common cause of Tinea nigra. Pigmented patches of Tinea nigra may be confused with other inflammatory or neoplastic aetiology. For Tinea nigra, skin scrapings are sufficient for diagnosis by KOH mount and culture on SDA. Generally, the lesions resolve within 2 weeks of adequate treatment. To conclude, a high suspicion should be maintained to timely diagnose the disease, avoid unnecessary invasive investigation and early administration of treatment.

Keywords: Dermatomycosis, *Hortaea werneckii*, Melanized fungus, Tinea nigra

INTRODUCTION

Millions of lives are affected by fungal diseases, globally.[1] But the epidemiology of fungal infections differs from place to place, depending on a number of variables such as socioeconomic status, the presence of fungal endemicity associated with particular geo-ecological traits, and at risk persons. These variables can have a significant negative influence on an individual's health.[2] The superficial fungal infections of the keratinized tissues of the skin, hair, and nails are known as dermatomycosis. Dermatophytosis manifests itself in various clinical forms, notably Tinea corporis, Tinea cruris, Tinea capitis, Tinea nigra, onychomycosis to name a few.[3] *Hortaea werneckii*, a melanized yeast-like fungus that was previously categorized in the literature as Exophiala, Phaeoannellomyces, and Cladosporium, is the cause of Tinea nigra, a superficial dermatomycosis.[4] Tinea nigra affects people in temperate or subtropical climates because it flourishes in high-temperature, low-oxygen, and humid settings.[5] Hyperhydrosis and being in coastal or hyper saline habitats, where the causative agent may be absorbed from the natural ecosystem, are predisposing variables linked to the disorder.[6,7] Individuals arrive with well-demarcated, expanding, hyperchromic plaques on the palms or soles having

uneven borders, in which the fungus may or may not live in commensalism with other organisms.[8] The lesions are differential diagnosis with melanocytic lesions.[9] Pigmented patches of Tinea nigra may be confused with junctional nevi, post inflammatory pigmentation, malignant melanoma, lentigines, Addison's disease, and melanosis of syphilis and pinta. Misdiagnosis of the lesions had led in the past to biopsies or surgical excisions.[10] Dermoscopy, Wood's light, and KOH preparation can be used to differentiate between these two illnesses.[11] Quick information is obtained through direct KOH mount, which shows short, twisted, thick, light brown hyphae. These hyphae can occasionally be darkened and occasionally exhibit short filaments and yeast-like cells. *Hortaea werneckii* colonies grow in 5–8 days on a normal medium. They appear creamy and black at first, and then subsequently develop filamentous characteristics.[4] The development of molecular diagnostics for *H. werneckii* which involves rDNA ITS sequencing as well as PCR-fingerprinting methods have also become available.[12] While the majority of Tinea nigra cases have been linked to *H. werneckii*, other dematiaceous fungal species, like *Stenella araguata*, may also cause a comparable clinical presentation.[13] Tinea nigra is easily and successfully treated. The majority of instances can be resolved with just keratinolytic treatments, such as Whitfield ointment, salicylic acid, and urea, used once or twice daily.[14] The isolation of *Hortaea werneckii* from blood and splenic abscess of neutropenic patients have been also reported.[15] This is an uncommon instance where the fungus caused a systemic phaeohyphomycosis and acted as an opportunistic pathogen.[4]

In the present study, objective was to show that *Hortaea werneckii* is an important cause of skin diseases caused by fungus.

METHODS

A prospective observational study was conducted in the Department of microbiology at Government Medical College, Aurangabad from 1st January 2023 to 31st December 2023.A total of 295 skin scrapings samples were collected and examined.

Inclusion criteria

All the skin scraping samples from both male and female of all age groups that were sent for microbiological examination in the Department of Microbiology within the time frame of 1st January to 31st December 2023 were included in this study.

Exclusion criteria

Samples other than skin scrapings were excluded from the study.

Procedure

Skin lesions were clinically assessed and skin scrapings were collected from the active edges of the lesion and transported to microbiology laboratory and processed as per standard guidelines. Every sample was subjected to laboratory examination and the causative organism was identified using macroscopic and microscopic features.

Specimens were first examined in wet mounts after partial digestion with 10% potassium hydroxide (KOH) and were also inoculated on Sabouraud dextrose agar (SDA with chloramphenicol to minimize bacterial contamination), and incubated at 25°C and 37°C for 28 days. After noting the macroscopic features of colony such as colony morphology, colour and texture of growth, slide mounts were prepared in lactophenol cotton blue (LPCB) to study microscopic features. Special techniques such as fungal slide culture on Corn Meal Agar were also used as they allow fungal growth to be examined in its original form without disturbing spore arrangement.

RESULTS

Out of 295 samples examined from 1st January 2023 to 31st December 2023, a total of 78 cases (26.4%) were culture positive for various fungi. The distribution of various dermatophytic and non dermatophytic fungi (*Aspergillus* spp, *Mucorales*, *Dematiceous* fungi and others) is given in Figure 1. The distribution of dematiceous fungi including Hortaea weneckii is given as follows in Figure 2.

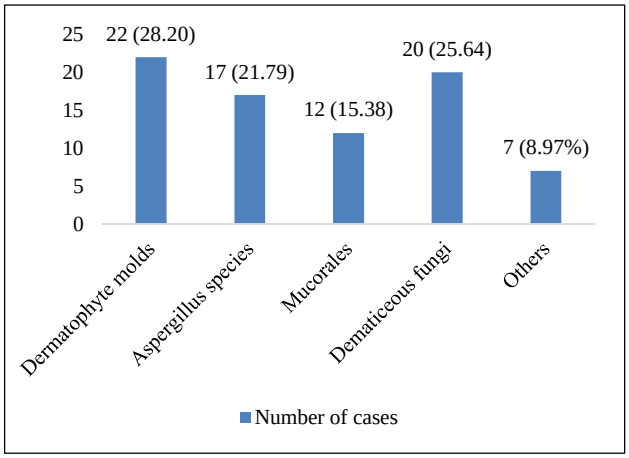

Figure 1: Distribution of various fungi in skin scrapings.

Non dermatophytes were the most common cause of fungal infections overall (71.8%) while the rest were caused by dermatophytes (28.2%). Among the non dermatophytes, dematiceous fungi were the commonest cause. *Hortaea werneckii* was the most common dematiceous fungus to cause skin diseases.

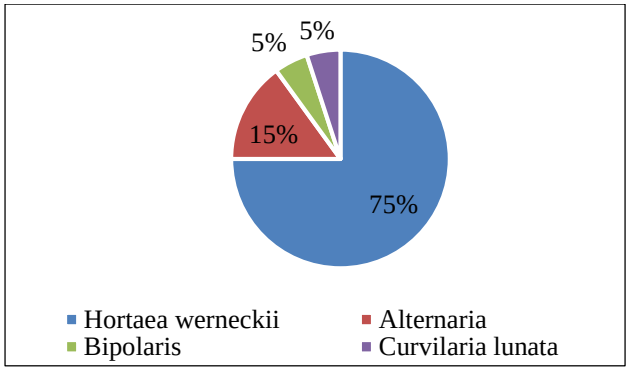

Figure 2: Distribution of various dematiceous fungi.

Out of 15 cases positive for *Hortaea werneckii*, 9 (60%) were male and 6 (40%) were female and M: F ratio was 1.5:1. Out of 15 patients, 11 (73.3%) were adults, 2 (13.3%) were children and 2 (13.3%) were adolescents as shown in the (Figure 3).

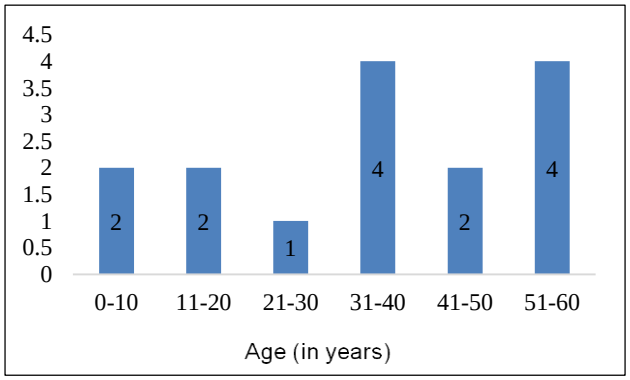

Figure 3: Age distribution of patients.

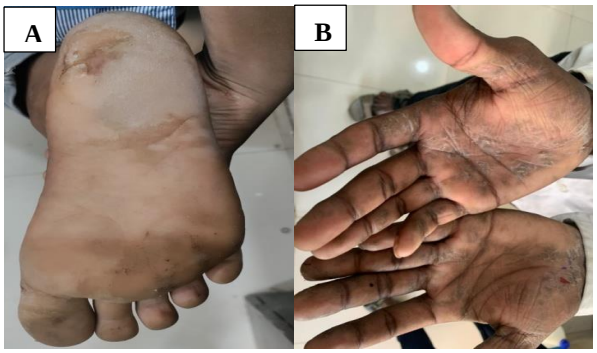

Figure 4: (A) Tinea nigra plantaris and (B) Tinea nigra palmaris

About 7 out of the 15 (46.66%) patients showed lesion on both palmar as well as plantar region, 3 (20%) patients only on palmar region, 3 (20%) only on plantar region, 1 (6.66%) patient showed lesion on scalp and face and 1 (6.66%) only on scalp as seen in (Figure 4 shows tinea nigra plantaris and shows lesions of tinea nigra palmaris (patient has scratched the lesion), 5a and 5b shows patient with the lesion on scalp and face). Figure 6 shows distribution of patients according to the area of involvement.

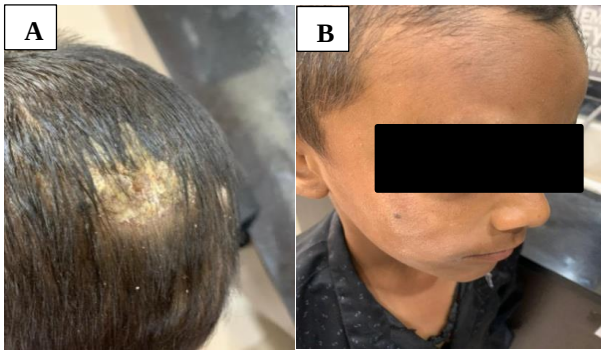

Figure 5: Patient with the lesion on scalp. B) Lesion on face.

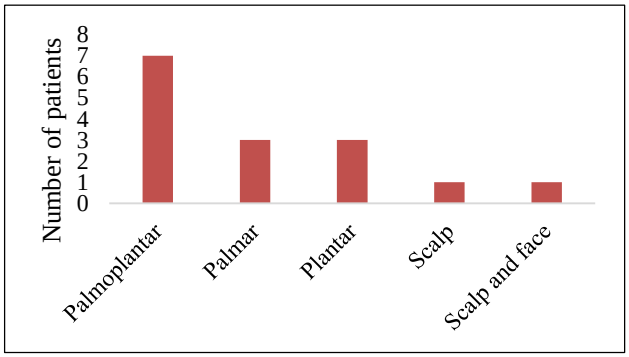

Figure 6: Distribution of patients according to the area of involvement.

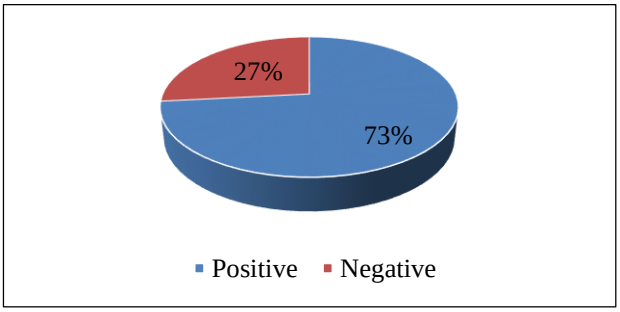

Figure 7: Pie chart showing percentage of patients with *Hortaea werneckii* showing KOH positivity.

The skin scrapings were taken and examined in 10% KOH wet mount which showed positivity of 11(73.33%) out of 15 samples as shown in (Figure 7). On direct microscopy, multibranched separate hyphae were seen as shown in (Figure 8).

The primary isolation of the fungus was done on SDA. The cultures were incubated at BOD 25°C and at 37°C. At 25°C colonies appear slowly and mature after about three weeks. Initially the surface is first light brown in color, wet, glossy, and yeast-like, but it quickly turns olive-black. A thin coating of mycelium in the middle

may cause it to lose its sheen and turn olive in colour later on. Greyish green hyphae may grow at the periphery as shown in the (Figure 9) while the reverse is black. No growth was seen on 37°C.

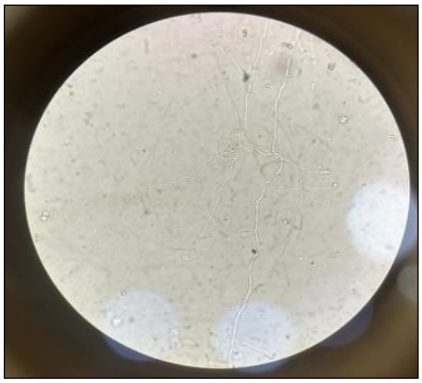

Figure 8: Multibranched and septate hyphae (KOH, 40×).

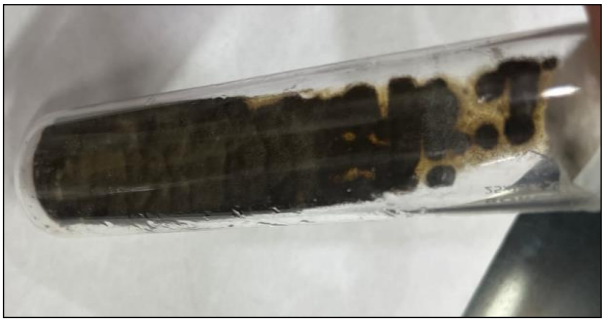

Figure 9: Colony morphology of *Hortaea werneckii.*

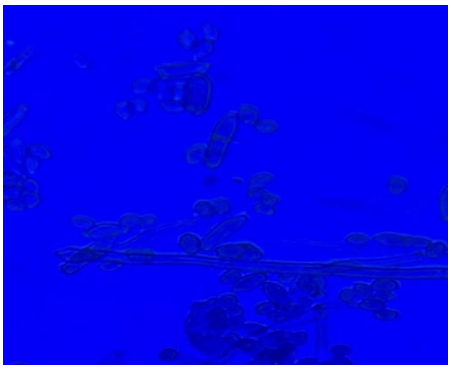

Figure 10: Annelloconidia and biclavate conidia (LPCB, 40X).

On microscopy, majority of the cells in the very early phase are yeast-like, pale or dark brown. In reality, mature forms are annellides, spherical at one end and tapering and elongated with striations at the other end where conidia are generated. Mature forms are one or two celled (two celled stage often being referred to as biclavate cells). Dark, finely divided, thick-walled hyphae appear with ageing. At annellidic sites along the hyphae, annelloconidia can develop and accumulate. Every conidium has the ability to divide into new conidia and act as an annellide. As the culture ages, chlamydoconidia may form. (Figure 10) shows annelloconidiae of *Hortaea werneckii.* On slide culture, there are light brown peanut shaped conidia comprising of 1-2 ampullaceous cells. The annellated zones are prominent, 1-2 micrometer wide with clearly visible annellations formed on the intercalary or lateral conidiogenous cells as shown in (Figure 11).

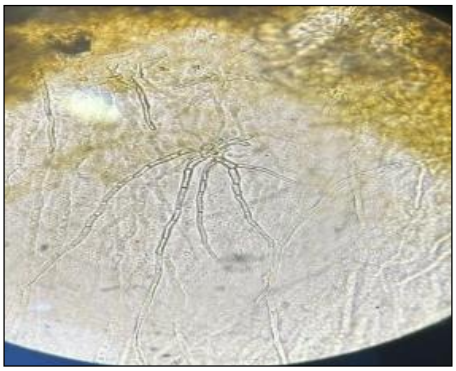

Figure 11: Slide culture of *Hortaea werneckii*.

DISCUSSION

Out of the 295 cases of skin lesions suspected to have been caused due to fungus, only 78 cases were caused by fungus in this study. Among these cases, dermatophytes were the cause in 22 patients while the rest 56 were due to non dermatophytes. In the study by Pathave et al, out of 113 skin samples, 25 were due to dermatophytes while 3 were due to non dermatophytes. The infections due to non dermatophytes are on rise which were previously considered as contaminants.[16]

Hortaea werneckii is a non dermatophytic dematiceous fungus which causes a rare type of skin lesion known as Tinea nigra. The majority of reports come from humid and tropical climatic zones. Cases from Asia were reported from India, Sri Lanka, Myanmar, and Polynesia by Sarangi et al, Uezato et al 2006, while cases from Latin America were recorded from Brazil, Mexico, Venezuela by Cabrera et al, Severo et al, and Perez et al.[6,13,17-19]

The cases are not particularly distributed according to the age or gender by Bonifaz et al, Pegas et al.[4, 20] In a study of 12 patients conducted in Venezuela between 1972 and 2002, Tinea nigra was found to be more prevalent among young people with fair skin who visited beaches.[13] But in our study we have seen that it affected more in adults as compared to adolescents and children, who were working in humid climate.

Most cases are unilateral but bilateral infections can also be observed, probably resulting from autoinoculation.[21] In the study by Bonifaz et al, 19 out of 22 cases of the disorder were located on the palms and 3 on the soles.[4]

In our study, we found 7 out of 15 cases to be involving both palmar and plantar surfaces. We also found bilateral cases palmar involvement in one patient and bilateral plantar involvement in another patient. We also found 2 cases of Tinea nigra from scalp. A case of Tinea nigra from scalp was also reported by Prasanna et al.[22]

It is not necessary to take biopsy of scaly lesions; a rapid diagnosis can be achieved by scraping the stratum corneum with a scalpel blade and KOH preparation, which shows pigmented hyphae, and later can be confirmed by the growth of a dematiaceous mold on culture media.[23] According to Gupta et al. and Hughes et al. Tinea nigra can be clearly identified from other dermatophytoses or skin diseases by the pigmentation of its hyphae.[8,24] Nearly all of the cases in the study by Bonafiz et al. had diagnoses by direct examination, which were then verified by culture.[4] In our study, we confirmed the cases with direct examination by KOH mount, culture on Soubaurod's dextrose agar, lactophenol cotton blue staining and slide culture. Out of 15 cases, 11 (73.33%) were KOH positive as compared to Bonafiz et al. which found all 22 cases to be KOH positive.[4] Culture positive in this study goes beyond KOH positivity. This might occur from the KOH smear missing the fungal hyphae. This may be due to the low fungal elements in the sample and drying out procedure.[16]

Tinea nigra is easily treated and has a high success rate. The majority of cases respond to keratinolytic treatments such as urea, salicylic acid, and Whitfield ointment, which are used once or twice a day. Miconazole, ketoconazole, bifonazole, terbinafine, and ciclopiroxolamine have also been shown to have good therapeutic results.[4]

The present study was a single institutional study with a limited number of patients. Molecular testing could have been done for furthering the diagnosis.

CONCLUSION

Hortaea werneckii is the most common cause of Tinea nigra and is one of the most common non dermatophytic fungal causes of skin infection. It is common in tropical, humid climate and in patients frequently visiting beach or with hyperhydrosis as it grows in hypersaline environment. The organism causes superficial infection by colonizing stratum corneum. Invasive disease is extremely rare with a single case report of fungemia with splenic abscess in a known neutropenic patient. The infection is generally asymptomatic, rarely itchy and causes blackish macular patch over palms, sole and rarely interdigital web, face and scalp. Patients generally ignore the lesion but sometimes may seek treatment especially when in doubt for a serious lesion like melanoma.

Pigmented patches of Tinea nigra may be confused with other inflammatory or neoplastic aetiology. In occasional cases, patients have to undergo a biopsy to rule out a naevus or melanoma. Otherwise skin scrapings are sufficient for diagnosis by KOH mount and culture on SDA. Treatment is by keeping hands or the affected areas dry and use of urea, salicylic acid and Whitfield ointment. Additionally, antifungal agents may be used. Generally, the lesions resolve within 2 weeks of adequate treatment. To conclude, a high index of suspicion should be maintained to timely diagnose the disease, avoid unnecessary invasive investigation and early administration of treatment as it is easily treatable.

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REFERENCES

1. Ray A, Aayilliath K A, Banerjee S, Chakrabarti A, Denning DW. Burden of Serious Fungal Infections in India. Open Forum Infect Dis. 2022;9(12):ofac603.
2. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4(165):165rv13.
3. Haldar N, Haldar N, Sharma MK, Dutta G, Haldar NR. Epidemiology of dermatomycosis in the Sub-Himalayan region of West Bengal, India. JEMDS. 2015;4(91):15619-23.
4. Bonifaz A, Badali H, de Hoog GS, Cruz M, Araiza J, Cruz MA, et al. Tinea nigra by Hortaea werneckii, a report of 22 cases from Mexico. Stud Mycol. 2008;61:77-82.
5. Ibraheim MK, McNally MA, Tschen J. Interdigital Tinea Nigra. Cureus. 2020;12(4):e7579.
6. Severo LC, Bassanesi MC, Londero AT. Tinea nigra: report of four cases observed in Rio Grande do Sul (Brazil) and a review of Brazilian literature. Mycopathol. 1994;126(3):157-62.
7. de Hoog GS, Gerrits van den Ende AH. Nutritional pattern and eco-physiology of Hortaea werneckii, agent of human tinea nigra. Antonie Van Leeuwenhoek. 1992;62(4):321-9.
8. Gupta AK, Chaudhry M, Elewski B. Tinea corporis, tinea cruris, tinea nigra, and piedra. Dermatol Clin. 2003;21(3):395-400.
9. Falcão EM, Trope BM, Martins NR, Barreiros Mda G, Ramos-E-Silva M. Bilateral tinea nigra plantaris with good response to isoconazole cream: a case report. Case Rep Dermatol. 2015;7(3):306-10.
10. Aroeira Neves J, Costa OG. Tinea nigra. Arch Derm Syphilol. 1947;55(1):67-84.
11. Criado PR, Delgado L, Pereira GA. Dermoscopy revealing a case of Tinea Nigra. An Bras Dermatol. 2013;88(1):128-9.
12. Uijthof JM, de Cock AW, de Hoog GS, Quint WG, van Belkum A. Polymerase chain reaction-mediated genotyping of Hortaea werneckii, causative agent of tinea nigra. Mycoses. 1994;37(9-10):307-12.
13. Perez C, Colella MT, Olaizola C, Hartung de Capriles C, Magaldi S, et al. Tinea nigra: report of

twelve cases in Venezuela. Mycopathol. 2005;160(3):235-8.
14. Sayegh-Carreño R, Abramovits-Ackerman W, Girón GP. Therapy of tinea nigra plantaris. Int J Dermatol. 1989;28(1):46-8.
15. Ng KP, Soo-Hoo TS, Na SL, Tay ST, Hamimah H, Lim PC, et al. The mycological and molecular study of Hortaea werneckii isolated from blood and splenic abscess. Mycopathol. 2005;159:495-500.
16. Pathave H, Dash M, Sarangi G, Mohanty P, Bisoyi D. Clinicomycological study of superficial mycoses: a changing trend of increasing non-dermatophyte mold infection. J Coll Medi Sciences-Nepal. 2022;18(1):49-59.
17. Sarangi G, Dash D, Chayani N, Patjoshi SK, Jena S. Bilateral Tinea Nigra of palm: a rare case report from Eastern India. Ind J Med Microbiol. 2014;32(1):86-8.
18. Uezato H, Gushi M, Hagiwara K, Kayo S, Hosokawa A, Nonaka S. A case of tinea nigra palmaris in Okinawa, Japan. J Dermatol. 2006;33(1):23-9.
19. Cabrera R, Sabatin N, Urrutia M, Sepúlveda R. Tiña negra (tinea nigra): comunicación de un caso alóctono en Chile [Tinea nigra: a allochthonous case report in Chile]. Rev Chilena Infectol. 2013;30(1):90-3.
20. Pegas JR, Criado PR, Lucena SK, de Oliveira MA. Tinea nigra: report of two cases in infants. Pediatr Dermatol. 2003;20(4):315-7.
21. Tseng SS, Whittier S, Miller SR, Zalar GL. Bilateral tinea nigra plantaris and tinea nigra plantaris mimicking melanoma. Cutis. 1999;64(4):265-8.
22. Prasanna S, Jada SK. Karthika by. Hortaea werneckii and Rhodotorula rubra. Int J Curr Res. 2016;8(10):39922-4.
23. Kelarestaghi A, Hashemi SJ, Rafat Z, Kelarestaghi H, Moqaddam ZR, Maboudi A, et al. The first report of tinea nigra from Iran. New Microbes New Infect. 2022;49-50:101032.
24. Hughes JR, Moore MK, Pembroke AC. Tinea nigra palmaris. Clin Experim Dermatol. 1993;18(5):481-2.

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