Review article

The Clinical Significance of Fungi in Atopic Dermatitis

Arsa Thammahong[1], Chanisa Kiatsurayanon[2], Steven W Edwards[3], Pawinee Rerknimitr[4], Direkrit Chiewchengchol[5]

[1]Antimicrobial Resistance and Stewardship Research Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University; [2]Institute of Dermatology, Bangkok, Thailand; [3]Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom; [4]Division of Dermatology, Skin and Allergy Research Unit, Department of Medicine, Faculty of Medicine, Chulalongkorn University; [5]Translational Research in Inflammation and Immunology Research Unit, Faculty of Medicine, Chulalongkorn University;

Corresponding author:

Direkrit Chiewchengchol, MD, PhD, Translational Research in Inflammation and Immunology Research Unit, Faculty of Medicine, Chulalongkorn University, Rama 4 Road, Pathumwan, Bangkok, Thailand, 10330

E-mail: *cdirekrit@live.com*

Telephone: 02-256-4470 ext 626 Fax: 02-252-5952

Conflict of interest

The authors have declared no conflicts of interest.

Keywords: atopic dermatitis, fungi, *Malassezia, Candida,* dermatophytes

Introduction

Atopic dermatitis (AD) is a chronic and recurrent inflammatory skin disease with a typical characteristic of eczematous lesions [1, 2]. This condition is usually found in patients during infancy and early childhood, but the symptoms sometimes persist until adulthood [2]. Although skin lesions with age-specific patterns are sufficient clues for the diagnosis of AD, the 1980 Hanafin and Rajka criteria are useful for making a diagnosis in general practice [3].

A pruritic rash is a common clinical presentation that is sometimes accompanied by skin infections in patients with AD [2], particularly *Staphylococcus. aureus* and herpes simplex virus (HSV) [4-10]. However, fungi (e.g. *Malassezia* or *Candida* species) usually colonize on the skin of AD patients but the role of these fungi in the pathogenesis of AD remains unclear. For example, *Malassezia* species are part of the normal flora residing on the skin, and while these are not normally associated with dermatological conditions, these organisms can sometimes cause skin diseases in AD patients [11, 12]. In contrast, *Candida* species, which are also present as normal flora on the skin and mucous membranes, are the most common pathogenic yeasts causing mucocutaneous candidiasis [13-15]. Other fungi (e.g. dermatophytes) can also cause fungal infections of skin, hair, and nails in AD patients [16]. In this review, the epidemiology, pathogenesis, clinical manifestations, diagnosis, and management of *Malassezia*, *Candida*, and dermatophyte infections in AD patients are discussed (Table 1).

Epidemiology of fungi and atopic dermatitis

There are a number of fungi associated with AD. *Malassezia* yeasts are one of the most common fungi associated with AD especially in the head-neck lesion of adult patients [11, 12, 17, 18]. In healthy individuals, *Malassezia globosa* and *Malassezia restricta* are the most common species [19, 20], but a number of studies have revealed no difference in the number of *Malassezia* species isolated from the skin of both healthy and AD patients [11, 21-23], suggesting that these

fungi are opportunistic organisms in AD. Nonetheless, the level of *Malassezia* colonization was different depending on the severity of AD, i.e. increased up to 2-5 fold in severe AD compared to mild or moderate cases [23]. Moreover, the *Malassezia* species that are detected on the lesions of AD patients are different depending on the geographical area [19, 20, 24, 25]. Sugita, *et al.* proposed that pathogenic strains of *M. restricta* may be associated with the exacerbation of AD [23, 26]. Interestingly, serum *Malassezia*-specific IgE is found in 30% of children with AD, and 70% of adult patients [27-30], particularly in patients with head and neck lesions [31]. It is believed that *Malassezia* sensitization is correlated with adults more than children with AD [30, 32], partly because of less sebum production in children.

The association between *Candida* species colonization or infections and the severity of AD is unknown [13, 33]. *Candida* cultures from the gastrointestinal tract and nasopharynx are found more in patients with AD than healthy controls [15, 34]. It is hypothesized that the inappropriate use of topical and systemic antibiotics and corticosteroids probably affects the skin and mucosal microbiota to favor the growth of *Candida* species [35]. However, some previous studies showed that *Candida* species colonization in the oral cavity of AD patients was not significantly different compared to healthy controls [36, 37]. Therefore, the epidemiology of *Candida* species and AD still needs further investigation.

The epidemiology of *Trichophyton, Epidermophyton,* and *Microsporum* species and their role in the pathology of AD patients are still largely unknown. It has been reported that 79% of AD patients with dermatophyte infections show an immediate-type hypersensitivity reaction against *Trichophyton* antigen (a purified trichophytin) in the skin prick test [38]. The association between atopic diseases and dermatophyte infections with increased total IgE and *Trichophyton*-specific IgE levels is known as the atopic-chronic dermatophytosis syndrome [39]. These reports suggest that AD patients are more sensitive to these fungi than healthy individuals.

Recent microbiome studies in AD patients showed that *Malassezia* species accounted for 63-86% of all fungi, followed by non-*Malassezia* yeasts, such as *Candida albicans, Cryptococcus* species, and other filamentous fungi [40, 41]. However, the species of non-*Malassezia* yeasts and other filamentous fungi detected are highly variable in individual AD patients compared to healthy individuals while *Malassezia* species are consistently detected [40, 41]. Furthermore, the species of *Malassezia* detected and the ratio between *M. restricta* and *M. globosa,* are different depending on the AD severity [41, 42]. It has been reported that the ratio of *M. restricta* and *M. globosa* is > 1:1 in mild to moderate AD patients, while this ratio is close to 1 in severe cases [41, 42], suggesting that this ratio could probably be used to predict disease severity in AD patients.

Pathogenesis of fungi in AD

As the pathogenesis of AD can be associated with genetic predisposition, dysregulation of the immune system and environmental exposure [43, 44], patients with AD usually have skin barrier defects and decreased production of antimicrobial peptides leading to skin infections [45]. The common pathogens found in AD patients are *S. aureus*, herpes simplex virus, molluscum contagiosum virus, and the fungi described above [46-50].

Malassezia species easily invade through the skin barrier of AD patients causing skin infections. These species contact directly or indirectly with human keratinocytes and other immune cells leading to immune activation [12, 51]. It has been reported that Toll-like receptors type 2 and 4 of human keratinocytes and dendritic cells are stimulated by *Malassezia* species causing secretion of antimicrobial peptides and cytokines (e.g. human β-defensin 2 and CXCL8) [52, 53]. *Malassezia* species also activate human dendritic cells via NLRP3 inflammasome leading to the production of IL-1β, IL-4, IL-5, and IL-13 [54]. Moreover, it has been demonstrated that nanovesicles secreted by *M. sympodialis* activate skin dendritic cells

and dermal mast cells to release TNF-α, IL-6, IL-8, IL-10, and IL-12p70 leading to disease exacerbation [55]. Taken together, skin barrier defects and immune activation by *Malassezia* species in AD patients are very likely to contribute to *Malassezia* infections and skin inflammation.

It has been demonstrated that patients with AD usually develop skin sensitization by *Candida* species as a positive reaction to *C. albicans* in the skin prick test is found in 27-94% of these patients [56, 57]. Moreover, total serum IgE levels are elevated in AD patients and include an increase in specific *C. albicans* IgE levels, which is higher than in other allergic diseases and healthy controls [13, 15, 58]. It has been reported that specific *C. albicans* IgE levels are associated with disease severity in adult patients but not in children [13, 27]. However, previous studies demonstrated that *C. albicans*-specific IgE could cross-react between *Malassezia* species, *S. cerevisiae* polysaccharides and other *Candida* species leading to a high incidence of specific IgE levels in AD patients [13, 59]. This cross-reaction could over-estimate the incidence of *C. albicans* and the association with AD.

Similarly, immune activation by dermatophytes has been reported in AD patients as an immediate-type hypersensitivity reaction to *Trichophyton* antigen is observed [60, 61] particularly in patients with chronic noninflammatory dermatophytosis [16, 60]. The immediate hypersensitivity type 1 and atopic diseases may be related because they both activate the Th2 signaling pathway [16, 62]. However, other factors (e.g. human leukocyte antigens or HLAs) may play a role in this immune activation, which needs to be further investigated [63]. Immediate hypersensitivity to other molds such as *Penicillium, Cladosporium* species has also been reported [64]. Nevertheless, no reports have demonstrated an actual relationship between dermatophytes (including *Epidermophyton* and *Microsporum)* and AD pathogenesis [65, 66].

Clinical manifestations of fungi and atopic dermatitis

As mentioned earlier, AD causes skin barrier defects and decreased antimicrobial peptide production [45]. Fungi that are usually normal flora are able to colonize and sometimes cause an infection on AD skin.

1. *Malassezia* species

Malassezia infection manifests as different skin diseases in AD, e.g. pityriasis versicolor of the skin, *Malassezia* folliculitis of the hair follicle. The clinical manifestations of pityriasis versicolor usually are multiple hyper or hypo-pigmented macules with fine white scales on the seborrheic area such as eyebrows, nasolabial folds, cheek, chin and upper chest [19]. However, some lesions at the upper back and shoulders could be overlooked and left untreated in AD (Fig. 1A). Moreover, pityriasis versicolor found in adolescents and young adults with high sebaceous activity commonly has no symptoms, which could be neglected by the patient themselves. Tropical climates, hyperhidrosis, and corticosteroid use are believed as other predisposing factors of pityriasis versicolor in AD patients [25].

Malassezia (*Pityrosporum*) folliculitis usually presents as mild pruritic monomorphous papules and/or pustules without comedones at the neck, upper chest and back, and upper arms (Fig. 1B) [25]. *Malassezia* folliculitis is often under-diagnosed or misdiagnosed as acne vulgaris [67]. It is now recognised that predisposing factors for *Malassezia* folliculitis in AD patients are hot and humid tropical climates, oral corticosteroid (over-)use, topical or oral antibiotic use and immunosuppression [67].

2. *Candida* species

Cutaneous candidiasis shows erythematous patches or plaques with satellite papules or pustules at the intertriginous area such as the neck, axillae, buttocks including genitalia [14, 15, 36]. As the clinical manifestations of cutaneous candidiasis at diaper areas (Fig. 1C) sometimes show eczematous lesions, the patients are often misdiagnosed with irritant contact dermatitis (diaper

rash) rather than AD and treated with topical corticosteroids that could favor further *Candida* infection upon the lesions.

The association between oral candidiasis and AD is still unknown. However, *Candida* was more frequently isolated from the oral cavity of AD patients compared to healthy controls (23% VS 6%) [36]. This suggests that AD patients may be prone to *Candida* colonization in both the skin and oral cavity. Moreover, dental caries associated *Candida* colonization was found to be more prevalent in children with AD compared to healthy controls [68].

3. Dermatophytes

Dermatophyte infections are found as classic annular or ringworm-like lesions with inflammation and a scaly active border [2]. As the clinical manifestations of dermatophytosis are scaly pruritic erythematous rashes, it is sometimes difficult to differentiate this infection from eczematous lesions in AD patients (Fig. 2). Moreover, dermatophytosis should be more suspected particularly in AD patients who have been treated with standard treatments without improvement (e.g. tinea incognita) (Fig. 1D). Tinea incognita occurs when corticosteroid therapy is used to treat dermatophyte infections, which can mask the lesion. Therefore, it is very important to emphasize to general practitioners and dermatologists that a simple direct examination (e.g. KOH preparation, Gram staining) is a very helpful but simple laboratory investigation to identify these fungal infections in AD patients.

Diagnosis of fungal infections in AD

Skin scraping with potassium hydroxide (KOH) preparation or Gram staining is the most common and easiest method to distinguish types of fungi under light microscopy. However, Periodic Acid Schiff (PAS) staining is sometimes helpful particularly in hair and nail fungal infections.

Malassezia colonization (normal flora) and *Malassezia* from the skin of *Malassezia* folliculitis lesions are seen as broad-based budding yeasts in KOH preparation under light microscopy from healthy skin whilst both budding yeasts and short hyphae are observed from pityriasis versicolor lesions (Fig. 3A) [25]. Cutaneous candidiasis shows narrow-based budding yeasts, pseudohyphae and septate hyphae using KOH preparation and Gram staining (Fig. 3C, 3D) [69] but hyaline septate hyphae with arthroconidia are found from the scales of active lesions in dermatophytosis (Fig. 3B) [69].

Calcofluor white and blankophor (0.1%, w/v) or Chicago sky blue stain, together with 20% KOH preparation (1:1, v/v) can also be utilized to assist detection of fungal elements in some reports [70, 71]. Calcofluor white and blankophor bind to chitin on the fungal cell wall and fungal elements are identified under fluorescent microscopy with 400-440 nm blue filter while Chicago sky blue directly binds to the fungal elements, which are easily seen under light microscopy (blue fungal elements with a pink background). Sensitivity and specificity of both stains are comparable [72-74], but the Chicago sky blue stain is more cost-effective . Therefore, the Chicago sky blue stain can be used as a novel rapid technique for diagnosis of fungal skin infections [72, 73, 75, 76].

Although fungal culture is important to confirm fungal species [2], it is not a routine laboratory test for AD patients [13, 77]. *Malassezia* species need long-chain fatty acids to grow in culture media while *Candida* species are cultured in conventional media and identified using biochemical tests and molecular identification. Dermatophytes can also be cultured and identified using their typical morphologies and molecular identification [69]. For *Malassezia* and *Candida* yeasts, biochemical tests are sugar assimilation assays that can differentiate the pattern of carbon utilization among yeasts [13, 77]. Molecular identification using PCR-sequencing with pan-fungal primers (ITS, internal transcribed spacer) is needed if the biochemical assays or morphological identifications are inconclusive [77].

Management of atopic dermatitis and fungi

Management of atopic dermatitis involves patient education, appropriate skin hydration, irritant removal, anti-inflammatory therapy, antipruritic medications, and treatment of infections [2]. The first-line treatment of AD is emollients for hydration and topical corticosteroid for anti-inflammation including topical immunomodulators [2, 78-80].

Emollients (e.g. fatty acids, cholesterol, squalene) improve skin texture and reduce water loss [81-83]. However, emollients with fragrances are not recommended because of skin irritation and contact dermatitis [83, 84]. Furthermore, certain types of emollients containing olive oil should be avoided as they may affect stratum corneum homeostasis and support *Malassezia* growth leading to further fungal infections [83, 85]. Humectants (e.g. urea, glycerol, panthenol) and occlusive agents (e.g. mineral oil, beeswax, petroleum jelly, silicones, zinc oxide) are beneficial and sometimes added for further skin hydration in AD patients [83, 84]. Regarding treatment with topical corticosteroids, long term use should be carefully considered as it suppresses the immune responses on the skin of patients and could lead to prolonged infections and complications [86].

AD patients with *Malassezia* infections, e.g. pityriasis versicolor, *Malassezia* folliculitis, can be treated with topical and/or systemic antifungal therapy but patients with *Malassezia*-exacerbated AD are usually refractory to the standard treatments (e.g. emollients and topical corticosteroids), especially with head and neck lesions [50, 87, 88]. The symptoms of these AD patients can be ameliorated by systemic therapy (e.g. azoles) [89, 90]. There are several types of antifungal agents used but azoles are usually the treatment of choice [13]. Furthermore, azoles also show an anti-inflammatory effect on the skin of AD patients as they inhibit IL-4 and IL-5 production by T cells [91]. Interestingly, topical calcineurin inhibitors (e.g. tacrolimus)

inhibit skin inflammation in AD patients and have an antifungal effect on *Malassezia* species together with a synergistic effect with azoles (i.e. ketoconazole and itraconazole) [92].

Treatment of *Candida* infections in AD patients is similar to *Malassezia*-associated AD treatment [93, 94]. Previous reports showed that antifungal treatment (e.g. ketoconazole and fluconazole) can decrease symptoms and serum IgE levels especially in refractory AD patients [90, 93, 95-97]. Importantly, when these *Malassezia* and *Candida* infections are found in AD patients, antifungal therapy should be used [86]. Anti-inflammatory therapy, such as tacrolimus, and emollients with antifungal therapy are preferred over topical corticosteroid therapy for *Malassezia* and *Candida* infections [13, 25]. However, clinical judgment and close follow-up are essential to effectively treat AD patients with *Malassezia* or *Candida* infections.

The effectiveness of antifungal treatment in AD patients with dermatophytosis has been demonstrated in many studies [13, 16]. Topical and systemic azoles, including topical allylamines, e.g. terbinafine, are still the first-line treatment of these skin infections but systemic therapy is selected once a large area of the skin or hair and nail are infected [2, 98, 99]. For example, it has been reported that recalcitrant AD patients with nail infections (tinea unguium) and *Trichophyton*-specific IgE are clinically improved after systemic antifungal treatment [100]. Furthermore, severe generalized deep dermatophytosis, trichophytic granuloma in AD patients were eliminated after 200 mg/day itraconazole treatment for 4 months [2, 100-102].

Conclusion

AD is a chronic skin disease and fungal skin infections, e.g. *Candida* species, *Malassezia* species, and dermatophytes, can deteriorate and exacerbate this condition in some patients. Fungal skin infections are probably not the cause of AD but skin barrier defects and decrease

in anti-microbial peptide production on the skin in AD patients allow fungi to penetrate easily into the epidermal layer and cause fungal allergen hypersensitivity in these patients. It is very important to emphasize that fungal infections are one of the differential diagnoses and need to be excluded particularly in recalcitrant cases. The standard antifungal treatment along with conventional AD therapy can result in clinical improvement in AD patients. However, further studies on the effectiveness of the antifungal treatment are required for the selection of the specific antifungal agents or the combination of treatment between immunomodulators and antifungal agents. This knowledge could improve the prognosis and quality of life of AD patients, especially with fungal skin infections.

Acknowledgement

We would like to thank all AD patients and their families for contributing to the pictures in this review. Special acknowledgements go to Translational Research in Inflammation and Immunology Research Unit, and Antimicrobial Resistance and Stewardship Research Unit, Department of Microbiology, and the Skin and Allergy Research Unit, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Funding

No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

Conflict of interest

The authors have declared no conflicts of interest.

References

1. Schmitt J, Schwarz K, Baurecht H, Hotze M, Folster-Holst R, Rodriguez E, et al. Atopic dermatitis is associated with an increased risk for rheumatoid arthritis and inflammatory bowel disease, and a decreased risk for type 1 diabetes. J Allergy Clin Immunol. 2016;137(1):130-6.

2. Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, et al. Fitzpatrick's Dermatology in General Medicine, Ninth Edition, 2 Volume set: McGraw-Hill Education; 2019.

3. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol Suppl (Stockh). 1980(92):44-7.

4. Abeck D, Mempel M. *Staphylococcus aureus* colonization in atopic dermatitis and its therapeutic implications. Br J Dermatol. 1998;139 Suppl 53:13-6.

5. Baker BS. The role of microorganisms in atopic dermatitis. Clin Exp Immunol. 2006;144(1):1-9.

6. Nowicka D, Grywalska E. The Role of Immune Defects and Colonization of *Staphylococcus aureus* in the Pathogenesis of Atopic Dermatitis. Anal Cell Pathol (Amst). 2018;2018:1956403.

7. Zollner TM, Wichelhaus TA, Hartung A, Von Mallinckrodt C, Wagner TO, Brade V, et al. Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. Clin Exp Allergy. 2000;30(7):994-1000.

8. Geoghegan JA, Irvine AD, Foster TJ. *Staphylococcus aureus* and Atopic Dermatitis: A Complex and Evolving Relationship. Trends Microbiol. 2018;26(6):484-97.

9. Blicharz L, Rudnicka L, Samochocki Z. *Staphylococcus aureus*: an underestimated factor in the pathogenesis of atopic dermatitis? Postepy Dermatol Alergol. 2019;36(1):11-7.

10. Kim J, Kim BE, Ahn K, Leung DYM. Interactions Between Atopic Dermatitis and *Staphylococcus aureus* Infection: Clinical Implications. Allergy Asthma Immunol Res. 2019;11(5):593-603.

11. Glatz M, Bosshard PP, Hoetzenecker W, Schmid-Grendelmeier P. The Role of *Malassezia* spp. in Atopic Dermatitis. J Clin Med. 2015;4(6):1217-28.

12. Nowicka D, Nawrot U. Contribution of *Malassezia* spp. to the development of atopic dermatitis. Mycoses. 2019;62(7):588-96.

13. Faergemann J. Atopic dermatitis and fungi. Clin Microbiol Rev. 2002;15(4):545-63.

14. Calderone RA, Clancy CJ. *Candida* and candidiasis. 2nd ed. ed. Washington, DC: ASM Press; 2012.

15. Savolainen J, Lammintausta K, Kalimo K, Viander M. *Candida albicans* and atopic dermatitis. Clin Exp Allergy. 1993;23(4):332-9.

16. Woodfolk JA. Allergy and dermatophytes. Clin Microbiol Rev. 2005;18(1):30-43.

17. Broberg A, Faergemann J, Johansson S, Johansson SG, Strannegard IL, Svejgaard E. *Pityrosporum ovale* and atopic dermatitis in children and young adults. Acta Derm Venereol. 1992;72(3):187-92.

18. Jensen-Jarolim E, Poulsen LK, With H, Kieffer M, Ottevanger V, Stahl Skov P. Atopic dermatitis of the face, scalp, and neck: type I reaction to the yeast *Pityrosporum ovale*? J Allergy Clin Immunol. 1992;89(1 Pt 1):44-51.

19. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A. The *Malassezia* genus in skin and systemic diseases. Clin Microbiol Rev. 2012;25(1):106-41.

20. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, et al. Topographic diversity of fungal and bacterial communities in human skin. Nature. 2013;498(7454):367-70.

21. Sandstrom Falk MH, Tengvall Linder M, Johansson C, Bartosik J, Back O, Sarnhult T, et al. The prevalence of *Malassezia* yeasts in patients with atopic dermatitis, seborrhoeic dermatitis and healthy controls. Acta Derm Venereol. 2005;85(1):17-23.

22. Yim SM, Kim JY, Ko JH, Lee YW, Choe YB, Ahn KJ. Molecular analysis of *Malassezia* microflora on the skin of the patients with atopic dermatitis. Ann Dermatol. 2010;22(1):41-7.

23. Kaga M, Sugita T, Nishikawa A, Wada Y, Hiruma M, Ikeda S. Molecular analysis of the cutaneous *Malassezia* microbiota from the skin of patients with atopic dermatitis of different severities. Mycoses. 2011;54(4):e24-8.

24. Prohic A, Jovovic Sadikovic T, Krupalija-Fazlic M, Kuskunovic-Vlahovljak S. *Malassezia* species in healthy skin and in dermatological conditions. Int J Dermatol. 2016;55(5):494-504.

25. Harada K, Saito M, Sugita T, Tsuboi R. *Malassezia* species and their associated skin diseases. J Dermatol. 2015;42(3):250-7.

26. Sugita T, Tajima M, Amaya M, Tsuboi R, Nishikawa A. Genotype analysis of *Malassezia restricta* as the major cutaneous flora in patients with atopic dermatitis and healthy subjects. Microbiol Immunol. 2004;48(10):755-9.

27. Scalabrin DM, Bavbek S, Perzanowski MS, Wilson BB, Platts-Mills TA, Wheatley LM. Use of specific IgE in assessing the relevance of fungal and dust mite allergens to atopic dermatitis: a comparison with asthmatic and nonasthmatic control subjects. J Allergy Clin Immunol. 1999;104(6):1273-9.

28. Johansson C, Eshaghi H, Linder MT, Jakobson E, Scheynius A. Positive atopy patch test reaction to *Malassezia furfur* in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. J Invest Dermatol. 2002;118(6):1044-51.

29. Lange L, Alter N, Keller T, Rietschel E. Sensitization to *Malassezia* in infants and children with atopic dermatitis: prevalence and clinical characteristics. Allergy. 2008;63(4):486-7.

30. Glatz M, Buchner M, von Bartenwerffer W, Schmid-Grendelmeier P, Worm M, Hedderich J, et al. *Malassezia* spp.-specific immunoglobulin E level is a marker for severity of atopic dermatitis in adults. Acta Derm Venereol. 2015;95(2):191-6.

31. Brodska P, Panzner P, Pizinger K, Schmid-Grendelmeier P. IgE-mediated sensitization to *Malassezia* in atopic dermatitis: more common in male patients and in head and neck type. Dermatitis. 2014;25(3):120-6.

32. Zhang E, Tanaka T, Tajima M, Tsuboi R, Kato H, Nishikawa A, et al. Anti-*Malassezia*-Specific IgE Antibodies Production in Japanese Patients with Head and Neck Atopic Dermatitis: Relationship between the Level of Specific IgE Antibody and the Colonization Frequency of Cutaneous *Malassezia* Species and Clinical Severity. J Allergy (Cairo). 2011;2011:645670.

33. Arzumanyan VG, Magarshak OO, Semenov BF. Yeast fungi in patients with allergic diseases: species variety and sensitivity to antifungal drugs. Bull Exp Biol Med. 2000;129(6):601-4.

34. Buslau M, Menzel I, Holzmann H. Fungal flora of human faeces in psoriasis and atopic dermatitis. Mycoses. 1990;33(2):90-4.

35. Oliveira ADT, Sodre CS, Ferreira DC, Abad ED, Saintive S, Ribeiro M, et al. Oral Aspects Identified in Atopic Dermatitis Patients: A Literature Review. Open Dent J. 2018;12:424-34.

36. Javad G, Taheri Sarvtin M, Hedayati MT, Hajheydari Z, Yazdani J, Shokohi T. Evaluation of *Candida* Colonization and Specific Humoral Responses against *Candida albicans* in Patients with Atopic Dermatitis. Biomed Res Int. 2015;2015:849206.

37. Leibovici V, Alkalay R, Hershko K, Ingber A, Westerman M, Leviatan-Strauss N, et al. Prevalence of *Candida* on the tongue and intertriginous areas of psoriatic and atopic dermatitis patients. Mycoses. 2008;51(1):63-6.

38. Jones HE, Reinhardt JH, Rinaldi MG. A clinical, mycological, and immunological survey for dermatophytosis. Arch Dermatol. 1973;108(1):61-5.

39. Jones H. The atopic-dermatophytosis syndrome. Acta Dermatovener. 1980;Suppl(92):81-5.

40. Zhang E, Tanaka T, Tajima M, Tsuboi R, Nishikawa A, Sugita T. Characterization of the skin fungal microbiota in patients with atopic dermatitis and in healthy subjects. Microbiol Immunol. 2011;55(9):625-32.

41. Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin microbiome in atopic dermatitis: a systematic review. Br J Dermatol. 2017;177(5):1272-8.

42. Kim JE, Kim HS. Microbiome of the Skin and Gut in Atopic Dermatitis (AD): Understanding the Pathophysiology and Finding Novel Management Strategies. J Clin Med. 2019;8(4).

43. Thomsen SF, Ulrik CS, Kyvik KO, Hjelmborg J, Skadhauge LR, Steffensen I, et al. Importance of genetic factors in the etiology of atopic dermatitis: a twin study. Allergy Asthma Proc. 2007;28(5):535-9.

44. Liang Y, Chang C, Lu Q. The Genetics and Epigenetics of Atopic Dermatitis-Filaggrin and Other Polymorphisms. Clin Rev Allergy Immunol. 2016;51(3):315-28.

45. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med. 2002;347(15):1151-60.

46. Oh J, Conlan S, Polley EC, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. Genome Med. 2012;4(10):77.

47. Saintive S, Abad E, D CF, Stambovsky M, Cavalcante FS, Goncalves LS, et al. What is the role of *Staphylococcus aureus* and herpes virus infections in the pathogenesis of atopic dermatitis? Future Microbiol. 2017.

48. Beck LA, Boguniewicz M, Hata T, Schneider LC, Hanifin J, Gallo R, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. J Allergy Clin Immunol. 2009;124(2):260-9, 9 e1-7.

49. Chen X, Anstey AV, Bugert JJ. Molluscum contagiosum virus infection. Lancet Infect Dis. 2013;13(10):877-88.

50. Darabi K, Hostetler SG, Bechtel MA, Zirwas M. The role of *Malassezia* in atopic dermatitis affecting the head and neck of adults. J Am Acad Dermatol. 2009;60(1):125-36.

51. Buentke E, Scheynius A. Dendritic cells and fungi. APMIS. 2003;111(7-8):789-96.

52. Brasch J, Morig A, Neumann B, Proksch E. Expression of antimicrobial peptides and toll-like receptors is increased in tinea and pityriasis versicolor. Mycoses. 2014;57(3):147-52.

53. Baroni A, Orlando M, Donnarumma G, Farro P, Iovene MR, Tufano MA, et al. Toll-like receptor 2 (TLR2) mediates intracellular signalling in human keratinocytes in response to *Malassezia furfur.* Arch Dermatol Res. 2006;297(7):280-8.

54. Kistowska M, Fenini G, Jankovic D, Feldmeyer L, Kerl K, Bosshard P, et al. *Malassezia* yeasts activate the NLRP3 inflammasome in antigen-presenting cells via Syk-kinase signalling. Exp Dermatol. 2014;23(12):884-9.

55. Gehrmann U, Qazi KR, Johansson C, Hultenby K, Karlsson M, Lundeberg L, et al. Nanovesicles from *Malassezia sympodialis* and host exosomes induce cytokine responses--novel mechanisms for host-microbe interactions in atopic eczema. PLoS One. 2011;6(7):e21480.

56. Kortekangas-Savolainen O, Kalimo K, Lammintausta K, Savolainen J. IgE-binding components of baker's yeast (*Saccharomyces cerevisiae*) recognized by immunoblotting

analysis. Simultaneous IgE binding to mannan and 46-48 kD allergens of *Saccharomyces cerevisiae* and *Candida albicans*. Clin Exp Allergy. 1993;23(3):179-84.

57. Nissen D, Petersen LJ, Esch R, Svejgaard E, Skov PS, Poulsen LK, et al. IgE-sensitization to cellular and culture filtrates of fungal extracts in patients with atopic dermatitis. Ann Allergy Asthma Immunol. 1998;81(3):247-55.

58. Tanaka M, Aiba S, Matsumura N, Aoyama H, Tabata N, Sekita Y, et al. IgE-mediated hypersensitivity and contact sensitivity to multiple environmental allergens in atopic dermatitis. Arch Dermatol. 1994;130(11):1393-401.

59. Nermes M, Falth-Magnusson K, Savolainen J, Viander M, Bjorksten B. A comparison of the development of antibody responses to the polysaccharide antigen (*Candida albicans* mannan) in atopic and healthy infants and children. Clin Exp Allergy. 1996;26(2):164-70.

60. Deuell B, Arruda LK, Hayden ML, Chapman MD, Platts-Mills TA. *Trichophyton tonsurans* allergen. I. Characterization of a protein that causes immediate but not delayed hypersensitivity. J Immunol. 1991;147(1):96-101.

61. Woodfolk JA, Slunt JB, Deuell B, Hayden ML, Platts-Mills TA. Definition of a *Trichophyton* protein associated with delayed hypersensitivity in humans. Evidence for immediate (IgE and IgG4) and delayed hypersensitivity to a single protein. J Immunol. 1996;156(4):1695-701.

62. Kivity S, Schwarz Y, Fireman E. The association of perennial rhinitis with *Trichophyton* infection. Clin Exp Allergy. 1992;22(4):498-500.

63. McGregor JM, Hamilton AJ, Hay RJ. Possible mechanisms of immune modulation in chronic dermatophytoses: an *in vitro* study. Br J Dermatol. 1992;128:233-8.

64. Rajka G, Barlinn C. On the significance of the trichophytin reactivity in atopic dermatitis. Acta Derm Venereol. 1979;59(1):45-7.

65. Mungan D, Bavbek S, Peksari V, Celik G, Gugey E, Misirligil Z. *Trichophyton* sensitivity in allergic and nonallergic asthma. Allergy. 2001;56(6):558-62.

66. Escalante MT, Sanchez-Borges M, Capriles-Hulett A, Belfort E, Di Biagio E, Gonzalez-Aveledo L. *Trichophyton*-specific IgE in patients with dermatophytosis is not associated with aeroallergen sensitivity. J Allergy Clin Immunol. 2000;105(3):547-51.

67. Rubenstein RM, Malerich SA. *Malassezia* (*Pityrosporum*) folliculitis. J Clin Aesthet Dermatol. 2014;7(3):37-41.

68. Perugia C, Saraceno R, Ventura A, Lore B, Chiaramonte C, Docimo R, et al. Atopic dermatitis and dental manifestations. G Ital Dermatol Venereol. 2017;152(2):122-5.

69. Nenoff P, Kruger C, Schaller J, Ginter-Hanselmayer G, Schulte-Beerbuhl R, Tietz HJ. Mycology - an update part 2: dermatomycoses: clinical picture and diagnostics. J Dtsch Dermatol Ges. 2014;12(9):749-77.

70. Pihet M, Le Govic Y. Reappraisal of Conventional Diagnosis for Dermatophytes. Mycopathologia. 2017;182(1-2):169-80.

71. Hamer EC, Moore CB, Denning DW. Comparison of two fluorescent whiteners, Calcofluor and Blankophor, for the detection of fungal elements in clinical specimens in the diagnostic laboratory. Clin Microbiol Infect. 2006;12(2):181-4.

72. Afshar P, Larijani LV, Rouhanizadeh H. A comparison of conventional rapid methods in diagnosis of superficial and cutaneous mycoses based on KOH, Chicago sky blue 6B and calcofluor white stains. Iran J Microbiol. 2018;10(6):433-40.

73. Prakash R, Prashanth HV, Ragunatha S, Kapoor M, Anitha TK, Krishnamurthy V. Comparative study of efficacy, rapidity of detection, and cost-effectiveness of potassium hydroxide, calcofluor white, and Chicago sky blue stains in the diagnosis of dermatophytoses. Int J Dermatol. 2016;55(4):e172-5.

74. Tambosis E, Lim C. A comparison of the contrast stains, Chicago blue, chlorazole black, and Parker ink, for the rapid diagnosis of skin and nail infections. Int J Dermatol. 2012;51(8):935-8.

75. Mourad B, Ismail M, Hawwam S, Msseha M, Hassan R. Evaluation Of The Efficacy Of Fluorescent Staining And Chicago Sky Blue Staining As Methods For Diagnosis Of Dermatophytosis In Hair And Nails. Clin Cosmet Investig Dermatol. 2019;12:751-8.

76. Baddireddy K, Poojary S. A Novel Contrast Stain for the Rapid Diagnosis of Dermatophytoses: A Cross-Sectional Comparative Study of Chicago Sky Blue 6B Stain, Potassium Hydroxide Mount and Culture. Indian J Dermatol. 2019;64(4):311-4.

77. Walsh TJMD, Hayden RT, Larone DH. Larone's medically important fungi : a guide to identification. 6th edition. ed. Washington, DC: ASM Press; 2018.

78. Eichenfield LF, Tom WL, Berger TG, Krol A, Paller AS, Schwarzenberger K, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. J Am Acad Dermatol. 2014;71(1):116-32.

79. Liu L, Ong G. A randomized, open-label study to evaluate an intermittent dosing regimen of fluticasone propionate 0.05% cream in combination with regular emollient skin care in reducing the risk of relapse in pediatric patients with stabilized atopic dermatitis. J Dermatolog Treat. 2018;29(5):501-9.

80. Hanifin J, Gupta AK, Rajagopalan R. Intermittent dosing of fluticasone propionate cream for reducing the risk of relapse in atopic dermatitis patients. Br J Dermatol. 2002;147(3):528-37.

81. Simpson EL, Chalmers JR, Hanifin JM, Thomas KS, Cork MJ, McLean WH, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. J Allergy Clin Immunol. 2014;134(4):818-23.

82. Loden M. Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders. Am J Clin Dermatol. 2003;4(11):771-88.

83. Purnamawati S, Indrastuti N, Danarti R, Saefudin T. The Role of Moisturizers in Addressing Various Kinds of Dermatitis: A Review. Clin Med Res. 2017;15(3-4):75-87.

84. Weidinger S, Novak N. Atopic dermatitis. Lancet. 2016;387(10023):1109-22.

85. Danby SG, AlEnezi T, Sultan A, Lavender T, Chittock J, Brown K, et al. Effect of olive and sunflower seed oil on the adult skin barrier: implications for neonatal skin care. Pediatr Dermatol. 2013;30(1):42-50.

86. Coondoo A, Phiske M, Verma S, Lahiri K. Side-effects of topical steroids: A long overdue revisit. Indian Dermatol Online J. 2014;5(4):416-25.

87. Back O, Faergemann J, Hornqvist R. *Pityrosporum* folliculitis: a common disease of the young and middle-aged. J Am Acad Dermatol. 1985;12(1 Pt 1):56-61.

88. White I, Blatchford N. The treatment of secondary bacterial infections in atopic eczema with miconazole plus hydrocortisone. Br J Clin Pract. 1983;37(6):215-6, 22.

89. Svejgaard E, Larsen PO, Deleuran M, Ternowitz T, Roed-Petersen J, Nilsson J. Treatment of head and neck dermatitis comparing itraconazole 200 mg and 400 mg daily for 1 week with placebo. J Eur Acad Dermatol Venereol. 2004;18(4):445-9.

90. Lintu P, Savolainen J, Kortekangas-Savolainen O, Kalimo K. Systemic ketoconazole is an effective treatment of atopic dermatitis with IgE-mediated hypersensitivity to yeasts. Allergy. 2001;56(6):512-7.

91. Kanda N, Enomoto U, Watanabe S. Anti-mycotics suppress interleukin-4 and interleukin-5 production in anti-CD3 plus anti-CD28-stimulated T cells from patients with atopic dermatitis. J Invest Dermatol. 2001;117(6):1635-46.

92. Sugita T, Tajima M, Ito T, Saito M, Tsuboi R, Nishikawa A. Antifungal activities of tacrolimus and azole agents against the eleven currently accepted *Malassezia* species. J Clin Microbiol. 2005;43(6):2824-9.

93. Edwards SK, Bunker CB, Ziller F, van der Meijden WI. 2013 European guideline for the management of balanoposthitis. Int J STD AIDS. 2014;25(9):615-26.

94. Stary A, Soeltz-Szoets J, Ziegler C, Kinghorn GR, Roy RB. Comparison of the efficacy and safety of oral fluconazole and topical clotrimazole in patients with candida balanitis. Genitourin Med. 1996;72(2):98-102.

95. Back O, Scheynius A, Johansson SG. Ketoconazole in atopic dermatitis: therapeutic response is correlated with decrease in serum IgE. Arch Dermatol Res. 1995;287(5):448-51.

96. Morita E, Hide M, Yoneya Y, Kannbe M, Tanaka A, Yamamoto S. An assessment of the role of *Candida albicans* antigen in atopic dermatitis. J Dermatol. 1999;26(5):282-7.

97. Adachi A, Horikawa T, Ichihashi M, Takashima T, Komura A. [Role of *Candida* allergen in atopic dermatitis and efficacy of oral therapy with various antifungal agents]. Arerugi. 1999;48(7):719-25.

98. El-Gohary M, van Zuuren EJ, Fedorowicz Z, Burgess H, Doney L, Stuart B, et al. Topical antifungal treatments for tinea cruris and tinea corporis. Cochrane Database Syst Rev. 2014(8):CD009992.

99. van Zuuren EJ, Fedorowicz Z, El-Gohary M. Evidence-based topical treatments for tinea cruris and tinea corporis: a summary of a Cochrane systematic review. Br J Dermatol. 2015;172(3):616-41.

100. Wilson BB, Deuell B, Mills TA. Atopic dermatitis associated with dermatophyte infection and *Trichophyton* hypersensitivity. Cutis. 1993;51(3):191-2.

101. Klein PA, Clark RA, Nicol NH. Acute infection with *Trichophyton rubrum* associated with flares of atopic dermatitis. Cutis. 1999;63(3):171-2.

102.Tateishi Y, Sato H, Akiyama M, Abe M, Kobayashi H, Umehara S, et al. Severe generalized deep dermatophytosis due to *Trichophyton rubrum* (trichophytic granuloma) in a patient with atopic dermatitis. Arch Dermatol. 2004;140(5):624-5.

Table 1. Clinical manifestations, diagnosis, and management of fungi associated with AD (KOH: potassium hydroxide preparation; AD: atopic dermatitis)

Fungi associated with AD	Clinical manifestations	Diagnosis	Management
***Malassezia* species**	hypo/hyperpigmented macules/patches	KOH, culture, biochemical tests, molecular tests (optional)	Topical azole treatment (e.g. ketoconazole 2%, econazole 1% , miconazole 2%), 50% propylene glycol in water twice-daily for 3-4 weeks [87, 88] Systemic azole treatment (200mg of itraconazole for 2 weeks) for head-and-neck lesions [2, 89, 90]
***Candida* species**	Red patches/plaques with satellite papules/pustules	KOH, culture, biochemical tests, molecular tests (optional)	Topical azole treatment (clotrimazole 1% or miconazole 2% twice daily for 1-2 weeks) (Nystatin cream 100,000 units/g in case of imidazole allergy) [93] Systemic azole treatment (fluconazole 150 mg single dose) in refractory cases [2, 90, 94-97]
Dermatophytes	Annular or ringworm-like lesion with scaly active border	KOH, culture with microscopic morphology, molecular tests (optional)	Topical antifungal drugs e.g. azoles, allylamines, butenafine, ciclopirox, and tolnaftate once or twice daily for 1-3 weeks [98, 99] Systemic azole treatment (200mg/day itraconazole) in recalcitrant AD with dermatophyte co-infections [2, 100-102]

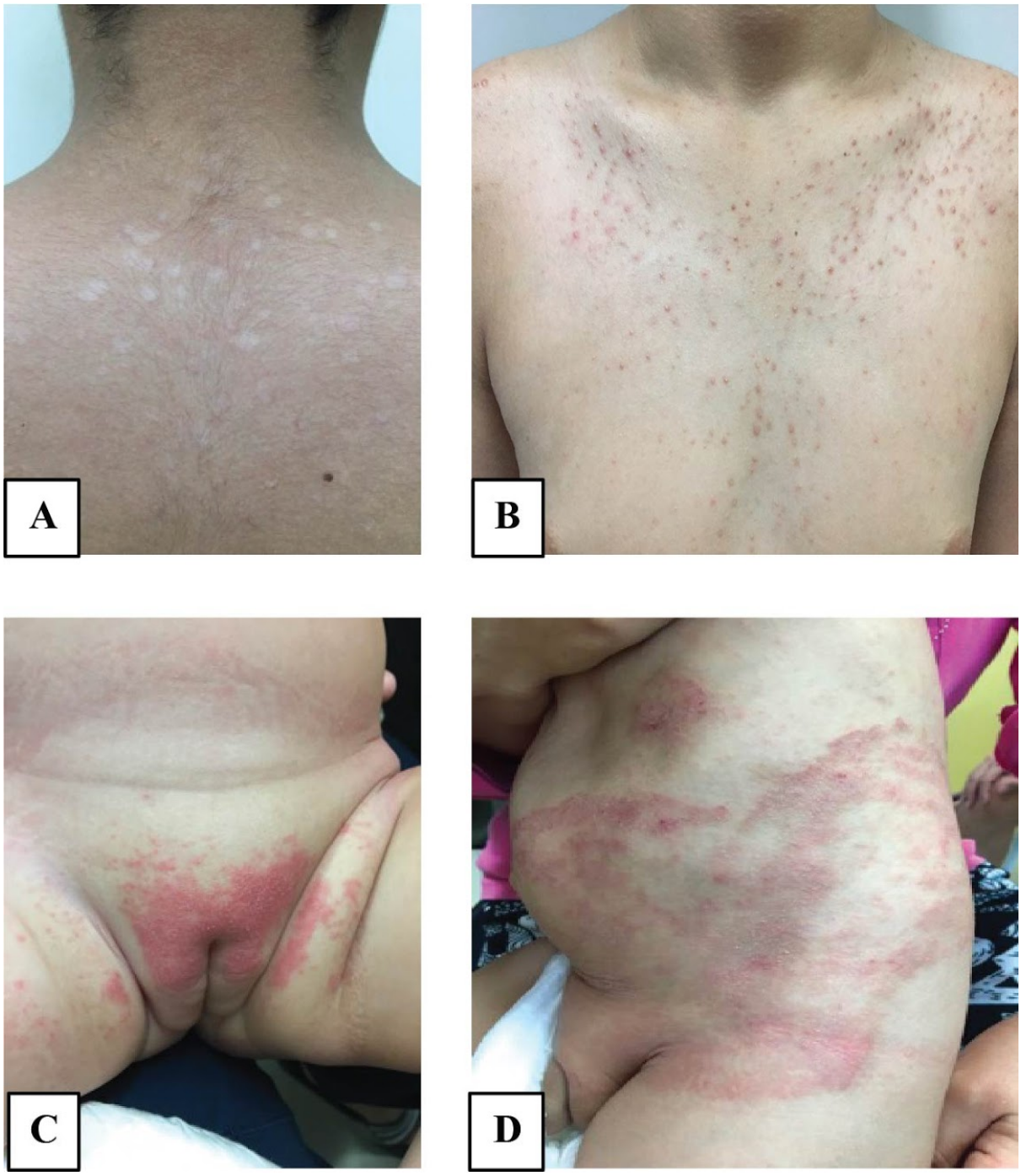

Figure 1.

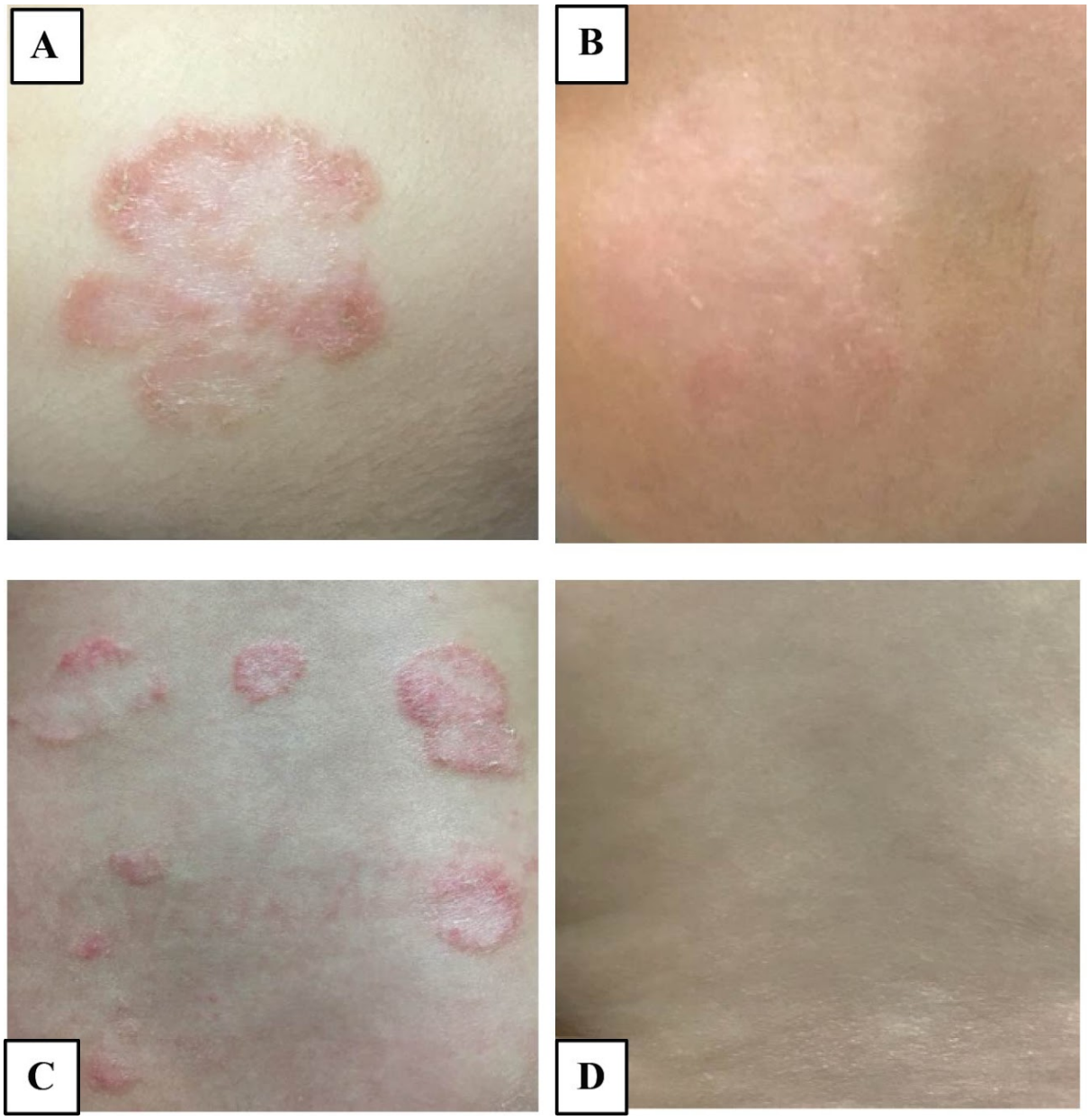

Figure 2.

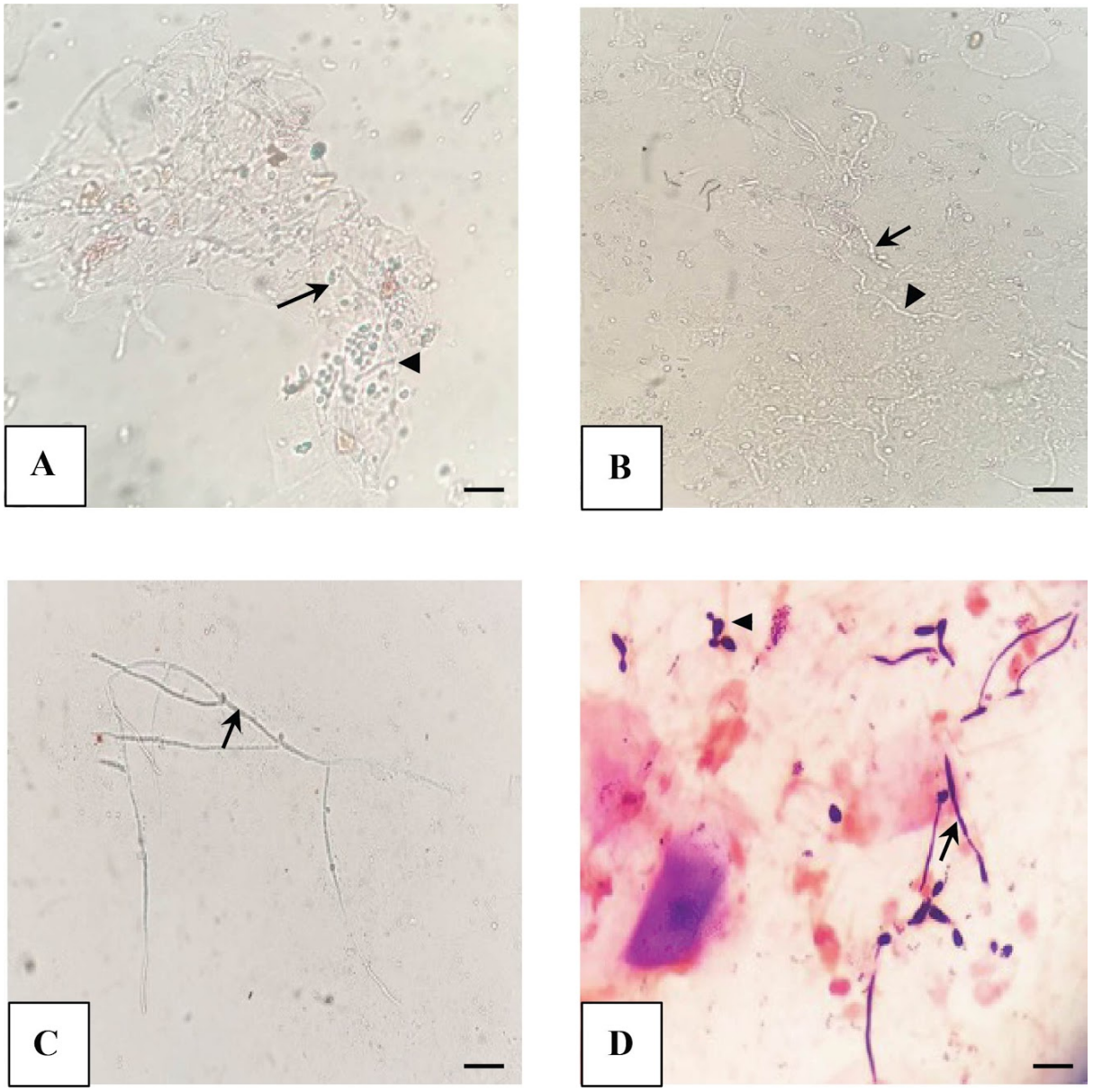

Figure 3.