E-ISSN 2228-8082
Volume 75, Number 2, February 2023

SMJ

Siriraj Medical Journal

The world-leading biomedical science of Thailand

SPECIAL ISSUE

MONTHLY

ORIGINAL ARTICLE

REVIEW ARTICLE

Indexed by Scopus®

PRINCE MAHIDOL AWARD CONFERENCE 2023

SETTING A NEW HEALTH AGENDA

Thai Association for Gastrointestinal

Thailand

COLLEGE INTERNATIONAL DE CHIRURGIENS THAILAND SECTION 1954

MESDA Mekong Endo-Surgery Development Association

https://he02.tci-thaijo.org/index.php/sirirajmedj/index

E-mail: sijournal92@gmail.com

Siriraj Medical Journal SMJ

Volume 75, Number 2, February 2023

ORIGINAL ARTICLE

62 **A 30-year Patch Testing Experience at Siriraj Dermatology**
Silada Kanokrungsee, et al.

70 **Patch Testing of Thai Children with Eczema**
Pailin Puangpet, et al.

76 **Perception and Management Practice of Onychomycosis among Thai Physicians**
Pichaya Limphoka, et al.

85 **Clinical Features of Adult Male Acne in a Tropical Country: A Prospective Cross- sectional Study**
Chayanee Likitwattananurak, et al.

92 **Human-pet Relationship, Pet Abandonment, and Clinical Correlation for Patients Infected with Dermatophytosis of the Glabrous Skin**
Sanchai Sombatmaithai, et al.

99 **Association Between the Level of Inflammation at Each Anatomical Sexual Activity from Gram Staining and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Infections**
Kittipoom Chinhiran, et al.

106 **Prevalence and Trend of Photodermatoses in Thailand: A 16-year Retrospective Study in a Tertiary Care Hospital**
Surachanee Likittanasombat, et al.

115 **Validity and Reliability of the Topical Corticosteroid Phobia (TOPICOP©) Questionnaire: Thai version**
Niorn Boonpuen, et al.

121 **Diphenylcyclopropenone Treatment Outcomes for Alopecia Areata**
Supenya Varothai, et al.

132 **Evaluation of Hair Follicle Counts of Occipital Scalp Biopsies from Male Hair Transplant Patients in Thailand**
Penvadee Pattanaprichakul, et al.

138 **Histopathological Diagnosis of Alopecia Clinically Relevant to Alopecia Areata**
Chinmanat Lekhavat, et al.

REVIEW ARTICLE

145 **Direct Immunofluorescence in Cutaneous and Systemic Lupus Erythematosus: A Literature Review**
Chuda Rujitharanawong, et al.

SIRIRAJ MEDICAL JOURNAL

www.smj.si.mahidol.ac.th

SIRIRAJ MEDICAL JOURNAL is published bimonthly, 6 issues a year (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec) and distributed by the end of the last month of that issue.
SIRIRAJ MEDICAL JOURNAL is listed as a journal following the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URM) by the International Committee of Medical Journal Editors (ICMJE) since 9 July 2010 [http://www.icmje.org/journals.html].

A 30-year Patch Testing Experience at Siriraj Dermatology

Silada Kanokrungsee, M.D., Pichanee Chaweekulrat, M.D., Chayada Chaiyabutr, M.D., Suthasanee Prasertsook, M.D., Chudapa Sereeaphinan, M.D., Monthathip Bunyavaree, B.Sc., Titinun Kumpangsin, B.Sc., Janista Thumrongtharadol, B.Sc., Waranya Boonchai, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To identify trends of contact allergy and patch testing amendments at the Contact Dermatitis Clinic, Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University.

Materials and Methods: Medical records of 6,862 patients referred to our clinic between January 1992 and December 2021 for patch testing were reviewed.

Results: The number of patients patch tested increased and reached a peak of 600 patients/year in 2019 before the COVID-19 pandemic. The most frequently used series was baseline, while the most used specific series was cosmetics. The overall positivity rate was 69%. The highest positivity rate was in the cosmetics series (70.2%). Nickel sulfate was the most common contact allergen found (24.2%).

Conclusion: Our patch test service has been growing in the last 30 years. The series of allergens used for patch testing has been amended every few years to be up-to-date with current global trends of contact allergies. Continual surveillance of contact prevalence and periodic updating of those series are necessary to enhance our ability to detect culprit contact allergens, which could help us improve care of our patients.

Keywords: Patch test; contact dermatitis; Siriraj; contact allergens; skin allergy; Thailand (Siriraj Med J 2023; 75: 62-69)

INTRODUCTION

Allergic contact dermatitis (ACD) is a common skin disease caused by immunological hypersensitivity to particular substances. Patch testing is considered the gold standard diagnostic procedure for identifying specific allergens that cause contact skin allergies. The baseline allergens, which contain common contact allergens found in certain populations is recommended for patch testing in every patient.[1] Furthermore, additional series are selected and used in testing based on a patient's rash and exposure history.[2] Specific allergens in each patch test series are updated, depending on the current trends of allergenicity at that time.[3]

History

Patch tests were introduced as a diagnostic tool for ACD in the late nineteenth century by German physician Joseph Jadassohn.[4] Contact Dermatitis Clinic, at Siriraj Hospital was established by Clinical Professor Emeritus Patcharee Sunthonpalin, M.D., who wrote the first and only *Contact Dermatitis* textbook in Thai. The clinic has been in operation since 1972 under the Department of Medicine, Faculty of Medicine Siriraj Hospital. The clinic started with six allergen series, including baseline, cosmetic, fragrance & botanical, textile, photoallergen & sunscreen, and the hair series. In 2010, our clinic hired more staff and expanded its services. In 2013, the name

Corresponding author: Waranya Boonchai
E-mail: waranya.boonchai@gmail.com
Received 10 June 2022 Revised 27 June 2022 Accepted 6 July 2022
ORCID ID:http://orcid.org/0000-0002-6673-6534
https://doi.org/10.33192/smj.v75i2.260755

of clinic was changed to the Occupational and Contact Dermatitis Clinic to broaden the range of disease focus. Since this time, many studies have been published in various international academic journals. There has also been improvement in our services, such as the creation of a own Nickel spot test (dimethylglyoxime),[5] cobalt test,[5] skin marking pen, Thai mite allergen for patch testing and a Thai database of Contact Allergen Avoidance Program or CAAP,[5,6] in accordance with Mahidol University's "Wisdom of the Land" to promote higher education and help society achieve a better quality of life. Currently, we have over 30 years of experience providing patch testing and treating contact and occupational-related dermatitis. Nowadays, we are the standard referral center for patch testing, and seventeen kinds of allergen series are available.[7]

As this year is the 30th anniversary of the founding of the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, we aimed to publish the outcomes and trends of patch testing in our clinic over its history to celebrate this memorable achievement.

MATERIALS AND METHODS

Study design and participants

Retrospective charts between January 1992 and December 2021 were reviewed at the Occupational and Contact Dermatitis Clinic, Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Patients' characteristics and patch test results were collected. This study was approved by the Siriraj Institutional Review Board (COA no. Si 808/2021).

Patch testing was carried out according to guidelines by the International Contact Dermatitis Research Group.[8] The allergens (Chemotechnique Diagnostics, Vellinge, Sweden) in aluminium Finn Chambers (SmartPractice, Phoenix, Arizona) were applied on the patients' upper back for 48 hours. The dermatologists measured the readings on day 2, 4 and 7, and the reaction was scored according to the guideline. Siriraj baseline series were adapted from the International standard and European baseline series.[7,9] Some patients were tested with additional series such as the cosmetics series, and hairdressing series, depending on patients' clinical histories.

Some series were not universally available at that period of time such as pigmented series that composed of 24 allergens; 13 allergens are allergen from baseline series such as paraphenylenediamine, fragrance etc. plus 11 particular allergens were jasmine oil, Ylang-ylang oil, benzyl salicylate, ammonium mercury, sudan I, disperse orange 3, disperse yellow 3, hydroquinone, coal tar, tricosan, and tretinoin. These allergens had been reported causing hyperpigmentation.

Statistical analysis

PASW Statistics for Windows, version 18 (SPSS Inc., Chicago, IL, USA), was used for data analysis. Categorical data was described using frequency and percentage. Continuous data was reported using mean and standard deviation for data with normal distribution or median and interquartile range for data with non-normal distribution.

RESULTS

Our clinic performed patch tests on a total 6,862 patients over a 30-year period. Most patients were female (78.6%), and the median age was 40 (29.0, 52.0) years old. Also, most patients were 18 – 59, or of working-age. Personal and family history of atopy was found in 42% and 32% of cases, respectively. Almost 40% of patients had a history of metal allergy, while only 15% had history of cosmetic allergy (Table 1).

The average number of patients who underwent patch testing was 229 (±158) patients/year. Between 1992 and 2006, the number of patients tested was approximately 100 patients/year, before it rose to almost 600 patients/year in 2019 (Fig 1). However, the number of patients tested decreased in 2020 and 2021 due to the COVID-19 pandemic. The positivity rate was 69%, and it ranged between 60% and 90% each year. Table 2 shows all positivity rates of each series in five-year intervals over the 30-year study period.

The most frequent additional allergen series used besides the baseline over the 30-year period were cosmetics, cheilitis and hairdressing in 1,203 (17.5%), 493 (7.2%) and 292 (4.3%) patients, respectively. The positivity rate varied in each series over time (Table 2). The cosmetic series had the highest positivity rate of 70.2%, and at times reached as high as 80%, followed by melasma (66.7%), baseline (66.5%) and pigmented cosmetic series (65.1%).

Regarding allergens in the baseline series, the one with the highest positivity rate over 30 years was nickel sulfate (24.2%), followed by gold sodium thiosulfate (19.9%), linalool hydroperoxide (13.0%) and fragrance mix I (12.95%). The top three most common allergens during each five-year period were three metals (nickel, gold sodium thiosulfate, potassium dichromate), two fragrances (fragrance mixed I, linalool hydroperoxide) and two preservatives (paraben mix, methylisothiazolinone) as displayed in Fig 2. Common sensitizers throughout the course of the study are summarized in Fig 2. The constant rate of nickel was approximately 20-30%, with the highest clinical relevance rate at 83.23% over the past 30 years. Fragrance allergens, including fragrance mix I, mix II and balsum of Peru gradually decreased after

TABLE 1. Demographic data.

Demographic data	n	(%)
Gender, n = 6,862		
Male	1,466	(21.4)
Female	5,396	(78.6)
Age, years (median; IQR), n= 6,851	40.0 (29.0 – 52.0)	
< 18 years, n (%)	221	(3.2)
18-59 years, n (%)	5,072	(83.1)
≥ 60 years, n (%)	928	(13.5)
Occupation, n= 6,702		
Blue collar workers*	1,470	21.9
White collar workersᵀ	3,792	56.6
Unemployed/retired	752	11.2
Student	688	10.3
Median duration of symptoms (months) (IQR)	12.0 (4.0 – 36.0)	
Personal history of atopy, n=6,859	2,908	(42.4)
Familial history of atopy, n=6,390	2,040	(31.9)
History of metal allergy, n=6,515	2,429	(37.3)
History of cosmetic allergy, n= 6,350	923	(14.5)
Positive for at least one allergen, n=6,862	4,738	(69.0)

*, Blue-collar worker refers to workers who engage in hard manual labor, typically agriculture, manufacturing, construction, homemaker, mechanic, maintenance, or another physically exhausting task that implies belonging to a lower social class.
ᵀ,White-collar workers are known as suit-and-tie workers who often avoid physical labor which implies belonging to a higher social class

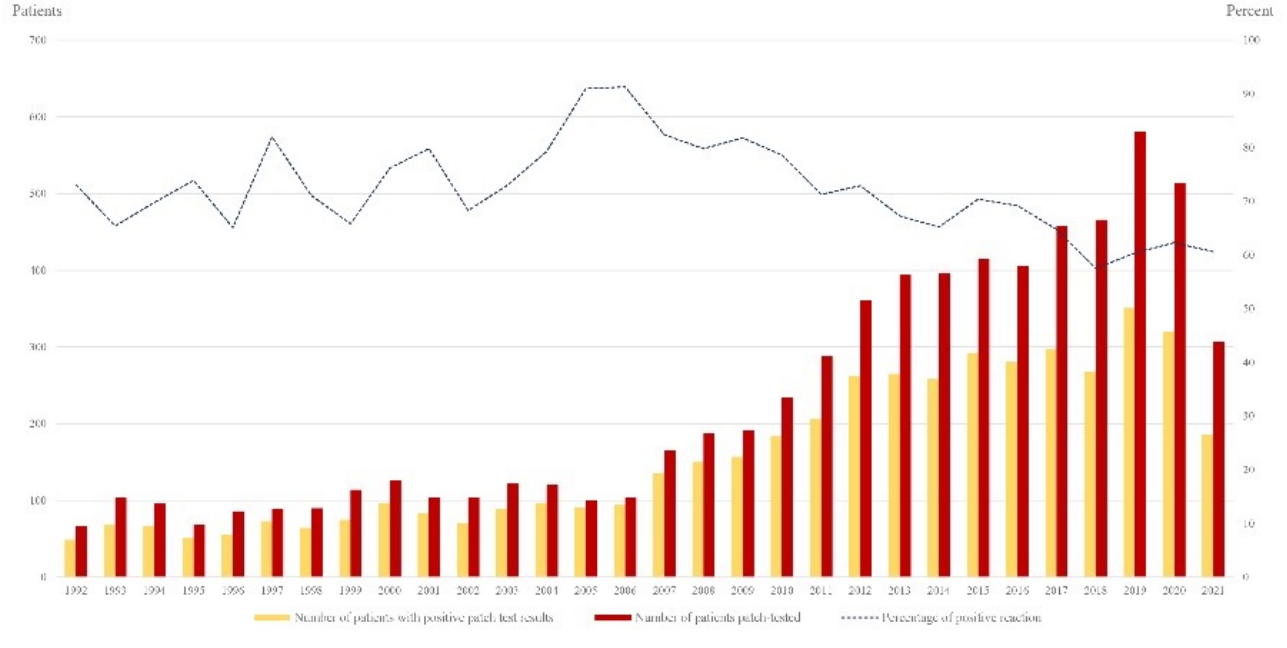


Fig 1. Number of patients referred for patch testing and percentage of positive reactions between 1992 and 2021.

TABLE 2. Positive patch test results in each patch test series.

Patch test series	Positive patch test results Total Number of patients tested	Number of patients with positive reaction n (%)	Year 1992-1996 n (%)	Year 1997-2001 n (%)	Year 2002-2006 n (%)	Year 2007-2011 n (%)	Year 2012-2016 n (%)	Year 2017-2021 n (%)
Baseline	5527	3673(66.5)	261/377(69.2)	289/371(77.9)	303/359(84.4)	553/701(78.9)	957/1427(67.1)	1310/2292(57.2)
Cosmetic	1203	845(70.2)	37/60(61.7)	91/121(75.2)	69/95(72.6)	147/176(83.5)	263/328(80.2)	238/423(56.3)
Cheilitis	493	292(59.2)	0/0(0.0)	0/0(0.0)	38/47(80.9)	64/94(68.1)	90/145(62.1)	100/207(48.3)
Hair dressing	292	125(42.8)	4/9(44.4)	15/22(68.2)	8/17(47.1)	21/25(84.0)	30/72(41.7)	47/147(32.0)
Photoallergen	207	27(13.0)	0/2(0.0)	6/39(15.4)	5/32(15.6)	2/23(8.7)	6/72(8.3)	8/39(20.5)
Eyelid	152	73(48.0)	0/0(0.0)	0/0(0.0)	1/1(100.0)	20/23(87.0)	32/59(54.2)	20/69(29.0)
Corticosteroids	151	10(6.6)	0/0(0.0)	4/46(8.7)	2/10(20.0)	1/26(3.8)	2/29(6.9)	1/40(2.5)
Plastic & Glue	144	27(18.8)	2/24(8.3)	5/13(38.5)	1/9(11.1)	10/23(43.5)	7/35(20.0)	2/40(5.0)
Textile	138	24(17.4)	0/15(0.0)	1/25(4.0)	6/19(31.6)	7/14(50.0)	6/30(20.0)	4/35(11.4)
Dental	128	72(56.3)	0/0(0.0)	0/0(0.0)	10/12 (83.3)	19/29(65.5)	27/52(51.9)	16/35(45.7)
Pigmented	126	82(65.1)	0/0(0.0)	0/0(0.0)	13/14(92.9)	18/26(69.2)	36/53(67.9)	15/33(45.5)
Fragrance & Botanical	61	31(50.8)	2/4(50.0)	0/3(0.0)	3/3(100.0)	7/10(70.0)	8/15(53.3)	11/29(37.9)
Prosthesis	14	7(50.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	3/7(42.9)	4/7(57.1)
Oil and cooling	13	2(15.4)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/6(0.0)	2/7(28.6%)
Melasma	12	8(66.7)	0/0(0.0)	0/0(0.0)	8/12(66.7)	0/0(0.0)	0/0(0.0)	0/0(0.0)
Shoes	9	1(11.1)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	1/9(11.1)
Bakery	9	0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/0(0.0)	0/9(0.0)
Overall	6862	4738(69.0)	291/422(69.0)	391/523(74.8)	442/551(80.2)	833/1068(78.0)	1359/1972(68.9)	1422/2326(61.1)

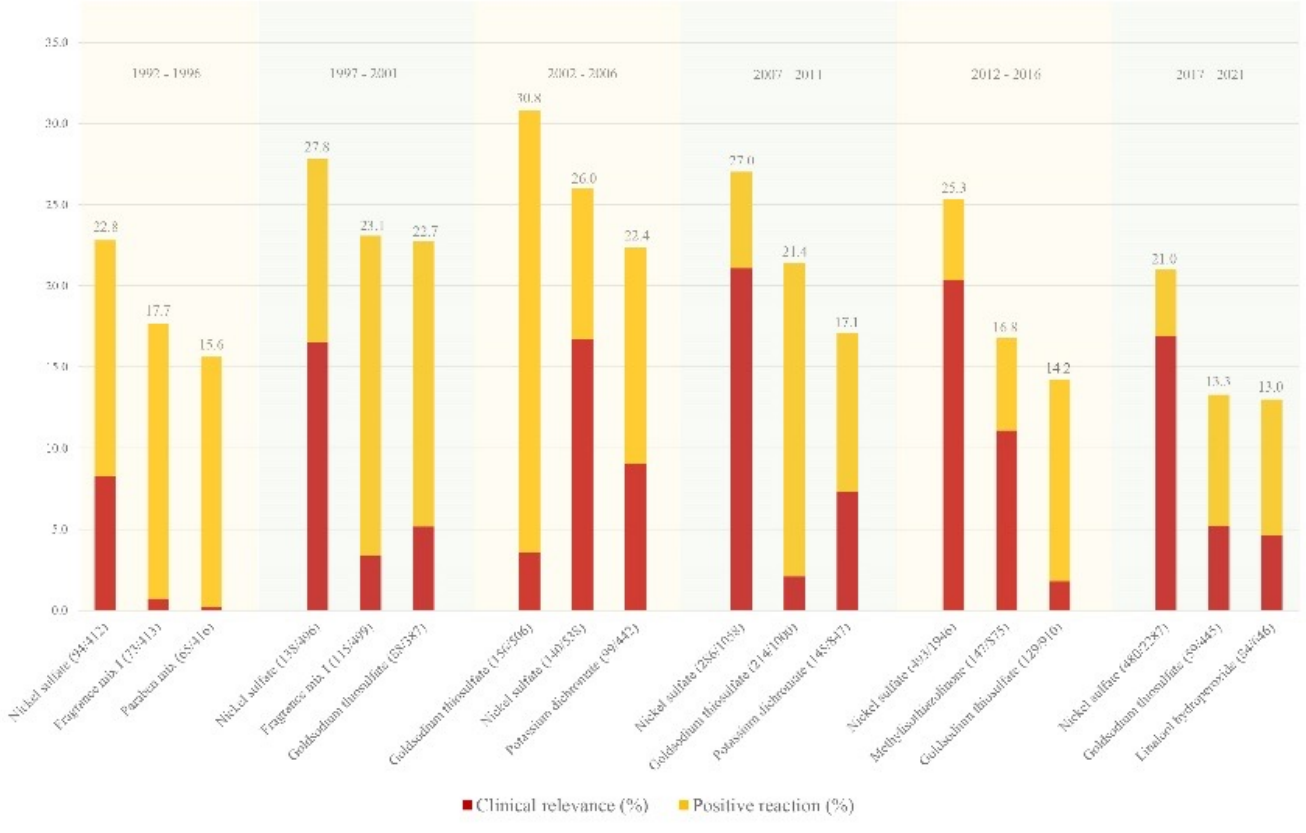


Fig 2. Common allergens in baseline series between 1992 and 2021.

2002, however, linalool hydroperoxide, a new fragrance allergen, had a high percentage of positive reactions and was a top three most common allergen in the last five years. Regarding preservative allergens, the paraben mix showed a high positivity rate in the early period of the study period (1992-1997), but it later declined. In contrast, isothiazolinone, including methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) and methylisothiazolinone (MI), saw a gradual rise in positive reactive rate, and a slight decrease after 2016.

DISCUSSION

This retrospective study uncovered big changes in the patch testing process, and allergen series over a 30-year period at the Occupational and Contact Dermatitis Clinic, Siriraj Hospital. We noticed growth in patch testing services along with changes in contact sensitizers over time. The number of patients referred for patch testing per year has progressively increased year-on-year, with a remarkable rise since 2006, and a five to six-fold increase in 2019. We believe the growth of our clinic is the result of several support systems such as our departments and faculties, along with ongoing encouragement of referring patients suspected of having ACD for patch testing. However, patch testing numbers decreased abruptly due to the COVID-19 outbreak from early 2020, like the rest of the world.[10]

The rate of positive patch test results in the baseline series, which are the standard set of allergens used in almost every patients suspected of having ACD in our clinic, was 66.5%, which is consistent with studies from the United states and Saudi Arabia as well as our previous study covering 2006-2018.[11-13] We also noticed a decline in the percentage of reactions after 2006, which coincided with an increase in the number of patients tested. This could be due to the fact that there were more patients interested in getting tested and that we were less stringent on which patients to include. There are discrepancies in patch testing positivity worldwide, ranging from 22%-55%.[14-16] This variation in positivity may be due to different exposures linked to local culture, industries, and regulations in each country. Furthermore, individual factors and the patch testing method are influential factors on the outcome as well.[17]

A chronological change in our contact allergen series was established around the world. Sensitizing allergens

in each patch test series were updated based on the current trends in allergenicity at that time.[3] Many sets of allergens are available in our clinic, and each series differs in number of substances. The cosmetic series is the second most common, after following baseline series, but it generated the highest yield. In addition, the percentage of positive results from the cosmetic series has been gradually rising. Despite a slightly downward trend in the last five years, it is worth using in patients suspected of having ACD because ninety percent of cosmetic products in the Thai market contain at least one allergen.[18] High rates of sensitization also reflect the growth of the cosmetic business and wide use of cosmetic products in our country. The melasma series was second, but it was only used between 2002-2004. Later, its allergens were merged into the cosmetic and pigmented series. Although reactions to the pigmented series have been declining, it remains the series with the fourth highest positivity rate. These findings suggest that although the baseline series already include common allergens from a specific time period, the addition of specialty allergen series relating to personal history or work products is still necessary for a complete evaluation, to increase the success rate of identifying the culprit, and to improve management of patients' allergic contact dermatitis.[11,19] However, the other series, such as, photoallergen, corticosteroid, plastic & glue, textile, oil & cooling and shoe, which have a small positivity rate, are more of a concern. This may indicate an inadequate set of allergens, which warrants a review.

Regarding individual allergens, nickel sulfate is known as the most common allergen worldwide.[11,20-22] Its prevalence in this study was steady over the time and comparable with a previous study from Thailand (24.0% in 2006-2018 vs 27.6% in 2000-2009).[9,21] However, the current study revealed a much higher share of positive reactions (24.2%) to nickel sulfate than studies from other Asian and European countries (13.9-20.4%).[11,14,22-24] A declining trend was noted in European countries following nickel regulations and introduction of novel substitute metals,[24] which contradicts results from our Thai study where there are no regulations on the specific amount of nickel allowed in nickel-containing accessories in the market.[5] The allergens, which displayed a downward trend in our study, include cobalt, gold sodium thiosulfate, fragrance mix I and II, *Myroxylon pereirae* and parabens, which were potent sensitizers in the past. This can potentially be explained by a decline in exposures to these sensitizers. Furthermore, many industries have substitutes to avoid this problem, resulting in relatively less prevalence.

For allergens showing more prevalence, hydroperoxide of linalool has been an emerging allergen since 2020. Linalool is the most common fragrance material in cosmetic products sold in the European market and has been increasingly responsible for contact allergy in Europe.[25] Our data showed that a positive reaction occurred in 13% of cases, compared to prior reports which reported a positivity rate ranging from 1.7-20%.[11,26,27] The variation depends on the exposure rate of the individual country, the popularity of the allergen in the environment, regional legislation, and the population tested. An increase in MCI/MI was also noted. MI is isothiazolinone preservative mainly used in cosmetic and personal care products. High concentrations of MCI/MI and MI caused a global epidemic in the mid-2010s.[21,28-30] Consequently, for leave-on cosmetics, the use of MCI/MI and MI as an ingredient in Europe has been banned since 2016 and 2017,[31,32] respectively, and the concentration of MI in rinse-off cosmetics has reduced since 2018 by the European Commission.[33] According to the legislation, the prevalence of isothiazolinone allergy considerably decreased after 2016. The change in MCI/MI and MI allergies in our study will likely follow global data as the Thai FDA usually takes a few years to adopt EU regulations.[34,35]

Limitation of our study was incomplete data due to long retrospective nature of study design e.g. clinical relevance of positive reaction information. Moreover, the study originated from single tertiary centre in Bangkok, Thailand, leading to lack of regional generality.

Siriraj Contact Dermatitis Clinic has been in operation for 30 years. This study reviewed 30-year outcomes at the clinic. Our patch testing service has grown over this period. The series of allergens used for patch testing is amended every few years to update it to the latest global trends of contact allergies. Continual surveillance of contact prevalence and periodic updates are necessary to enhance our ability to detect culprit contact allergens, and improve care of our patients.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Assistant Professor Dr.Chulaluk Komoltri for statistical advice and support.

Conflicts of interest: All authors have no conflicts of interest or financial support to declare.

Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit-sectors.

REFERENCES

1. Nelson JL, Mowad CM. Allergic Contact Dermatitis: Patch Testing Beyond the TRUE Test. J Clin Aesthet Dermatol. 2010;3(10):36-41.
2. Davis MD, Scalf LA, Yiannias JA, Cheng JF, El-Azhary RA, Rohlinger AL, et al. Changing trends and allergens in the patch test standard series: a mayo clinic 5-year retrospective review, January 1, 2001, through December 31, 2005. Arch Dermatol. 2008;144(1):67-72.
3. Zug KA, Warshaw EM, Fowler JF, Jr., Maibach HI, Belsito DL, Pratt MD, et al. Patch-test results of the North American Contact Dermatitis Group 2005-2006. Dermatitis. 2009;20(3):149-60.
4. Lachapelle JM. Patch testing: historical aspects. Ann Dermatol Venereol. 2009;136(8-9):575-7.
5. Boonchai W, Maneeprasopchoke P, Suiwongsa B, Kasemsarn P. Assessment of nickel and cobalt release from jewelry from a non-nickel directive country. Dermatitis. 2015;26(1):44-8.
6. Bunyavaree M, Limphoka P, Kumpangsin T, Boonchai W. Contact Allergen Avoidance Program (CAAP). Thai Journal of Dermatology. 2018;34(3):183-91.
7. Boonchai W, Kasemsarn P. Suitability of patch test allergens for standard series in Thai patients: ten-year retrospective review of patch test results. J Dermatol. 2013;40(1):65-7.
8. Pongpairoj K, Ale I, Andersen KE, Bruze M, Diepgen TL, Elsner PU, et al. Proposed ICDRG Classification of the Clinical Presentation of Contact Allergy. Dermatitis. 2016;27(5):248-58.
9. Boonchai W, Iamtharachai P. Risk factors for common contact allergens and patch test results using a modified European baseline series in patients tested during between 2000 and 2009 at Siriraj Hospital. Asian Pac J Allergy Immunol. 2014;32(1):60-5.
10. Gisondi P, Piaserico S, Conti A, Naldi L. Dermatologists and SARS-CoV-2: the impact of the pandemic on daily practice. J Eur Acad Dermatol Venereol. 2020;34(6):1196-201.
11. DeKoven JG, Silverberg JI, Warshaw EM, Atwater AR, Reeder MJ, Sasseville D, et al. North American Contact Dermatitis Group Patch Test Results: 2017-2018. Dermatitis. 2021;32(2):111-23.
12. Wentworth AB, Yiannias JA, Keeling JH, Hall MR, Camilleri MJ, Drage LA, et al. Trends in patch-test results and allergen changes in the standard series: a Mayo Clinic 5-year retrospective review (January 1, 2006, to December 31, 2010). J Am Acad Dermatol. 2014;70(2):269-75.e4.
13. El-Rab M, Al-Sheikh O. Is the European standard series suitable for patch testing in Riyadh, Saudi Arabia? Contact Dermatitis. 1995;33(5):310-4.
14. Boyvat A, Kalay-Yildizhan I. Patch test results of the European baseline series among 1309 patients in Turkey between 2013 and 2019. Contact Dermatitis. 2021;84(1):15-23.
15. Keragala BSDP, Herath HMMTB, Keragala TS, Malavi MAMH, Rodrigo C, Gunasekera CN. A seven-year retrospective analysis of patch test data in a cohort of patients with contact dermatitis in Sri Lanka. BMC Dermatol. 2019;19(1):10.
16. Bilcha KD, Ayele A, Shibeshi D, Lovell C. Patch testing and contact allergens in Ethiopia--results of 514 contact dermatitis patients using the European baseline series. Contact Dermatitis. 2010;63(3):140-5.
17. Schnuch A, Geier J, Uter W, Frosch PJ, Lehmacher W, Aberer W, et al. National rates and regional differences in sensitization to allergens of the standard series. Population-adjusted frequencies of sensitization (PAFS) in 40,000 patients from a multicenter study (IVDK). Contact Dermatitis. 1997;37(5):200-9.
18. Sukakul T, Pruksaeakanan C, Bunyavaree M, Boonchai W. Contact allergens in natural cosmetics-A market survey. J Cosmet Dermatol. 2022;26(6):2671-3.
19. Warshaw EM, Buonomo M, DeKoven JG, Pratt MD, Reeder MJ, Silverberg JI, et al. Importance of Supplemental Patch Testing Beyond a Screening Series for Patients With Dermatitis: The North American Contact Dermatitis Group Experience. JAMA Dermatol. 2021;157(12):1456-65.
20. Boonchai W, Iamtharachai P, Sunthonpalin P. Prevalence of allergic contact dermatitis in Thailand. Dermatitis. 2008;19(3):142-5.
21. Sukakul T, Chaweekulrat P, Limphoka P, Boonchai W. Changing trends of contact allergens in Thailand: A 12-year retrospective study. Contact Dermatitis. 2019;81(2):124-9.
22. Beliauskiene A, Valiukeviciene S, Uter W, Schnuch A. The European baseline series in Lithuania: results of patch testing in consecutive adult patients. J Eur Acad Dermatol Venereol. 2011;25(1):59-63.
23. Uter W, Bauer A, Belloni Fortina A, Bircher AJ, Brans R, Buhl T, et al. Patch test results with the European baseline series and additions thereof in the ESSCA network, 2015-2018. Contact Dermatitis. 2021;84(2):109-20.
24. Ahlström MG, Thyssen JP, Menné T, Johansen JD. Prevalence of nickel allergy in Europe following the EU Nickel Directive - a review. Contact Dermatitis. 2017;77(4):193-200.
25. Sukakul T, Bruze M, Mowitz M, Antelmi A, Bergendorff O, Björk J, et al. Contact allergy to oxidized linalool and oxidized limonene: Patch testing in consecutive patients with dermatitis. Contact Dermatitis. 2022;86(1):15-24.
26. Nath NS, Liu B, Green C, Atwater AR. Contact Allergy to Hydroperoxides of Linalool and D-Limonene in a US Population. Dermatitis. 2017;28(5):313-6.
27. Bråred Christensson J, Andersen KE, Bruze M, Johansen JD, Garcia-Bravo B, Giménez-Arnau A, et al. An international multicentre study on the allergenic activity of air-oxidized R-limonene. Contact Dermatitis. 2013;68(4):214-23.
28. Madsen JT, Andersen KE. Further evidence of the methylisothiazolinone epidemic. Contact Dermatitis. 2014;70(4):246-7.
29. Mahler V, Geier J, Schnuch A. Current trends in patch testing - new data from the German Contact Dermatitis Research Group (DKG) and the Information Network of Departments of Dermatology (IVDK). J Dtsch Dermatol Ges. 2014;12(7):583-92.
30. Uter W, Aalto-Korte K, Agner T, Andersen KE, Bircher AJ, Brans R, et al. The epidemic of methylisothiazolinone contact allergy in Europe: follow-up on changing exposures. J Eur Acad Dermatol Venereol. 2020;34(2):333-9.
31. The European Union Commission. Commission Regulation (EU) No 1003/2014 of 18 September 2014 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. 2014. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1003&from=FR.

32. The European Union Commission. Commission Regulation (EU) 2016/1198 of 22 July 2016 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. 2016. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R1198&from=FR.
33. The European Union Commission. Commission Regulation (EU) 2017/1224 of 6 July 2017 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. 2017. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1224&from=EN.
34. Sukakul T, Limphoka P, Boonchai W. Methylchloroisothiazolinone and/or Methylisothiazolinone Contact Allergies in Thailand. Dermatitis. 2021;32(6):375-80.
35. Sukakul T, Kanchanapenkul D, Bunyavaree M, Limphoka P, Kumpangsin T, Boonchai W. Methylchloroisothiazolinone and/or methylisothiazolinone in cosmetic products-A market survey. Contact Dermatitis. 2019;80(2):110-3.

Patch Testing of Thai Children with Eczema

Pailin Puangpet, M.D.*, Niorn Boonpuen, M.D.*, Kasira Saipornchai, M.D.*, Pattarida Poompakdeepan, M.D.*, Rungrat Suchaoin, M.D.*, John McFadden, M.D.**

**Occupational and Contact Dermatology Clinic, Institute of Dermatology, Bangkok, Thailand, **St John's Institute of Dermatology, King's College, London, UK.*

ABSTRACT

Objective: To detect contact allergy rate and common allergens in Thai children presented with eczema.

Materials and Methods: A total of 124 children, aged 1-15 years, were patch tested using a pediatric screening series of 16 allergens and relevant additional allergens. Data on clinical presentation, atopic history and test results were collected.

Results: Contact allergy was found in 51 of 124 children (41.1%) presented with all forms of eczema. The common allergens were lanolin alcohol (8.9%), cocamidopropyl betaine (8.1%), nickel sulfate (7.3%), fragrance mix I (5.6%), formaldehyde (5.6%), thimerosal (5.6%), fragrance mix II (4.8%), cobalt chloride (4.0%), methylchloroisothiazolinone/methylisothiazolinone (2.4%), methylisothiazolinone (2.4%) and thiuram mix (2.4%). Nineteen of 50 atopic dermatitis patients (38%) showed positive patch test reactions.

Conclusion: Allergic contact dermatitis is common in children. Both atopic and non-atopic patients can develop contact dermatitis. Patch testing should be performed in children presented with eczema regardless of contact dermatitis history.

Keywords: Patch test; children; pediatric; eczema; allergic contact dermatitis (Siriraj Med J 2023; 75: 70-75)

INTRODUCTION

Allergic contact dermatitis (ACD) is a cell-mediated hypersensitivity (type IV) reaction of the skin. The prevalence of ACD increases with age. Both ACD and irritant contact dermatitis in children seems to be important problems over the last years. The diagnosis of ACD is obtained with history, physical examination, and patch testing. The dermatitis observed can be both flare of the existing dermatitis and difficult-to-treat eczema. The location may be not only the direct contact sites, but also distal skin areas from 'secondary spread'.

From a systematic review, studied the data from January 1997 to May 2012, the common allergens in children and adolescents were nickel, thimerosal, cobalt, fragrance mix I, lanolin, neomycin, potassium dichromate and *Myroxylon pereirae*.[1] Frequent sources of allergens in children and adolescents are fragrances, creams, makeup, toys, hair dyes, nail polish, henna tattoos, and piercings. Therefore, the sensitization rate among these age groups can be significantly rising.[1] Previous studies showed prevalence rates of 13.3-24.5%.[2]

Nowadays, there is a wide selection of personal products that are used by children. Beauty trends in teenagers depend on cultures, social media, and influencers. Children and teenagers use cosmetics earlier and more than in the past. The contact allergens at present may not be the same.

Patch testing is an uncomfortable procedure for both children and parents. At least 3 visits are needed and patients have to avoid water on the patch test sites. These inconvenient factors might cause the clinicians being reluctant to refer patients for patch testing. Moreover, ACD can be easily misdiagnosed particularly in children who have existing atopic dermatitis. Patch testing is the

Corresponding author: Pailin Puangpet
E-mail: pailin.samutrapong@gmail.com
Received 31 July 2022 Revised 31 August 2022 Accepted 4 October 2022
ORCID ID:http://orcid.org/0000-0002-3993-6434
https://doi.org/10.33192/smj.v75i2.260740

gold standard to identify this hidden second diagnosis. If the patients appropriately avoid the causative contact allergens, the recalcitrant dermatitis can improve leading to better quality of life.[3]

The aims of this study were to detect the frequency of contact sensitization in Thai children diagnosed with all forms of eczema regardless of the contact dermatitis history and to find the common contact allergens.

MATERIALS AND METHODS

This prospective study was conducted at the Occupational and Contact Dermatitis Clinic, Institute of Dermatology, Bangkok, Thailand over 19 months. Children, aged 1-15 years, diagnosed with all forms of eczema for more than 1 month were enrolled. Children who have contraindication to patch test procedure such as active widespread eczema or taking oral corticosteroid were excluded. Demographic data: age, sex, triggered factors, history of atopy, location and duration of lesions were recorded.

All patients were patch tested with the pediatric screening series of 16 allergens. (Table 1) Screening allergens, provided by AllergEAZE®, in AllergEAZE patch test chamber® and supplemental relevant allergens (in selective cases), provided by Chemotechnique Diagnostics, Sweden, were applied on the upper back. Patch test results were interpreted at day (D) 2 and D4 according to International Contact Dermatitis Research Group (ICDRG) criteria. The number of positive patch test reactions and clinical relevance were recorded.

RESULTS

One hundred and twenty-four children, 61 females and 63 males, were included. The average age was 8 years. Forty-eight cases (38.7%) were 1–7 years old, 76 cases (61.3%) were 8-15 years old. The mean duration of eczema before patch testing was 28.5 months (1-144 months). The mean recurrent episodes of eczema flares were 5 times per year. The legs and arms were the most common site involved (Table 2). The most commonly reported trigger factors in subjects with positive patch test reaction were heat, dust, and seafood. Insomnia was reported by 20.2% of cases.

The results showed 84 positive patch test reactions in 51 patients (41.1%). Current or past relevance was detected in 18 of 75 positive patch test reactions (24%). Lanolin alcohol was the most common contact allergen in our cohort, followed by cocamidopropyl betaine, nickel sulfate, fragrance mix I, formaldehyde, thimerosal, fragrance mix II, cobalt chloride, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), methylisothiazolinone (MI) and thiuram mix. (Fig 1 and Table 3) There were 21 irritant patch test reactions from cocamidopropyl betaine, formaldehyde, cobalt chloride, MI, potassium dichromate and MCI/MI.

The additional relevant positive allergens found in 9 patients were house dust mite, benzalkonium chloride, an unknown topical corticosteroid cream and personal care products. Ten patients were patch tested with house dust mite allergen and 5 patients had a positive result (50%).

TABLE 1. Pediatric baseline series.

Substances	Concentration and vehicle
Nickel sulfate	2.5 % Petrolatum
Balsam of peru	25 % Petrolatum
Fragrance mix I	8 % Petrolatum
Fragrance mix II	14 % Petrolatum
p-tert-Butylphenol formaldehyde resin	1 % Petrolatum
Formaldehyde	1% Water
Colophony	20 % Petrolatum
Potassium dichromate	0.25 % Petrolatum
Cobalt (II) chloride	1 % Petrolatum
Thimerosal	0.1 % Petrolatum
Lanolin alcohol	30 % Petrolatum
Methylchloroisothiazolinone/methylisothiazolinone	0.02 % Water
Methylisothiazolinone	0.2 % Water
Cocamidopropyl betaine	1 % Water
Thiuram mix	1 % Petrolatum
Mercapto mix	2 % Petrolatum

TABLE 2. Anatomic sites of involvement.

Anatomic sites of involvement	Frequency
Leg	60 (48.4%)
Arm	44 (35.5%)
Hand	30 (24.2%)
Cubital fossa	29 (23.4%)
Foot	28 (22.6%)
Abdomen	19 (15.3%)
Back	18 (14.5%)
Face	15 (12.1%)
Neck	12 (9.7%)
Elbow	12 (9.7%)
Eyelid	8 (6.5%)
Lip	7 (5.6%)
Knee	4 (3.2%)
Axillae	4 (3.2%)

In addition, two or more positive allergic reactions were found in 24 children (19.4%). Of these, 7 patients had positive reaction to 3 allergens and 1 patient had positive reactions to 4 allergens (formaldehyde, thimerosal, nickel and personal product).

History of atopic dermatitis was presented in 50 of 124 subjects (40.3%), followed by allergic rhinitis (35.5%), asthma (3.2%) and allergic conjunctivitis (1.6%). Among patients with positive patch test reaction, allergic rhinitis and atopic eczema were found in 48.7% and 46.1% respectively. According to family history, allergic rhinitis was found in 46.8%, followed by atopic dermatitis 15.3%, asthma 8.1% and allergic conjunctivitis 5.6%.

Among 50 children who had atopic dermatitis history, 19 cases (38%) showed at least one positive patch test reactions. The common contact allergens in atopic dermatitis group were nickel (15.8%), lanolin alcohol (15.8%), cocamidopropyl betaine (10.5%).

DISCUSSION

ACD in children has been estimated as being uncommon. This study shows that contact sensitization

TABLE 3. Patch test reactions in children with eczema (N=124).

Allergens	Positive reaction (Total = 84)	Percent	Relevance (Total = 18)	Irritant reaction (Total = 21)
Lanolin alcohol	11	8.9	0	0
Cocamidopropyl betaine	10	8.1	2 (20%)	8
Nickel sulfate	9	7.3	4 (44.4%)	0
Fragrance mix I	7	5.6	2 (28.6%)	0
Formaldehyde	7	5.6	1 (14.3%)	6
Thimerosal	7	5.6	0	0
Fragrance mix II	6	4.8	2 (33.3%)	0
Cobalt chloride	5	4.0	2 (40%)	3
Methylchloroisothiazolinone/methylisothiazolinone	3	2.4	2 (66.7%)	1
Methylisothiazolinone	3	2.4	3 (100%)	2
Thiuram mix	3	2.4	0	0
Myroxylon pereirae	2	1.6	0	0
Mercapto mix	1	0.8	0	0
p-tert-butylphenol formaldehyde resin	1	0.8	0	0
Colophonium	0	0	0	0
Potassium dichromate	0	0	0	1
Allergens tested in selective cases				
House dust mite	5			
Personal care products	2			
Benzalkonium chloride	1			
Topical corticosteroid cream (unknown)	1			

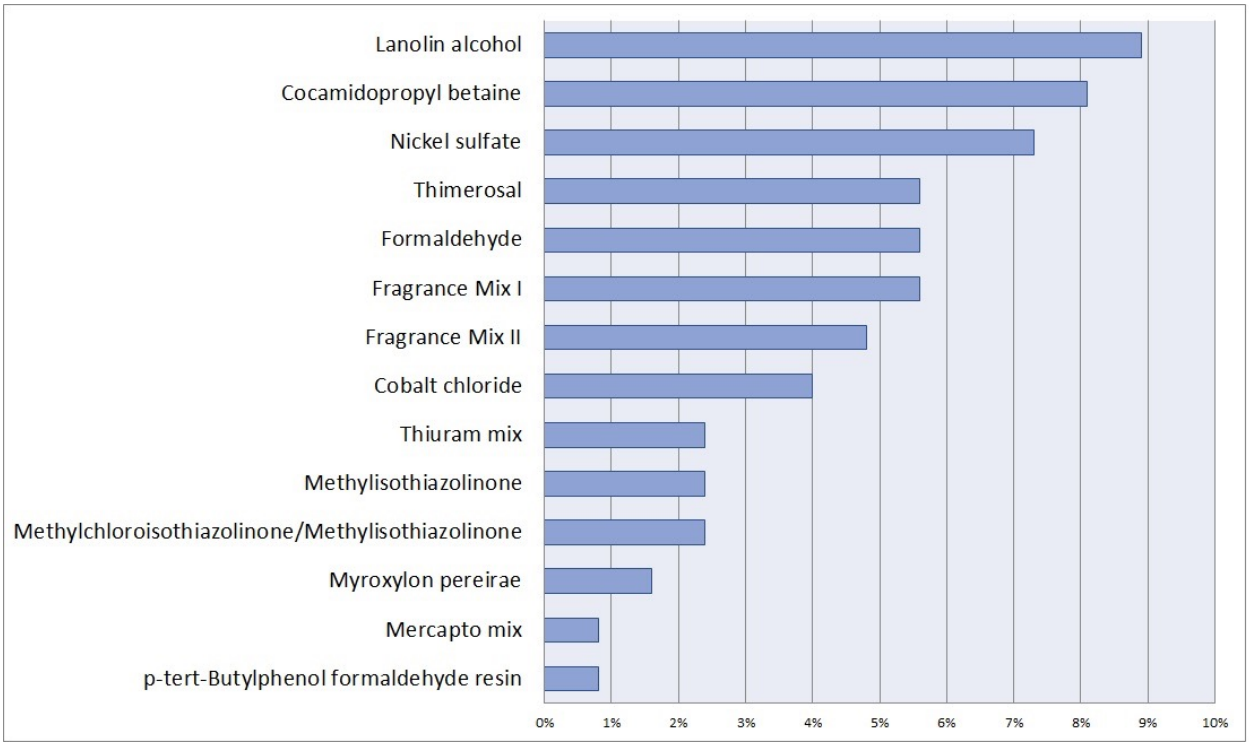


Fig 1. Percentage of positive patch test reactions in 124 children with eczema.

was found in 51/124 children (41.1%) corroborating the results reported in a study from the UK which has shown the prevalence of positive patch test reaction in 110 children with eczema aged 2-18 years to be 44%.[4] The rates were 59.2% of 125 children and 51% of 79 children in Brazil and The Netherlands respectively.[5,6]

Patch test reaction rate in current study is lower than the literature that revealed 26.6-95.6%.[6] The reported clinical relevance of 51.7-100%[7] was also higher than the in this study (24%). This could be because our study carried out in unselected children with eczema regardless of contact dermatitis history while most previous studies performed in patients suspected ACD and tested a larger number of allergens than in this study. Zug et al. reported 62.3% positive patch test results in North American children aged 18 years or younger during 2005 and 2012. These patients were suspicious of ACD and were patch tested with up to 70-allergen series.[8]

Our results highlight the significance of patch testing as an investigation of a child with chronic atopic dermatitis. Contact allergy coexists in 38% of children with atopic dermatitis (AD), most commonly to nickel sulfate (15.8%). Cattani et al.[9] recently patch tested 54 Brazilian children, 4 -18 years old, with recalcitrant atopic dermatitis, positive reactions were found in 27.7%, most commonly to nickel sulfate, disperse blue, and fragrance mix I. ACD occurrence in children with atopic eczema can be described by the impaired epidermal barrier that could enhance allergen penetration and the exposure to sensitizing chemicals in personal care products.[10] Boonstra et al. reported that concomitant contact dermatitis may be a cause of AD becoming a difficult-to-treat disease.[11]

A retrospective study by Boonchai et al. showed a positive patch test reaction rate of 35.5% among 112 Thai children, aged less than 18 years, suspected ACD. The common allergens were nickel, potassium dichromate, methylisothiazolinone. These results may not be used to compare with our study because there are many differences in the study methodology: retrospective chart review vs prospective study, mean ages (14.5 vs 8 years), inclusion criteria (suspected ACD vs all forms of eczema) and the allergens routinely tested that did not include our common allergens such as cocamidopropyl betaine and thimerosal.[12]

In this study, the most common allergen was lanolin alcohol. Exposure to lanolin can come from emollients, ointments, cosmetics, toiletries and topical medicaments.[7] In a previous study in North America, prevalence of lanolin allergy was 4.6%.[13] The results of TRUE (Thin-layer Rapid Use Epicutaneous Test showed lanolin allergy rate of 15.8% in 101 children and adolescents aged 6-18 years who were patch tested on suspicion of having ACD.[14] Lanolin allergy was reported being more common in children than in adults (4.5% vs 3.2%).[9] In our center,

the sensitization rate of lanolin alcohol in all age group including adults is approximately 1.5%. However, Uldahl et al. reported that patients allergic to lanolin may use lanolin-containing products on intact skin without problem[15], but may develop allergic contact dermatitis after applying lanolin-containing topical medicaments to damaged or ulcerated skin. This phenomenon is called 'lanolin paradox'. The difficulty in determining clinical relevance of a positive patch test reaction can cause by lanolin paradox. Moreover, it can be difficult to distinguish between true allergic reactions and irritant reaction.

Exposure sources of cocamidopropyl betaine include shampoos, cleansers, toothpaste, detergents, liquid soaps, bath gels, skin care products and antiseptics.[16] Cocamidopropyl betaine was noted to be an important allergen in younger age group.[17] In a retrospective study of 1142 children aged less than 18 years, there was a higher frequency of positive patch test reactions to cocamidopropyl betaine in patients with AD when compared to non-AD group.[18]

Nickel was high in our child cohort (7.3%). Most previous studies reported that nickel is the most common allergen causing ACD in children.[5,6,7,19] Sensitization to nickel may begin in infancy. One patient was related to wearing necklace with metal sacred pendant amulet since birth. It is also a common practice to pierce ears at a very young age in Thailand, especially in girls.

One of our cases presented with upper lip dermatitis. Patch test revealed positive reactions to fragrance mix I, fragrance mix II and *Myroxylon pereirae* which possibly present in her favorite bottled soft drink. Very young children can be sensitized to contact allergen such as fragrance. Apart from direct contact in relation to the use of perfumed products, airborne contact of volatile perfume used by a family member can cause recurrent eyelid dermatitis. The mother of a young child bought a flameless stone burner that heats and diffuses aromatic oils containing fragrance in order to clean air in the home. Eyelid dermatitis improved after the mother stop using the burner. However, airborne dermatitis recurred when the child's grandmother used perfume on herself in the same room.

A 7 year-old atopic boy presented with recalcitrant conjunctivitis and photophobia showed a relevant positive patch test to benzalkonium chloride. Benzalkonium chloride is a preservative in several eye medicaments for allergic conjunctivitis. Avoidance of benzalkonium chloride containing eye drops resulted in dramatic improvement.

CONCLUSION

This study indicates that allergic contact dermatitis is common in children. Lanolin, cocamidopropyl betaine and nickel are the top three common contact allergens in Thai pediatric population. Both atopic and non-atopic patients can develop contact dermatitis. Patch testing should be performed in children presented with eczema regardless of contact dermatitis history.

Limitation

The small number of allergens in our pediatric screening series could partly decrease the sensitivity of patch test. Further study testing with larger number of screening allergens for children is recommended.

REFERENCES

1. Rodrigues DF, Goulart EM. Patch-test results in children and adolescents: systematic review of a 15-year period. An Bras Dermatol. 2016;91(1):64-72.
2. Pigatto P, Martelli A, Marsili C, Fiocchi A. Contact dermatitis in children. Ital J Pediatr. 2010;13;36:2.
3. Kasemsarn P, Boonchai W. Usefulness of Patch Testing in Dermatology. Siriraj Med J. 2012;64(2):73-7.
4. Moustafa M, Holden CR, Athavale P, Cork MJ, Messenger AG, Gawkrodger DJ. Patch testing is a useful investigation in children with eczema. Contact Dermatitis. 2011;65:208-12.
5. Rodrigues DF, Goulart EM. Patch test results in children and adolescents. Study from the Santa Casa de Belo Horizonte Dermatology Clinic, Brazil, from 2003 to 2010. An Bras Dermatol. 2015;90(5):671-83.
6. de Waard-van der Spek FB, Oranje AP. Patch tests in children with suspected allergic contact dermatitis: a prospective study and review of the literature. Dermatology. 2009; 218:119-25.
7. Simonsen AB, Deleuran M, Johansen JD, Sommerlund M. Contact allergy and allergic contact dermatitis in children - a review of current data. Contact Dermatitis. 2011;65(5):254-65.
8. Zug KA, Pham AK, Belsito DV, DeKoven JG, DeLeo VA, Fowler Jr JF, et al. Patch testing in children from 2005 to 2012: results from the North American Contact Dermatitis Group. Dermatitis. 2014;25(6):345-55.
9. Cattani CAS, Oppermann K, Perazzoli S, Guarda NH, Barea P, Bonamigo RR. Sensitizing agents found in children and adolescents with recalcitrant atopic dermatitis: a cross-sectional study with a pediatric battery. An Bras Dermatol. 2022;97(3):307-14.
10. Mortz CG, Andersen KE. Allergic contact dermatitis in children and adolescents. Contact Dermatitis. 1999;41:121-30.
11. Boonstra M, Rustemeyer T, Middelkamp-Hup MA. Both children and adult patients with difficult-to-treat atopic dermatitis have high prevalences of concomitant allergic contact dermatitis and are frequently polysensitized. J Eur Acad Dermatol Venereol. 2018;32(9):1554-61.
12. Boonchai W, Chaiyabutr C, Charoenpipatsin N, Sukakul T. Pediatric contact allergy: A comparative study with adults. Contact Dermatitis. 2021;84(1):34-40.
13. Silverberg JI, Patel N, Warshaw EM, DeKoven JG, Atwater AR, Belsito DV, et al. Lanolin allergic reactions: North American

Contact Dermatitis Group experience, 2001 to 2018. Dermatitis. 2022;33(3):193-99.
14. Jacob SE, Herro EM, Sullivan K, Matiz C, Eichenfield L, Hamann C. Safety and efficacy evaluation of TRUE TEST panels 1.1, 2.1, and 3.1 in children and adolescents. Dermatitis. 2011;22(4): 204-10.
15. Uldahl A, Engfeldt M, Svedman C. Clinical relevance of positive patch test reactions to lanolin: a ROAT study. Contact Dermatitis. 2021;84(1):41-9.
16. Jacob SE, Amini S. Cocamidopropyl betaine. Dermatitis. 2008;19(3):157-60.
17. Belloni Fortina A, Fontana E, Peserico A. Contact sensitization in children: a retrospective study of 2,614 children from a single center. Pediatr Dermatol. 2016;33(4):399-404.
18. Jacob SE, McGowan M, Silverberg NB, Pelletier JL, Fonacier L, Mousdicas N, et al. Pediatric contact dermatitis registry data on contact allergy in children with atopic dermatitis. JAMA Dermatol. 2017;153(8):765-70.
19. Mortazavi H, Ehsani A, Sajjadi SS, Aghazadeh N, Arian E. Patch testing in Iranian children with allergic contact dermatitis. BMC Dermatol. 2016;16(1):10.

Perceptions and Management Practices of Onychomycosis Among Thai Physicians

Pichaya Limphoka, M.D.*, Charussri Leeyaphan, M.D.*, Chudapa Sereeaphinan, M.D.*, Pattriya Chanyachailert, M.D.*, Sumanas Bunyaratavej, M.D.*, Carren Hau, M.D., Ph.D.**

**Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, **Section of Dermatology, Department of Internal Medicine, Manila Doctors Hospital, Manila, Philippines.*

ABSTRACT

Objective: To examine the proportion of physicians who conducted mycological laboratory procedures to confirm a diagnosis of onychomycosis. The secondary purpose was to evaluate the practical management of physicians, comparing general practitioners and dermatology-related physicians.

Materials and Methods: This cross-sectional study and questionnaire-based research was conducted during 2021-2022. The questionnaire was composed of questions related to the practical management of onychomycosis, including diagnosis and treatment.

Results: Overall, 143 physicians were recruited to take part in this study. The number of physicians who conducted direct examination with potassium hydroxide was 99 (69.2%). The number of dermatology-related physicians who conducted mycological laboratory examinations to confirm the diagnosis was significantly higher than among general physicians (95.8% vs. 52.2%; p<0.001). Feet examination and determination of poor prognostic factors, such as elderly age, nail thickness, presence of dermatophytoma and nondermatophytes infection, were done by the dermatology-related physicians in significantly higher numbers. Blood testing before starting treatment for onychomycosis with oral antifungal medications seemed to be higher (87.5%) in the dermatology-related group. Moreover, mycological re-evaluation after treatment cessation was more significantly requested by the dermatology-related participants (75% vs. 15.8%, p<0.001).

Conclusion: Laboratory confirmation, feet examination, and the recognition of poor prognostic factors were significantly lower in the general practitioner group. These findings should raise awareness for improving further education about onychomycosis management in medical students, since mycological laboratory examination is crucial for diagnosis and it is helpful in guiding the proper disease management for complete disease remission.

Keywords: Mycological laboratory; general practitioners (Siriraj Med J 2023; 75: 76-84)

INTRODUCTION

Onychomycosis is one of the most common nail diseases worldwide.[1] The prevalence of onychomycosis in the Asian population is approximately 10%.[1] The diagnosis of onychomycosis requires both characteristic clinical manifestations and laboratory investigation.[2] Clinical manifestations that are suggestive of onychomycosis are subungual hyperkeratosis, onycholysis, nail discoloration, or nail dystrophy. However, there are a number of diseases, including chronic trauma, lichen planus, psoriasis, and subungual malignant melanoma, that mimic the features of onychomycosis.[2,3,14] A previous study showed that about 65% of general practitioners misdiagnosed psoriatic nails as onychomycosis.[4] Therefore, laboratory investigations,

Corresponding author: Sumanas Bunyaratavej
E-mail: consultskin@yahoo.com
Received 24 May 2022 Revised 28 June 2022 Accepted 29 June 2022
ORCID ID:http://orcid.org/0000-0002-0852-7742
https://doi.org/10.33192/smj.v75i2.260741

involving either direct microscopic examination with potassium hydroxide or fungal culture, are essential to confirm the diagnosis of onychomycosis. Fungal culture is also beneficial in identifying the specific species of pathogen, and can help physicians to make the correct decision and to adjust the proper treatments to individual patients.[2,5]

The treatment of onychomycosis often requires long-term treatment.[6] Oral antifungal treatments, such as allylamine terbinafine and the triazole group, have potential systemic side effects, such as hepatic involvement.[7,8] Several studies have revealed an inappropriate management of onychomycosis among physicians.[5] For example, a survey in the United Kingdom found that only a small proportion of physicians conducted laboratory confirmation before treating onychomycosis with oral terbinafine.[7] One research study found that only 30% of dermatologists performed laboratory monitoring during oral terbinafine treatment.[5]

The objective of the present study was to determine the proportion of physicians who conducted mycological laboratory examination prior to a diagnosis of onychomycosis. We also aimed to evaluate the practical management of physicians, by comparing general practitioners with dermatology-related physicians.

MATERIALS AND METHODS

Study design

This cross-sectional study was a questionnaire-based research and was conducted during 2021-2022. This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital (COA no. Si 733/2021). The questionnaire was developed and divided into four parts. The initial part aimed to collect the participants' demographic data, including age, sex, marital status, workplace, working experience, and level of confidence in onychomycosis management. The other three parts were: 1) the opinions regarding the laboratory investigation request, covering 5 items; 2) the prognostic evaluation of onychomycosis, covering 10 items; and 3) the knowledge and practice for onychomycosis treatment, covering 6 items. Each variable item was recorded as categorical data. Google Forms was used as a channel to send the questionnaire. The study participants must be physicians who practice in Thailand.

Statistical analysis

Data were analyzed using PASW Statistics for Windows, version 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as frequency and percentage. The Chi-square test or Fisher's exact test were used to compare the differences between the general practitioners and dermatology-related participants. A p-value less than 0.05 was considered as statistically significant.

RESULTS

In total, 143 physicians were recruited to participate in the study. Their demographic data are shown in Table 1. Their mean (SD) age was 33.9 (7.8) years old, while the minimum and maximum ages were 21 and 62 years old, respectively. Among the participants, 48 (33.6%) were dermatology-related physicians, comprising 23 (16.1%) who were board-certified dermatologists (16.1%) and 25 (17.5%) who were MSc, diploma, or in-training dermatology residents. Most participants worked in a university-based hospital. The minimum working experience was 1 year, while the maximum was 36 years. Most participants saw 0-5 patients per week (50%) and had low to moderate confidence in skin disease management. Regarding nail disease, 47 (32.9%) participants encountered patients with nail problems in 2-5 cases per year. The majority of participants (41.3%) reported that they had a moderate level of confidence in onychomycosis management.

Table 2 shows the information about onychomycosis diagnosis when comparing the dermatology-related physicians with general practitioners. The proportion of physicians who conducted direct examination with potassium hydroxide was 69.2%. The opinion that laboratory investigations were necessary for onychomycosis diagnosis was significantly higher in the dermatology-related physicians (97.9% vs. 54.7%, $p < 0.001$). In practical management, 30 (62.5%) dermatology-related participants requested laboratory investigations almost or every time, which was significantly higher than for the other group (62.5% vs. 13.7%, $p < 0.001$). The presence of distinct clinical features of onychomycosis were the major cause for not requesting laboratory examinations. The significant reason those general practitioners did not request laboratory examinations to confirm diagnosis was the lack of knowledge of specimen processing ($p < 0.001$). Nail fungal culture was requested significantly more by the dermatology-related participants (79.2% vs. 37.9%, $p < 0.001$). The opinion that fungal culture was necessary was also significantly higher among dermatology-related physicians (89.6% vs. 50.5%, $p < 0.001$).

Table 3 demonstrates the prognostic evaluations of onychomycosis. Most participants had an awareness of poor prognosis factors, especially in the dermatologist-related group. An older age > 70 years old, nail thickness > 2 mm, presence of dermatophytoma and *Neoscytalidium* spp. infection were factors that the dermatology-related

TABLE 1. Demographic data of the participants.

Demographic data	Total physicians n = 143 (%)
Age, years (mean, SD)	33.9 (7.7)
Status	
General practitioner	45 (31.5)
Specialist other than dermatologist	45 (31.5)
MSc, diploma, dermatology resident in training	25 (17.5)
Board-certified dermatologist	23 (16.1)
Other specialists	5 (3.5)
Workplace*	
University hospital	60 (42.0)
General hospital	35 (24.5)
Community hospital	16 (11.2)
Private hospital	31 (21.7)
Private clinic	28(19.6)
Working experience, years (mean, SD)	9.2 (6.9)
Number of skin patients during last one year (patients per week)	
0–5	72 (50.3)
6–15	18 (12.6)
16–30	17 (11.9)
31–50	13 (9.1)
>50	23 (16.1)
Confidence in management of skin disease	
Very low confidence	17 (11.9)
Low confidence	51 (35.7)
Moderate confidence	53 (37.1)
Very high confidence	22 (15.4)
Number of patients with nail diseases during last one year (patients per year)	
0–1	25 (17.5)
2–5	47 (32.9)
6–10	22 (15.4)
11–40	34 (23.8)
>40	15 (10.5)
Confidence in management of nail disease	
Very low confidence	30 (21.0)
Low confidence	48 (33.6)
Moderate confidence	59 (41.3)
Very high confidence	6 (4.2)

*One physician could have more than one workplace.
Abbreviation: SD, standard deviation

TABLE 2. Comparison of the proportion between Thai general practitioners and dermatologic-related physicians regarding requesting laboratory investigations for onychomycosis diagnosis and treatment.

Items	Overall n = 143 (%)	Thai general practitioners n = 95 (%)	Dermatology-related physicians n = 48 (%)	P-value
Opinion regarding the necessity of requesting laboratory investigations of nail specimens for diagnosis				<0.001*
Not necessary	44 (30.8)	43 (45.3)	1 (2.1)	
Necessary	99 (69.2)	52 (54.7)	47 (97.9)	
Requesting laboratory investigation of nail specimens before starting treatment				<0.001*
Never	47 (32.9)	45 (47.4)	2 (4.2)	
Less than half of cases	38 (26.6)	31 (32.6)	7 (14.6)	
More than half of cases	15 (10.5)	6 (6.3)	9 (18.8)	
Almost or every time	43 (30.1)	13 (13.7)	30 (62.5)	
Reasons for not requesting laboratory investigations of nail specimens before starting treatment[a,b]	**n = 100**	**n = 82**	**n = 18**	
Distinct clinical manifestations	64 (64.0)	51 (62.2)	13 (72.2)	0.422
Unapproachable to available laboratory	56 (56.0)	48 (58.5)	8 (44.4)	0.275
Lack of knowledge in specimen processing	39 (39.0)	38 (46.3)	1 (5.6)	0.001*
Long turnaround time	38 (38.0)	30 (36.6)	8 (44.4)	0.534
Patients' financial problems	14 (14.0)	9 (11.0)	5 (27.8)	0.125
Investigations requested by physicians for onychomycosis diagnosis and treatment[a]				
Direct KOH examination	136 (95.1)	89 (93.7)	47 (97.9)	0.424
Nail fungal culture	74 (51.7)	36 (37.9)	38 (79.2)	<0.001*
Nail pathology	32 (22.4)	17 (17.9)	15 (31.3)	0.070
Molecular testing	2 (1.4)	2 (2.1)	0	0.551
Opinion regarding necessity of fungal culture				<0.001*
Necessary	91 (63.6)	48 (50.5)	43 (89.6)	
Not necessary	52 (36.4)	47 (49.5)	5 (10.4)	

*A p-value less than 0.05 indicated statistical significance, Chi-squared test.

[a]One physician could have more than one reason for not requesting laboratory investigations or one investigation that could be requested for the diagnosis and treatment of onychomycosis.

[b]Some participants responded to the questionaries in this topic.

TABLE 3. Prognostic evaluation of onychomycosis.

Questions	Overall n = 143 (%)	Thai general practitioners n = 95 (%)	Dermatology-related physicians n = 48 (%)	P-value
Expectation of nail condition after complete treatment				0.446
Normal or near normal	69 (48.3)	43 (45.3)	26 (54.2)	
Improved but not normal	72 (50.3)	50 (52.6)	22 (45.8)	
Stable or worse	2 (1.4)	2 (2.1)	0	
Awareness of poor prognostic factors				<0.001*
No	29 (20.3)	29 (30.5)	0	
Yes	114 (79.7)	66 (69.5)	48 (100.0)	
What are the prognostic factors?[a]	**n = 144**	**n = 66**	**n = 48**	
Peripheral vascular disease	97 (85.1)	57 (86.4)	40 (83.3)	0.654
Age > 70 years old	88 (77.2)	46 (69.7)	42 (87.5)	0.025*
Area involvement > 2/3	86 (75.4)	50 (75.8)	36 (75.0)	0.926
Nail thickness > 2 mm	84 (73.7)	44 (66.7)	40 (83.3)	0.046*
Dermatophytoma	67 (58.8)	28 (42.4)	39 (81.3)	<0.001*
Neoscytalidium spp. infection	61 (53.5)	24 (36.4)	37 (77.1)	<0.001*
Lateral nail fungal infection	51 (44.7)	26 (39.4)	25 (52.1)	0.179
Distal nail fungal infection	21 (18.4)	15 (22.7)	6 (12.5)	0.164
Do they know NDM?				<0.001*
Not know	39 (27.3)	39 (41.1)	0	
Not sure	37 (25.9)	31 (32.6)	6 (12.5)	
Know	67 (46.9)	25 (26.3)	42 (87.5)	
How often do they do foot examination?				<0.001*
Never	20 (14.0)	20 (21.1)	0	
Less than half of cases	44 (30.8)	40 (42.1)	4 (8.3)	
More than half of cases	25 (17.5)	20 (21.1)	5 (10.4)	
Almost or every time	54 (37.8)	15 (15.8)	39 (81.3)	
How often do they do examine for peripheral vascular disease?				0.047*
Never	68 (47.6)	52 (54.7)	16 (33.3)	
Less than half of cases	43 (30.1)	27 (28.4)	16 (33.3)	
More than half of cases	24 (16.8)	13 (13.7)	11 (22.9)	
Almost or every time	8 (5.6)	3 (3.2)	5 (10.4)	
How often do they do measure nail thickness?				<0.001*
Never	75 (52.4)	64 (67.4)	11 (22.9)	
Less than half of cases	38 (26.6)	25 (26.3)	13 (27.1)	
More than half of cases	13 (9.1)	3 (3.2)	10 (20.8)	
Almost or every time	17 (11.9)	3 (3.2)	14 (29.2)	

TABLE 3. Prognostic evaluation of onychomycosis. (Continued)

Questions	Overall n = 143 (%)	Thai general practitioners n = 95 (%)	Dermatology-related physicians n = 48 (%)	P-value
Opinion regarding importance of requesting laboratory investigations of nail specimens after treatment				0.012*
Not important	9 (6.3)	6 (6.3)	3 (6.3)	
Not sure	47 (32.9)	39 (41.1)	8 (16.7)	
Important	87 (60.8)	50 (52.6)	37 (77.1)	
How often do they request laboratory investigations of nail specimens after complete treatment?				
Never	92 (64.3)	80 (84.2)	12 (25.0)	<0.001*
Less than half of cases	24 (16.8)	9 (9.5)	15 (31.3)	
More than half of cases	10 (7.0)	2 (2.1)	8 (16.7)	
Almost or every time	17 (11.9)	4 (4.2)	13 (27.1)	
How often do they request blood testing before prescribing oral antifungal drugs?				0.130
Never	32 (22.4)	26 (27.4)	6 (12.5)	
Less than half of cases	28 (19.6)	20 (21.1)	8 (16.7)	
More than half of cases	25 (17.5)	14 (14.7)	11 (22.9)	
Almost or every time	58 (40.6)	35 (36.8)	23 (47.9)	

*A p-value less than 0.05 indicated statistical significance, Chi-squared test.

[a]One physician could consider more than one factor to be a prognostic factor.

participants significantly had more awareness of ($p < 0.001$). *Neoscytalidium* spp. was significantly more well known in the dermatology-related group (87.5% vs. 26.3%, $p < 0.001$). Feet examination was significantly done every time by the dermatology-related participants (81.3%, $p < 0.001$). Among the general practitioners, nail thickness measurement and laboratory request after treatment were less frequently done ($p < 0.001$). Before starting treatment for onychomycosis with oral antifungal agents, blood testing seemed to be more frequently ordered in the dermatology-related group.

Treatment of the onychomycosis aspects are shown in Table 4. The use of topical therapy was significantly higher in the dermatology-related group (81.3% vs. 34.7%, $p < 0.001$). Also, 41.7% of the dermatology-related group participants used oral antifungal agents almost or every time. Most participants did not agree with surgical nail avulsion as a treatment option. However, the indications for surgical nail avulsion were not recognized in the non-dermatology-related group (70.5%). Chemical nail avulsion was used significantly more by the dermatology-related participants (62.5% vs. 3.2%, $p < 0.001$).

DISCUSSION

The results provide interesting information about onychomycosis practice in Thailand. Most participants in this survey had encountered only a small number of patients suspected of onychomycosis. However, almost half of the participants had a moderate level of confidence in managing onychomycosis.

We found that nearly all the dermatology-related physicians used laboratory investigations to confirm

TABLE 4. Knowledge and practice for onychomycosis treatment.

Questions	Overall n = 143 (%)	Thai general practitioners n = 95 (%)	Dermatology-related physicians n = 48 (%)	P-value
Role of topical therapy for onychomycosis				<0.001*
Not know	30 (21.0)	29 (30.5)	1 (2.1)	
Know but never use	41 (28.7)	33 (34.7)	8 (16.7)	
Know and used to treat with topical therapy	72 (50.3)	33 (34.7)	39 (81.3)	
Experience in treating onychomycosis with oral antifungal medication				0.002*
Never	21 (14.7)	20 (21.1)	1 (2.1)	
Less than half of cases	41 (28.7)	31 (32.6)	10 (20.8)	
More than half of cases	37 (25.9)	20 (21.1)	17 (35.4)	
Almost or every time	44 (30.8)	24 (25.3)	20 (41.7)	
Opinion regarding roles of surgical nail avulsion in treatment				<0.001*
Not agree	108 (75.5)	63 (66.3)	45 (93.8)	
Not sure	30 (21.0)	28 (29.5)	2 (4.2)	
Agree	5 (3.5)	4 (4.2)	1 (2.1)	
Indications for surgical nail avulsion				<0.001*
Not know	79 (55.2)	67 (70.5)	12 (25.0)	
Know but never use	51 (35.7)	23 (24.2)	28 (58.3)	
Know and used to treat patient with surgical nail avulsion	13 (9.1)	5 (5.3)	8 (16.7)	
Role of chemical nail avulsion for onychomycosis				<0.001*
Not know	66 (46.2)	65 (68.4)	1 (2.1)	
Know but never use	44 (30.8)	27 (28.4)	17 (35.4)	
Know and used to treat patient with chemical avulsion	33 (23.1)	3 (3.2)	30 (62.5)	
Role of partial nail avulsion for onychomycosis				<0.001*
Not know	62 (43.4)	58 (61.1)	4 (8.3)	
Know but never use	65 (45.5)	30 (31.6)	35 (72.9)	
Know and used to treat patient with partial nail avulsion	16 (11.2)	7 (7.4)	8 (18.8)	

*A p-value less than 0.05 indicated statistical significance, Chi-squared test.

the diagnosis, which was consistent with the result of a study in Canada.[9] Unfortunately, almost half of the general practitioners never used mycological laboratory examinations to confirm the diagnosis of onychomycosis. These results were similar to several studies that reported that general practitioners tended to treat onychomycosis without the mycological evidence of fungal infection[5,6] This study showed that the most common reason for not performing mycological laboratory examinations was the belief that the clinical manifestations were sufficient for diagnosis. Nevertheless, the lack of knowledge in specimen processing was found to be significantly different between the two groups. More than half of the participants conducted fungal culture. However, the proportion who conducted fungal culture was significantly higher among the dermatology-related physicians. This emphasized that knowledge played a key role in onychomycosis management. In addition, this study revealed that the second most-common reason for general practitioners not to perform mycological investigations was the lack of accessibility to laboratory facilities. Therefore, easily accessible mycological laboratory facilities may motivate physicians to use mycological investigations to confirm the diagnosis. Several studies also demonstrated other reasons, such as general practitioners had insufficient skills or it was time-consuming to collect specimens, or they felt familiar through previous individual practice.[5,9]

Cases of concurrent onychomycosis and tinea pedis were also reported.[10] Our study revealed that a significantly lower number of general practitioners did feet examination compared to the dermatology-related physicians. Examination of the feet was recommended in patients diagnosed with onychomycosis, since tinea pedis could be reservoirs of infection, resulting in unsuccessful treatment.[10] Poor prognostic factors, including elderly age, longer onset, presence of cardiovascular diseases, widespread dermatophyte infection, and multiple nail infection, were found to be associated with a poorer outcome.[11,12] In this study, the poor prognostic factors that were significantly underrecognized by the general practitioners were an elderly age, subungual hyperkeratosis (>2 mm), presence of dermatophytoma, and NDM infection. Greater education for general practitioners to recognize the prognostic factors would result in a better treatment outcome.[12]

According to the results from this study, oral antifungal drugs were often used by dermatology-related physicians more than by general practitioners. This study showed the same percentage of general practitioners who never used oral antifungal drugs as a treatment option.[7] Almost half of the physicians performed blood tests before starting oral antifungal agents, but the proportion was not statistically significant between the two groups. Oral terbinafine is an effective drug in treating dermatophyte onychomycosis. However, terbinafine is expensive and can cause adverse side effects, such as hepatic involvement (elevated hepatic enzymes).[5] It is thus recommended that oral antifungal agents should be prescribed only when physicians see characteristic clinical manifestations and when mycological laboratory examinations confirm the diagnosis of onychomycosis.[5,7] Our study showed that the percentage of general practitioners who never conducted blood testing prior to prescribing oral antifungal agents was 27.4%. This proportion was lower compared to a previous study that showed 36% of general practitioners had never done a blood test.[7] Blood testing for complete blood count and liver function prior to starting oral antifungals was suggested by some experts.[6] Monitoring the laboratory results during treatment with oral antifungal drugs is still controversial. Unsurprisingly, in one study it was found that the percentage of dermatologists who monitored blood tests during using oral antifungal therapy was only 30%.[9] Some authors suggested that the careful monitoring of blood testing is recommended in patients with underlying hepatic disease.[6]

The opinion regarding performing follow-up laboratory examination after the cessation of treatment was significantly higher in the dermatology-related group. When practicing, almost all the general practitioners never performed laboratory investigations after the completion of treatment. The reasons for this were that they may perform mycological investigations only in cases with no response or in suboptimal treatment or recurrent disease cases.[6,7]

A 43-year retrospective study of lawsuits involving nail conditions in the United States found that the most common reason was inadequate treatment causing undesirable sequalae, which was found in 19 cases (51%).[13] Unfortunately, misdiagnosis leading to a serious issue was the major reason why lawsuits were won by the plaintiff.[13]

In conclusion, the laboratory confirmation of onychomycosis was significantly lower in the general practitioner group. However, in all cases of suspected onychomycosis, mycological laboratory examinations to confirm the diagnosis are crucial.[6,7,14] A clear guideline for onychomycosis management and concrete evidence supporting mycological laboratory examinations as essential for onychomycosis diagnosis may be helpful to guide the proper management.[7] Laboratory investigations prior and during the administration of oral antifungal drugs are still inconclusive. Further studies about the benefits and cost

effectiveness are needed. Easily accessible mycological laboratory facilities will also motivate physicians to conduct proper investigations. This study also emphasizes that education is important for all physicians in order to ensure they can provide the proper management of onychomycosis, reduce adverse events, and provide the best care for patients.

Conflict of interests: All authors have no conflicts of interest or financial support to declare.

Funding: None

REFERENCES

1. Sigurgeirsson B, Baran R. The prevalence of onychomycosis in the global population: a literature study. J Eur Acad Dermatol Venereol. 2014;28(11):1480-91.
2. Ameen M, Lear JT, Madan V, Mohd Mustapa MF, Richardson M. British Association of Dermatologists' guidelines for the management of onychomycosis 2014. Br J Dermatol. 2014; 171(5):937-58.
3. Denning DW, Evans EG, Kibbler CC, Richardson MD, Roberts MM, Rogers TR, et al. Fungal nail disease: a guide to good practice (report of a Working Group of the British Society for Medical Mycology). BMJ. 1995;311(7015):1277-81.
4. Bunyaratavej S, Prasertworonun N, Chaiwanon O, Pattanaprichakul P. Alarming trend towards deviation of clinical diagnosis and management of psoriatic nails by Thai general practitioners and non-dermatologist specialists. J Eur Acad Dermatol Venereol. 2015;29(2):398-9.
5. Wilcock M, Hartley J, Gould D. Inappropriate use of oral terbinafine in family practice. Pharm World Sci. 2003;25(1): 25-6.
6. Gupta AK, Mays RR, Versteeg SG, Piraccini BM, Takwale A, Shemer A, et al. Global perspectives for the management of onychomycosis. Int J Dermatol. 2019;58(10):1118-29.
7. Lasseter G, McNulty CA, Palmer M, Yoxall H, Kibbler C, Health Protection Agency GPMLUG. Developing best practice for fungal specimen submission--fungal audit of general practice. Mycoses. 2012;55(6):476-82.
8. Wharry S. FDA issues warnings about drugs used to treat fungal nail infections. CMAJ. 2001;164(12):1738.
9. Gupta AK, Shear NH. A questionnaire study on the management of onychomycosis: a Canadian perspective. Int J Dermatol. 1998;37(6):457-60.
10. Walling HW. Subclinical onychomycosis is associated with tinea pedis. Br J Dermatol. 2009;161(4):746-9.
11. Loo DS. Onychomycosis in the elderly: drug treatment options. Drugs Aging. 2007;24(4):293-302.
12. Widaty S, Miranda E, Bramono K, Menaldi SL, Marissa M, Oktarina C, et al. Prognostic factors influencing the treatment outcome of onychomycosis Candida. Mycoses. 2020;63(1):71-7.
13. Xiang L, Lipner SR. Characteristics of malpractice lawsuits involving nail disorders in the United States from 1977 to 2019. J Am Acad Dermatol. 2020;83(4):1202-4.
14. Leeyaphan C, Bunyaratavej S, Chadchavalpanichaya N, Rujitharanawong C, Phaitoonwattanakij S, Matthapan L. Clinical and Laboratory Findings in Trauma-Induced Nail Dystrophy versus Onychomycosis. Siriraj Med J. 2018;70(6): 490-5.

Clinical Features of Adult Male Acne in a Tropical Country: A Prospective Cross-sectional Study

Chayanee Likitwattananurak, M.D., Leena Chularojanamontri, M.D., Papapit Tuchinda, M.D., Kanokvalai Kulthanan, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To evaluate the characteristics of post-adolescent male patients with acne in terms of the onset of the condition, its clinical course and severity, and the behaviors associated with its severity.

Materials and Methods: A prospective, cross-sectional study was conducted on adult males with acne who visited Siriraj Hospital, Thailand. All male acne patients aged 21 years and older were enrolled. Diagnoses and physical examinations were performed by dermatologists.

Results: Seventy-two patients (mean age, 26.9 [± 4.3] years) were included. Persistent acne, relapse acne, and late-onset acne (onset at age ≥ 21 years) were reported in 62.5%, 33.3%, and 4.2% cases, respectively. Persistent acne tended to subside at 26 years of age, whereas late-onset acne tended to start at 28 years of age. The acne severity was mild in most cases. Pimple-picking, followed by frequent face washing, were common habits among male acne patients. Shaving influenced the severity in some adult male with acne.

Conclusion: Adult male acne commonly presented as inflammatory lesions and comedones on the cheeks. They commonly had an onset earlier than 21 years old and continued into adulthood, but the post-adolescent severity tended to be mild. While several factors have been reported elsewhere to be involved in the severity of acne, this study found that only shaving influenced severity.

Keywords: Acne vulgaris; adult acne; male (Siriraj Med J 2023; 75: 85-91)

INTRODUCTION

Acne vulgaris is chronic inflammatory skin disease characterized by open and closed comedones, inflammatory papules, pustules, and nodules in seborrheic areas.[1,2] It is one of the most troublesome skin diseases. The pathogenesis of acne involves the colonization and proliferation of *Corynebacterium acnes*, resulting in the inflammatory process, hyperkeratinization, and sebum hyperproduction.[2,3] The negative effects of acne vulgaris include depression, antisocial behavior, and even unemployment. The prevalence of acne vulgaris is 90%-95% of the population.[3,4] Acne commonly occurs during adolescence, and it is found less often during the pre- and post-adolescent periods.[3]

Adolescent acne predominantly occurs in males, while post-adolescent acne is usually found in females.[5] However, post-adolescent acne or adult acne can also affect male patients. Adult patients with acne have many challenging problems. For instance, the acne may develop therapeutic resistance or respond slowly to treatment; the skin can also easily become allergic to topical treatments, leading to drug discontinuation. Previous studies have identified a wide variety of factors related to acne severity in adolescent acne rather than post-adolescent acne.[3-5]

Corresponding author: Kanokvalai Kulthanan
E-mail: kanokvalai.kul@mahidol.ac.th
Received 18 March 2022 Revised 7 July 2022 Accepted 9 July 2022
ORCID ID:http://orcid.org/0000-0002-7618-821X
https://doi.org/10.33192/smj.v75i2.260742

Based on our literature review, data from prospective studies focusing on post-adolescent male patients with acne is limited. The present study aimed to evaluate the characteristics of post-adolescent male patients with acne in terms of its onset, clinical course, and severity, and the behaviors associated with the severity of acne.

MATERIAL AND METHODS

This study was ethically approved by the Institutional Review Board SIRB 796/2018 (EC4), COA no Si 018/2019. Adult male patients were recruited if they had acne, were aged 21 years or older,[6] and attended the Outpatient Dermatologic Clinic, Siriraj Hospital, between January 2019 and June 2020. Informed consent was obtained from all of the patients. The demographic data collected included the patients' present ages, their age at acne onset, and aggravating factors. The acne was classified into 3 types: persistent, late-onset, and relapse. Persistent acne was defined as acne that had an onset earlier than 21 years of age and which continued into adult life. Late-onset acne was defined as acne with an onset of ≥ 21 years of age (usually between 21 and 25 years of age). Relapse acne referred to acne that had an onset earlier than 21 years of age, but subsequently subsided before recurring sometime after remission.[6]

A record was made of each patient's skin type (oily; normal; dry; and mixed [oily nose, and normal-to-dry skin on the cheeks]), which was determined by a combination of history taking and physical examination. All of the physical examinations were conducted by the one dermatologist (CL). Severity of acne was recorded throughout all affected areas such as the whole face, neck, chest and back. The severity of the acne, and its location and characteristics were characterized using the Investigator's Global Assessment (IGA) severity scale. IGA offers a composite score to define acne severity, and the tool has been accepted by the U.S. Food and Drug Administration. The acne severity can be assessed using two methods. With the first method, the acne severity is classed as "mild", "moderate", or "severe", depending on the number of comedones, inflammatory lesions, lesions, and pseudocysts. Mild acne has < 20 comedones, < 15 inflammatory papules, a total lesion count of < 30, and no pseudocysts. Moderate acne has 20–100 comedones, 15–50 inflammatory lesions, a total lesion count of 30–125, and no pseudocysts. Severe acne has > 100 comedones, > 50 inflammatory lesions, a total lesion count of > 125, and > 5 pseudocysts. The second classification method utilizes a five-point scale: 0 = "clear", 1 = "almost clear", 2 = "mild", 3 = "moderate", and 4 = "severe". Clear acne means that there are no lesions, while almost-clear acne describes the condition in which there are rare noninflammatory lesions and only one inflammatory lesion. Mild acne has some noninflammatory lesions and 2–3 inflammatory lesions. Moderate acne has many noninflammatory lesions, some inflammatory lesions, and one nodular lesion. Severe acne has many noninflammatory and inflammatory lesions, as well as 2–3 nodular lesions.[7]

An evaluation was made of the patients' acne complications, such as post-inflammatory hypo/hyperpigmentation, pitted (atrophic) scarring, and hypertrophic scarring. Additionally, the Global Scale for Acne Scar Severity (SCAR-S) system was used to categorize the severity of scars as "macular", "mild", "moderate", and "severe". A macular scar is defined as an erythematous, post-inflammatory hypo/hyperpigmentation scar without any textural change. A mild scar presents a mild atrophic appearance, and the scar can be covered by makeup or facial hair. A moderate scar can be covered by manually stretching the skin, but not by makeup. A severe scar is a permanent scar that cannot be removed by stretching the skin.[8]

Data were analyzed using PASW Statistics for Windows (version 18.0; SPSS Inc., Chicago, Ill., USA). *P*-values less than 0.05 indicates statistical significance.

RESULTS

Seventy-two male patients with acne were enrolled. Their mean age was 26.9 ± 4.3 years, and their mean body mass index was 21.9 ± 3.0 kilogram/m^2. The mean age of acne onset was 15.3 ± 2.5 years. Onset of the patients' secondary sex characteristics occurred between 12 and 18 years of age, with the mean age of onset of voice cracks, shin-hair growth, and beard growth being 14.4 ± 1.6, 15.1 ± 2.3, and 15.6 ± 2.1 years, respectively. Seventy-two percent had a positive family history of acne; of those, 94.2% had first-degree relatives with acne while the rest (5.8%) had second-degree relatives with acne. Table 1 details the skin and acne types, acne severities, habits related to acne, and previous treatments. Over half of the patients (55.6%) had the oily skin type, while 37.5% had the mixed type. Most cases were of the persistent acne type, followed by relapse acne and then late-onset acne. With the persistent acne group, the condition first developed at the mean age of 15.1 ± 1.9 years, continued to 26.4 ± 5.7 years of age, and then subsided. As to the relapse group, the acne first appeared at the mean age of 15.4 ± 1.9 years; it subsequently subsided for a duration of 9.7 ± 8.2 years before recurring at the age of 24.3 ± 4.4 years. The late-onset acne commenced at 27.7 ± 2.5 years of age.

TABLE 1. Demographic data and previous treatments of adult male acne patients.

Characteristics	N/72 (%)
Skin type	
Oily	40 (55.6)
Mixed	27 (37.5)
Normal	5 (6.9)
Type of adult male acne	
Persistent	45 (62.5)
Relapse	24 (33.3)
Late-onset	3 (4.2)
Severity based on IGA-scale	
Mild	60 (83.3)
Moderate	11 (15.3)
Severe	1 (1.4)
Severity based on 5-scale IGA	
0 = clear	0 (0)
1 = almost clear	21 (29.2)
2 = mild	33 (45.8)
3 = moderate	17 (23.6)
4 = severe	1 (1.4)
Behavior during acne period	
Picking pimples	50 (69.4)
Frequent face washing	17 (23.6)
Using facial sheet masks	3 (4.2)
Previous treatment	
Topical medications	
2.5%-5% benzoyl peroxide	35 (48.6)
1% clindamycin	30 (41.7)
Adapalene	13 (18.1)
0.025%-0.05% tretinoin	12 (16.7)
4% erythromycin	8 (11.1)
Azaleic acid	6 (8.3)
Systemic medications	
Antibiotics	20 (27.8)
Isotretinoin	16 (22.2)

Abbreviation: IGA, Investigator's Global Assessment

According to the IGA-scale severity assessments, the majority of our patients had mild acne. Pimple picking, followed by frequent facial washing, were common habits during the acne period of the male patients. The advice of dermatologists was sought by 45.8% of the 72 patients, and a comparable proportion (43.0%) consulted general practitioners. A sizeable minority (19.4%) bought over-the-counter medications, but very few patients (1.4%) sought treatment at beauty salons. Most used several therapies for their acne. The most common topical treatment was benzoyl peroxide in conjunction with topical antibiotics. Doxycycline was the most frequently used antibiotic; the 2 antibiotics, sulfamethoxazole and trimethoprim, were used in combination by a minority of patients.

The most common locations on the face were the

cheeks (100%), the chin (68.1%), and the forehead (54.7%). The most frequently observed clinical presentation was inflammatory lesions, followed by whitehead comedones, blackhead comedones, and nodular lesions. Our study revealed the types of acne lesions that were present at various locations of the face. (Fig 1) Inflammatory acne was usually found around the cheeks and perioral areas, whereas whitehead comedones typically occurred on the forehead. One-third of the patients had acne on both facial and non facial areas (such as the chest and back).

Post-inflammatory hypo/hyperpigmentation (75%) and pitted (atrophic) scarring (72.2%) were common complications, but hypertrophic scarring was found in only 12.5% of the cohort. Based on the SCAR-S system, 40.3%, 29.2%, 29.2%, and 1.3% had macular, mild, moderate, and severe scarring, respectively. One patient with severe scarring had a history of anabolic steroid use for body building during the acne period. Table 2 lists the factors that were deemed to be potentially associated with acne severity. The proportion of patients who frequently shaved their beard was significantly higher for patients with moderate-to-severe acne than patients with mild acne. However, it should be noted that of the 29 patients who frequently shaved their beard, 20 (69%) had acne lesions on the chin.

DISCUSSION

Acne is a chronic inflammatory skin disease affecting all generations of the population. The results of our study are consistent with those of previous studies on females in that persistent acne was found to be the most common type of post-adolescent acne.[6] However, the common acne locations in our male patients (the forehead and both cheeks) differed from those reported for post-adolescent females (the lower face: chin, jawline, and neck).[9,10] The data on the predominant type of lesions found in female acne are controversial. A review by Holzmann et al. showed that comedones were usually minimal or absent, yet Bagatin et al. reported that comedones were the most common type. Our study showed that inflammatory papules and comedones were common types of acne in post-adolescent male patients, which is similar to the adolescent-acne patterns found for both genders.[6,9-10]

Regarding acne complications, an Indian study by Khunger et al. claimed that post-acne scarring is more frequent in adults than adolescents due to treatment resistance and delayed therapy.[14] It has also been reported elsewhere that adult male patients tend to have more acne scarring than adult female patients.[3] The acne complications found by Khunger and colleagues were-in descending order of frequency-pigmentary changes, icepicks, rolling,

Fig 1. Clinical presentation of adult male acne on different locations of the face.
Abbreviations: B, blackheads: **C**, closed comedones: **I**, inflammatory papules: **N**, nodular lesions

TABLE 2. Factors associated with the severity of male acne in this study.

Factors associated with severity of acne	Mild (n = 60) N (%)	Moderate-to-severe (n = 12) N (%)	*P*-value
Inadequate sleep	41 (68.3)	11 (91.7)	0.092
Stress	38 (63.3)	7 (58.3)	0.492
Exercise	8 (13.3)	4 (33.3)	0.106
Exposure to sunlight	18 (30)	5 (41.7)	0.318
Shaving	21 (35)	8 (66.7)	0.044*
Hormonal therapy	1 (1.7)	1 (8.3)	0.308
Smoking	5 (8.3)	2 (16.7)	0.330
Chocolate	11 (18.3)	2 (16.7)	0.629
Sugary diet	15 (9)	2 (16.7)	0.587
Dairy diet	5 (8.3)	2 (16.7)	0.330
Whey protein	4 (6.7)	0 (0)	0.474
Face massage	2 (3.3)	1 (8.3)	0.426
Face scrub	3 (5)	2 (16.7)	0.191
Hairspray/oil	4 (6.7)	0 (0)	0.357
Family history	39 (65.0)	11 (91.7)	0.185

Remark: a *p*-value less than 0.05 indicates statistical significance.

atrophic scarring, and keloidal scarring; this sequence is consistent with the findings of our study.[14] One Polish population-based observational study conducted by Chlebus et al. found that 53.9% of the adult patients with persistent acne had scarring.[15] Our study revealed a higher prevalence of scarring than that reported by Chlebus and colleagues. Rawling et al. reviewed that the Asian skin type has a thinner stratum corneum which is the epidermal outermost layer and protective layer, resulting in more skin barrier defects.[16] This may explain that the Asian skin type is more vulnerable to forming scar tissue than Caucasian skin. Thus, Asian skin may require different care methods.

Table 3 summarizes the factors identified by previous studies as being associated with acne severity in post-adolescent male and female patients. The likelihood of having the more severe forms of acne was reported to increase with family history of acne; the consumption of high-dairy, fatty, or sugary diets; the drinking of milk and sugary beverages; the presence of hirsutism, acanthosis nigricans, or excessive seborrhea; chemical substance exposure; smoking; acne onset during adolescence; and the male gender.[1,5,9,11-12,15,18,21,23-25] Smoking has been shown to be able to increase and decrease acne severity, especially in male patients.[1,13,16,19] Due to the limited number of patients, our study did not find any significant differences between the mild and moderate-to-severe acne groups in terms of the aforementioned factors. However, shaving was significantly found more often in patients with the more severe forms of acne. We assumed that the shaving technique employed and the use, or non-use, of a shaving gel or cream might contribute to this finding. It should be noted that our study and the work by Klaz and colleague comprised only male patients.[17]

There are some limitations to our study. Firstly, a limited number of patients were enrolled. Moreover, this work was conducted at a tertiary hospital in Thailand, and not on the general population; the results may therefore not be generalizable. Lastly, this research did not include male-to-female transgender individuals, who may receive

TABLE 3. Comparison of previous studies on post-adolescent acne with our study.

Study	Goulden et al. 1999[11]	Schafer et al. 2001[13]	Klaz et al. 2006[17]	Xu et al. 2007[20]	Wei et al. 2010[18]	Ismail et al. 2012[12]	Khunger et al. 2012[14]	Wolkenstein et al. 2015[21]	Di Landro et al. 2017[22]	Karadag et al. 2019[19]	Wolkenstein et al. 2018[24]	Aalemi et al. 2019[23]	Kaminsky et al. 2019[9]	Ibrahim et al. 2019[25]	Penso et al. 2020[5]	Our study
Type of study	Case-control	Cross sectional	Cross sectional	Cross sectional	Cross sectional, case-control	Case-control	Cross sectional	Cross sectional	Case-control	Prospective, case control	Cross sectional	Case-control	Observational study	Case-control	Cross sectional	Prospective, cross sectional
Nationality	British	German	Israelian	Chinese Han	Chinese	Malaysian (79.5%) Non-Malay (20.5%)	Indian	French	Italian	Turkish	Belgian Czechian Slovakian, French, Italian, Polish, Spanish	Afghan	Latin, American, Iberian	Egyptian	French	Thai
No of cases **Male** **Female** **Age (yrs)**	204 – N/A – N/A – > 25	896 – 49.2% – 50.8% – 1 to 87	27,083 – 100% – 0% – 21 to 22	975 – 51% – 49% – 16 to 25	2,920 – 51.26% – 49.65% – 17 to 25	44 – 34.1% – 65.9% – 18 to 30	280 – 17.9% – 82.1% – > 25	2,266 – 41.2% – 58.8% – 15 to 24	205 – 50.2% – 49.8% – 10 to 24	3,826 – 31.4 – 68.6 – > 25	6,063 – 50.42% – 49.58% – 15 to 24	279 – 54.1% – 45.9% – 10 to 24	1,384 – 20% – 80% – 25 to 60	100 – 19% – 81% – N/A	24,452 – 25% – 75% – > 18	72 – 100% – 0% – > 21
Mean age (yrs)	N/A	42 (median)	21.85 ± 1.16	18.4 (median)	21.56 ± 1.57	18–30	30.5	19.0 ± 2.6	17.2 ± 3.1	20.4 ± 4.52	19.9 ± 2.8	18.7 ± 3.2	33.35 ± 8.42	19.4 ± 4.5	57 ± 14	26.9 ± 4.3
Age at acne onset (yrs)	N/A	12–15 female< male	N/A	N/A	16.24 ± 2.32	N/A	N/A	N/A	N/A	15.92 ± 3.68	N/A	17.4 ± 2.9	N/A	N/A	N/A	15.3 ± 2.5
Types **Persistent** **Late-onset** **Relapse**	N/A	N/A	N/A	N/A	N/A	N/A	– 73.2% – 26.8% – N/A	N/A	N/A	N/A	N/A	N/A	– 66% – 32% – N/A	N/A	N/A	– 62.5% – 33.3% – 4.2 %
Severity **Mild** **Moderate** **Severe**	N/A	– 75.4% – 21.7% – 2.9%	– N/A – N/A – 0.88%	N/A	N/A	N/A	– 61% – 28% – 12%	– 65.2% – 31.2% – 3.6%	N/A	– 12.6% – 42.3% – 45.1%	N/A	N/A	– 36.9% – 48% – 15%	– 35% – 39% – 26%	N/A	– 83.3% – 15.3% – 1.4%
Factors increasing acne severity	Family history of acne	Active smoker	Number of cigarettes smoked per day was a protective factor of acne	Family history of acne	Family history; psycho-logical disorders; insomnia; mental stress; high-caloric diets; oily skin	Family history; high glycemic load; ice-cream; milk	Topical steroid use; drug use	Cannabis; chocolate; sweets	Family history; milk; high BMI (> 18.5)	Family history; smoking; high BMI; chocolate; fruit juice intake	Family history; chocolate intake	Family history; diet (milk, chocolate, eggs, low fat milk, potato chips)	Male	Family history; diet; smoking; sun exposure	Milk; sugary beverages; sugary and fatty products	Shaving

Abbreviations: BMI, body mass index (kg/m^2); EU, European; N/A, not applicable; yrs, years

estrogens and/or antiandrogens. Thus, our findings may not be applicable to this group of patients.

To summarize, in our tropical country, the male patients with post-adolescent acne usually presented with inflammatory lesions and comedones, which were predominantly located on the cheeks. They commonly had an onset earlier than 21 years of age and continued into adulthood, but the severity during the post-adolescent period tended to be mild. While several factors have been reported elsewhere to be involved in the severity of acne, this study found that only shaving influenced severity.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Ms. Saowalak Hunnangkul for her assistance with the statistical analyses. This was an unfunded study.

Conflict of interest: The authors declare that there are no personal or professional conflicts of interest regarding any aspect of this study.

REFERENCES

1. Heng AHS, Chew FT. Systematic review of the epidemiology of acne vulgaris. Sci Rep. 2020;10:5754.
2. Thiboutot DM, Dréno B, Abanmi A, Alexis AF, Araviiskaia E, Barona Cabal MI, et al. Practical management of acne for clinicians: An international consensus from the Global Alliance to Improve Outcome in Acne. J Am Acad Dermatol. 2018;78:S1-S23.e1.
3. Skroza N, Tolino E, Mambrin A, Zuber S, Balduzzi V, Marchesiello A, et al. Adult acne versus adolescent acne: a retrospective study of 1,167 patients. J Clin Aesthet Dermatol. 2018;11:21-5.
4. Chlebus E, Chlebus M. Factor affecting the course and severity of adult acne: Observational cohort study. J Dermatolog Treat. 2017;28:737-44.
5. Penso L, Touvier M, Deschasaux M, Szabo de Edelenyi F, Hercberg S, Ezzedine K, et al. Association between adult acne and dietary behaviors findings from the Nutrinet-Sante' Prospective cohort study. JAMA Dermatol. 2020;156:854-62.
6. Holzmann R, Shakery K. Postadolescent acne in females. Skin Pharmacol Physiol. 2014; 27 Suppl 1:3-8.
7. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Acne Vulgaris: establishing effectiveness of drugs intended for treatment guidance for industry [Internet]. 2018 [cited 2021 Jan 21]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/acne-vulgaris-establishing-effectiveness-drugs-intended-treatment.
8. Clark AK, Saric S, Sivamani RK. Acne Scars: How Do We Grade Them? Am J Clin Dermatol. 2018;19:139-44.
9. Kaminsky A, Florez-White M, Bagatin E, Arias MI. Large prospective study on adult acne in Latin America and the Iberian Peninsula: risk factors, demographics, and clinical characteristics. Int J Dermatol. 2019;58:1277-82.
10. Bagatin E, Freitas THP, Rivitti-Machado MC, Machado MCR, Ribeiro BM, Nunes S, et al. Adult female acne: a guide to clinical practice. An Bras Dermatol. 2019;94:62-75.
11. Goulden V, McGeown H, Cunliffe WJ. The familial risk of adult acne: comparison between first-degree relatives of affected and unaffected individuals. Br J Dermatol. 1999;141:297-300.
12. Ismail NH, Manaf ZA, Azizan NZ. High glycemic load diet, milk and ice cream consumption are related to acne vulgaris in Malaysian young adults: a case control study. BMC Dermatol. 2012;12:13.
13. Schäfer T, Nienhaus A, Vieluf D, Berger J, Ring J. Epidemiology of acne in the general population: the risk of smoking. Br J Dermatol. 2001;145:100-4.
14. Khunger N, Kumar C. A clinico-epidemiological study of adult acne: is it different from adolescent acne? Indian J Dermatol Venereol Leprol. 2012;78:335-41.
15. Chlebus E, Chlebus M. The role of adolescent acne treatment in formation of scars Among patients with persistent adult acne: evidence from an observational study. Cutis. 2019;104:57-61.
16. Rawlings AV. Ethnic skin types: are there differences in skin structure and function? Int J Cosmet Sci. 2006;28:79-93.
17. Klaz I, Kochba I, Shohat T, Zarka S, Brenner S. Severe acne vulgaris and tobacco smoking in young men. J Invest Dermatol. 2006;126:1749-52.
18. Wei B, Pang Y, Zhu H, Qu L, Xiao T, Wei HC, et al. The epidemiology of adolescent acne in North East China. J Eur Acad Dermatol Venereol. 2010;24:953-7.
19. Karadağ AS, Balta I, Saricaoğlu H, Kiliç S, Kelekçi KH, Yildirim M, et al. The effect of personal, familial, and environmental characteristics on acne vulgaris: a prospective, multicenter, case controlled study. G Ital Dermatol Venereol. 2019;154:177-85.
20. Xu SX, Wang HL, Fan X, Sun LD, Yang S, Wang PG, et al. The familial risk of acne vulgaris in Chinese Han—a case-control study. J Eur Acad Dermatol Venereol. 2007;21:602-5.
21. Wolkenstein P, Misery L, Amici JM, Maghia R, Branchoux S, Cazeau C, et al. Smoking and dietary factors associated with moderate-to-severe acne in French adolescents and young adults: results of a survey using a representative sample. Dermatology. 2015;230:34-9.
22. Di Landro A, Cazzaniga S, Parazzini F, Ingordo V, Cusano F, Atzori L, et al. Family history, body mass index, selected dietary factors, menstrual history, and risk of moderate to severe acne in adolescents and young adults. J Am Acad Dermatol. 2012;67:1129-35.
23. Aalemi AK, Anwar I, Chen H. Dairy consumption and acne: a case control study in Kabul, Afghanistan. Clin Cosmet Investig Dermatol. 2019;12:481-7.
24. Wolkenstein P, Machovcová A, Szepietowski JC, Tennstedt D, Veraldi S, Delarue A. Acne prevalence and associations with lifestyle: a cross-sectional online survey of adolescents/young adults in 7 European countries. J Eur Acad Dermatol Venereol. 2018;32:298-306.
25. Ibrahim AA, Salem RM, El-Shimi OS, Baghdady SMA, Hussein S. IL1A (-889) gene polymorphism is associated with the effect of diet as a risk factor in acne vulgaris. J Cosmet Dermatol. 2019;18:333-6.

Human-pet Relationship, Pet Abandonment, and Clinical Correlation for Patients Infected with Dermatophytosis of the Glabrous Skin

Sanchai Sombatmaithai, M.D.*, Sumanas Bunyaratavej, M.D., Pattriya Chanyachailert, M.D.**, Pichaya Limphoka, M.D.**, Chuda Rujitharanawong, M.D.**, Suthasanee Prasertsook, M.D.**, Charussri Leeyaphan, M.D.**, Carren S Hau, M.D., Ph.D.*****

**Department of Dermatology, Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand, **Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, ***Section of Dermatology, Department of Internal Medicine, Manila Doctors Hospital, Manila, Philippines.*

ABSTRACT

Objective: The study on human-pet relationship and pet abandonment among dermatophytosis patients is limited. This study aims to review these correlations.

Materials and Methods: A two-year retrospective cross-sectional study was conducted. Case record forms were reviewed for clinical manifestations, fungal identification, human-pet relationships, and changes in the relationships after dermatophytosis diagnosis.

Results: A total of 230 dermatophytosis patients from the Dermatology outpatient clinic, Siriraj Hospital, were included. The mean age was 41.9 ± 19.1 years and 51.3% were female. Among 170 cases with positive fungal culture, zoophilic dermatophytosis from *M. canis* infection was identified in 15.9% which was predominately found in females and manifested as shorter duration of onset, and higher involvement on exposed areas when compared to anthropophilic dermatophytosis. Most (71%) of patients with *M. canis* infection classified themselves as pet-lovers. The relationship with pets had changed after the dermatophytosis diagnosis in 41% of them which was statistically different from 8.8% in non-pet lovers ($P = 0.001$). The overall pet abandonment rate was 26.6%. The abandonment rate was 40.9% among non-pet lovers, while 30.6% was reported among pet lovers.

Conclusion: Zoophilic *M. canis* infection was associated with rapid onset and on predominant-exposed areas. Some pets could be asymptomatic, so identification of the reservoirs of dermatophytosis is important in the treatment process and helps prevent future recurrence. Paying attention to human-pet relationships and pet abandonment is critical. Knowledge about dermatophytosis transmission, and appropriate pet management should be advised to decrease abandonment.

Keywords: Dermatophyte; animals; Trichophyton; Microsporum; pet (Siriraj Med J 2023; 75: 92-98)

INTRODUCTION

Dermatophytosis is a common skin disease affecting humans. Depending on their reservoirs, the causative pathogens are classified into 3 groups: anthropophilic, zoophilic, and geophilic dermatophytes.[1] The anthropophilic dermatophytes are comprised of *Trichophyton rubrum, Epidermophyton floccosum, Trichophyton violaceum, Trichophyton tonsurans, Trichophyton mentagrophytes* var.

Corresponding author: Sumanas Bunyaratavej
E-mail: consultskin@yahoo.com
Received 25 April 2022 Revised 18 June 2022 Accepted 20 June 2022
ORCID ID: https://orcid.org/0000-0002-0852-7742
http://dx.doi.org/10.33192/smj.v75i2.260745

interdigitale etc. The zoophilic dermatophytes consisted of *Microsporum canis, Trichophyton mentagrophytes* var. *mentagrophytes (T. mentagrophytes), Trichophyton verrucosum etc.*[2] Zoophilic dermatophytes are transmitted to humans from animals, which are major reservoirs. These forms of dermatophytosis tend to cause inflammatory infections and atypical skin lesions in humans.[3,4] The causative organisms vary geographically. *M. canis* and *T. verrucosum* are the most common causative pathogens of zoophilic dermatophytosis in Southern European and Arabic countries.[5,6] Moreover, the incidence of superficial skin infections with *M. canis* has significantly increased, suggesting that zoophilic dermatophytosis will eventually become a major health problem.[5-8] Animal specificity has been reported for the zoophilic species of dermatophytes. Cats and dogs, for example, are the main reservoirs of *M. canis*, which is common in Thailand. In tropical countries, people usually wear short sleeves and pants, which may increase the area exposed to infection. Contact between humans and animals is the chief influence in cases of zoophilic dermatophytosis.[9,10] The animal-borne diseases could result in owners abandoning their pets.

The primary objective of this study was to investigate the human-pet relationship and pet abandonment in terms of the former emotional bonds and the psychological consequences after a diagnosis of a fungal infection. In addition, the clinical manifestations of dermatophytosis caused by zoophilic pathogens were also compared with anthropophilic dermatophytes.

MATERIALS AND METHODS

Study design

The study protocol was approved by the Siriraj Institutional Review Board (COA no. Si 564/2017). This was a 2-year, retrospective cross-sectional chart review. All data were recruited from the case record form of the outpatient fungal infection clinic, Department of Dermatology Siriraj Hospital, a tertiary hospital in Thailand, as every patient who visited our clinic was normally asked the questions via face-to-face interviews and then recorded in the case record form. Those patients diagnosed with dermatophytosis were included in this study. The diagnosis had been confirmed by clinical manifestation and mycological laboratory investigations, including potassium hydroxide preparation and/or fungal culture of dermatophytosis. All specimens were obtained from suspected glabrous skin lesions.

The clinical manifestation and history of having pets were reviewed. The relationship between the owners and their pets was described in 4 aspects, including the affection of the owners for their pets before the dermatophytosis diagnosis, the area where the owners let their pets live in the house, the frequency of animal contact, and physical interaction with their pets. The emotional affection for the pets was graded subjectively by the owners as no, minimal, moderate, high, and extreme affection. Those who labeled themselves to have no or minimal affection for pets were grouped as "non-pet lovers", and those labeled to have moderate, high, and extreme affection were "pet-lovers". The areas where the pets live were classified as the bedroom, outdoor, indoor (any other areas out of the bedroom), and a cage. The frequency of animal contact was graded by the owners as never, sometimes, almost every day, and every day. The physical interaction with the pets was categorized as touch, and hug or kiss.

Their relationship with pets changed after the dermatophytosis diagnosis and the management of the pets after the skin infection diagnosis were also routinely recorded in the case record form of our clinic. Pet abandonment was divided into 2 groups: "no abandonment", when the owners showered their pets more frequently and/or took their pets to visit vets, and "abandonment", when the owners never made physical contact with their pets again.

Statistical analysis

Chi-squared and Fisher's exact tests were used to compare the sites of skin lesions and the relationships between the animals and the owners. Logistic regression was used to analyze the factors associated with dermatophytosis. The statistical analyses were performed with the program IBM SPSS Statistics for Windows, version 18 (IBM Corp., Armonk, N.Y., USA). A significance *P* value of 0.05 was used.

RESULTS

Two hundred and thirty patients were included in this study. The mean age was 41.9 ± 19.1 years, with approximately half of the patients being female (51.3%). Among 130 current pet owners, the most common type of pets reported was dogs-only (46.9%), followed by cats-only (19.2%), and both dogs and cats (13.9%). Based on the 170 confirmed fungal cultures, 27 (15.9%) patients were definitely diagnosed with zoophilic dermatophytosis from *M. canis* infection, whereas 143 patients (84.1%) were reported as anthropophilic dermatophytosis. Comparisons of the demographic and clinical data were presented in Table 1. Females (88.9%) accounted for a significantly higher proportion of zoophilic infections from *M. canis* than males (11.1%; $P = 0.006$). In the case of zoophilic infections, the median duration from

TABLE 1. Factors associated with anthropophilic and zoophilic *M. canis* infections.

Factors	Dermatophytes		Crude Odd Ratio (95% CI)	*P* value	Adjusted Odd Ratio (95% CI)	*P* value
	Anthropophilic N = 143	Zoophilic caused by *M. canis* N = 27				
Sex: n (%)						
Female	60 (42.0)	24 (88.9)	11.07 (3.19–38.45)	< 0.001	11.49 (2.02–65.19)	0.006
Male	83 (58.0)	3 (11.1)	1		1	
Age: years ± SD	38.0 ± 18.0	27.9 ±19.2	0.97 (0.95–0.99)	0.011	0.99 (0.95–1.03)	0.564
Having pets: n (%)						
No	74 (54.8)	2 (7.7)	1		1	
Yes	61 (45.2)	24 (92.3)	14.56 (3.31–64.06)	< 0.001	12.31 (2.21–68.66)	0.004
Unknown	8	1				
Sites: n (%)						
Unexposed areas	77 (67.0)	5 (20.0)	1		1	
Exposed areas	38 (33.0)	20 (80.0)	8.11 (2.82–23.26)	< 0.001	6.41 (1.63–25.25)	0.008
Undetermined	28	2				
Duration of onset of lesion: n (%)						
≤ 1 month	56 (39.7)	23 (92.0)	17.46 (3.96–76.97)	< 0.001	14.24 (2.36–85.81)	0.004
> 1 month	85 (60.3)	2 (8.0)	1		1	
Uncertain	2	2				

"Unexposed areas" of glabrous skin is defined as the trunk, upper arms, and upper legs; "exposed areas" of glabrous skin is defined as the face, neck, forearms, and lower legs; "undetermined areas" is defined as non-glabrous skin or thickened and hard keratin, including the palms, soles, hair, and scalp.

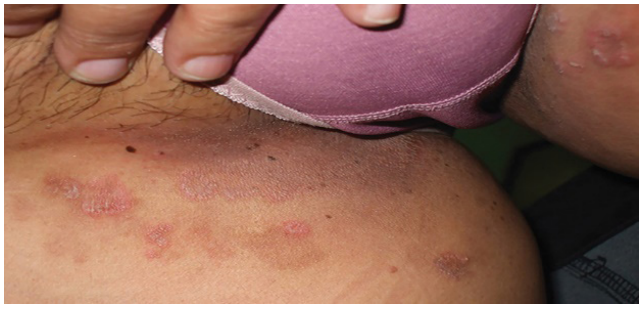


Fig 1. Tinea cruris, presenting with well-defined scaly erythematous annular plaques with hyperpigmentation on non-exposed areas. Anthropophilic dermatophyte *T. rubrum* was identified.

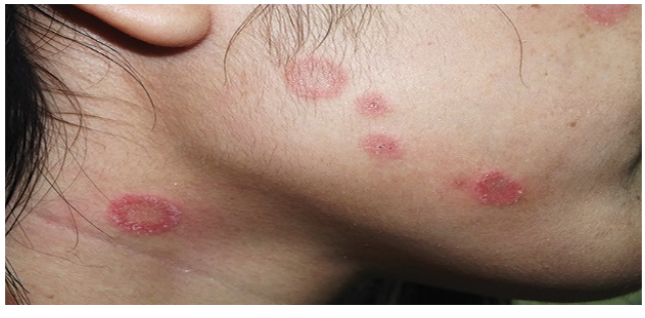


Fig 2. Tinea faciei from animal-transmitted infection, presenting with multiple, discrete, well-defined, erythematous annular plaques with excoriation on exposed areas. Zoophilic dermatophyte *M. canis* from her cat was the causative organism.

the onset of symptoms to the hospital-visit date was 10 days IQR (7, 14) which was statistically shorter than that for anthropophilic infections (median 90 days, IQR 30, 365, $P < 0.001$). Zoophilic dermatophytes were found at exposed areas (80%) of the body more than anthropophilic dermatophytes (33%; $P = 0.008$).

The pet and owner relationships were compared between anthropophilic and zoophilic infection, as shown in Table 2. The results demonstrated that the closer their relationship was, defining as the display of most/extreme affection, a high frequency of contact, and behavior of hugging or kissing their own pets, the higher the owners

TABLE 2. Relationship between the pet and patient associated with dermatophyte infections.

Relationships	Dermatophytes		Odds ratio (95% CI)	*P* value
	Anthropophilic N = 143	Zoophilic caused by *M. canis* N = 27		
Affection for the pet, n (%)				
Minimal, none	21 (47.7)	1 (5.0)	1	
Moderate	16 (36.4)	8 (40.0)	10.5 (1.2–92.7)	0.034
Most, extreme	7 (15.9)	11 (55.0)	33.0 (3.6–303.4)	0.002
NA	99	7		
Frequency of contact, n (%)				
Never, sometimes	28 (62.2)	3 (15.8)	1	
Every day, almost	17 (37.8)	16 (84.2)	8.8 (2.2–34.7)	0.002
NA	98	8		
Type of touch, n (%)				
Touch	29 (74.4)	8 (42.1)	1	
Hugging/kissing	10 (25.6)	11 (57.9)	4.0 (1.3–12.7)	0.019
NA	104	8		
Location of the animals, n (%)				
Outdoor	20 (44.4)	4 (21.1)	1	
Indoors (general)	18 (40.0)	11 (57.9)	3.1 (0.8–11.3)	0.095
Indoors (bedroom)	5 (11.1)	4 (21.1)	4.0 (0.7–21.8)	0.109
In a cage	2 (4.4)	0 (0.0)	–	–
NA	98	8		

NA = not available.

took a zoophilic infection risk. The causative organisms were categorized and compared by the patients' gender. As a result, *T. rubrum, T. mentagrophytes* complex, and *M. canis* were the three most common dermatophytes found (53.4%, 26.1% and 11.7%, respectively). More female patients were infected with the zoophilic *M. canis* than males ($P < 0.001$) whereas the anthropophilic *T. rubrum* was mostly found in males ($P < 0.001$).

There were 161 patients with documented data regarding pets. Seventy-six patients (47.2%) did not have pets. The number of patients who had pets in the zoophilic dermatophytosis disease group (92.3%) was significantly higher than those with anthropophilic dermatophytosis (45.2%; $P = 0.005$). Dermatophyte species isolated from the owners of many kinds of pets were demonstrated in Table 3. It was also found that 61.9% of the patients infected with *M. canis* reported that their pets got hair loss or skin inflammation.

After the diagnosis of dermatophytosis, the overall patients reported showering their pets more frequently was 60.7%, took their pets to visit the vet 16.1%, and never made physical contact with their pets again 23.2%. Sixty-one patients were classified as pet-lovers who reported their status change with pets. Twenty-five of them (41.0%) reported that the relationship with pets changed after the dermatophytosis diagnosis which was statistically different from 8.8% in non-pet lovers ($P = 0.001$), as shown in Table 3. One of the factors which contributed to these significant changes in the relationship might be due to the management of pets after the diagnosis. The patients who classified themselves as pet lovers took their pets to visit the vet more than those non-pet lovers. As a result, non-pet lovers demonstrated a 40.9% abandonment rate while the abandonment rate of pet-lovers was only 30.6% ($P = 0.421$). Overall recurrence rate was 24.7%, and pet-lovers (17.5%) had a significantly lower rate than non-pet lovers (38.2%; $P = 0.024$).

DISCUSSION

Similar to the previous study, *T. rubrum* was found to have the highest prevalence, followed by *T. mentagrophytes* complex and *M. canis*.[11] Dermatophyte

TABLE 3. Behavior of owner after the diagnosis of dermatophytosis.

Management after diagnosis of dermatophytosis	Affection with pet		*P* value
	Pet-lover	Non-pet lover	
Relationship with pets changed, n (%)	(N= 61)	(N=34)	0.001
Yes	25 (41.0)	3 (8.8)	
No	36 (59.0)	31 (91.2)	
Management of pets, n (%)	(N= 37)	(N=22)	0.003
Shower pets more frequently	9 (24.3)	12 (54.5)	
Took pets to visit the vet	17 (45.9)	1 (4.5)	
Never made any more physical contact	11 (29.7)	9 (40.9)	
Abandonment of pets, n (%)	(N= 36)	(N= 22)	0.421
Yes	11 (30.6)	9 (40.9)	
No	25 (69.4)	13 (59.1)	
Recurrence, n (%)	(N= 63)	(N=34)	0.024
Yes	11 (17.5)	13 (38.2)	
No	52 (82.5)	21 (61.8)	

infections transmitted via animals are common. Nowadays, dermatophytosis transmitted by pets and livestock through contact is likely to affect children and young adults.[12] Zoophilic dermatophytes are considered to be causative pathogens, especially *M. canis*, of which cats and dogs are the main reservoirs.[12-18] In this study, 92.3% of the patients infected with *M. canis* owned pets. Among these pets, the majority were reported to be symptomatic, but not all of them. As reported in previous studies, the infected pets could be totally asymptomatic.[19] The correlation between zoophilic infections and the factors of sex and age depends on the dermatophyte species and the type of animal, such as a pet or livestock.[5,20] Female owners had the highest prevalence of zoophilic dermatophytosis, correlated with previous studies.[21] In addition, the more intimacy between patients and their pets, the more chance patients would be infected with zoophilic *M. canis*, as this current study showed that pet-lovers were significantly closer to their pet than non-pet lovers, and *M. canis* was also found significantly in them. In the current study, the owners of cats-only had a significantly higher prevalence of *M. canis* (60.0%) than those with dogs-only (11.4%), with $P < 0.001$. This corresponds well with the previous study: cats infected by *M. canis* caused significant environmental contamination, shedding viable, airborne, fungal elements.[16,22] Dogs have been reported to play a lesser role in the environmental spreading of *M. canis*, contaminating surfaces but never the air.[13,23,24]

Previous studies have investigated the relationship between causative pathogens and tinea infections. *T. rubrum* was found to predominately cause tinea corporis, tinea cruris, and tinea pedis, whereas *M. canis* commonly caused tinea capitis because exposed areas (such as the scalp, beard, face, and arms) are prone to zoophilic dermatophyte infection.[21,25] As with our investigation, those findings demonstrated significant correlations between exposed areas and zoophilic dermatophytosis, and between unexposed areas and anthropophilic dermatophytosis.

The research on the human-animal relationship in patients with zoophilic dermatophytosis was limited. Most patients (70.5%) showed no changes in the human-pet relationship after the diagnosis of an animal-transmitted infection. However, the relationship status was influenced by how the patients behaved with their animals. The change in relationship rate for non-pet lovers was significantly lower than those of pet lovers. It may be due to self-awareness and knowledge of the infection in pet-lovers. They had a better understanding of the transmission of zoophilic infections so they kept more distance between themselves and their pets, as shown in the recurrence rate of pet-lovers which was less than non-pet lovers. Therefore, it is essential for patients to be educated that zoophilic dermatophytosis is curable,[26] and the level of awareness of animal abandonment should be raised. Treatment of pets by veterinarians is needed because the infected pets are reservoirs of dermatophytes and can spread the infection to both humans and other pets.

The study had several limitations. Firstly, since this was a retrospective chart review, some data were incomplete or derived from recall histories. Secondly, Because *T. mentagrophytes* complex is a species complex consisting of 5 species, including *T. interdigitale* as the anthropophilic species and *T. mentagrophytes* as the zoophilic species, the diagnosis can be ambiguous due to phenotypic variations. The molecular technique that helps to differentiate *T. interdigitale* from *T. mentagrophytes* was not routinely used to reveal the identity of the causative species. They were therefore grouped as *T. mentagrophytes* complex and were included only in the descriptive analysis of the prevalence of the dermatophyte species. Regarding sample size calculation, it was calculated based on prevalence of zoophilic dermatophytosis among those with dermatophytosis. However, sample size of subgroup population was not evaluated. Finally, fungal cultures from the pets were not obtained to prove the causative relationship.

In summary, the nature of the relationship between the patients and the pets was the chief influence in the type of dermatophytosis. Dermatologists should be aware that zoophilic infections can have a rapid onset and mainly affect the exposed body parts. Although some pets were reported to be asymptomatic, identification of the reservoirs of dermatophytosis is important in the treatment process and helps prevent future recurrence. A close relationship between humans and pets is also an important factor that leads to appropriate pet management after diagnosis of zoophilic dermatophytosis, resulting in good treatment outcomes and minimizing the risk of recurrence.

ACKNOWLEDGMENTS

All authors declare that there were no conflicts of interest. We thank Dr. Chulaluk Komoltri for her statistical advice. We also thank Rungsima Kiratiwongwan, MD and Supisara Wongdama, MD, for their kind support.

Conflicts of interest: None declared

Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit-sectors.

REFERENCES

1. Pires CA, Cruz NF, Lobato AM, Sousa PO, Carneiro FR, Mendes AM. Clinical, epidemiological, and therapeutic profile of dermatophytosis. An Bras Dermatol 2014;89(2):259-64.
2. Craddock LN, SM S. Superficial Fungal Infection. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, et al., eds. Fitzpatrick's Dermatology, 9th ed: McGraw-Hill, 2019. p. 2927.
3. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995;8(2):240-59.
4. Bunyaratavej S, Kiratiwongwan R, Limphoka P, Lertrujiwanit K, Leeyaphan C. Pattern Recognition using Morphologies of Anthropophilic and Zoophilic Dermatophytosis Lesions: Comparison between Final-Year Medical Students and Dermatology Residents. Siriraj Med J 2020;72(6):488-91.
5. Aghamirian MR, Ghiasian SA. Dermatophytoses in outpatients attending the Dermatology Center of Avicenna Hospital in Qazvin, Iran. Mycoses 2008;51(2):155-60.
6. Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. Mycopathologia 2008;166(5-6):335-52.
7. Cai W, Lu C, Li X, Zhang J, Zhan P, Xi L, et al. Epidemiology of Superficial Fungal Infections in Guangdong, Southern China: A Retrospective Study from 2004 to 2014. Mycopathologia 2016;181(5-6):387-95.
8. Rashidian S, Falahati M, Kordbacheh P, Mahmoudi M, Safara M, Sadeghi Tafti H, et al. A study on etiologic agents and clinical manifestations of dermatophytosis in Yazd, Iran. Curr Med Mycol 2015;1(4):20-5.
9. Chermette R, Ferreiro L, Guillot J. Dermatophytoses in animals. Mycopathologia 2008;166(5-6):385-405.
10. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses 2008;51 Suppl 4:2-15.
11. Bunyaratavej S, Limphoka P, Kiratiwongwan R, Leeyaphan C. Survey of skin and nail fungal infections by subject age among thai adults and the etiological organisms. Southeast Asian J Trop Med Public Health SE 2019;50(6):1118-31.
12. Nenoff P, Handrick W, Krüger C, Vissiennon T, Wichmann K, Gräser Y, et al. Dermatomykosen durch Haus- und Nutztiere. Der Hautarzt 2012;63(11):848-58.
13. Cafarchia C, Romito D, Sasanelli M, Lia R, Capelli G, Otranto D. The epidemiology of canine and feline dermatophytoses in southern Italy. Mycoses 2004;47(11-12):508-13.
14. Halsby KD, Walsh AL, Campbell C, Hewitt K, Morgan D. Healthy animals, healthy people: zoonosis risk from animal contact in pet shops, a systematic review of the literature. PLoS One 2014;9(2):e89309.
15. Katoh T, Maruyama R, Nishioka K, Sano T. Tinea corporis due to *Microsporum canis* from an asymptomatic dog. J Dermatol 1991;18(6):356-9.
16. Mancianti F, Nardoni S, Corazza M, D'Achille P, Ponticelli C. Environmental detection of *Microsporum canis* arthrospores in the households of infected cats and dogs. J Feline Med Surg 2003;5(6):323-8.
17. Shiraki Y, Hiruma M, Matsuba Y, Kano R, Makimura K, Ikeda S, et al. A case of tinea corporis caused by *Arthroderma benhamiae* (teleomorph of *Tinea mentagrophytes*) in a pet shop employee. J Am Acad Dermatol 2006;55(1):153-4.
18. Stull JW, Peregrine AS, Sargeant JM, Weese JS. Household knowledge, attitudes and practices related to pet contact and associated zoonoses in Ontario, Canada. BMC Public Health 2012;12(1):553.
19. Kobwanthanakun W, Bunyaratavej S, Leeyaphan C. Tinea corporis from *Microsporum canis*: A case report in 2 patients from 1 asymptomatic feline. Thai J Dermatol 2018;34(4):299-304.
20. Iorio R, Cafarchia C, Capelli G, Fasciocco D, Otranto D,

GiangasperoA. Dermatophytoses in cats and humans in central Italy: epidemiological aspects. Mycoses 2007;50(6):491-5.
21. Bunyaratavej S, Limphoka P, Kiratiwongwan R, Leeyaphan C. Comparison of patient and clinical differences between superficial skin infections due to *Microsporum canis* and *Trichophyton rubrum*. Southeast Asian J Trop Med Public Health SE 2020;51(1).
22. Limphoka P, Bunyaratavej S, Leeyaphan C. Fingernail onychomycosis caused by *Microsporum canis* in a teenager. Pediatr Dermatol 2021;38(2):524-5.
23. Cafarchia C, Romito D, Capelli G, Guillot J, Otranto D. Isolation of *Microsporum canis* from the hair coat of pet dogs and cats belonging to owners diagnosed with *M. canis* tinea corporis. Vet Dermatol 2006;17(5):327-31.
24. Moriello KA, Coyner K, Paterson S, Mignon B. Diagnosis and treatment of dermatophytosis in dogs and cats.: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol 2017;28(3):266-e68.
25. Ali-Shtayeh MS, Yaish S, Jamous RM, Arda H, Husein EI. Updating the epidemiology of dermatophyte infections in Palestine with special reference to concomitant dermatophytosis. J Mycol Med 2015;25(2):116-22.
26. Bunyaratavej S, Kiratiwongwan R, Suphatsathienkul P, Munprom K, Matthapan L, Supcharoenkul S, et al. Effect of Different Shampoos and Contact Time on *Microsporum canis* Infected Hair: In vitro Model Study. Thai J Dermatol 2020;36(4):150-6.

Association Between the Level of Inflammation at Each Anatomical Sexual Activity from Gram Staining and *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Infections

Kittipoom Chinhiran, M.D.*, Rossaphorn Kittiyaowamarn, M.D.*, Busara Bamrungsak, M.Sc.*, Narissara Yuchui, M.Sc.*, Kornsiri Boonprathueng, B.Sc.*, Natnaree Girdthep, M.Sc.*, Thanyaporn Karnpanich, B.Sc.*, Phichayut Phinyo, M.D., Ph.D.*,*, Pakpoom Wongyikul, M.D.**, Teodora Elvira Wi, M.D.****

**Bangrak STIs Center, Division of AIDS and STIs, Department of Disease Control, Ministry of Public Health, Bangkok, Thailand. **Center for Clinical Epidemiology and Clinical Statistics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. ***Department of Family Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. ****Department of the Global HIV, Hepatitis and STI Programmes, World Health Organization, Geneva, Switzerland.*

ABSTRACT

Objective: This study aimed to determine the association between the severity of inflammation at each anatomical sexual activity from gram staining with *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections.

Materials and Methods: This study was conducted using laboratory test data from patients at the Bangrak Sexually Transmitted Infections Center. The data obtained consisted of gram staining, which was divided by the number of polymorphonuclear leukocytes (PMNL), NG culture, and Nucleic Acid Amplification Test (NAAT) for NG and CT results.

Results: For the diagnostic association between PMNL and NG infection, the results revealed that samples with urethral PMNL 3+ or 4+ carried a significant likelihood ratio (LR) for positive infection, LR 5.61 (P<0.001) and LR 59.66 (P<0.001), respectively. Cervical, rectal, and pharyngeal PMNL was not related to infection. For CT infection, urethral gram stains with PMNL levels were greater than or equal to 2+ and cervical specimens with PMNL 4+ were associated with CT infection. Rectal and pharyngeal PMNL showed no significant association with CT infection.

Conclusion: Determination of PMNL levels from gram staining contributes to the diagnosis of patients with NG and CT in the urethra, particularly for patients with a high degree of inflammation.

Keywords: Gram staining; culture; nucleic acid amplification test; *Neisseria gonorrhoeae*; *Chlamydia trachomatis*
(Siriraj Med J 2023; 75: 99-105)

INTRODUCTION

Gonorrhea and nongonococcal urethritis (NGU) are sexually transmitted infections (STIs) that remain major public health problems in many countries around the world. According to an estimation from the World Health Organization in 2020, there will be 374 million new STIs in all regions around the world, with 82 million being gonorrhea, and 129 million being chlamydia.[1]

From epidemiological surveillance data in Thailand, it was found that STIs increased the morbidity rate from 28.8 per 100,000 people in 2017 to 33.6 per 100,000 people in 2020, which then slightly decreased to 29.2

Corresponding author: Kittipoom Chinhiran
E-mail: x_destiny_war@hotmail.com
Received 5 July 2022 Revised 14 August 2022 Accepted 17 August 2022
ORCID ID:http://orcid.org/0000-0001-8233-0000
https://doi.org/10.33192/smj.v75i2.260747

per 100,000 people in 2021. The most common disease was gonorrhea, followed by NGU. In 2021, the rate of gonorrhea was 9.2 per 100,000 people, while NGU was 2.7 per 100,000 people. It was found that the age group that experienced the most STIs comprised those aged 15-24 years.[2]

Gonorrhea and NGU are STIs that cause inflammation at the site of infection. Gonorrhea is caused by *Neisseria gonorrhoeae* (NG), while NGU is caused by several types of organisms such as *Chlamydia trachomatis* (CT), *Mycoplasma genitalium, Ureaplasma urealyticum*, and *Trichomonas vaginalis*.[3,4] The most common organism that causes NGU is CT.[3,5,6] In addition, gonorrhea can also be found in coinfection with other sexually transmitted diseases.[5,7] Screening for gonorrhea and NGU can be carried out by gram staining, which can detect pathogens that are gram-negative intracellular diplococci in polymorphonuclear leukocytes (PMNL) in gonorrhea[3,8] and PMNL can be detected in both gonorrhea and nongonococcal urethritis.[3]

Previous studies found that urethral gram staining had sensitivity to the diagnosis of gonorrhea in 95% of symptomatic male patients, with 97% specificity, and cervical gram staining had 40-60% sensitivity.[9] The sensitivity of gram staining is reduced in the rectum, pharynx, and in those who are asymptomatic.[10] The sensitivity of gram staining in diagnosing chlamydia in males is 23%.[11] In females, it was found that using only gram staining had low sensitivity.[12]

Studies that address the association between the markers of inflammation (i.e. PMNL levels) and NG and CT infections are limited. This study aimed to determine the association between the severity of inflammation from gram staining with NG and CT.

MATERIALS AND METHODS

This study was conducted using laboratory test data from patients at the Bangrak STIs Center during the period from January 1, 2019, to December 31, 2021. The data were obtained from routine services consisting of gram staining, NG culture, and Nucleic Acid Amplification Test (NAAT) for NG and CT results. The data were obtained with permission from the Division of AIDS and STIs, and the Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP).

The data used for analysis consisted of gram staining results, which were divided by the number of PMNL/oil immersion field (OIF). They were categorized as no PMNL, rare (1-4 cells/OIF), 1+ (5-10 cells/OIF), 2+ (11-20 cells/OIF), 3+ (21-40 cells/OIF) and 4+ (≥41 cells/OIF).[13] Results of NG culture and NAAT for NG and CT, which are both standard tests for the diagnosis[8] of urethra, cervix, rectum, and pharynx, were collected. Gram staining data that yielded no culture or NAAT results were excluded.

Statistical methods

Data were analyzed using STATA version 17.0. Categorical data were reported as numbers and percentages. Diagnostic accuracy was measured by sensitivity, specificity, likelihood ratio (LR), and area under the receiving operating characteristics (ROC) curve. Confidence intervals for each diagnostic parameter were also estimated at a 95% level. A p-value < 0.05 was considered statistically significant.

RESULTS

Of the 1,474 gram-stained patients, 883 cases were tested for NG by cultures or NAAT and 225 cases were identified as NG infection: urethra (145 cases; 40.7%), cervix (54 cases; 13.8%), rectum (20 cases; 18.5%), pharynx (6 cases; 21.4%); 1,179 cases were tested for CT by NAAT and 431 cases were identified as CT infection: urethra (267 cases; 53.8%), cervix (102 cases; 22.5%), rectum (31 cases; 25.0%), pharynx (31 cases; 29.5%) (Table 1).

TABLE 1. Number of samples with NG and CT infections.

Gram stain samples	Total samples n	Total samples with culture/NAAT for NG n	Positive for NG n (%)	Total samples with NAAT for CT n	Positive for CT n (%)
Urethra	660	356	145 (40.7)	496	267 (53.8)
Cervix	553	391	54 (13.8)	454	102 (22.5)
Rectum	139	108	20 (18.5)	124	31 (25.0)
Pharynx	122	28	6 (21.4)	105	31 (29.5)

Abbreviations: CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; NAAT, Nucleic Acid Amplification Test

For the diagnostic association between PMNL and NG infection, our results revealed that samples with urethral PMNL 3+ or 4+ carried a significant likelihood ratio for positive infection, LR 5.61 (95% CI 2.57 to 12.24, P<0.001) and LR 59.66 (95% CI 25.37 to 140.27, P<0.001), respectively. At a PMNL cutoff point equal to or more than 3+, the sensitivity and specificity for NG infection were 75.2% (95% CI 67.4 to 82.0) and 95.7% (95% CI 92.1 to 98.0), respectively, whereas the sensitivity and specificity were 56.5% (95% CI 48.1 to 64.8) and 99.1% (95% CI 96.6 to 99.9), respectively, for a cutoff point at 4+. Cervical, rectal, and pharyngeal PMNL were not related to infection (Table 2).

For CT infection, urethral gram stain with PMNL levels 2+, 3+, and 4+, the LRs were significantly higher than 1 [LR 1.89 (95% CI 1.09 to 3.27, P 0.024) for PMNL 2+, LR 2.24 (95% CI 1.28 to 3.92, P 0.005) for PMNL 3+, and LR 6.00 (95% CI 3.02 to 11.91, P <0.001) for PMNL 4+]

TABLE 2. Diagnostic accuracy for the number of PMNL for NG infections.

	Positive for NG		Negative for NG		Sensitivity % (95%CI)	Specificity % (95%CI)	LR (95%CI)	P-value	AuROC (95%CI)
	n	(%)	n	(%)					
Urethra	(n=145)		(n=211)						
No PMNL	4	(2.8)	18	(8.5)	NA	NA	0.32 (0.08, 1.01)	0.036	0.89 (0.85, 0.93)
Rare	13	(9.0)	133	(63.0)	97.2 (93.1, 99.2)	8.5 (5.1, 13.1)	0.14 (0.08, 0.25)	<0.001	
1+	12	(8.3)	28	(13.3)	88.3 (81.9, 93.0)	71.6 (65.0, 77.5)	0.62 (0.31, 1.26)	0.188	
2+	7	(4.8)	23	(10.9)	80.0 (72.6, 86.2)	84.8 (79.3, 89.4)	0.44 (0.19, 1.04)	0.061	
3+	27	(18.6)	7	(3.3)	75.2 (67.4, 82.0)	95.7 (92.1, 98.0)	5.61 (2.57, 12.24)	<0.001	
4+	82	(56.6)	2	(1.0)	56.5 (48.1, 64.8)	99.1 (96.6, 99.9)	59.66 (25.37, 140.27)	<0.001	
Cervix	(n=54)		(n=337)						
No PMNL	0	(0.0)	7	(2.1)	NA	NA	NE	NE	0.64 (0.56, 0.72)
Rare	7	(13.0)	80	(23.7)	100.0 (93.4, 100.0)	2.1 (0.8,4.2)	0.29 (0.13,0.62)	0.001	
1+	2	(3.7)	45	(13.4)	87.0 (75.1, 94.6)	25.8 (21.2, 30.8)	0.15 (0.42, 0.51)	0.003	
2+	12	(22.2)	61	(18.1)	83.3 (70.7, 92.1)	39.2 (33.9, 44.6)	0.65 (0.34, 1.25)	0.196	
3+	16	(29.6)	94	(27.9)	61.1 (46.9, 74.1)	57.3 (51.8, 62.6)	0.56 (0.32, 0.99)	0.047	
4+	17	(31.5)	50	(14.8)	31.5 (19.5, 45.6)	85.2 (80.9, 88.8)	1.12 (0.62, 2.03)	0.701	
Rectum	(n=20)		(n=88)						
No PMNL	5	(25.0)	44	(46.6)	NA	NA			0.65 (0.52, 0.78)
Rare	8	(40.0)	36	(40.9)	75.0 (50.9, 91.3)	46.6 (35.9, 57.5)	0.5 (0.18, 1.40)	0.187	
1+	3	(15.0)	4	(4.6)	35.0 (15.4, 59.2)	87.5 (78.7, 93.6)	0.98 (0.39, 2.43)	0.961	
2+	2	(10.0)	4	(4.6)	20.0 (5.7, 43.7)	92.0 (84.3, 96.7)	3.30 (0.73, 14.89)	0.119	
3+	1	(5.0)	3	(3.4)	10.0 (1.2, 31.7)	96.6 (90.4, 99.3)	2.20 (0.39, 12.46)	0.371	
4+	1	(5.0)	0	(0.0)	5.0 (0.1, 24.9)	100.0 (95.9, 100.0)	1.47 (0.15, 14.81)	0.744	
Pharynx	(n=6)		(n=22)						
No PMNL	1	(16.7)	4	(18.2)	NA	NA	0.92 (0.83, 10.17)	0.943	0.61 (0.34,0.89)
Rare	3	(50.0)	16	(72.7)	83.3 (35.9, 99.6)	18.2 (5.2, 40.3)	0.69 (0.15, 3.21)	0.630	
1+	1	(16.7)	2	(9.1)	33.3 (4.3, 77.7)	90.1 (70.8, 98.9)	1.83 (0.14, 24.11)	0.639	
2+	0	(0.0)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	
3+	0	(0.0)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	
4+	1	(16.7)	0	(0.0)	16.7 (0.4, 64.1)	100.0 (84.6, 100.0)	NE	NE	

Abbreviations: AuROC, area under the receiving operating characteristics curve; CI, confidence interval; LR, likelihood ratio; NA, not applicable; NE: not estimable; NG, *Neisseria gonorrhoeae*; PMNL: polymorphonuclear leukocytes

and, thus, were associated with the infection. At a PMNL cutoff point equal to or more than 2+, the sensitivity was 55.1% (95% CI 48.9 to 61.1), and specificity was 79.9% (95% CI 74.1 to 84.9). At PMNL 3+, the sensitivity was 38.6. % (95% CI 32.7 to 44.7), and specificity was 88.6% (95% CI 83.8 to 92.4). At PMNL 4+, sensitivity was 21.0% (95% CI 16.2 to 26.4), and specificity was 96.5% (95% CI 93.2 to 98.5). In cervical specimens, PMNL 4+ was modestly associated with CT infection, with LR 2.03 (95% CI 1.27 to 3.25, P 0.003). At a PMNL cutoff point equal to or more than 4+, sensitivity was 33.3% (95%CI 24.3 to 43.4) and specificity was 83.5% (95% CI 79.2 to 87.2) for diagnosis of CT infection. Rectal and pharyngeal PMNL showed no significant association with CT infection (Table 3).

In terms of discriminative ability, only urethral PMNL levels had an area under ROC higher than 0.70, the generally defined acceptable threshold, for both NG and CT infections (Figs 1 and 2).

TABLE 3. Diagnostic accuracy for the number of PMNL for CT infections.

	Positive for CT		Negative for CT		Sensitivity % (95%CI)	Specificity % (95%CI)	LR (95%CI)	P-value	AuROC (95%CI)
	n	(%)	n	(%)					
Urethra	(n=267)		(n=229)						
No PMNL	9	(3.4)	19	(8.3)	NA	NA	0.41 (0.18, 0.90)	0.025	0.72 (0.68, 0.76)
Rare	75	(28.1)	139	(60.7)	96.6 (93.7, 98.4)	8.3 (5.1, 12.7)	0.46 (0.33, 0.64)	<0.001	
1+	36	(13.5)	25	(10.9)	68.5 (62.6, 74.1)	69.0 (62.6, 74.9)	1.23 (0.72, 2.12)	0.443	
2+	44	(16.5)	20	(8.7)	55.1 (48.9, 61.1)	79.9 (74.1, 84.9)	1.89 (1.09, 3.27)	0.024	
3+	47	(17.6)	18	(7.9)	38.6 (32.7, 44.7)	88.6 (83.8, 92.4)	2.24 (1.28, 3.92)	0.005	
4+	56	(21.0)	8	(3.5)	21.0 (16.2, 26.4)	96.5 (93.2, 98.5)	6.00 (3.02, 11.91)	<0.001	
Cervix	(n=102)		(n=352)						
No PMNL	0	(0.0)	7	(2.0)	NA	NA	NE	NE	0.63 (0.57 ,0.69)
Rare	10	(9.8)	83	(23.6)	100.0 (96.4, 100.0)	2.0 (0.8, 4.1)	0.42 (0.21, 0.82)	0.011	
1+	12	(11.8)	45	(12.8)	90.2 (82.7, 95.2)	25.6 (21.1, 30.5)	0.92 (0.47, 1.81)	0.815	
2+	18	(17.7)	64	(18.2)	78.4 (69.2, 86.0)	38.4 (33.2, 43.7)	0.97 (0.55, 1.71)	0.926	
3+	28	(27.5)	95	(27.0)	60.8 (50.6, 70.3)	56.5 (51.2, 61.8)	1.02 (0.63, 1.64)	0.935	
4+	34	(33.3)	58	(16.5)	33.3 (24.3, 43.4)	83.5 (79.2, 87.2)	2.03 (1.27, 3.25)	0.003	
Rectum	(n=31)		(n=93)						
No PMNL	7	(22.6)	44	(48.8)	NA	NA	0.48 (0.20, 1.16)	0.100	0.63 (0.53, 0.73)
Rare	17	(54.8)	36	(38.4)	77.4 (58.9, 90.4)	47.3 (36.9, 57.9)	1.42 (0.70, 2.87)	0.332	
1+	3	(9.7)	4	(4.7)	22.6 (9.6, 41.1)	86.0 (77.3, 92.3)	2.25 (0.49, 10.30)	0.294	
2+	2	(6.5)	5	(4.7)	12.9 (3.6, 29.8)	90.3 (82.4, 95.5)	1.20 (0.22, 6.53)	0.832	
3+	2	(6.5)	3	(2.3)	6.5 (0.8, 21.4)	95.7 (89.4, 98.8)	2.00 (0.33, 12.22)	0.451	
4+	0	(0.0)	1	(1.2)	0.0 (0.0, 11.2)	98.9 (94.2, 100.0)	NE	NE	
Pharynx	(n=31)		(n=74)						
No PMNL	6	(19.4)	25	(33.8)	NA	NA	0.57 (0.21, 1.53)	0.264	0.61 (0.50, 0.71)
Rare	18	(58.1)	41	(55.4)	80.6 (62.5, 92.5)	33.8 (23.2, 45.7)	1.05 (0.52, 2.10)	0.895	
1+	4	(12.9)	6	(8.1)	22.6 (9.6, 41.1)	89.2 (79.8, 95.2)	1.59 (0.42, 6.01)	0.492	
2+	2	(6.5)	2	(2.7)	9.7 (2.0, 25.8)	97.3 (90.6, 99.7)	2.39 (0.34, 16.92)	0.382	
3+	1	(3.2)	0	(0.0)	3.2 (0.1, 16.7)	100.0 (95.1, 100.0)	NE	NE	
4+	0	(0.0)	0	(0.0)			NE	NE	

Abbreviations: AuROC, area under the receiving operating characteristics curve; CI, confidence interval; LR, likelihood ratio; NA, not applicable; NE: not estimable; CT *Chlamydia trachomatis*; PMNL: polymorphonuclear leukocytes

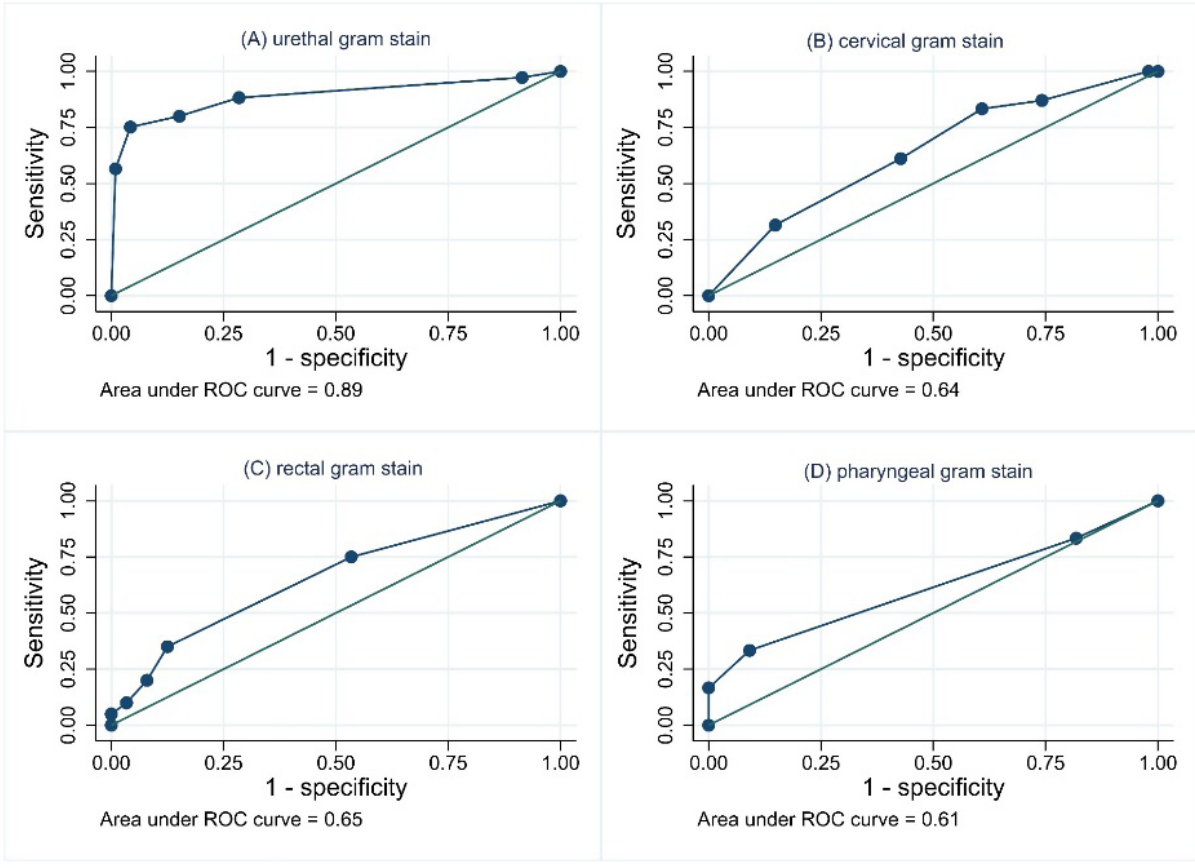


Fig 1. ROC curve for diagnosis of NG from each specimen.

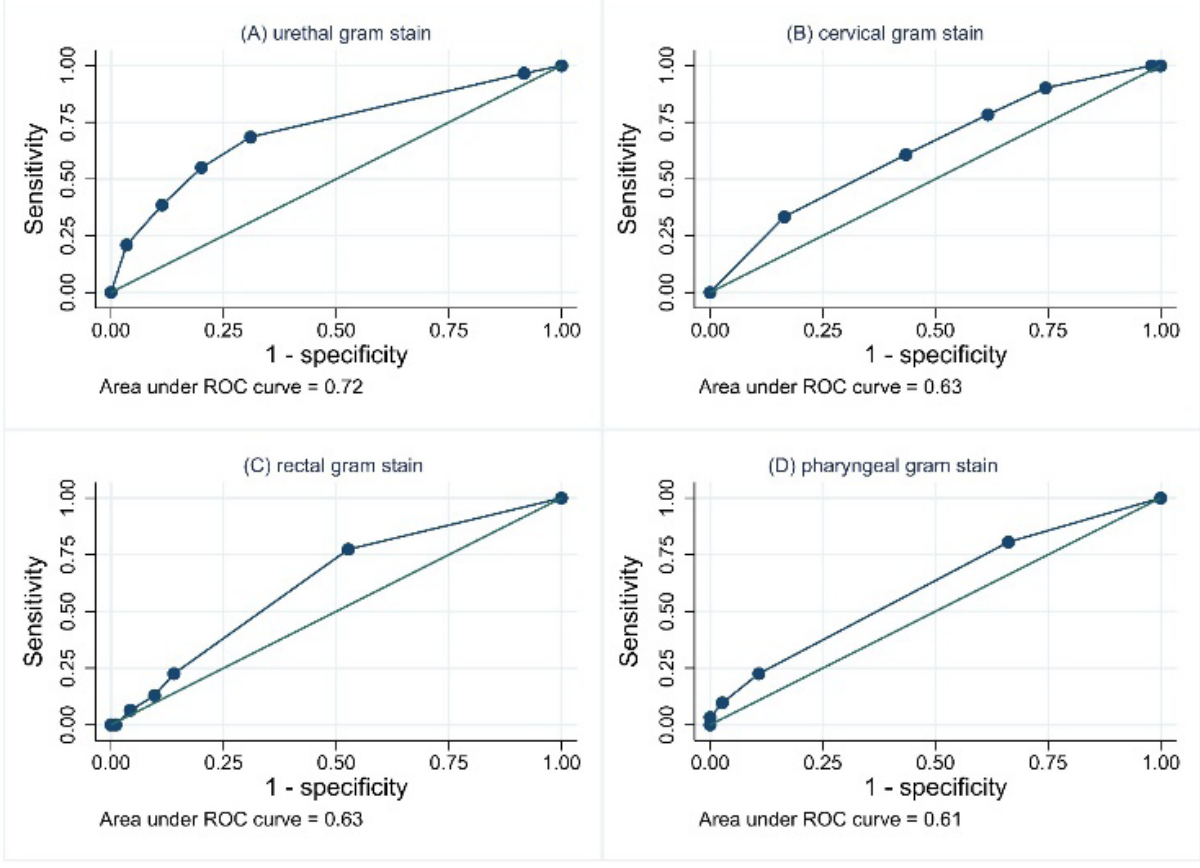


Fig 2. ROC curve for diagnosis of CT from each specimen.

DISCUSSION

Gonorrhea and chlamydia can cause inflammation at the site of the infection. The patient may or may not have symptoms. Screening for inflammation from gram staining can aid in diagnosis and treatment in cases where a culture test or NAAT is not available. The US Center for Disease Control defined PMNL ≥2 WBC/OIF as urethritis and PMNL >10 WBC/high-power field as cervicitis.[3] However, some studies used PMNL values >30 WBC per high-power field for cervicitis.[14,15]

From this study, the inflammation defined by PMNL from gram staining at the urethra was significantly associated with NG and CT infections. Those infected with NG were associated with higher PMNL levels than those with CT (3+ or above in NG and 2+ or above in CT). Inflammation of the cervix was statistically associated with CT infection at the PMNL level 4+. The severity of anal and pharyngeal inflammation was not associated with NG and CT infection.

Therefore, the detection of inflammation from PMNL levels may help in the diagnosis of gonorrhea and chlamydia in the urethra. The high levels of PMNL will have a relatively high specificity found in urethral and cervical infections, so it can help in the diagnosis of infection at the urethra and cervix. However, it may not be used to aid in the diagnosis of gonorrhea or chlamydia in the rectal and pharyngeal region. However, higher PMNL levels have been found to have low sensitivity, while lower PMNL levels have high sensitivity but low specificity, so other factors such as the patient's symptoms, and history of sexual behavior should be used to support the diagnosis.

Usually, gonorrhea is diagnosed using culture or NAAT, while chlamydia uses NAAT as the standard test. However, NAAT for chlamydia is not widely used in Thailand due to the high cost. The syndromic management is more common in gonorrhea and will lead to overtreatment which can cause antimicrobial-resistant microorganisms.[16] Therefore, it is necessary to step forward the diagnosis of gonorrhea and chlamydia from a syndromic approach to laboratory diagnosis by using at least gram staining which is feasible in all levels of hospitals.

From this study, the cutoff of PMNL from gram staining can be used for decision-making in the diagnosis and treatment of gonorrhea and chlamydia. This will reduce the rate of drug resistance from the overuse of antibiotics.

This study had several limitations. First, the number of rectal and pharyngeal samples was too low to draw rigorous conclusions on the diagnostic association between PMNL levels and infections. Second, there was no standardized protocol to collect the specimens from all sites from each included patient, and not all patients were verified for both infections. Therefore, we could not accurately estimate the proportion of patients with isolated infection of NG and CT or patients with co-infection from both organisms.

CONCLUSION

The determination of PMNL levels from gram staining contributes to the diagnosis of patients with NG and CT in the urethra, particularly patients with a high degree of inflammation. If the amount of inflammation is lower, however, other factors such as a history of sexual behavior or additional patient symptoms should be considered to support the diagnosis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Division of AIDS and STIs, and the Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) for permitting the use of data.

Conflict of interest: none

REFERENCES

1. World Health Organization (WHO) [Internet]. Sexually transmitted infections (STIs); 2021 [cited 2022 Mar 22]. Available from: https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis).
2. HIV INFO HUB [Internet]. Rate of five main STIs reported case in Thailand, 2009-2020; 2021 [cited 2022 May 5]. Available from: https://hivhub.ddc.moph.go.th/epidemic.php.
3. Centers for Disease Control and Prevention. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep. 2021;70(4):51-68.
4. Wada K, Hamasuna R, Sadahira T, Araki M, Yamamoto S. UAA-AAUS guideline for M. genitalium and non-chlamydial non-gonococcal urethritis. J Infect Chemother. 2021;27(10):1384-8.
5. Bellinato F, Maurelli M, Gisondi P, Fernandez ML, Girolomoni G. Clinical profile and co-infections of urethritis in males. Ital J Dermatol Venerol. 2021;156(6):681-5.
6. Mostafa MM, Mahdy A, Ghoniem G. Updates on Sexually Transmitted Urethro-cystitis. Current Bladder Dysfunction Reports. 2022.p.1-6.
7. Surawan TM, Jiamton S. Three Co-Existing Sexually Transmitted Diseases in a Heterosexual Male Youth: A Case Report. Siriraj Med J. 2016;68:117-8.
8. Centers for Disease Control and Prevention. Screening Tests To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections 2002. MMWR Recomm Rep. 2002;51(RR-15):6.
9. Unemo M, Ballard R, Ison C, Lewis D, Ndowa F, Peeling R, editors Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. Geneva: WHO

Document Production Services; 2013.
10. Meyer T, Buder S. The Laboratory Diagnosis of Neisseria gonorrhoeae: Current Testing and Future Demands. Pathogens. 2020;9(91):1-19.
11. Orellana MA, Gómez-Lus ML, Lora D. Sensitivity of Gram stain in the diagnosis of urethritis in men. Sex Transm Infect. 2012;88:284-7.
12. Myziuk L, Romanowski B, Brown M. Endocervical Gram stain smears and their usefulness in the diagnosis of *Chlamydia trachomatis*. Sex Transm Inf. 2001;77:103-6.
13. Charoenwattanachokchai A, Lokpichart S, editors. Laboratory Manual for STIs Diagnosis. Nakhon Pathom: National Office of Buddhism; 2010.
14. Randjelovic I, Moghaddam A, De Blasio BF, Moi H. The Role of Polymorphonuclear Leukocyte Counts from Urethra, Cervix, and Vaginal Wet Mount in Diagnosis of Nongonococcal Lower Genital Tract Infection. Infect Dis Obstet Gynecol. 2018; 2018:8236575.
15. De la Tablaa VO, Gutiérrezb F. Cervicitis: Etiology, diagnosis and treatment. Enferm Infecc Microbiol Clin (Engl Ed). 2019;37(10): 661-7.
16. Sirivongrangson P, Girdthep N, Sukwicha W, Buasakul P, Tongtoyai J, Weston E, et al. The first year of the global Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) in Bangkok, Thailand, 2015-2016. PLoS One. 2018;13(11): e0206419.

Prevalence and Trend of Photodermatoses in Thailand: A 16-year Retrospective Study at Siriraj Hospital

Surachanee Likittanasombat, M.D.*, Narumol Silpa-archa, M.D.*, Chayada Chaiyabutr, M.D.*, Rattanavalai Nitiyarom, M.D., Wanee Wisuthsarewong, M.D.**, Chanisada Wongpraparut, M.D.***

**Department of Dermatology, **Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: Photodermatoses are a group of cutaneous disorders with abnormal reactions triggered by exposure to sunlight. Previous studies reported varying photodermatoses prevalence in Caucasians and African-Americans; however, it was seldom reported in the Asian population. The aim of our study was to determine the prevalence, clinical characteristics and trend of photodermatoses in Thailand.

Materials and Methods: A retrospective chart review was performed at the Faculty of Medicine Siriraj Hospital, Mahidol University using diagnoses from the International Classification of Disease (ICD), Tenth Revisions codes, between January 2005 and September 2021.

Results: A total of 561 patients with definite diagnoses of photodermatoses were identified. The prevalence of photodermatoses in the outpatient dermatology clinic was 3 cases per 1,000. The most common photodermatoses were chemical and drug-induced photosensitivity (39.4%), followed by immunologically-mediated photodermatoses (30.1%), photo-aggravated dermatoses (29.4%) and genophotodermatoses (1.1%). Overall phototesting was performed in 276 cases (49.2%). In our study, some photodermatoses had unique clinical characteristics including a pinpoint popular variant of polymorphous light eruption and adult-onset actinic prurigo. Over 16 years, the trend of patients being diagnosed with photodermatoses has continued to rise gradually with an increment of 1.67 times.

Conclusion: Photodermatoses are uncommon in Thailand. Some photodermatoses have distinctive clinical features in Asian populations. The trend of photodermatoses in Thailand is continually rising, reflecting an increase in physicians' awareness and knowledge of these cutaneous conditions.

Keywords: Photodermatoses; drug-induced photosensitivity; immunologically-mediated photodermatoses; photo-aggravated dermatoses; genophotodermatoses; polymorphous light eruption (Siriraj Med J 2023; 75: 106-114)

INTRODUCTION

Photodermatoses are a group of skin disorders caused by exposure to sunlight. Photodermatoses are generally classified into four categories: immunologically-mediated photodermatoses, chemical and drug-induced photosensitivity, photoexacerbated dermatoses, and genophotodermatoses.[1-3] Immunologically-mediated photodermatoses are further sub-divided into five diseases: polymorphous light eruption (PMLE), actinic prurigo (AP), chronic actinic dermatitis (CAD), solar urticaria (SU), and hydroa vacciniforme (HV). The pathophysiology of these diseases is still unclear, but it varies depending on different immune dysregulation. Most of these diseases require phototesting to confirm the diagnosis.[1,3,4] Chemical

Corresponding author: Chanisada Wongpraparut
E-mail: chanisada@hotmail.com
Received 26 June 2022 Revised 23 August 2022 Accepted 30 August 2022
ORCID ID:http://orcid.org/0000-0002-9014-3229
https://doi.org/10.33192/smj.v75i2.260748

and drug-induced photosensitivity can be triggered by both external and internal causes. The external causes can be further split into photoallergic and phototoxic reactions. Moreover, various exogenous chemical agents can lead to phytophotodermatitis and photoallergic contact dermatitis. An example of photodermatoses caused by endogenous agents is porphyrias, which are caused by an abnormal heme biosynthetic pathway.[1,3] Meanwhile, photoexacerbated dermatoses such as photoexacerbated eczema, photoexacerbated atopic dermatitis, systemic lupus erythematosus, autoimmune connective tissue diseases, vesiculobullous diseases, HIV photosensitivity, are the main groups of photodermatoses.[1] Last but not least, genophotodermatoses are rare genetic disorders that cause severe photosensitivity and other manifestations, and examples include xeroderma pigmentosum (XP), Bloom syndrome, and Rothmund–Thomson syndrome.[1,2]

To diagnose photodermatoses, the history, clinical presentation, phototesting, an laboratory investigations (e.g. porphyrins, lupus serology) are necessary in each individual case. Phototesting can help in evaluating the degree of photosensitivity and the specific wavelength which elicits a cutaneous response in an individual patient. Moreover, some cases also require a photoprovocation test, photopatch test and skin biopsy.

According to the previous studies, genetics, race, skin phototypes and climate are the factors associated with different prevalence of photodermatoses.[5-9] In Europe, the prevalences of immunologically-mediated photodermatoses is reported to be about 10-20% in PMLE,[10] 16.5 cases per 100,000 for CAD,[11] 3.9 cases per 100,000 for solar urticaria,[11] and 3.3 cases per 100,000 for actinic prurigo.[11] Most of previous studies have been conducted on the African-American and Caucasian populations.[7-9,11] Proportion of photodermatoses was also different between races. PMLE has more prevalence in Africans-Americans and Caucasians compared to other races. Drug-induced photosensitivity and photo-aggravated dermatoses are more prevalent in Caucasians.[9] However, the research that looks at the overall proportion of photodermatoses in the Thai population is still limited. Determining the prevalence, clinical features, and trend of photodermatoses in Thailand was the purpose of this study.

MATERIALS AND METHODS

This study was approved by the ethics committee of the Siriraj Institutional Review Board (COA no. Si 909/2021). A 16-year retrospective chart review was performed at the Faculty of Medicine Siriraj Hospital, Mahidol University, a tertiary care hospital in Thailand, using diagnoses from the International Classification of Disease (ICD), Tenth Revisions codes related to photodermatoses between January 2005 and September 2021. In our study, cases of photodermatoses were selected if the diagnosis included one of the following ICD-10 codes: E52 (Niacin deficiency [pellagra]), E80 (Disorders of porphyrin and bilirubin metabolism), E80.0 (Hereditary erythropoietic porphyria), E80.1 (Porphyria cutanea tarda), E80.2 (Other porphyria), L29.9 (Actinic prurigo), L56.0 (Drug phototoxic response), L56.1 (Drug photoallergic response), L56.2 (Photocontact dermatitis [berloque dermatitis])., L56.3 (Solar urticaria), L56.4 (Polymorphous light eruption), L56.8 (Photoallergic contact dermatitis), L57.8 (Chronic actinic dermatitis), Q82.1 (Xeroderma pigmentosum), Q82.88 (Other specified congenital malformations of skin) and Q87.1 (Congenital malformation syndromes predominantly associated with short stature). All medical records were manually reviewed. The data were collected and included patients' clinical chart data upon further investigations correlated with the ICD code. Phototesting with or without photoprovocation test was required in the diagnoses of immunologically-mediated photodermatoses. Photopatch test was required in the diagnosis of photoallergic contact dermatitis. Diagnosis of porphyrias was made based on clinical presentations and porphyrin profiles. Other photodermatoses can be diagnosed by clinical presentation, phototesting with or without photoprovocation. The exclusion criteria were patients whose clinical history and examination were not suggestive of photodermatoses. Demographic data including age, sex, age onset of skin lesions, skin phototype, photosensitizing agents, area of skin involvement and characteristics of skin lesions were collected. Additional investigations such as phototesting, photoprovocation test, photopatch test, skin biopsy and laboratory investigations were also recorded.

Phototesting by determination of minimal erythema dose (MED) for UVA and UVB was performed on the lower back areas or the buttocks. UVA radiation was delivered by UVA 700 L (Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany); UVB radiation was delivered by a bank of UV 802 L (Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany); and visible light delivered for 30 minutes by a Kodak Carousel S-AV 2020 projector (Kodak AG, Stuttgart, Germany). For the visible light testing, a glass of water was placed in front of the projector to absorb infrared. Another visible light machine included the Fiber-Lite Mi-152 High Intensity Illuminator (Dolan-Jenner industries, Boxborough, MA, USA). An IL1700 radiometer (International light Inc. Newburyport, MA, USA) was used to measure UVA and UVB irradiance.

Minimal Erythema Dose was defined as the lowest UV dose which produced perceptible erythema reading at 24 hours after UVA and UVB irradiation. The result will be interpreted as UVA and UVB photosensitivity if the MED-UVA < 30 J/cm^2 and MED-UVB < 50 mJ/cm^2. The photoprovocation test was done to reproduce skin lesions if the phototest was negative but still clinically related to photodermatoses using an energy of 60-100 J/cm^2 of UVA and 1.5 times MED of UVB for 3 consecutive days. Results of the photoprovocation test were measured at 24, 48 and 72 hours. Our photoallergen set used for photopatch testing in this study was developed in accordance with previous literature of photoallergic contact dermatitis. It is mainly composed of ultraviolet filters, topical medications, fragrances and preservatives. Both sets were removed after 48 hours but one set was irradiated with UVA 10 J/cm^2. Results were interpreted at 48 and 96 hours.

Statistical analysis

Descriptive analysis was used for demographic data presented as frequency and percentage, or as a mean with standard deviation (SD) and median with interquartile range (IQR). Statistical analysis was carried out using a statistical software (SPSS version 18.0; SPSS Inc., Chicago, USA).

RESULTS

A total of 4,371 medical records matching ICD10 codes from the dermatology clinic were collected and a chart review was performed. Our study confirmed the diagnosis of 561 patients with photodermatoses out of a total of 189,806 cases who had visited the dermatology clinic from 2005-2021. The calculated prevalence of photodermatoses in our hospital was 3 cases per 1,000. Of the 561 cases, the majority were females (n=335, 59.7%). The mean age ± SD and mean age to onset ± SD of photodermatoses was 49.1±17.8 years and 47.9±18.4 years, respectively. The median disease duration was 4 months (0.75, 12.0). The majority of patients had skin type IV and V. The most frequently affected areas in photodermatoses were the extensor surface of the upper extremities (n=362, 64.8%), face (n=255, 45.5%) and the V-shape of the neck (n=231, 41.3%). Meanwhile, papules and plaques (n=297, 52.9%), macules and patches (n=254, 45.3%) and wheals and flares (n=29, 5.2%) were the most commonly reported. Examples of photo-distributed lesions are shown in Fig 1. Overall, phototesting, photoprovocation, and photopatch tests were conducted in 276 (49.2%), 37 (6.6%) and 43 (7.7%) patients, respectively. Of the 276 patients who underwent phototesting, positive results were seen in 148 (53.6%) patients. Most of the cases with positive phototesting results were diagnosed with CAD (n=53, 35.8%), SU (n=27, 18.2%) and AP (n=17, 11.5%). The details of photodermatoses, phototesting, photoprovocation, and photopatch test results are summarized in Table 1.

Immunologically-mediated photodermatoses

Of 561 photodermatoses patients, 169 (30.1%) patients were diagnosed with immunologically-mediated photodermatoses. CAD (n=67, 11.9%) was the most prevalent followed by AP (n=38, 6.8%), PMLE (n=35, 6.2%) and SU (n=29, 5.2%). No cases of HV were found.

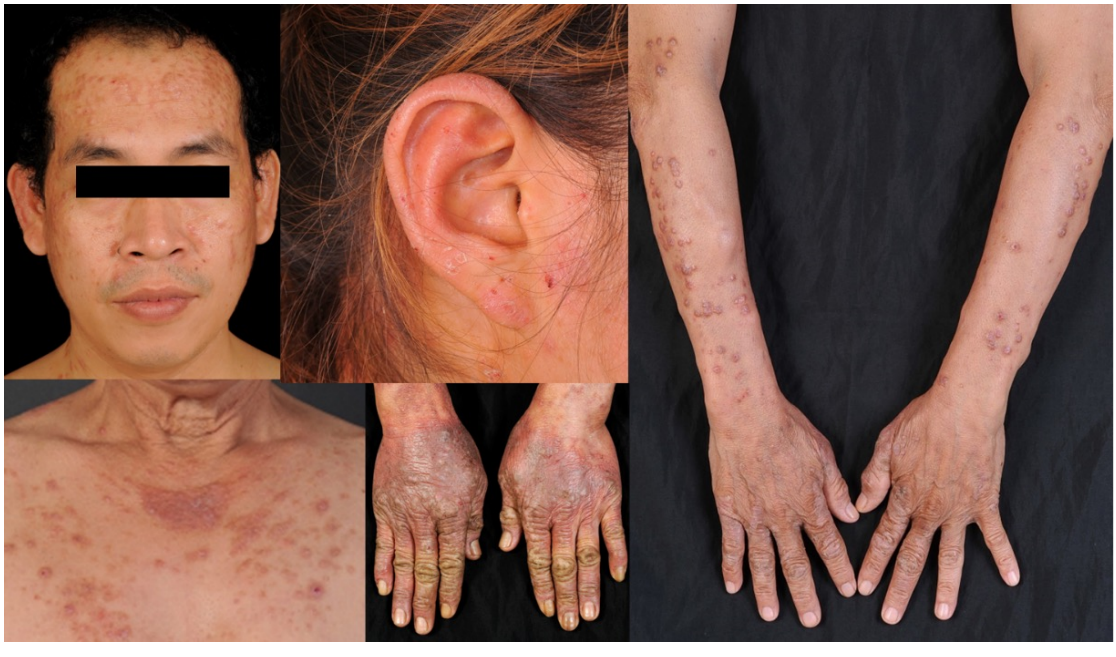


Fig 1. Skin lesions on photodistributed areas.

TABLE 1. Prevalence and photobiological characteristics of each photodermatoses (N=561).

Diagnosis	N (%)	Phototesting (N)					Photoprovocation test (N)				Photopatch test (N)	
		Done	Positive	Positive UVA	Positive UVB	Positive UVA and UVB	Done	Positive UVA	Positive UVB	Positive UVA and UVB	Done	Positive
Immunologically-mediated photodermatoses	169 (30.1%)											
Chronic actinic dermatitis	67 (11.9%)	67	53	20	15	18	1	1	0	0	6	0
Actinic prurigo	38 (6.8%)	37	17	11	2	4	15	7	1	5	1	0
Polymorphous light eruption	35 (6.2%)	20	8	6	2	0	8	3	1	3	1	0
Solar urticaria*	29 (5.2%)	29	27*	7	0	0	-	-	-	-	-	-
Chemical and drug-induced photosensitivity	221 (39.4%)											
Phytophotodermatitis	94 (16.8%)	-	-	-	-	-	-	-	-	-	-	-
Drug-induced photosensitivity	87 (15.5%)	19	11	7	1	3	1	0	0	0	2	0
Photoallergic contact dermatitis	25 (4.5%)	12	5	4	1	0	1	0	0	0	25	25
Porphyrias	15 (2.7%)	1	1	1	0	0	1	1	0	0	-	-
Porphyria cutanea tarda	12 (2.1%)	-	-	-	-	-	-	-	-	-	-	-
Erythropoietic protoporphyria	1 (0.2%)	1#	1	1	0	0	1	1	0	0	-	-
Hepatoerythropoietic porphyria	1 (0.2%)	-	-	-	-	-	-	-	-	-	-	-
Variegate porphyria	1 0.2%)	-	-	-	-	-	-	-	-	-	-	-
Photoexacerbated dermatoses	165 (29.4%)											
Photoexacerbated dermatoses	109 (19.4%)	74	12	7	3	2	7	0	0	0	6	0
Eczema	53 (9.4%)	50	4	2	0	2	5	0	0	0	4	0
Atopic dermatitis	11 (2.0%)	9	3	2	1	0	-	-	-	-	1	0
Dermatomyositis	11 (2.0%)	4	1	0	1	0	-	-	-	-	1	0
Others	34 (6.1%)	11	4	3	1	0	2	0	0	0	-	-
Other unspecified photosensitive dermatitis and photosensitivity	56 (10.0%)	16	13	7	3	3	3	0	0	0	2	0
Genophotodermatoses	6 (1.1%)											
Xeroderma pigmentosum	4 (0.7%)	1	1	0	1	0	-	-	-	-	-	-
Bloom syndrome	1 (0.2%)	-	-	-	-	-	-	-	-	-	-	-
Cockayne syndrome	1 (0.2%)	-	-	-	-	-	-	-	-	-	-	-

*Visible light testing was positive in 9 cases of solar urticaria. Visible light testing and UVA were positive in 11 cases of solar urticaria.
Visible light testing was positive in Erythropoietic protoporphyria

Most of the CAD patients were male (n=55, 82.1%) with a mean age of 57.8±13.0 years. The disease duration before diagnosis varied from one month to 20 years. The upper extremities and face were mostly affected with lesions such as eczematous plaques and papules. Phototesting in CAD showed an abnormal MED in 53 (79.1%) out of 67 patients. Of this group, 20 (37.7%) cases had low MED to UVA, 15 (28.3%) cases had low MED to UVB, and 18 (34.0%) cases had low MED to both UVA and UVB. Other negative phototesting cases can be explained by the early or mild disease. In our institute, visible light is not routinely tested for patients suspected of CAD; however, visible light may be the cause. (Table 1)

AP was the second most prevalent diagnosis of 38 cases. The male to female ratio was 1.9:1, with a mean age of 53.2±11.7 years. The median duration of AP was 1 year (0.5, 2.5). All cases were adult-onset. Clinical presentations were chronic pruritic nodules on faces, extensor surfaces of forearms, and dorsum of hands. None of our cases had cheilitis and conjunctivitis. Of all 38 AP cases, 17 (44.7%) patients had abnormal MED, 11 (28.9%) patients had reduced MED to UVA, 2 (5.3%) patients had reduced MED to UVB and 4 (10.5%) cases had reduced MED to both UVA and UVB. Photoprovocation with UVA and UVB was carried out in 15 patients, with a positive result in 13 (86.7%) cases: 7 (46.7%) patients had UVA positive, 1 (6.7%) patient had UVB positive and 5 (33.3%) patients had both UVA and UVB positive. A high yield was observed on day 3 of the provocation test in both UVA and UVB.

PMLE was the third most prevalent type of immunologically-mediated photodermatoses. The female to male ratio was 4:1, and the mean age was 42.1±14.3 years. The median duration of disease before the diagnosis was 6 (2, 27) months. Lesions were mostly small papules on the extensor surface of the upper extremities compatible with a pinpoint papular variant. Phototesting was carried out in 20 patients and positive results were reported in 8 (40.0%) cases. Among 8 positive phototesting cases, a low MED to UVA and low MED to UVB were observed in 6 (75.0%) and 2 (25.0%) patients, respectively. Further photoprovocation test was done in 8 cases with positive results in 7 (87.5%) cases. Another 15 patients were diagnosed with PMLE clinically.

SU was noted in 29 patients. Of these, 65.5% were female with a mean age ± SD of 32.2±12.0 years, and the median duration of symptoms before diagnosis was 2 (1,4) years. Phototesting with UVA, UVB and visible light was performed and revealed wheals and flares in 27 (93.1%) patients. Of all 29 SU cases, visible light alone and UVA alone were positive in 9 (31.0%) patients and 7 (24.1%) patients respectively, while positive results from both visible light and UVA were noted in 11 (37.9%) patients.

Chemical and drug-induced photosensitivity

Chemical and drug-induced photosensitivity were the most prevalent among the four photodermatoses groups (n=221, 39.4%). These included drug-induced photosensitivity (n=87, 15.5%), phytophotodermatitis (n=94, 16.8%), photoallergic contact dermatitis (n=25, 4.5%) and porphyrias (n=15, 2.7%).

Of the 87 patients diagnosed with drug-induced photosensitivity, phototesting was performed in 19 (21.8%) patients. Culprit drugs responsible for photosensitivity were simvastatin, fenofibrate, piroxicam, thiazides, amiodarone and losartan which were confirmed with abnormal MED in phototesting in 11 patients. None of the patients underwent an oral drug challenge test.

Photoallergic contact dermatitis was reported in 25 (4.5%) patients. A photopatch test was done in all cases and common responsible agents were benzophenone-3 (n=5, 20%) and fragrance mix I (n=3, 12%). The third most common photoallergens were balsam of peru, cobalt, ethylhexyl salicylate, homosalate, PABA, 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoic, 3,4-methylbenzyliden (camphor) which were found in one case each.

Fifteen patients were also diagnosed with porphyrias, based on a clinical, skin biopsy, immunofluorescence and biochemical investigation. Porphyria cutanea tarda (PCT) was reported in 12 patients, followed by a single case of erythropoietic protoporphyria (EPP), hepatoerythropoietic porphyria (HEP) and variegate porphyria.

Photoexacerbated dermatoses

Photoexacerbated dermatoses were reported in 165 (29.4%) patients. Details of each dermatosis are shown in Table 1. Other photoexacerbated dermatoses include lupus erythematosus, T-cell proliferative disorder, HV-like lymphoproliferative disease, actinic granuloma, erythema multiforme-induced photosensitivity, HIV- induced photosensitivity, lichen planus pigmentosus, overlapping syndrome, pseudoporphyria and undifferentiated connective tissue disease. The patients initially presented with abnormal skin lesions on sun-exposed areas or photosensitivity and were referred for photodermatology consultation. An intensive study of their history, physical examination, collection of laboratory data (e.g. porphyrin plasma scan, lupus serology) and photobiological testing was performed for each patient. Phototesting was conducted in 90 patients with some showing abnormal MED. There were some patients whose diagnosis could not be confirmed; however,

skin lesions were presented on sun-exposed areas and associated with sunlight exposure so a diagnosis of other unspecified photosensitive dermatitis and photosensitivity was assigned.

Childhood photodermatoses and genophotodermatoses

In this study, there were 18 patients aged below 18 years, and 12 (66.7%) of them were females. In this childhood group, phytophotodermatitis (33.3%) was the most frequent diagnosis. Xeroderma Pigmentosum (XP) was noted in four patients while Cockayne syndrome and Bloom syndrome were noted in one patient each. Erythropoietic Protoporphyria (EPP) was also diagnosed in one (5.6%) patient.

Trend of photodermatoses

The trend of photodermatoses in our dermatology outpatient clinic is shown in Fig 2. Over the past 16 years, the number of patients diagnosed with photodermatoses, except for genophotodermatoses, has risen gradually. The overall increment between 2005 to 2021 is about 1.67 times based on the trend line. The incidence of photodermatoses was highest in 2019, with a total of 60 cases. The number of cases has fallen during 2020-2021 due to the COVID-19 pandemic.

DISCUSSION

The authors conducted a large retrospective photodermatoses study over 16 years. We confirmed diagnosis in 561 patients and found a low prevalence of 0.3% in our outpatient dermatology clinic at a tertiary care hospital in Thailand. Our study demonstrates a high prevalence of chemical and drug-induced photosensitivity (39.4%), followed by immunologically-mediated photodermatoses (30.1%), photoexacerbated dermatoses (29.4%) and a few cases of genophotodermatoses (1.1%). A comparison of the prevalence of each photodermatosis reported is summarized in Table 2.

The overall prevalence of photodermatoses in previous studies of Asians, Caucasians and African-Americans was congruent.[7] However, a higher proportion of PMLE was noted in African-Americans, while higher proportions of photoallergic contact dermatitis, phototoxic drug eruptions, phytophotodermatitis, porphyrias, and solar urticaria were noted in Caucasians.[9] The mean age and mean age to onset in our study were 42 years, which is similar to previous reports.[7]

In this study, phytophotodermatitis was the most prevalent in the chemical and drug-induced photosensitivity group. This can be explained by common plant-based chemicals called furocoumarins found in Thai recipes e.g. lime, and bergamot. The skin lesions usually develop when patients contact with plant chemicals and then sunlight. Drug-induced photosensitivity was found in 15.5% of all patients, which is similar to other studies from Singapore and the United States, which reported a higher prevalence in Caucasians compared to darker-skinned patients.[6,7,12] Various drugs such as fluoroquinolones, tetracyclines, thiazides, retinoids, diltiazem and nonsteroidal anti-inflammatory drugs are common oral medications that lead to drug-induced photosensitivity.[13] Similar to the previous studies, drugs responsible for photosensitivity

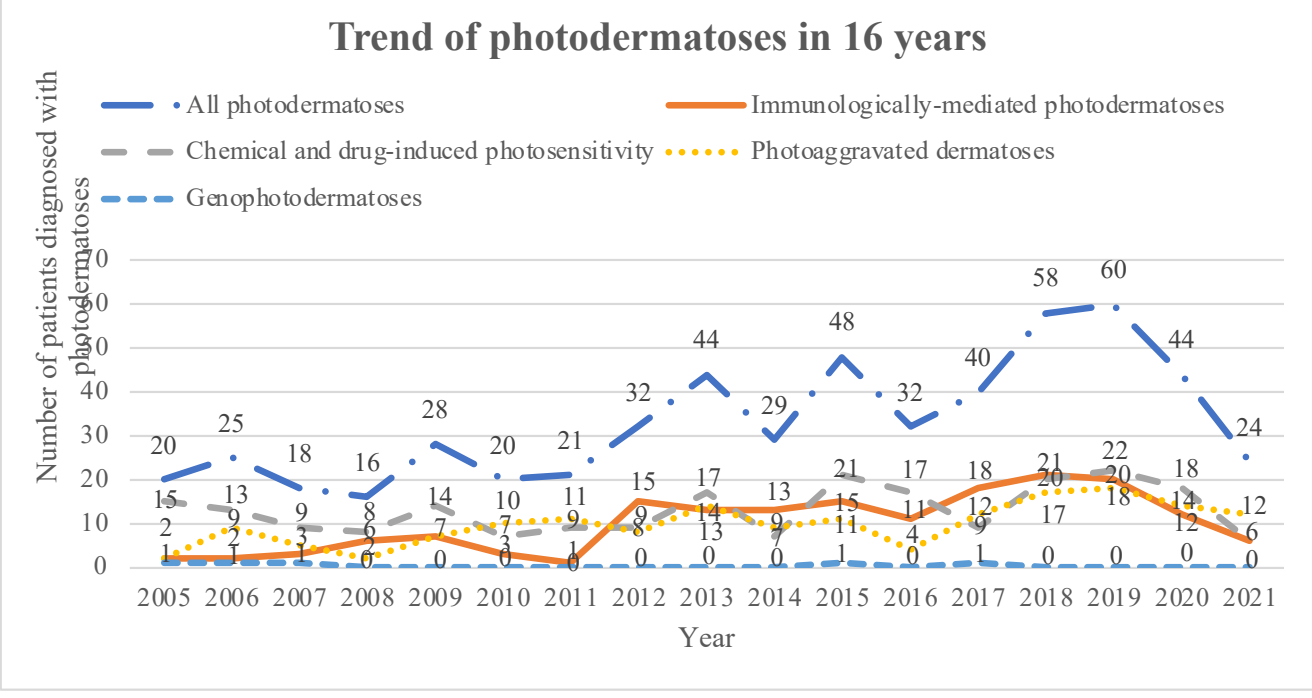


Fig 2. The trend of photodermatoses from 2005 to 2021.

TABLE 2. Prevalence of photodermatoses in previous studies.

Diagnosis	Our study	Fotiades et al.[13]	Khoo et al.[11]	Wong and Khoo[6]	Nakamura et al.[7]		Hamel R et al.[9]		
Country	Thailand	United States	Singapore	Singapore	United States		United States		
Study period	2005-2021	1986-1993	1991-1993	2000-2001	2004-2012		2006-2016		
Number of cases (n)	561 (Asians)	203	152	141	138 (AAF)	63 (Caucasians)	572 (AAF)	378 (Caucasians)	130 (Other races)
Immunologically-mediated photodermatoses (%)									
Polymorphous light eruption	6.2	26	13	25	86.2	54	74.3	43.9	62.3
Chronic actinic dermatitis	11.9	17	5	14	2.9	1.6	4.4	2.9	1.5
Actinic prurigo	6.8	NR	4	4	NR	NR	0	0.8	0.8
Solar urticaria	5.2	4	5	6	0.7	1.6	0.7	3.2	3.8
Hydroa vacciniforme	0	NR	NR	NR	NR	NR	0	0.5	1.5
Chemical and drug-induced photosensitivity (%)									
Porphyrias	2.7	NR	NR	NR	0	7.9	0.3	8.2	2.3
Drug-induced photosensitivity	15.5	7	11	13	0.7	15.9	2.8	13.0	6.2
Phytophotodermatitis	16.8	NR	NR	NR	0	6.3	0	2.1	2.3
Photoallergic contact dermatitis	4.5	8	3	4	0	1.6	1.2	3.7	3.8
Photoexacerbated dermatoses	29.4	NR	32	23	9.4	11.1	15.9	20.9	14.6
Genophotodermatoses	1.1	NR	3	0	NR	NR	NR	NR	NR

Abbreviations: NR= Not reported, AAF= African-Americans

in our study were simvastatin, fenofibrate, and thiazides. The diagnosis of drug-induced photosensitivity was mainly based on the history of suspected drug intake, photodistributed rash, and improvement of rash after drug discontinuation. Only 19 patients underwent phototesting as a result, and 7 of them tested positive for UVA, 1 for UVB, and 3 for both UVA and UVB.

Regarding immunologically-mediated photodermatoses, CAD was the most common disorder detected in 11.9% of all cases. The prevalence of Thai CAD corresponded with other studies.[14] CAD was commonly seen in males (82.1%), with a mean age of 58 years. AP was the second most common immunologically-mediated photodermatoses. All cases were adult-onset AP correlated with previous studies that adult-onset AP was more common in Asians while AP in American individuals usually started in childhood.[15,16] Contrast to the classic AP, cheilitis and conjunctivitis are rare in adult-onset AP. Patients with AP in our study were more likely to have normal MED similar to a study by *Akaraphanth R et al.*[15] In order to confirm the diagnosis, a photoprovocation test should be carried out. Our study showed lower PMLE percentages than those in earlier studies.[6,7,9,12,14] This can be explained by the fact that Thailand experiences a lot of sunlight throughout the year, which can cause skin hardening. Diagnosis of PMLE was mainly based on history and clinical diagnoses and not referred for phototesting. Clinical characteristics of PMLE in our study were tiny papules which were similar to pinpoint papular variant of PMLE. This was different from erythematous papules and plaques, a classical prototype of PMLE in fair-skinned populations.[17] Moreover, the mild symptoms of PMLE could be overlooked by physicians. Prevalence of SU was diagnosed in 5.2%, which is similar to the values previously reported.[6,12] Our study demonstrated that visible light spectrum and UVA were common in SU, making physical photoprotection or tinted sunscreen essential components of the therapy.

Photoexacerbated dermatoses were the third most prevalent group in our study. Most cases were photoaggravated eczema, photosensitive atopic dermatitis, dermatomyositis, lupus erythematosus and other unspecified photosensitive dermatitis and photosensitivity. Photosensitive atopic dermatitis (PhAD) was detected in 11 patients. Nine patients also underwent phototesting, one patient reported an abnormal response to UVB and two patients reported an abnormal response to UVA. PhAD is an uncommon condition and seems to affect women more than men. These patients present photodistributed rash and fulfilled the criteria of atopic dermatitis. Photosensitivity is also often observed during summer, during exposure to artificial UV phototherapy or may appear after diagnosis of AD.[18] To confirm the diagnosis, photoprovocation can be performed and may induce papular or eczematous reactions.[18]

Genophotodermatoses are a rare conditions with an incidence of 2.3 per million in Western Europe.[19] However, we had four cases of Xeroderma Pigmentosum, one case of Cockayne syndrome and one case of Bloom syndrome. Corresponding to a study by *Khoo et al* and *Wong and Khoo*, our study demonstrated a low percentage of genophotodermatoses compared to other groups of photodermatoses.[6,12]

The number of patients diagnosed with photodermatoses varied each year. Notwithstanding, the overall trend of photodermatoses in our study showed a steady increase which corresponded with *Nassan H et al's* study[20], except in 2020-2021 when the diagnosis of photodermatoses declined due to the COVID-19 pandemic.

Our study had some limitations. First, this was a retrospective study in which some important data such as Fitzpatrick skin phototype and patients' current medication was missing. Furthermore, some patients who were clinically suspected to have photodermatoses were excluded because phototesting was not performed. Additionally, the ICD coding approach might not be entirely covered. Patients with photodermatoses who have ICD10 codes other than those for photodermatoses would not be included in this study. Last but not least, as the majority of patients in our study were sent to this single-center tertiary care facility, the prevalence of photodermatoses may differ from that of the general Thai community. To estimate the prevalence of photodermatoses, comprehensive national data that have been integrated from every Thai hospital are required.

CONCLUSION

In Thailand's general dermatology practice, photodermatoses are infrequent. Our study showed larger proportions of drug-induced photosensitivity and phytophotodermatitis, compared to immunologically-mediated photodermatoses and photoaggravated dermatoses. Some photodermatoses have distinctive clinical features in Asian populations. Recent trends in the diagnosis of photodermatoses suggest that either the prevalence of these diseases is actually rising or that physicians are becoming more aware of and knowledgeable about these photodermatoses.

Conflict of interests: All authors do not have any conflicts of interest or financial support to declare.

REFERENCES

1. Gutierrez D, Gaulding JV, Motta Beltran AF, Lim HW, Pritchett EN. Photodermatoses in skin of colour. J Eur Acad Dermatol Venereol. 2018;32(11):1879-86.
2. Bylaite M, Grigaitiene J, Lapinskaite GS. Photodermatoses: classification, evaluation and management. Br J Dermatol. 2009;161 Suppl 3:61-8.
3. Choi D, Kannan S, Lim HW. Evaluation of patients with photodermatoses. Dermatol Clin. 2014;32(3):267-75.
4. Hönigsmann H, Hojyo-Tomoka MT. Polymorphous light eruption, hydroa vacciniforme, and actinic prurigo. Photodermatol: CRC Press; 2007.p.149-68.
5. Deng D, Hang Y, Chen H, Li H. Prevalence of photodermatosis in four regions at different altitudes in Yunnan province, China. J Dermatol. 2006;33(8):537-40.
6. Wong SN, Khoo LS. Analysis of photodermatoses seen in a predominantly Asian population at a photodermatology clinic in Singapore. Photodermatol Photoimmunol Photomed. 2005;21(1): 40-4.
7. Nakamura M, Henderson M, Jacobsen G, Lim HW. Comparison of photodermatoses in African-Americans and Caucasians: a follow-up study. Photodermatol Photoimmunol Photomed. 2014;30(5):231-6.
8. Kerr HA, Lim HW. Photodermatoses in African Americans: a retrospective analysis of 135 patients over a 7-year period. J Am Acad Dermatol. 2007;57(4):638-43.
9. Hamel R, Mohammad TF, Chahine A, Joselow A, Vick G, Radosta S, et al. Comparison of racial distribution of photodermatoses in USA academic dermatology clinics: A multicenter retrospective analysis of 1,080 patients over a 10-year period. Photodermatol Photoimmunol Photomed. 2020;36(3):233-40.
10. Ibbotson S, Dawe R. Cutaneous Photosensitivity Diseases. In: Griffiths CEM, Barker J, Bleiker T, Chalmers R, Creamer D, editors. In Rook's Textbook of Dermatology. 9th ed. John Wiley & Sons; 2016.p.127.1-127.35.
11. Dawe RS. Prevalences of chronic photodermatoses in Scotland. Photodermatol Photoimmunol Photomed. 2009;25(1):59-60.
12. Khoo SW, Tay YK, Tham SN. Photodermatoses in a Singapore skin referral centre. Clin Exp Dermatol. 1996;21(4):263-8.
13. Blakely KM, Drucker AM, Rosen CF. Drug-induced photosensitivity-an update: culprit drugs, prevention and management. Drug Saf. 2019;42(7):827-47.
14. Fotiades J, Soter NA, Lim HW. Results of evaluation of 203 patients for photosensitivity in a 7.3-year period. J Am Acad Dermatol. 1995;33(4):597-602.
15. Akaraphanth R, Sindhavananda J, Gritiyarangsan P. Adult-onset actinic prurigo in Thailand. Photodermatol Photoimmunol Photomed. 2007;23(6):234-7.
16. Lestarini D, Khoo LS, Goh CL. The clinical features and management of actinic prurigo: A retrospective study. Photodermatol Photoimmunol Photomed 1999;15:183-7.
17. Gruber-Wackernagel A, Wolf P. Polymorphic Light Eruption. In: Kang S, Amagai M, Bruckner AL, et al., eds. Fitzpatrick's Dermatology, 9th ed. New York: McGraw-Hill Education; 2019.
18. Ellenbogen E, Wesselmann U, Hofmann SC, Lehmann P. Photosensitive atopic dermatitis-a neglected subset: Clinical, laboratory, histological and photobiological workup. J Eur Acad Dermatol Venereol. 2016;30(2):270-5.
19. Lehmann AR, McGibbon D, Stefanini M. Xeroderma pigmentosum. Orphanet J Rare Dis. 2011;6:70.
20. Nassan H, Dawe RS, Moseley H, Ibbotson SH. A review of photodiagnostic investigations over 26 years: experience of the National Scottish Photobiology Service (1989-2015). J R Coll Physicians Edinb. 2017;47(4):345-50.

Validity and Reliability of the Topical Corticosteroid Phobia (TOPICOP©) Questionnaire: Thai Version

Niorn Boonpuen, M.D.*,, Areeya Srimuang, M.D.*, Pailin Puangpet, M.D.*,**, Chavalit Supsrisunjai, M.D.*,****

**Institute of Dermatology, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand, **Rangsit University, Bangkok, Thailand.*

ABSTRACT

Objective: To determine the validity and reliability of the Thai version of the Topical Corticosteroid Phobia (TOPICOP©) questionnaire, which is used to evaluate topical corticosteroid (TCS) phobia in atopic dermatitis (AD).

Materials and Methods: The translation process and assessment of content validity were undertaken step-by-step. Adult patients with AD and the guardians of pediatric patients with AD completed the questionnaire twice, 2 weeks apart to test the reliability.

Results: We enrolled 30 adult patients with AD and 30 caretakers of pediatric patients with AD in this study. The Index of Item-Objective Congruence (IOC) was 0.9, indicating high validity. Test-retest reliability using Pearson correlation coefficient was tested, with r 0.938 ($p < 0.001$). The Cronbach's alpha coefficient showed reliable internal consistency, with 0.691 and 0.734, respectively.

Conclusion: The Thai version of the TOPICOP© is valid, reliable, and feasible for assessing TCS phobia in patients with AD and their caretakers of pediatric patients with AD.

Keywords: Atopic dermatitis; questionnaire; reliability; topical corticosteroid phobia; TOPICOP (Siriraj Med J 2023; 75: 115-120)

INTRODUCTION

Topical corticosteroids (TCS) have been a cornerstone of treatment for various skin conditions. However, steroid phobia is an important issue in clinical practice in the field of cooperation and therapeutic outcomes for skin diseases that require steroid treatment. The local and serious systemic side effects are widely discussed and are concerns among patients and caregivers, often resulting in limited use of TCS in medical practice.[1-3]

The term of steroid phobia was firstly termed within the context of asthma and eczema in 1987.[4] This phenomenon is common, complex, and widespread. Steroid phobia has broad implications and ranges from worry, fear, and excessive anxiety to an irrational fear of using TCS.[5-7] Steroid phobia can arise for a variety of reasons including a negative personal experience with side effects, misunderstandings, polypharmacy, frequent changes in clinics, and misinformed advice.[5,6,8,9]

In a systematic review of the literature from 1946 to 2016, the prevalence of TCS phobia ranged from 21% to 83.7%.[7] TCS phobia may be associated with a higher rate of noncompliance.[3,7] Recently, a systematic review including studies published between May 2000 and February 2021, found that the prevalence of steroid phobia found that was higher, ranged from 31% to 95.7%.[8]

Prior to 2013, no standardized tools were available to evaluate steroid phobia. Additionally, each relevant study used a different definition of steroid phobia.[6,7]

TOPICOP© is a questionnaire for the assessment of TCS phobia in atopic dermatitis (AD), which was conducted in 2010. They can be assessed by answering the questionnaire themselves. The questionnaire comprises

Corresponding author: Niorn Boonpuen
E-mail: niornmink@gmail.com
Received 20 June 2022 Revised 17 August 2022 Accepted 29 August 2022
ORCID ID:http://orcid.org/0000-0001-8041-3363
https://doi.org/10.33192/smj.v75i2.260750

12 questions, divided into two assessments: Six items address knowledge and beliefs, and six items address fears and behaviors. The responses for each item are scored from 0 to 3 on a 4-point-Likert scale), with a total score of 36, which can be converted to a percentage for easier calculation. A high score is associated with greater severity of steroid phobia. The TOPICOP© has been tested for its psychometric properties and had a Cronbach's a-coefficients of 0.81. The French version of the TOPICOP© scale was released in 2013, with subsequent translations into English and other languages.[6] The TOPICOP© was used in a multi-institutional feasibility study in 15 countries among 1,564 volunteers. The results showed that 81% of respondents rated the questionnaire as being very clear and to the point, and 79% could complete the questionnaire in less than 5 minutes.[10]

We aimed to translate the English version of the TOPICOP© scale into Thai language and to test the validity and reliability of the Thai version of the TOPICOP© to obtain a standard for application in further studies.

MATERIALS AND METHODS

In this cross-sectional study, we collected data on TCS phobia among adult patients with AD and parents of pediatric patients with AD who visited the outpatient department at the Institute of Dermatology, Bangkok, Thailand between April, and October 2021 were recruited.

The authors requested permission from Dr. Sebastien Barbarot, the copyright owner of the TOPICOP© questionnaire, to translate the scale into Thai according to the suggestion and use it in this study. This research was approved by the Institutional Review Board of the Institute of Dermatology and the Department of Medical Services, Ministry of Public Health, Thailand (certification of approval number IRB/IEB 006/2021).

We coordinated with standardized document translation centers to translate the English version of the TOPICOP© questionnaire into Thai. The preliminary Thai-translated questionnaire was corrected and adjusted to confirm that the Thai version of TOPICOP© covered all aspects of the concept being measured. We used a process of forward and backward translations. To assess the content validity of the Thai version of TOPICOP, three bilingual dermatologists assessed the content validity. The Index of Item-Objective Congruence (IOC) was used to evaluate the questionnaire items based on a score range from -1 to +1. On the basis, we developed the Thai version of the TOPICOP© (Fig 1).

แบบสอบถามเรื่องความกลัวในการใช้ยาทาสเตียรอยด์

แพทย์ได้สั่งยาทาสเตียรอยด์ให้คุณหรือเด็กในปกครองของคุณเพื่อทาบริเวณผื่น
แบบสอบถามนี้ต้องการที่จะทราบเกี่ยวกับความรู้ ความเชื่อ ความกังวล และพฤติกรรมเกี่ยวกับการรักษาด้วยวิธีนี้
โปรดเลือกคำตอบที่ตรงกับความคิดเห็นของคุณมากที่สุด

1. ยาทาสเตียรอยด์สามารถดูดซึมเข้าสู่กระแสเลือด
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
2. ยาทาสเตียรอยด์ทำให้เกิดการติดเชื้อ
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
3. ยาทาสเตียรอยด์ทำให้คุณอ้วน
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
4. ยาทาสเตียรอยด์ทำลายผิวหนังของคุณ
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
5. ยาทาสเตียรอยด์ส่งผลเสียต่อสุขภาพในอนาคต
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
6. ยาทาสเตียรอยด์ทำให้เป็นโรคหืด
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
7. ฉันกลัวการทายาสเตียรอยด์บนผิวหนังบางตำแหน่ง เช่น รอบดวงตา
❑ ไม่เลยสักนิด ❑ ไม่จริง ❑ เล็กน้อย ❑ แน่นอน
8. ฉันไม่แน่ใจเรื่องผลข้างเคียงของยาทาสเตียรอยด์แต่ฉันยังรู้สึกกลัวที่จะใช้ยา
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
9. ฉันกลัวว่าจะใช้ยาทาสเตียรอยด์มากเกินไป
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง
10. ฉันรอนานที่สุดเท่าที่จะทำได้ก่อนที่จะรักษาตัวเองหรือเด็กในปกครองด้วยยาทาสเตียรอยด์
❑ ไม่เคย ❑ บางครั้ง ❑ บ่อยครั้ง ❑ ตลอดเวลา
11. ฉันหยุดยาทาสเตียรอยด์โดยเร็วที่สุดเท่าที่จะทำได้
❑ ไม่เคย ❑ บางครั้ง ❑ บ่อยครั้ง ❑ ตลอดเวลา
12. ฉันต้องการคำแนะนำให้มั่นใจเพิ่มขึ้นเกี่ยวกับยาทาสเตียรอยด์
❑ ไม่เห็นด้วยอย่างยิ่ง ❑ ไม่เห็นด้วย ❑ เห็นด้วย ❑ เห็นด้วยอย่างยิ่ง

Fig 1. Thai version of the Topical Corticosteroid Phobia (TOPICOP©) questionnaire.

Adult patients with AD and the parents of pediatric patients with AD aged younger than 18 years old who attended our outpatient unit were asked to join the study. Sixty participants were selected according to the inclusion and exclusion criteria. Those who were unable to read Thai were excluded. To assess the reliability, the test-retest method was performed, and the volunteers were asked to complete the TOPICOP© twice, two weeks apart.

Statistical analyses

Demographic data are presented as descriptive statistics with means ± standard deviations (SD), and the median for continuous variables and frequency, and percentages for categorical variables. The test-retest method using Pearson correlation was used to test the reliability of the translated version over time, which participants asked to complete the TOPICOP© twice, 2 weeks apart. A Pearson correlation coefficient more than 0.8 was considered to indicate reliability. Cronbach's alpha method was used to measure the internal consistency within each subscale to check whether all questions were measured in the same way. IBM SPSS Statistics for Mac, version 28.0.1.1 was used for data management and statistical analyses (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 60 participants (30 adult patients with AD and 30 parents of pediatric patients with AD) completed the questionnaire, with 68.3% females, and mean age 33.7±10.7 years. Most respondents had a bachelor's degree or higher (83.3%), and 43.3% of patients had moderate severity of AD. The median duration of AD was 3.6 years. Among the total respondents, 10% reported that they themselves or their children had previous complications from TCS. Compared with pediatric patients, adult patients with AD had more comorbidities, especially AD concomitant with allergic rhinitis. Doctors were the most common source of information about TCS, followed by the internet, pharmacists, nurses, friends/family, and television or radio. Seventy percent of respondents admitted buying topical steroids without a prescription. Demographic data, knowledge, behaviors, and disease characteristics are shown in Table 1.

The mean TOPICOP scores among participants was respectively 21.1±4.2 (58.6±11.7%) and 21.4±4.4 (59.4±1.22%) on the two testing occasions. There was no difference in scores between adult patients with AD and parents of pediatric patients with AD (Table 2).

In terms of content validity, an acceptable threshold of the IOC is considered to be more than 0.5 for all items. In the Thai version of TOPICOP©, the IOC for all items was 0.9; r=0.938 (p <0.001), and Cronbach's alpha coefficients for the test-retest was 0.691, and 0.734, respectively (Table 3).

Responses to each item of the TOPICOP© scale were shown in Table 4. We identified some areas of concern. Most participants reported that they were afraid of applying too much TCS, that they felt afraid of applying TCS to the areas where the skin is very delicate, and that they needed reassurance about TCS.

The median (range) time to complete 12 questions in all participants was 2 (0.66-9) minutes. Ninety-three percent of participants completed the questionnaire in less than 5 minutes.

DISCUSSION

Steroid phobia has never been explored in Thailand. The prevalence of and reasons for TCS phobia should be addressed to improve adherence, intervention, and improved quality of life of the patients and caregivers. The TOPICOP© can help clinicians and researchers to identify the fears relating to TCS.

In a cross-cultural study using a questionnaire, the validity and reliability of translation must be assessed to ensure that the translated questionnaire properly addresses cultural and linguistic differences. In the present study, the validity of the TOPICOP©, Thai version was statistically approved. Evidence of reliability using the Pearson correlation coefficient of the test-retest data was strongly high and the Cronbach's alpha coefficient was acceptable. The results of the assessment were similar to those of previous reports.[6,10] The time spent on the questionnaire completion was only 2 minutes. Thus, this self-assessment tool can be useful in clinical practice owing to its short completion time.

Scores on the TOPICOP© were moderately high in our population (mean, 58.6%); however, it is not possible to determine the magnitude and severity of TCS phobia among Thais. Further studies on the prevalence of TCS phobia, correlation with disease severity, and compliance should be discussed in larger population.

Healthcare workers have important roles in providing information that can affect concerns about TCS. However, online sources of information regarding TCS are increasingly common. A high rate of buying TCS over the counter without a prescription was reported among participants. Awareness and knowledge about TCS treatment are crucial topics to be considered.

Limitations

The main limitation in our study was the small sample

TABLE 1. Demographic data, knowledge, behaviors, and disease characteristics.

Demographic data	All (n=60)	Adult patients (n=30)	Parents of pediatric patients (n=30)
Age (years): mean (SD)	33.7 (10.7)	28.2 (8.3)	39.2 (10.2)
Sex: n (%)			
Male	19 (31.7%)	17 (56.7%)	2 (6.7%)
Female	41 (68.3%)	13 (43.3%)	28 (93.3%)
Education: n (%)			
Primary	-	-	-
Secondary	10 (16.7%)	2 (6.7%)	8 (26.7%)
Graduate	38 (63.3%)	24 (80.0%)	14 (46.7%)
Post-graduate	12 (20.0%)	4 (13.3%)	8 (26.7%)
Occupation: n (%)			
Non-medical professionals	54 (90.0%)	27 (90.0%)	27 (90.0%)
Medical professionals	6 (10.0%)	3 (10.0%)	3 (10.0%)
Income (Baht/month): n (%)			
< 15,000	8 (13.3%)	4 (13.3%)	4 (13.3%)
15,001 – 30,000	20 (33.3%)	11 (36.7%)	9 (30.0%)
30,001 – 50,000	10 (16.7%)	5 (16.7%)	5 (16.7%)
>50,000	22 (36.7%)	10 (33.3%)	12 (40.0%)
Know the advantages of TCS	41 (68.3%)	20 (66.7%)	21 (70.0%)
Know the disadvantages of TCS	34 (56.7%)	16 (53.3%)	18 (60.0%)
Source of information about TCS			
Doctors	51 (85.0%)	24 (80.0%)	27 (90.0%)
Nurses	12 (20.0%)	5 (16.7%)	7 (23.3%)
Pharmacists	25 (41.7%)	11 (36.7%)	14 (46.7%)
Friends/family	11 (18.3%)	6 (20.0%)	5 (16.7%)
Television/radio	8 (13.3%)	3 (10.0%)	5 (16.7%)
Internet	25 (41.7%)	13 (43.3%)	12 (40.0%)
History of buying topical corticosteroids without a prescription	42 (70.0%)	21 (70.0%)	21 (70.0%)
Duration of disease (years): median (range)	3.6 (0.1-33.7)	14.5 (0.1-33.7)	2.0 (0.2-15.0)
Baseline SCORAD score	34.7 (20.0)	38.6 (22.7)	30.8 (16.3)
AD severity: n (%)			
Mild	23 (38.3)	10 (33.3)	13 (43.3)
Moderate	26 (43.3)	14 (46.7)	12 (40.0)
Severe	11 (18.3)	6 (20.0)	5 (16.7)
Comorbidities: n (%)			
None	26 (43.3)	9 (30.0)	17 (56.7)
Food allergy	8 (13.3)	5 (16.7)	3 (10.0)
Asthma	2 (3.3)	2 (6.7)	0 (0.0)
Allergic rhinitis	27 (45.0)	18 (60.0)	9 (30.0)
Other	3 (5.0)	2 (6.7)	1 (3.3)
Number of relapses in 1 month: median (range)	2 (0-5)	2 (0-5)	2 (0-4)
Duration of exacerbation cycle (days/cycle): median (range)	5.5 (0-30)	6.5 (1-30)	4.5 (0-30)
Hospital admission: Yes	32 (53.3)	22 (73.3)	10 (33.3)
Complications: Yes	6 (10.0)	3 (10.0)	3 (10.0)

Abbreviations: SCORAD, scoring atopic dermatitis; SD, standard deviation; AD, atopic dermatitis; TCS, topical corticosteroids.

TABLE 2. Scores for the Topical Corticosteroid Phobia (TOPICOP©), Thai version.

Global TOPICOP score	All (n = 60)	Patients (n=30)	Parents (n=30)
Sum score: mean (SD)			
First time	21.1 (4.2)	21.2 (4.1)	21.0 (4.4)
Second time	21.4 (4.4)	21.8 (4.7)	21.1 (4.2)

TABLE 3. Validity and Reliability.

Content validity	IOC
Q1: TCS can be absorbed into the bloodstream.	1.0
Q2: TCS can lead to infections.	1.0
Q3: TCS can make you fat.	1.0
Q4: TCS damage your skin.	0.7
Q5: TCS will affect your future negatively.	1.0
Q6: Using TCS can lead to asthma.	1.0
Q7: I am afraid of putting TCS on certain skin areas such as eyelids.	1.0
Q8: I do not know of any side effects, but I am still afraid of using TCS.	1.0
Q9: I am afraid of applying too much TCS.	0.7
Q10: I wait as long as I can before treating myself or my child with TCS.	0.7
Q11: I stop TCS treatment as soon as I can.	1.0
Q12: I need reassurance about topical corticosteroids.	0.7
Mean	0.9
Reliability	
Test-retest reliability: Pearson correlation coefficient	r = 0.938
Internal consistency: Cronbach's alpha coefficient	
First test	α = 0.691
Second test	α = 0.734

Abbreviations: IOC, The Index of Item-Objective Congruence; TCS, topical corticosteroid.

size, as a result, we could not estimate the relationship between TCS phobia and related patient or caretaker factors. Additionally, in this study, we did not review more detailed data of TOPICOP subscores addressing aspects of worry and beliefs. Use of the TOPICOP© is appropriate for the identification of patients' or parents' fears regarding TCS but cannot be used to assess patient adherence.

CONCLUSION

The Thai version of the TOPICOP© is valid, reliable, and feasible for self-assessment of topical corticosteroid phobia in Thai AD patients or their caretakers. Further studies among a large number of patients will be informative and beneficial for identifying topical corticosteroid phobia among Thais. These data will help to improve compliance with AD therapy and therapeutic outcomes.

TABLE 4. Responses for Topical Corticosteroid Phobia (TOPICOP©) scale items.

Items of the TOPICOP© n (%)	Totally disagree/ never	Not really agree/ sometimes	Almost agree/often	Totally agree/always
Q1: TCS can be absorbed into bloodstream.	2 (3.3)	24 (40)	25 (41.7)	9 (15)
Q2: TCS can lead to infection.	5 (8.3)	29 (48.3)	21 (35)	5 (8.3)
Q3: TCS can make you fat.	9 (15)	40 (66.7)	7 (11.7)	4 (6.7)
Q4: TCS damage your skin.	2 (3.3)	9 (15)	41 (68.3)	8 (13.3)
Q5: TCS will affect your future health negatively.	2 (3.3)	8 (13.3)	37 (61.7)	13 (21.7)
Q6: Using TCS can lead to asthma.	14 (23.3)	41 (68.3)	4 (6.7)	1 (1.7)
Q7: I am afraid of putting TCS on certain skin areas such as eyelids.	1 (1.7)	4 (6.7)	24 (40)	31 (51.7)
Q8: I do not know of any side effects, but I am still afraid of using TCS.	5 (8.3)	13 (21.7)	38 (63.3)	4 (6.7)
Q9: I am afraid of applying too much TCS.	0 (0)	3 (5)	30 (50)	27 (45)
Q10: I wait as long as I can before treating myself or my child with TCS.	13 (21.7)	28 (46.7)	13 (21.7)	6 (10)
Q11: I stop TCS treatment as soon as I can.	5 (8.3)	20 (33.3)	16 (26.7)	19 (31.7)
Q12: I need reassurance about TCS.	0 (0)	5 (8.3)	16 (26.7)	39 (65)

Abbreviation: TCS, topical corticosteroid.

ACKNOWLEDGMENTS

The study was supported by the Institute of Dermatology. The authors would like to acknowledge Dr. Sebastian Barbarot for the permission to translate TOPICOP© into Thai and use it in this study. We specially thank Dr. Tanongkiet Tienthavorn, Dr. Voraphol Vejjabhinanta, and Dr. Walaiorn Pratchyapruit for giving comments and scoring validation of TOPICOP©, Thai version. We thank Julaporn Pooliam for statistical analysis and Analisa Avila, MPH, ELS, of Edanz (www.edanz.com/ac) for editing a draft of this manuscript.

Funding support: This work was supported by the Institute of Dermatology, Department of Medical Services, Ministry of Public Health, Thailand.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. Charman C, Williams H. The use of corticosteroids and corticosteroid phobia in atopic dermatitis. Clin Dermatol. 2003;21(3):193-200.
2. Charman CR, Morris AD, Williams HC. Topical corticosteroid phobia in patients with atopic eczema. Br J Dermatol. 2000;142(5):931-6.
3. Mueller SM, Tomaschett D, Vogt DR, Itin P, Cozzio A, Surber C. Topical corticosteroid concerns from the clinicians' perspective. J Dermatolog Treat. 2017;28(5):464-8.
4. David TJ. Steroid scare. Arch Dis Child. 1987;62(9):876-8.
5. Aubert-Wastiaux H, Moret L, Le Rhun A, Fontenoy AM, Nguyen JM, Leux C, et al. Topical corticosteroid phobia in atopic dermatitis: a study of its nature, origins and frequency. Br J Dermatol. 2011;165(4):808-14.
6. Moret L, Anthoine E, Aubert-Wastiaux H, Le Rhun A, Leux C, Mazereeuw-Hautier J, et al. TOPICOP©: a new scale evaluating topical corticosteroid phobia among atopic dermatitis outpatients and their parents. PLoS One. 2013;8(10):e76493.
7. Li AW, Yin ES, Antaya RJ. Topical corticosteroid phobia in atopic dermatitis: a systematic review. JAMA Dermatol. 2017;153(10):1036-42.
8. Contento M, Cline A, Russo M. Steroid phobia: a review of prevalence, risk factors, and interventions. Am J Clin Dermatol. 2021;22(6):837-51.
9. Kojima R, Fujiwara T, Matsuda A, Narita M, Matsubara O, Nonoyama S, et al. Factors associated with steroid phobia in caregivers of children with atopic dermatitis. Pediatr Dermatol. 2013;30(1):29-35.
10. Stalder JF, Aubert H, Anthoine E, Futamura M, Marcoux D, Morren MA, et al. Topical corticosteroid phobia in atopic dermatitis: International feasibility study of the TOPICOP score. Allergy. 2017;72(11):1713-9.

Diphenylcyclopropenone Treatment Outcomes for Alopecia Areata

Supenya Varothai, M.D., Rattapon Thuangtong, M.D., Daranee Sonmek, B.N.S., Thanawan Iamphonrat, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To ascertain (1) Diphenylcyclopropenone (DCP)'s efficacy in treating alopecia areata (AA), alopecia totalis (AT), and alopecia universalis (AU) in Thai patients; and (2) prognostic factors.

Materials and Methods: We retrospectively reviewed the medical records of patients with AA, AT, and AU who were administered DCP at Siriraj Hospital, Bangkok, Thailand. The median response and relapse times of the 3 groups were evaluated. Factors affecting outcomes were investigated.

Results: Fifty-nine cases were enrolled (AA, 22; AT, 9; AU, 28), with women predominating in each group. The overall response was 61% (AA, 78.6%; AT, 66.7%; AU, 50%). The median response time was 58 weeks, with a significantly longer time for AU than AA ($P = 0.006$). Factors significantly influencing response to DCP, evaluated by multivariate analysis, were older age at onset ($P = 0.02$), disease duration before DCP initiation ($P = 0.003$), and treatment duration to initial hair regrowth ($P = 0.001$). The overall relapse rate was 63.9%, with a median of 39 weeks between response and relapse. The most common side effect was blistering (73.7%).

Conclusion: DCP is effective and safe for treating extensive AA. Favorable prognostic factors are low disease severity, late disease onset, short duration before DCP treatment, and short duration to initial response. As the relapse rate is high, maintenance therapy should be considered.

Keywords: Alopecia areata; Diphenylcyclopropenone; Diphencyprone; DCP; DPCP; Topical immunotherapy; Prognosis (Siriraj Med J 2023; 75: 121-131)

INTRODUCTION

Alopecia areata (AA) is an autoimmune disorder affecting hair follicles. There are several types of nonscarring alopecia, consisting of a focal area of patchy hair loss (patchy AA), complete scalp hair loss (alopecia totalis, AT), and entire scalp hair with body hair loss (alopecia universalis, AU). While limited forms of the disease may recover spontaneously or respond well to intralesional corticosteroid therapy, the extensive forms (AT, AU) often resist treatment modalities. Topical immunotherapy is the most effective therapeutic option with the best safety profile for treating severe, chronic AA. The mechanism of action of topical immunotherapy is inconclusive. It is supposed that contact sensitizers induce a new population of inflammatory cells. These cells have an inhibitory effect on the preexisting autoimmune reaction of hair, which promotes the regrowth of hair.

Three contact sensitizers have been used exclusively to treat AA: dinitrochlorobenzene, squaric acid dibutyl ester, and diphenylcyclopropenone (DCP). In our hair clinic, due to the mutagenic effects of dinitrochlorobenzene and the instability of squaric acid dibutyl ester, we use DCP as the standard treatment for AT, AU, and recalcitrant cases of extensive AA. "Recalcitrant cases of extensive AA" are defined as the presence of either more than a 30% loss of scalp hair or the ophiasis type of AA ("ophiasis AA") and failure of intralesional corticosteroid therapy. The application method was adopted from the standard

Corresponding author: Supenya Varothai
E-mail: supenya_var@gmail.com
Received 13 August 2022 Revised 9 September 2022 Accepted 13 September 2022
ORCID ID:http://orcid.org/0000-0002-3740-0425
https://doi.org/10.33192/smj.v75i2. 260751

protocol described previously.[1] Unlike the unilateral application of the original regimen, we painted DCP on all of the affected areas. The literature[2] has reported that the efficacy of DCP varies widely, from 5% to 85%, and the published evidence for prognostic factors (especially in Asians) is limited. In addition, the application of DCP is time-consuming. It is therefore essential to have certainty of treatment outcomes and prognostic factors. Treatment can be adjusted with that knowledge, and patients can be adequately advised.

The primary purpose of this study was to ascertain the efficacy of DCP treatment for patients with AA, AT, and AU. The secondary objectives were (1) to determine the side effects and relapse rate of the therapy and (2) to identify factors influencing its outcomes.

MATERIALS AND METHODS

We retrospectively reviewed the medical records of patients diagnosed with AA, AT, and AU who initiated DCP treatment at the Hair Clinic of the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, between January 1999 and July 2007. Before this research began, its protocol was approved by the Siriraj Institutional Review Board, Siriraj Hospital, Mahidol University, Bangkok, Thailand, in August 2011. Patients who received treatment for at least 6 months were included, and the data were continuously recorded for up to 2 years. Patients were excluded if they were lost to follow-up for more than 3 months.

Demographic data were recorded. Personal data (history of atopy, vitiligo, autoimmune thyroid diseases, and family history of AA) were reviewed. Details of the following were collected:

1. The course and degree of hair loss
2. Age at onset
3. Previous treatment modalities
4. Disease duration when treatment began
5. Treatment duration to a proper eczematous reaction
6. Duration and DCP concentration for the initial response.

The number of treatments corresponded to the duration of therapy as the treatment protocol required DCP to be applied weekly. Details of examinations of the area of involvement, nail changes, and general skin were evaluated.

The patients were classified into 4 groups based on disease severity: AA, AT, AU, and ophiasis AA (the recalcitrant form of AA). All cases were also classified as "responder" or "nonresponder." "Responders" were patients who achieved cosmetically acceptable hair regrowth of more than 75% of the affected area. Consentaneously, all reviewed cases were assessed under two board-certified dermatologists (SV and RT) with united approval. Further investigations were conducted on the duration needed to achieve a response and the time to relapse for the responders. Factors associated with the responders were also investigated.

Statistical analysis

Descriptive statistics were applied to demographic and personal data and treatment history. Kaplan–Meier survival analysis was used to calculate the median response and median relapse times. Univariate and multivariate analyses were used to determine factors affecting treatment. To this end, the following were considered using Kaplan–Meier and Cox regression: sex, presence of atopy, previous treatment modalities, age at onset, severity of scalp involvement, nail involvement, duration of hair loss before DCP application, duration of treatment to reach proper eczematous reaction, duration of treatment to obtain initial hair regrowth, and concomitant treatment.

RESULTS

Demographic data

A summary of the demographic data is presented in Tables 1 and 2. Data were collected from 59 patients; most (50) were women. After classifying the severity of scalp involvement as AA, AT, AU, and ophiasis AA, AU was found most frequently (47.5%), followed by AA (23.7%), AT (15.3%), and ophiasis AA (13.6%). According to the medical records, none had a positive family history of AA. Seven patients had a history of atopy, and approximately half had AU. Two patients had vitiligo, and both were classified as having ophiasis AA. According to their histories, thyroid function tests, or thyroid antibody tests, no patients had autoimmune thyroiditis. Approximately 60% of the cases had nail abnormalities in the form of nail pits.

Most of the patients had previously received other treatments, and 2 had undergone DCP treatment prior to the episode studied. The age at onset of the disease ranged from 3.8 to 68.8 years (median = 26.6), and the age at the initiation of DCP treatment ranged from 11.9 to 63.4 years (median = 29.4). The duration from disease onset to initiation of DCP treatment was 0.1 to 19.9 years (median = 2.3). Generally, there were no statistically significant differences in the demographic data of the 4 clinical groups. One exception was a history of previous intralesional steroid injections ($P = 0.01$). The other was the age at initial DCP treatment of the AU group; it was significantly younger than that of the ophiasis AA group ($P = 0.02$).

TABLE 1. Demographic data.

Factors	Groups; number (%)					*P* value
	Total	AA	AT	AU	Ophiasis AA	
	59 (100.0)	14 (23.7)	9 (15.3)	28 (47.5)	8 (13.6)	
Female	50 (84.7)	11 (22.0)	7 (14.0)	25 (50.0)	7 (14.0)	0.74
Family history of AA	0	0	0	0	0	-
A history of atopy	7 (11.9)	0	2 (28.6)	4 (57.1)	1 (14.3)	-
Vitiligo	2 (3.4)	0	0	0	2 (100.0)	-
Autoimmune thyroiditis	0	0	0	0	0	-
Pitting nail	11 (57.9)	1 (9.1)	3 (27.3)	6 (54.5)	1 (9.1)	0.49
Previous IL steroids	21 (35.6)	7 (33.3)	6 (28.6)	4 (19.0)	4 (19.0)	0.01*
Previous topical steroids	31 (52.5)	8 (25.8)	5 (16.1)	14 (45.2)	4 (12.9)	0.98
Previous oral steroids	27 (45.8)	9 (33.3)	4 (14.8)	12 (44.4)	2 (7.4)	0.35
Previous IM steroids	29 (49.2)	5 (17.2)	5 (17.2)	16 (55.2)	3 (10.3)	0.54
Previous topical minoxidil	12 (20.3)	1 (8.3)	1 (8.3)	8 (66.7)	2 (16.7)	0.42
Previous DCP	2 (3.4)	0	0	2 (100.0)	0	0.78
Median age of onset (min, max; years)	26.6 (3.8,62.8)	26.3 (8.1,62.8)	27 (14.7,35.5)	24.7 (3.8,44.4)	42.9 (9.0,54.7)	0.08
Median age at initial DCP treatment (min, max; years)	29.4 (11.9,63.4)	28.7 (14.0,63.4)	31.5 (18.5,35.8)	27.2 (11.9,47.2)	46.4 (19.3,62.1)[3]	0.02*
Median duration from onset to initial DCP treatment (min, max; years)	2.3 (0.1,19.9)	0.9 (0.1,19.9)	2.4 (0.4,13.6)	2.7 (0.2,13.5)	4.2 (0.2,13.9)	0.39

* $P < 0.05$ was statistically significant.

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; DCP, diphenylcyclopropenone; IL, intralesional; IM, intramuscular

Notes: 1: Statistically significant compared with AA
2: Statistically significant compared with AT
3: Statistically significant compared with AU
4: Statistically significant compared with ophiasis AA

Treatment data

A "proper eczematous reaction" was defined as at least 2 days of eczematous reaction. The duration of treatment to reach a proper eczematous reaction varied from 1 to 50.6 weeks (median = 5 weeks). The median time to achieve a proper eczematous reaction was the shortest for patients with AU (3.3 weeks), followed by AT (5 weeks) and AA (6.9 weeks). It was the longest for patients with ophiasis AA (7.4 weeks). There were no statistically significant differences between the groups (P = 0.48). The median DCP concentration at the proper eczematous reaction was 0.001% for all groups.

The treatment duration needed to obtain initial hair regrowth ranged from 4 to 70.1 weeks (median = 11 weeks). The median durations for AA, AT, AU, and ophiasis AA were 8, 10.1, 14.1, and 22.5 weeks, respectively. The duration of treatment required to obtain initial hair regrowth for the AA group was significantly shorter than that for the ophiasis AA group (P = 0.03). The maximum DCP concentration was 1% for the AT and AU groups, but it was lower for the AA and ophiasis AA groups (0.5% and 0.35%, respectively). However, there were no significant differences among the 4 groups (P = 0.06). Almost all patients did not receive any other treatment

TABLE 2. Treatment data.

Factors	Groups; number (%)					*P* value
	Total	AA	AT	AU	Ophiasis AA	
	59 (100)	14 (23.7)	9 (15.3)	28 (47.5)	8 (13.6)	
Median duration of treatment to first eczema (min, max; weeks)	5 (1.0,50.6)	6.9 (1.0,10.0)	5 (1.1,19.9)	3.3 (1.0,50.6)	7.4 (1.0,29.6)	0.48
Median DCP concentration at first eczema (min, max)	0.001 (0.00001,1.0)	0.001 (0.00010,0.35)	0.001 (0.00001,0.1)	0.001 (0.00001,1.0)	0.00055 (0.0001,0.35)	0.41
Median duration of treatment to initial hair regrowth (min, max; weeks)	11 (4.0,70.1)	8 (4.0,50.7)	10.1 (6.1,21.9)	14.1 (5.0,64.3)	22.5 (9.9,70.1)[1]	0.03*
Median Maximum DCP concentration	1 (0.001,2.0)	0.5 (0.001,2.0)	1 (0.01,1.5)	1 (0.01,2.0)	0.35 (0.001,1.0)	0.06
Median DCP concentration at cosmetic hair regrowth (min, max)	0.1 (0.0001,2.0)	0.1 (0.0001,1.5)	0.1 (0.0001,1)	0.1 (0.0001,2.0)	0.1 (0.001,0.5)	0.85

* $P < 0.05$ was statistically significant.

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis; DCP, diphenylcyclopropenone

Notes: 1: Statistically significant compared with AA
2: Statistically significant compared with AT
3: Statistically significant compared with AU
4: Statistically significant compared with ophiasis AA

modality during DCP treatment. The exceptions were 7 patients (11.9%) who received topical minoxidil (Table 2).

Response to treatment

As mentioned above, the "response to treatment" was defined as cosmetically acceptable hair regrowth or a hair regrowth of more than 75%. Sixty-one percent of cases responded to treatment. The treatment duration to obtain a response ranged from 5 weeks to 24 months, with a median response time of 58 weeks after the initiation of treatment. In terms of disease severity, 78.6% of the AA cases responded to DCP with a 28.9-week median response time. For AT, 66.7% responded with a 52.1-week median response time. For patients with AU and ophiasis AA, 50.0% and 62.5% responded, with median response times of 93.4 and 58 weeks, respectively. The median response time of the AU group was significantly longer than that of the AA group (P = 0.006; Table 3 and Figs 1a and 1b). The DCP concentration required to achieve a treatment response was 0.1% for all groups (Table 2).

Prognostic factors

Univariate analysis—demographic data

Age at onset of alopecia and duration from disease onset until the initiation of DCP treatment had significant effects on treatment responses (P = 0.04 and 0.003, respectively). After being categorized, late-onset patients (20 years or older) had a significantly better response rate than younger patients. The duration from disease onset to the initial DCP treatment was divided into 3 groups: less than 1 year, 1 to 3 years, and more than 3 years. A shorter duration was found to be associated with better outcomes. The other demographic factors of the responders and nonresponders (sex, history of atopy, nail abnormality, and age at initial DCP treatment) did not differ significantly. The previous treatment modalities also did not generally affect the outcomes. However, an exception was that the patients who had received prior intralesional steroids were significantly more responsive to DCP than those who had not (P = 0.02; Tables 4a and 4b).

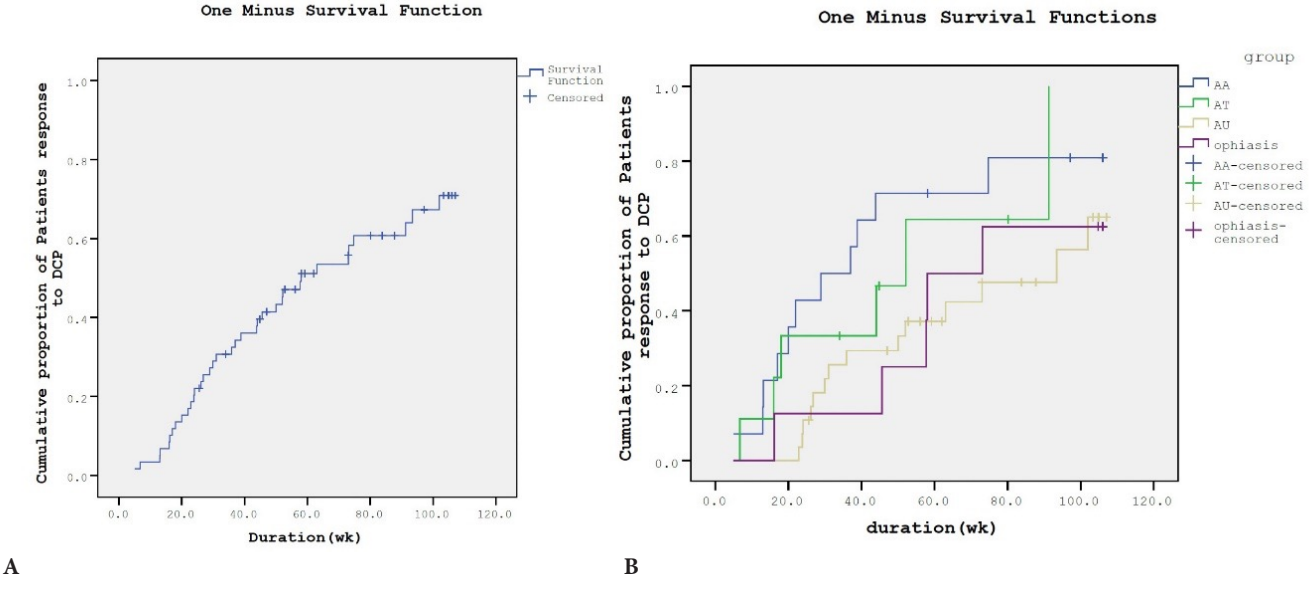
TABLE 3. Median response times.

Groups	Total N	Response N (%)	Median response time (weeks)	*P* value
Overall	59	36 (61.0)	58	0.03*
AA	14	11 (78.6)	28.9[3]	0.006*
AT	9	6 (66.7)	52.1	
AU	28	14 (50.0)	93.4	
Ophiasis AA	8	5 (62.5)	58	

* P < 0.05 was statistically significant.

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis

Notes: 1: Statistically significant compared with AA
2: Statistically significant compared with AT
3: Statistically significant compared with AU
4: Statistically significant compared with ophiasis AA



A B

Fig 1. **(A)** The overall cumulative proportions of responses. **(B)** The cumulative proportions of response, classified by disease severity.

Univariate analysis—treatment data

One factor was found to influence the treatment outcomes: the duration of treatment needed to obtain initial hair regrowth (P = 0.01). Patients who achieved initial hair regrowth before 12 weeks of treatment had significantly better outcomes (Tables 4a and 4b). The duration of treatment to reach a proper eczematous reaction did not affect the treatment outcomes. The responder and nonresponder groups also had no significant difference in their DCP concentrations at the proper eczematous response (P = 0.16, 0.19, respectively).

Multivariate analysis

Multivariate analysis was performed using Cox regression to determine the prognostic factors that independently affected treatment outcomes and to calculate the hazard ratio of each factor. Factors that significantly influenced the response to DCP were age at onset (P = 0.02), disease duration before the initial DCP treatment (P = 0.003), and treatment duration until initial hair regrowth (P = 0.001). The hazard ratio was 3.13 for patients with ages at onset of 20 or older. This finding meant that these patients responded to treatment

TABLE 4a. Univariate analysis of categorical variables.

Factors	Total	Response	Median survival time				*P* value
			Median	Standard error	95% Confidence interval Lower Bound	Upper Bound	
Sex							0.41
Female	50	29	73.0	13.5	46.5	99.5	
Male	9	7	52.0	12.1	28.2	75.8	
A history of atopy							0.54
No	11	8	52.1	22.6	7.9	96.4	
Yes	7	4	44.0	14.8	15.0	73.0	
Unknown	41	24	73.0	13.4	46.7	99.3	
Nail							0.41
Normal	8	5	50.0	17.8	15.2	84.8	
Pitting	11	5	73.1	12.2	49.2	97.0	
Unknown	40	26	52.0	8.5	35.3	68.7	
Previous intralesional steroids							0.02*
No	38	20	73.1	9.3	55.0	91.3	
Yes	21	16	38.9	10.0	19.2	58.5	
Previous topical steroids							0.19
No	28	16	74.7	11.7	51.8	97.7	
Yes	31	20	50.0	9.5	31.4	68.6	
Previous oral steroids							0.90
No	32	18	58.0	8.4	41.6	74.4	
Yes	27	18	73.0	14.9	43.7	102.3	
Previous intramuscular steroids							0.52
No	30	19	52.1	7.6	37.3	67.0	
Yes	29	17	63.0	24.1	15.7	110.3	
Previous topical minoxidil							0.52
No	47	31	57.7	8.7	40.6	74.8	
Yes**	12	5	-	-	-	-	
Previous DCP							0.26
No	57	36	57.7	8.7	40.6	74.8	
Yes**	2	0	-	-	-	-	
Concomitant minoxidil							0.40
No	52	31	58.0	13.3	31.8	84.2	
Yes	7	5	52.1	39.5	0.0	129.5	
Age at onsetγ							0.02*
< 20 years**	17	7	102.0	-	-	-	
≥ 20 years	40	27	44.0	8.9	26.5	61.5	
Duration from onset to initial DCP treatmentγ							<0.001*
< 1 year	15	14	23.9	5.1	13.9	33.8	
1–3 years	18	11	57.7	23.8	11.1	104.3	
> 3 years**	24	9	-	-	-	-	
Duration of treatment to initial hair regrowthγ							0.007*
≤ 12 weeks	30	24	38.9	5.6	27.9	49.8	
> 12 weeks	22	10	93.4	16.7	60.6	126.2	

* P < 0.05 was statistically significant.
**The number of responders were less than half the total number of patients; thus, the median survival time could not be evaluated.
γ The data for this factor were incomplete.
Abbreviation: DCP, diphenylcyclopropenone

TABLE 4b. Univariate analysis of continuous variables.

Factors	HR	95.0% CI for HR		*P* value
		Lower	Upper	
Age at onset (years)	1.03	1.00	1.06	0.04*
Age at initial DCP treatment (years)	1.01	0.99	1.04	0.30
Duration from onset to initial DCP treatment (years)	0.84	0.75	0.94	0.003*
Duration of treatment to first eczema (weeks)	0.95	0.88	1.02	0.16
Number of treatments at first eczema	0.94	0.86	1.03	0.19
DCP concentration at first eczema	0.43	0.04	5.05	0.50
Duration of treatment to initial hair regrowth (weeks)	0.97	0.95	0.99	0.02*
Number of treatments at initial hair regrowth (weeks)	0.94	0.91	0.98	0.01*

*P < 0.05 was statistically significant.
Abbreviations: DCP, diphenylcyclopropenone; HR, hazard ratio

3.13 times better than younger patients. Durations of disease before the initial DCP treatment of less than 1 year were associated with the best prognoses, while longer durations corresponded to worse outcomes. The hazard ratio was 3.81 for durations less than 1 year but 1.07 for 1 to 2 years. Finally, patients who needed less than 12 weeks to obtain initial hair regrowth responded significantly better than those who needed more than 12 weeks (hazard ratio = 4.66; Table 5 and Figs 2-4).

Side effects

Approximately 30% of the patients experienced side effects from the therapy. The most common side effect was blistering (23.7%); all other effects were uncommon (1%-4%; Table 6). Localized effects consisted of blistering and lymphadenopathy, while generalized effects were generalized eczema, angioedema, and urticaria.

Relapse

The overall relapse rate was 63.9%. In our group analysis, the relapse rate was highest for ophiasis AA (100%), followed by AT (66.7%), AU (57.6%), and AA (54.5%). The overall median relapse time was 39 weeks after achieving a response to DCP. There were no statistically significant differences in the median relapse times of the 4 groups (Table 7).

TABLE 5. Multivariate analysis.

Factors	HR	95.0% CI for HR		*P* value
		Lower	Upper	
Age at onset (years)				
< 20	1.00 (reference)			
≥ 20	3.13	1.16	8.39	0.02*
Duration from onset to initial DCP treatment (years)				
< 1	3.81	1.56	9.28	0.003*
1–2	1.07	0.43	2.68	0.89
≥ 3	1.00 (reference)			
Duration of treatment to initial hair regrowth (weeks)				
≤ 12	4.66	1.89	11.52	0.001*
> 12	1.00 (reference)			
Previous intralesional steroids				
No	1.00 (reference)			
Yes	1.38	0.65	2.96	0.40

*P < 0.05 was statistically significant.

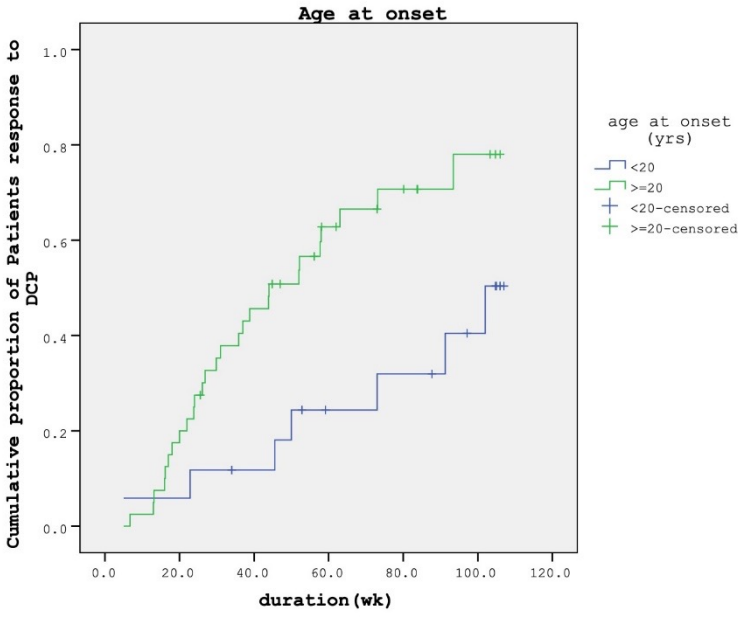


Fig 2. The cumulative proportions of response, classified by age at onset

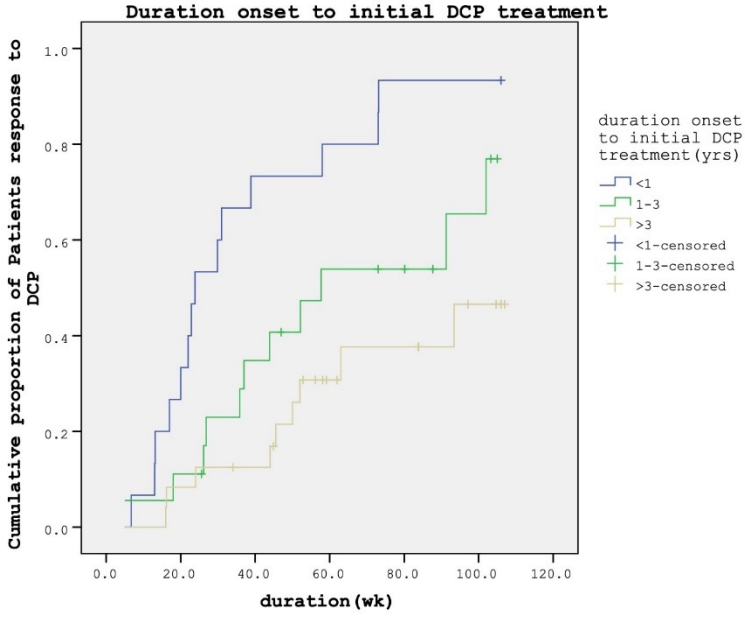


Fig 3. The cumulative proportions of response, classified by disease duration before initial DCP treatment

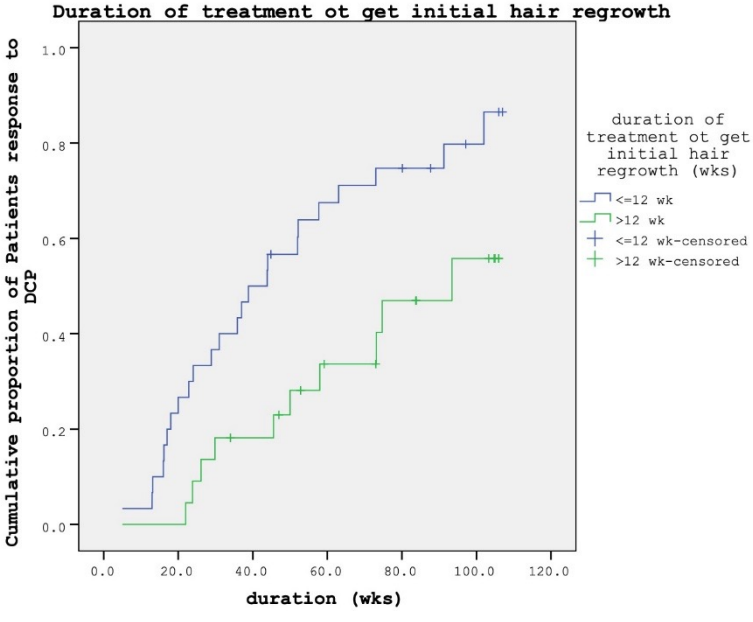


Fig 4. The cumulative proportions of response, classified by duration of treatment needed to obtain initial hair regrowth

TABLE 6. Side effects.

Side effects	Number (%)
No	40 (67.7)
Yes	19 (32.3)
Blistering	14 (23.7)
Generalized eczema	2 (3.4)
Angioedema	1 (1.7)
Lymphadenopathy	1 (1.7)
Urticaria	1 (1.7)

TABLE 7. Relapse rate.

Group	Total	Response N (%)	Median relapse time				*P* value
			Median	Standard error	95% Confidence interval Lower Bound	Upper Bound	
Overall	36	23 (63.9)	39.0	4.5	30.1	47.9	0.27
AA	11	6 (54.5)	52.0	4.1	44.0	60.0	
AT	6	4 (66.7)	30.0	14.9	0.8	59.2	
AU	14	8 (57.1)	39.7	11.2	17.8	61.6	
Ophiasis AA	5	5 (100)	29.9	10.8	8.7	51.0	

Abbreviations: AA, alopecia areata; AT, alopecia totalis; AU, alopecia universalis

DISCUSSION

The organ-specific autoimmune disease AA is associated with the activation of T lymphocytes around hair follicles. The disease causes various degrees of nonscarring alopecia and significant psychosocial problems. The underlying mechanism of DCP is not yet fully understood. However, many hypotheses explain its action, such as antigenic competition, perifollicular lymphocyte apoptosis, and peribulbar CD4/CD8 lymphocyte ratio changes. Effective treatment modalities are intralesional corticosteroids, systemic corticosteroids, and contact immunotherapy with DCP. Alkhalifah et al. reviewed the therapeutic options and found that contact immunotherapy was effective for extensive AA in an intracontrolled, half-head study.[2] At our clinic, DCP contact immunotherapy is the standard treatment for patients with AT, AU, and recalcitrant extensive AA (more than a 30% loss of scalp hair) who have not responded to systemic corticosteroids for at least 12 weeks.

According to the demographic data of our study, women greatly predominated (84.7%). This finding was inconsistent with most other studies, which reported that women and men were equally susceptible to the disease.[6] The epidemiological study by Kyriakis et al. showed that women had a higher prevalence of AA than men, but only marginally (women = 53.7%; men = 46.3%).[7] Our results possibly indicate that Thai women are considerably more concerned about appearance than Thai men.

The reported efficacy of DCP has varied from 5% to 85%.[1] This extensive range may result from differences in patient demographics, the baseline extent of hair loss, treatment guidelines and protocols, the definitions of response to treatment, and the duration of the follow-up period to evaluate treatment outcomes. Consequently, we used the definition of a response given by published guidelines.[8] In our study, the overall cumulative response was 61% within 24 months of follow-up, and the median response period was 58 weeks. This overall response was

close to the rate recently reported by El-Zawahry et al. (55.7%).[9] In a group of studies with baseline extents of hair loss and response definitions similar to our work, we noted that investigations with short evaluation periods had lower response rates. Hull et al. found a 35.7% rate in research that defined a response as terminal hair with patchy AA, cosmetic acceptance, or terminal hair over the scalp at 8 months.[10] A study by Sotiriadis et al. revealed that 39.5% of patients achieved greater than 80% hair regrowth at the 6-month follow-up visit.[11]

In contrast, studies with longer evaluation durations (12-32 months) had noticeably higher treatment response rates (38%-77.9%).[9,12–15] The cumulative response also increased over time, as found by the current investigation and 2 other studies.[9,13] Therefore, we propose that a 6-month evaluation period may be too short to effectively determine the response to treatment. We also suggest that an extended duration of treatment should be used to avoid prematurely denoting patients as nonresponders. This approach would be particularly beneficial for patients with AU, who had the longest median response time in the present work.

Moreover, our analysis showed that the duration and number of treatments needed to obtain initial hair regrowth in patients with ophiasis AA were longer than those of the other patients. The duration was also statistically significantly longer than that for patients with AA. Therefore, it is essential to inform patients with ophiasis AA that they will need to be prepared to wait a minimum of 5 months after treatment before hair regrowth becomes apparent.

Some studies have investigated prognostic factors for treatment outcomes. A large prospective study by van der Steen et al. demonstrated that the type of AA before treatment, the disease duration before therapy, and the presence of nail changes were significant prognostic factors.[16] However, when these investigators performed re-evaluations at the 19-month follow-up, only the type of AA and the disease duration remained significant.[17] A study by Gordon et al. found that the presence of nail changes, a long duration of AA, and a history of atopy were adverse prognostic factors.[14] A large retrospective study by Wiseman et al. also found a positive correlation between the degree of AA and the age at onset of the disease.[13] In contrast, Avgerinou et al. did not identify any significant prognostic factors.[12]

According to the disease severity classifications used in our study, the AA group had a significantly better prognosis than the AU group. Initially, we set out to analyze the prognostic factors for each of the 4 groups. Unfortunately, the number of patients in each group was too small to allow accurate calculations. Consequently, we performed univariate and multivariate analyses to determine the overall prognostic factors. The multivariate analysis found that outcomes were significantly affected by age at onset of the disease, the duration before the initiation of DCP treatment, and the duration of treatment needed to obtain initial hair regrowth. Our results were consistent with previous studies. However, more information was obtained by the present work, especially in terms of the DCP concentration required for a proper eczematous response and the finding that the maximum concentration did not affect treatment outcomes. Furthermore, we calculated the hazard ratios of these factors to obtain more information. Earlier studies did not use this approach.

Approximately one-third of our patients suffered side effects from DCP, most of which were blistering. All cases improved after discontinuing DCP and topical steroid and oral antihistamine treatments. The other side effects were generalized eczema, angioedema, lymphadenopathy, and urticaria. Approximately 60% of the responders had relapses, with a median relapse duration of 39 weeks. We observed that relapses usually occurred when we stopped DCP treatment. The relapse rate of approximately 60% was close to those of other studies.[12,13] Ohlmeier et al. found that the relapse rate once DCP therapy was stopped was higher if DCP was not tapered. These investigators recommended that DCP should not be abruptly stopped but instead be reduced gradually to maintain remission and avoid the risk of relapse.[18] Further extensive studies should be performed to explore this aspect.

In conclusion, DCP is an effective and safe treatment modality for extensive AA, with an overall response rate of 61%. The factors associated with a good prognosis are low disease severity, older age at disease onset (20 or older), a short period from onset to initial DCP treatment, and a short duration of treatment to obtain initial hair regrowth. However, as the relapse rate is high, maintenance therapy should be considered.

ACKNOWLEDGEMENTS

None

Conflicts of interest: There are no conflicts of interest related to any aspect of this study.

Funding/financial support: This study did not receive any grants from funding agencies.

REFERENCES

1. Rokhsar CK, Shupack JL, Vafai JJ, Washenik K. Efficacy of topical sensitizers in the treatment of alopecia areata. J Am Acad Dermatol 1998;39:751-61.
2. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part II. Treatment. J Am Acad Dermatol 2010;62:191-202.
3. Happle R. Antigenic competition as a therapeutic concept for alopecia areata. Arch Dermatol Res 1980;267:109-14.
4. Herbst V, Zoller M, Kissling S, Wenzel E, Stutz N, Freyschmidt-Paul P. Diphenylcyclopropenone treatment of alopecia areata induces apoptosis of perifollicular lymphocytes. Eur J Dermatol 2006;16:537-42.
5. Happle R, Klein HM, Macher E. Topical immunotherapy changes the composition of the peribulbar infiltrate in alopecia areata. Arch Dermatol Res 1986;278:214-8.
6. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. J Am Acad Dermatol 2010;62:177-88.
7. Kyriakis KP, Paltatzidou K, Kosma E, Sofouri E, Tadros A, Rachioti E. Alopecia areata prevalence by gender and age. J Eur Acad Dermatol Venereol 2009;23:572-3.
8. Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines--Part II. National Alopecia Areata Foundation. J Am Acad Dermatol 2004;51:440-7.
9. El-Zawahry BM, Bassiouny DA, Khella A, Zaki NS. Five-year experience in the treatment of alopecia areata with DPC. J Eur Acad Dermatol Venereol 2010;24:264-9.
10. Hull SM, Norris JF. Diphencyprone in the treatment of long-standing alopecia areata. Br J Dermatol 1988;119:367-74.
11. Sotiriadis D, Patsatsi A, Lazaridou E, Kastanis A, Vakirlis E, Chrysomallis F. Topical immunotherapy with diphenylcyclopropenone in the treatment of chronic extensive alopecia areata. Clin Exp Dermatol 2007;32:48-51.
12. Avgerinou G, Gregoriou S, Rigopoulos D, Stratigos A, Kalogeromitros D, Katsambas A. Alopecia areata: topical immunotherapy treatment with diphencyprone. J Eur Acad Dermatol Venereol 2008;22:320-3.
13. Wiseman MC, Shapiro J, MacDonald N, Lui H. Predictive model for immunotherapy of alopecia areata with diphencyprone. Arch Dermatol 2001;137:1063-8.
14. Gordon PM, Aldrige RD, McVittie E, Hunter JA. Topical diphencyprone for alopecia areata: evaluation of 48 cases after 30 months' follow-up. Br J Dermatol 1996;134:869-71.
15. Cotellessa C, Peris K, Caracciolo E, Mordenti C, Chimenti S. The use of topical diphenylcyclopropenone for the treatment of extensive alopecia areata. J Am Acad Dermatol 2001;44:73-6.
16. van der Steen PH, van Baar HM, Happle R, Boezeman JB, Perret CM. Prognostic factors in the treatment of alopecia areata with diphenylcyclopropenone. J Am Acad Dermatol 1991;24:227-30.
17. van der Steen PH, Boezeman JB, Happle R. Topical immunotherapy for alopecia areata: re-evaluation of 139 cases after an additional follow-up period of 19 months. Dermatology 1992;184:198-201.
18. Ohlmeier MC, Traupe H, Luger TA, Bohm M. Topical immunotherapy with diphenylcyclopropenone of patients with alopecia areata - a large retrospective study on 142 patients with a self-controlled design. J Eur Acad Dermatol Venereol 2012;26:503-7.

Evaluation of Hair Follicle Counts of Occipital Scalp Biopsies from Male Hair Transplant Patients in Thailand

Penvadee Pattanaprichakul, M.D.*, Rattapon Thuangtong, M.D.*, Kanchalit Thanomkitti, M.D.*, Supenya Varothai, M.D.*, Daranporn Triwongwaranat, M.D.*, Panitta Sitthinamsuwan, M.D., Suchanan Hanamornroongruang, M.D.**, Kumpol Aiempanakit, M.D.***, Maria Franchesca Quinio, M.D.******

**Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, **Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand, ***Department of Dermatology, Bangkok Hospital Hat Yai, Bangkok Dusit Medical Service, Hat Yai, Songkhla, 90110, Thailand, ****East Avenue Medical Center, Metro Manila, 1100, Philippines.*

ABSTRACT

Objective: To evaluate the average hair follicle count from the occipital scalp of Thai males with AGA who were candidates for hair transplantation.

Materials and Methods: A cross-sectional study of 47 male with AGA undergoing hair transplantation surgery was conducted. The 4-mm punch biopsies from the occipital scalp were evaluated for hair count parameters. The results were compared to prior studies.

Results: The average counts of total hair follicles and the density of hair follicle per square millimeter were 18.6±1.2, and 1.5±0.1, respectively. The terminal-to-vellus ratio was 11.1, and the percent ratio of anagen-to-telogen ratio was 91.9:8.1. The hair count number is significantly lower than other ethnicities including Thais in general population ($P < 0.001$), but greater than the Thai males with AGA in the previous study. ($P < 0.001$).

Conclusion: Our study showed a lower average hair density as compared to the other normal Asian population. The total hair count in the occipital area from this study is less when compared to the previous studies conducted in Thai normal controls but higher than those with more advanced AGA. This result supported the evidence of hormonal effect involving the occipital scalp of male AGA.

Keywords: Hair counts; hair density; occipital area; androgenetic alopecia (Siriraj Med J 2023; 75: 132-137)

INTRODUCTION

The occipital scalp has been the common donor site for hair transplantation as treatment for androgenetic alopecia (AGA). It is considered androgen insensitive because the growth cycle and the density of the hair follicle originating from this area is not affected by androgen even after transferring to the baldness zone.[1-2] Therefore, the hair counts from the occipital area should represent a normal reference for hair density in the general population. However, recent published data regarding the androgenetic effect on this nonbalding scalp area in AGA cases have been reported in both males and females.[3-5] Evaluation of hair density from the occipital scalp in individuals with AGA could reassure the appropriate quality and quantity of hair from the donor site suitable for hair transplantation surgery.

AGA is the universal cause of non-scarring alopecia among general population. It is characterized by the

Corresponding author: Rattapon Thuangtong
E-mail: rattaponthuangtong@yahoo.com
Received 30 July 2022 Revised 1 September 2022 Accepted 17 September 2022
ORCID ID:http://orcid.org/0000-0001-5639-8984
https://doi.org/10.33192/smj.v75i2. 260752

gradual and progressive reduction in the hair density and hair diameter in both genders. Male AGA shows the characteristic features of patterned alopecia in both temporal and anterior hairline as well as hair thinning at the vertex region of the scalp. Assessment of the hair counts from the donor-site, or occipital area has been previously reported in a few studies.[4-6] Therefore, we aim to evaluate the average number of hair follicles and follicular density at the occipital scalp in Thai males with AGA for the reference record and to compare with the previous studies in Thailand.[4] Furthermore, we aim to compare the hair count parameters with the previous published data of hair counts in general Thai population and among other ethnics worldwide.[1,7-13]

MATERIALS AND METHODS

This descriptive, cross-sectional study enrolled 47 male participants who were candidates for hair transplantation for the treatment of AGA at Siriraj Hair Transplantation Clinic, Department of Dermatology, Faculty of Medicine Siriraj Hospital. This study was approved by Siriraj Institutional Review Board with written informed consents signed by all the participants prior to the study enrollment. (COA no. Si 581/2010) Demographic data including age, age at onset of hair loss, duration of the disease, family history of AGA, grading of the disease severity by Hamilton and Norwood classification, treatment, and underlying medical conditions was recorded. The scalp involvement of other skin diseases such as psoriasis, seborrheic dermatitis, atopic dermatitis, and superficial fungal infection were excluded by history taking and clinical evaluation.

Biopsy methods and tissue sample processing

Two pieces of 4-millimeter (mm) punch biopsy specimens were performed at the external occipital protuberance of clinically normal-looking occipital scalp before the beginning of hair transplantation surgery. One specimen was bisected vertically and the other specimen was transversely bisected at 1 mm below the dermo-epidermal junction with the cut surface embedded side-by-side in the paraffin cassette. All paraffin-embedded-tissue blocks were routinely processed and serially sectioned by an experienced technician. Ten consecutive sections with a 5-micrometer thickness were stained with hematoxylin and eosin (H&E) and prepared for microscopic evaluation by two independent dermatopathologists (PP and PS) in the blinded fashion. Histological records included the total number of the following hair parameters; follicular units, hair follicles, follicular stelae, type of hair (terminal or vellus/miniaturized hair), phase of hair cycle (anagen and telogen/catagen), terminal-to-vellus ratio, and anagen-to-catagen ratio percentage. We did not differentiate between intermediate and vellus hair but we grouped them together with miniaturized hair. We also combined the number of catagen and telogen hair in the same parameter. The maximum number of each hair count parameter was noted by each dermatopathologist and the average counts between both evaluators were recorded for statistical analysis.

Statistical analysis

All statistical analyses were analyzed and reported as descriptive statistics values by SPSS v.18 (SPSS Inc., Chicago, IL, USA). Categorical data was reported as numbers and percentages, and continuous data was reported as mean (range) or mean ± SD. The mean hair count was compared with the previous published data using the one-sample t-test for continuous variables and Chi-square for categorical data. P-value of < 0.001 was considered statistically significant.

RESULTS

A total of 47 male participants, who were candidates for hair transplantation for the treatment of AGA, were enrolled in this study. The demographic data (gender, age, age at disease onset, disease duration before transplantation, disease classification, previous treatment for AGA, and underlying medical conditions) of the study participants was demonstrated in Table 1. The mean age was 35.2±8.3 years, ranging from 21-53 years. The average age at the disease onset was 26.2±6.7 years, ranging from 18-40 years. The average disease duration before the transplantation was 9.0±4.9 years, ranging from 2-22 years. Thirty-seven participants (78.7%) reported the family history of AGA. The number of participants categorized by Hamilton-Norwood classification were 8 cases in type II (17%), 26 cases in type III (55.3%), 9 cases in type IV (19.1%), and 4 cases in type V (8.5%), respectively. Previous treatments recorded for AGA were monotherapy of oral finasteride in 8 cases (17%), topical minoxidil in 2 cases (4.3%), and a combination of oral finasteride and topical minoxidil in 37 cases (78.7%). There were 6 participants (12.8%) with known underlying medical diseases. The participants under 40 years old tend to present with less disease severity when compared with the age group above 40 years old ($P = 0.04$). Moreover, the presence of family history of AGA, the previous or current treatment of AGA, and underlying medical conditions were not associated with the grading of AGA severity ($P = 0.242$, 0.106, 0.602, respectively).

The hair count parameters from the transverse section

TABLE 1. Demographic data.

Demographic characteristics (N = 47)	
Gender: Male, n (%)	47 (100)
Age; years, mean (range)	35.2 (58 - 21)
Age at onset of AGA; years, mean (range)	26.2 (18 - 40)
Disease duration; years, mean (range)	9.0 (2 - 22)
Family history of AGA, n (%)	37 (78.7)
Hamilton-Norwood classification, n (%)	
Type II	8 (17.0)
Type III	26 (55.3)
Type IV	9 (19.1)
Type V	4 (8.5)
Treatment history for AGA, n (%)	
Oral finasteride	8 (17.0)
Topical minoxidil	2 (4.3)
Oral finasteride and topical minoxidil	37 (78.7)
Presence of underlying systemic diseases, n (%) *	
Dyslipidemia	4 (8.5)
Diabetes mellitus type 2	1 (2.1)
Hypertension	2 (4.3)
Chronic hepatitis B infection	1 (2.1)

Abbreviations: AGA: Androgenetic alopecia, SD: Standard deviation
*Three participants with more than one underlying diseases

of 4-mm punch biopsy were presented in Table 2. The mean number of the following hair count parameters was calculated: follicular units was 8.2±1.3 and total hair follicles were 18.6±1.2. The ratio between terminal and vellus hair (T:V ratio) was 11.1:1. The average percentage of anagen to telogen (A:T) ratio was 91.1:8.1. The mean count of follicular stelae was 0.4±0.7 and follicular density was 1.5±0.1 follicle per mm^2.

Comparison of hair count parameters from the transverse section of 4-mm punch biopsy between the present study and the previous published data among Asians including Thais, Caucasians, African-Americans, and Hispanics was also shown in Table 2. The number of hair follicles and follicular density was significantly less than the previous studies from normal Thai subjects, other Asians (Taiwan and Iran), Caucasians, African Americans, and Hispanics ($P < 0.001$). In contrast, this study showed a significantly higher number of hair count and follicular density as compared to the Korean subjects with hair diseases in the previous study. ($P < 0.001$).

The comparison of the data with the previous study in Thai male AGA and the control group was shown in Table 3. The average age of the participants in this study was not significantly different from the control group but was significantly less than the AGA group from that study ($P = 0.007$ and < 0.001, respectively). When compared to normal controls of the previous study, the mean number of total hair follicles, terminal hair count and follicular unit of our study were significantly less ($P < 0.001$). On the other hand, there were no statistical differences between vellus hair count, T:V ratio and A:T ratio ($P = 0.001$, $P = 0.001$ and $P = 0.249$, respectively) between the two studies. When compared to the male AGA group of the previous study, there were significantly higher number of total hair follicles and terminal hairs, as well as T:V ratio, and A:T ratio in the present study. ($P < 0.001$). In contrast, the median number of vellus hair was significantly lower in the present study as compared to the previous study ($P < 0.001$).

TABLE 2. Hair counts in transverse biopsy sections and comparison with previous reports among different ethnic populations.

Ethnic				Asians			Caucasians		African American	Hispanic
Country and study	**Present study**	**Thailand 1** (Ref 9)	**Thailand 2** (Ref 10)	**Taiwan** (Ref 11)	**Korea** (Ref 12)	**Iran** (Ref 13)	**USA** (Ref 1)	**USA** (Ref 14)	**USA** (Ref 1)	**Mexico** (Ref 15)
Number of cases	47	90	20	31	35	30	12	22	22	50
Hair conditions	AGA	Normal	Normal	Normal	AGA>AA>Normal	Normal	Normal	AGA	Normal	Normal
Biopsy site	Occipital	Occipital	Vertex	Vary	Occipital	Vary	Vary	Vertex	Vary	Occipital
Gender (M:F)	47:0	51:39	16:4	17:14	19:16	21:9	4:8	13:9	12:10	25:25
Age (years)	35.2 (8.3)	36.5 (15.1)	55.1 (15.8) *	37 (15.3)	33.1 (10)	35.5 (14.7)	34.7 (12.2)	43 (3.5) *	31.7 (8.5) *	34.14 (10.57)
Total hair follicles	18.6 (1.2)	20.5 (5.2) *	28.3 (9.2) *	21.3 (4.8) *	16.1 (3.6) *	36.4 (7.2) *	35.5 (5.5) *	40 (2.2) *	21.5 (5) *	23.2 (4.29) *
Follicular density (per mm^2)	1.5 (0.1)	NA	2.2 (0.7) *	1.7 (0.4) *	1.2 (0.3) *	NA	2.7 (0.4) *	3.1 (0.8) *	1.7 (0.4) *	1.75 (0.6) *
Terminal hairs	16.9 (1.4)	18.2 (4.1) *	16.5 (8.4)	20.5 (4.6) *	14.9 (3.2) *	34 (6.4) *	30.4 (6.4) *	35 (2.1) *	18.4 (5) *	21.08 (4.1) *
Vellus hairs	1.7 (0.6)	2 (0-7) **	6.9 (7) *	0.8 (1) *	1.1 (1.3) *	2.4 (1.2) *	5.1 (3.5) *	5 (0.6) *	3 (2.1) *	2.12 (1.04) *
T:V ratio	11.1:1	8.9:1*	2:1*	25.3:1*	13.5:1*	17.4:1*	6:1*	7:1*	6.1:1*	11.37:1
A:T ratio percent	91.9:8.1	91.9:7.9	NA	91.6:8.4*	93.6:6.4*	93.7:6.3*	94.5:5.5*	93.5:6.5*	93.9:6.1*	90.7:7.84
Follicular units	8.2 (1.3)	9.1 (1.6) *	10.7 (2.6) *	9.4 (1.9) *	7.8 (1.7)	NA	NA	14 (0.5) *	NA	7.56 (1.63) *

Numbers indicate mean (standard deviation), AGA; Androgenetic alopecia, M:F; male to female, T:V; terminal to vellus, A:T; anagen to telogen,
NA; not available, Ref; reference number
* Statistical significance ($p < 0.001$)
** Median (max-min), no comparison with the present study

TABLE 3. Comparison with occipital hair count in normal Thai male and Thai male with AGA.[6]

Hair counts from occipital scalp	Present study	Control	P-value	AGA	P-value
Number of cases	47	82		82	
Age, years, mean (SD)	35.2 (8.3)	38.6 (10.5)	0.007	40.1 (8.9)	< 0.001
Total hair follicles, mean (SD)	18.6 (1.2)	19.9 (6.1)	< 0.001	17.6 (4.2)	<0.001
Terminal hairs, mean (SD)	16.9 (14.)	17.9 (4.2)	< 0.001	15.9 (3.8)	<0.001
Vellus hairs, median (range)	2 (1-3)	2 (0-7)	0.001	3 (0-14)	<0.001
T:V ratio	11.1:1	8.9:1	0.001	7.4:1	<0.001
A:T ratio	91.9:8.1	92.2:7.8	0.249	87.6:12.4	<0.001
Follicular units, mean (SD)	8.2 (1.3)	9.3 (1.9)	<0.001	8.4 (1.8)	0.264

Abbreviations: AGA; androgenetic alopecia, SD; standard deviation, T:V; terminal-to-vellus, A:T; anagen-to-telogen
Statistical significance ($p < 0.001$)

DISCUSSION

The occipital scalp is considered the common and appropriate donor site for hair transplantation surgery for the treatment of AGA due to the follicular unresponsiveness to the androgenetic effects.[1-2] The scalp biopsy is a useful tool for the diagnosis of various hair and scalp diseases.[14] Different reference values of hair count parameters have been reported among various ethnic groups worldwide. The differences of hair density in each ethnic population have been established and a few similar studies reported that Asian individuals have less amount of hair follicles than that of whites, African-Americans, and Hispanics.[1, 7-13] We reported the average hair count parameters from the occipital scalp biopsy of male AGA in transverse sections using a 4-millimeter punch biopsy. Our study result shows significantly lower hair density than normal populations of previously mentioned ethnic groups including Thais, with an exceptionally greater amount of hair counts than the Korean cohort. This present study has a limitation of obtaining skin punch biopsies at the occipital area of Thai male subjects with AGA, because it was assumed that the samples acquiring from the patients mentioned above were comparable to the average hair parameters of the normal Thai male populations. However, the hormonal effects of androgen in AGA patients could influence the decreased amount of occipital hair as observed in the results of both present and previous studies.[10] In the aspect of T:V ratio, the ratio in the present study is higher than the previous reported data in Thai AGA males. The results in this study might be contrast with the results and analysis of the previous studies regarding the androgenetic effect upon the occipital area, due to the lack of evidence of miniaturization.[4,7,8] But it is important to take note that, the majority of the population in this study belonged to the less disease-severity groups (Hamilton-Norwood classification type II and III) and was relatively younger than the Thai male with AGA subjects in the previous study.[4] These observations could explain the less androgenetic effect in the population included in the present study.

The total hair counts from this study is less when compared to previous studies conducted in Thai normal controls.[4,7,8] This could be due to the fact that the biopsy specimen of the present studies was taken from live patients with AGA while those of the previous studies were taken from cadavers who did not have scalp or hair diseases especially AGA.

We do not report nor discuss the histological findings of vertical scalp biopsy section due to previously documented data that vertical section would be inappropriate and is not contributable for the diagnosis of non-scarring alopecia. In contrast, transverse section provides more information of the hair cycle phase, hair density and diameter than vertical section which would be very useful in the diagnosis of non-scarring alopecia especially AGA.[14-15]

CONCLUSION

This study reported the average hair follicular parameters from the donor site of the occipital scalp in Thai male patients with AGA who were undergoing hair

transplantation surgery. These results aimed to be utilized as a reference of the average hair density of the donor site in Thais. Hair density was shown to be distinctive among different races and fluctuates among Asians, of which, our study supported lower average hair density among Asians. In addition, the total hair counts in the occipital area from this study is less when compared to the previous studies conducted in Thai normal controls but higher than those with more advanced AGA. This result supports the evidence that hormonal effects may play a major role in the determination of the hair density even in normal looking, uninvolved occipital region. Further studies are recommended to emphasize the androgenetic effect in the different regions of the scalp, especially those which are known to be non-hormonal affected areas.

ACKNOWLEDGEMENTS

We would like to thank Rattiya Techakajornkeart, MD, for her assistance in data collection and Miss Julaporn Pooliam for statistical analysis of the data.

Funding support: The present study was financially supported by the Siriraj Grant for Research and Development Fund, Faculty of Medicine Siriraj Hospital, Mahidol University (Grant number R015432035).

Full disclosure of all authors: The authors do not have conflict of interest to declare.

REFERENCES

1. Sperling LC. Hair density in African Americans. Arch Dermatol. 1999;135(6):656-8.
2. Sawaya ME, Price VH. Different levels of 5alpha-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. J Invest Dermatol. 1997; 109:296-300.
3. Ekmekci TR, Sakiz D, Koslu A. Occipital involvement in female pattern hair loss: histopathological evidences. J Eur Acad Dermatol Venereol. 2010;24:299-301.
4. Khunkhet S, Chanprapaph K, Rutnin S, Suchonwanit P. Histopathological Evidence of Occipital Involvement in Male Androgenetic Alopecia. Front Med (Lausanne). 2021;22:790597.
5. Watanabe-Okada E, Amagai M, Ohyama M. Histopathological investigation of clinically non-affected perilesional scalp in alopecias detected unexpected spread of disease activities. J Dermatol. 2014;41:802-7.
6. Park JH, Park JM, Kim NR, Manonukul K. Hair diameter evaluation in different regions of the safe donor area in Asian populations. Int J Dermatol. 2017;56:784-7.
7. Visessiri Y, Pakornphadungsit K, Leerunyakul K, Rutnin S, Srisont S, Suchonwanit P. The study of hair follicle counts from scalp histopathology in the Thai population. Int J Dermatol. 2020;59:978-81.
8. Yaprohm P, Manonukul J, Sontichai V, Pooliam J, Srettabunjong S. Hair follicle counts in Thai population: a study on the vertex scalp area. J Med Assoc Thai. 2013;96:1578-82.
9. Ko JH, Huang YH, Kuo TT. Hair counts from normal scalp biopsy in Taiwan. Dermatol Surg. 2012;38:1516-20.
10. Lee HJ, Ha SJ, Lee JH, Kim JW, Kim HO, Whiting DA. Hair counts from scalp biopsy specimens in Asians. J Am Acad Dermatol. 2002;46:218-21.
11. Aslani FS, Dastgheib L, Banihashemi BM. Hair counts in scalp biopsy of males and females with androgenetic alopecia compared with normal subjects. J Cutan Pathol. 2009; 36:734-9.
12. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. J Am Acad Dermatol. 1993;28:755-63.
13. Martínez-Luna E, Rodríguez-Lobato E, Vázquez-Velo JA, Cuevas-González JC, Martínez Velasco MA, Toussaint Caire S. Quantification of hair follicles in the scalp in Mexican Mestizo population. Skin Appendage Disord. 2018;5:27-31.
14. Palo S, Biligi DS. Utility of horizontal and vertical sections of scalp biopsies in various forms of primary alopecias. J Lab Physicians. 2018;10:95-100.
15. Solomon AR. The transversely sectioned scalp biopsy specimen: the technique and an algorithm for its use in the diagnosis of alopecia. Adv Dermatol. 1994;9:127-57.

Histopathological Diagnosis of Alopecia Clinically Relevant to Alopecia Areata

Chinmanat Lekhavat, M.D.*,, Pinyo Rattanaumpawan, M.D., MSCE, Ph.D.***, Antonella Tosti, M.D.****, Tarinee Korviriyakamol, M.D.*****, Suthep Jerasutat, M.D.******, Poonnawis Sudtikoonaseth, M.D.*,**, Penvadee Pattanaprichakul, M.D.*******

**Institute of Dermatology, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand, **Science in Dermatology and Dermatosurgery Curriculum, Rangsit University, Pathum Thani, Thailand, ***Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, ****Dr Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, USA, *****Asoke Skin Hospital, Bangkok, Thailand, ******Lions Supannahong Clinic, Bangkok, Thailand, *******Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

ABSTRACT

Objective: To study the histopathological diagnosis of alopecia clinically relevant to AA and to compare the histopathology between acute and chronic AA divided by time to onset at three and six months.

Materials and Methods: We conducted a cross-sectional study of 113 patients with typical manifestation of AA. Two scalp biopsies were done horizontally and vertically to confirm diagnosis. Histological findings of AA in the acute group were subsequently compared with the chronic group.

Results: Of the 113 eligible patients, 109 (96.5%) were pathologically diagnosed with AA. Other diagnoses included lichen planopilaris, lupus panniculitis, and unspecified scarring alopecia. The percentage of terminal telogen hairs in the acute group was significantly higher than the chronic group (mean % anagen: % telogen ratio = 21.2%:78.8% vs. 36.0%:64.0%; $p = 0.016$), while the chronic group had a significantly higher number of follicular streamers (mean ± SD; 2.5 ± 2.2 vs. 3.7 ± 2.6; $p = 0.023$). The number of vellus hairs significantly increased in the acute group at the six-month onset (p = 0.006). The number of nanogen hairs also increased significantly in the chronic group at both the three- and six-month onset ($p = 0.020$ and $p = 0.007$).

Conclusion: Typical manifestations of AA are not always diagnosed as AA. Acute AA has more terminal telogens and vellus hairs, while chronic AA has more follicular streamers and nanogen hairs.

Keywords: Alopecia areata; scarring alopecia; lichen planopilaris; lupus erythematosus panniculitis; hair disorders; histopathology (Siriraj Med J 2023; 75: 138-144)

INTRODUCTION

Alopecia areata (AA) is a common hair loss condition that affects both men and women between the ages of 15 and 29. The incidence of AA is close to 2% of patients who visit dermatology clinics each year, with an estimated prevalence of 0.2%.[1,2] A previous study showed that the incidence rate of AA was higher in Asians at 3.8%, and that 85% of AA patients in Asia developed the disease before the age of 40.[3] The pathogenesis of AA is believed to be an autoimmune disorder caused by an abnormal immune response to hair follicle correlated antigens and genetic factors.[4-6] Interferon-gamma (IFN-γ) is an important inflammatory cytokine that tampers with the normal immune privilege of anagen hair bulbs and causes it to collapse and damage the hair follicles.[4,5] However, an association between AA and atopic diseases,

Corresponding author: Penvadee Pattanaprichakul
E-mail: penvadeep@yahoo.com
Received 9 June 2022 Revised 9 September 2022 Accepted 15 September 2022
ORCID ID:http://orcid.org/0000-0002-3293-7813
https://doi.org/10.33192/smj.v75i2.260753

metabolic syndrome, *Helicobacter pylori* infection, lupus erythematosus, iron deficiency anemia, thyroid diseases, vitamin D deficiency, and audiologic and ophthalmic abnormalities, and psychological problems have also been reported.[7-11]

The diagnosis of AA is usually based on typical clinical presentations, or well-defined round or ovoid non-scarring alopecia in the hair-baring area of the body, usually on the scalp.[12] The presence of exclamation point hairs provides sufficient discriminatory value to make a proper diagnosis.[12] However, diseases that can cause clinical patches of non-scarring alopecia and broken hairs, such as trichotillomania, secondary syphilis, and tinea capitis, may be confused with AA.[13] Several studies have shown clinical similarity between AA and early lesions of scarring alopecia, such as lupus erythematosus panniculitis and frontal fibrosing alopecia.[14,15] In addition, trichotillomania, telogen effluvium, or androgenetic alopecia can also mimic the diffuse variant of AA.[16,17] In those cases, the clinical presentation can be misleading, resulting in improper diagnosis and treatments. Therefore, a histopathological examination is essential for diagnosing AA, especially in ambiguous cases.

The objective of our study was to determine the diagnosis of alopecia that clinically mimicked AA and to compare the histopathology of AA between the acute stage (disease onset ≤ 3 months) and the chronic stage (disease onset >3 months).

MATERIALS AND METHODS

This cross-sectional study was conducted at the Institute of Dermatology, Bangkok, Thailand, from February 2012 to November 2013. The study protocol was approved by the Institutional Review Board of the Institute of Dermatology and the Department of Medical Services, Ministry of Public Health, Thailand (certification of approval number 011/2012). Patients with clinical presentation of AA on the scalp were eligible for the study if they were 18 or older and allowed two scalp biopsies (horizontal and vertical sections). Patients were excluded if they had androgenetic alopecia, trichotillomania, telogen effluvium, anagen effluvium, tinea capitis, secondary syphilis, and scarring alopecia.

Tissue histopathology

According to the institute's standard of care, all patients with clinical diagnosis of AA underwent a horizontal and vertical section biopsy of the scalp with four millimeters (mm) punch at the advancing edge of an area with active hair loss, recent hair loss, or hair regrowth. Biopsy specimens were embedded in 10% formalin and processed routinely. At least 15 horizontal and 10 vertical sections were cut and stained with hematoxylin and eosin and periodic acid-Schiff (PAS) to exclude fungal elements.

The horizontal sections of all cases were independently examined by two dermatopathologists (PP and PS), and the vertical sections individually also by two dermatopathologists (SJ and PS). For the diagnosis of alopecia areata; all dermatopathologists followed the combination of characteristic histopathologic findings for the diagnosis of alopecia areata as followed;

1. Non-scarring alopecia with normal or nearly normal number of hair follicles
2. Peribulbar lymphocytic infiltrate (with occasional eosinophils)
3. Increased number of terminal catagen and telogen hairs equal or greater than 50% of total hairs (or anagen-to-telogen ratio equal or less than 1:1)
4. Presence of miniaturized (nanogen) hairs, pigmented hair casts, melanin pigment deposits in fibrous tracts

Any disagreements were resolved via discussion among the authors. The histopathological findings of each case were recorded whether AA was present in the vertical or horizontal section. Other histologic features in the horizontal sections were recorded as follows: 1) the number of follicular units, 2) presence of terminal anagen follicles, 3) presence of terminal catagen/telogen follicles, 4) presence of total terminal follicles, 5) presence of vellus follicles, 6) presence of total follicles, 7) presence of nanogen follicles, 8) presence of follicular stelae (streamers), 9) presence of pigmented hair casts, 10) lymphocytes around follicular papillae and stelae, and 11) eosinophils around follicular papillae and stelae. Histological findings of patients with AA in the acute group were subsequently compared with those in the chronic group. For the presence of inflammatory infiltrate, fibrosis, and pigmented hair cast; the histologic results were gathered from both vertical and horizontal sections.

Statistical analysis

Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as mean ± SD. The histologic characteristics between the acute and chronic groups were compared using the two-sample t-test for continuous variables and the Fisher's exact test for categorical variables. A two-tailed test with a p-value of <0.05 was considered statistically significant. All statistical calculations were done using STATA version 14.0 (STATA Corp, College Station, TX).

RESULTS

A total of 113 patients were enrolled in the study. Of these, 109 patients (96.5%) were pathologically diagnosed with AA, and four patients (3.5%) were identified as having scarring alopecia. Of these four patients with scarring alopecia, two had lichen planopilaris (1.7%), one of them had lupus erythematosus panniculitis (0.9%), and one had unspecified scarring alopecia (0.9%). Figs 1, 2, 3 and 4 show the clinical presentation and histopathologic findings of the four patients with scarring alopecia.

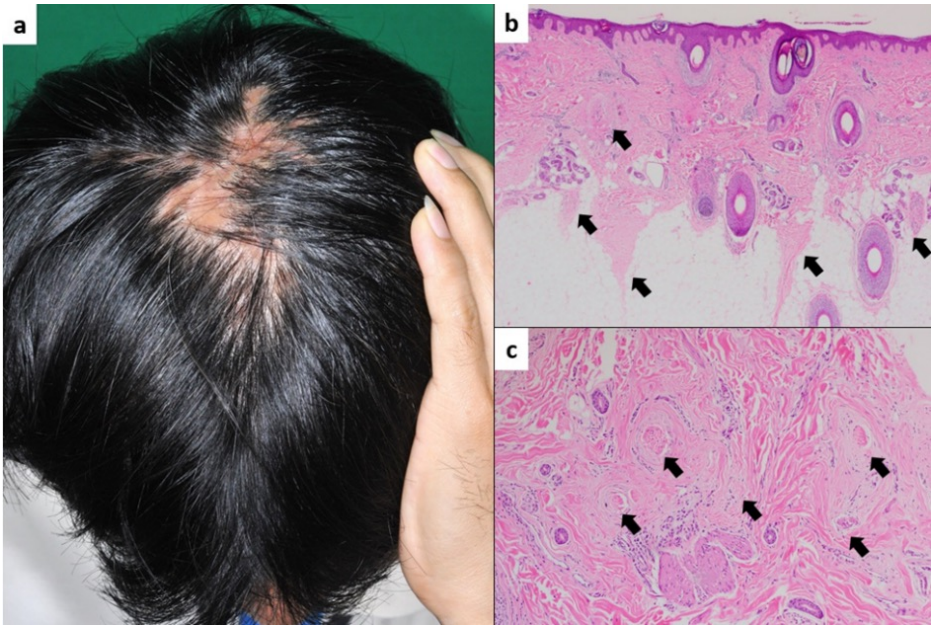


Fig 1. Lichen planopilaris; **(a)** Male who had circumscribed area of hair loss for one year, **(b)** Fibrotic tracts (arrows) in the vertical section, HE x40 magnification, **(c)** Fibrotic tracts (arrows) in the transverse section, HE x100 magnification.

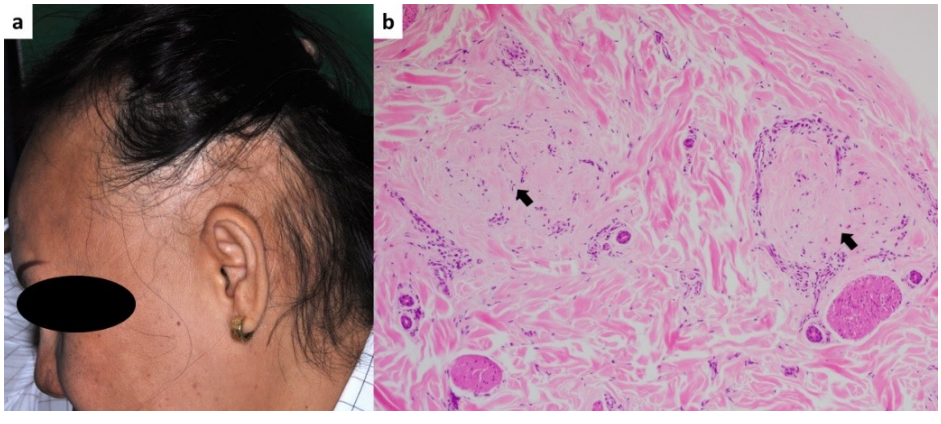


Fig 2. Lichen planopilaris; **(a)** Female who had ophiasis-like alopecia for four years, **(b)**, Fibrotic tracts (arrows), HE x200 magnification

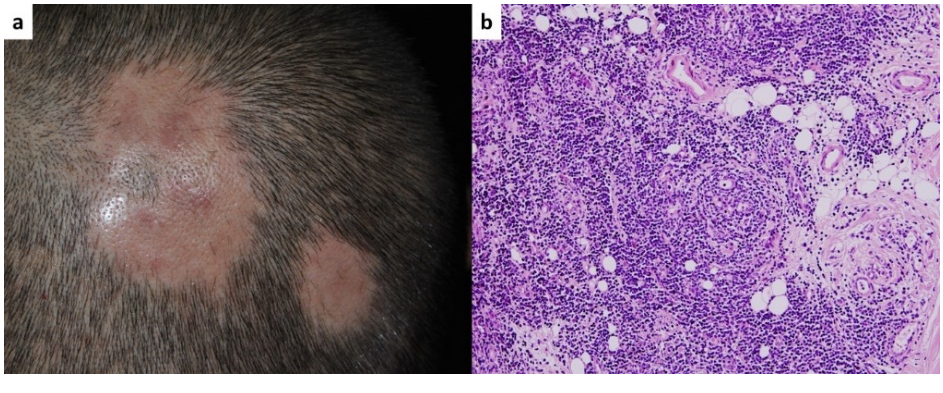


Fig 3. Lupus erythematosus panniculitis; **(a)** Male with multiple patches of alopecia over three months, **(b)** Dense lymphoplasmacytic infiltrates in the subcutaneous fat lobules, HE x200 magnification

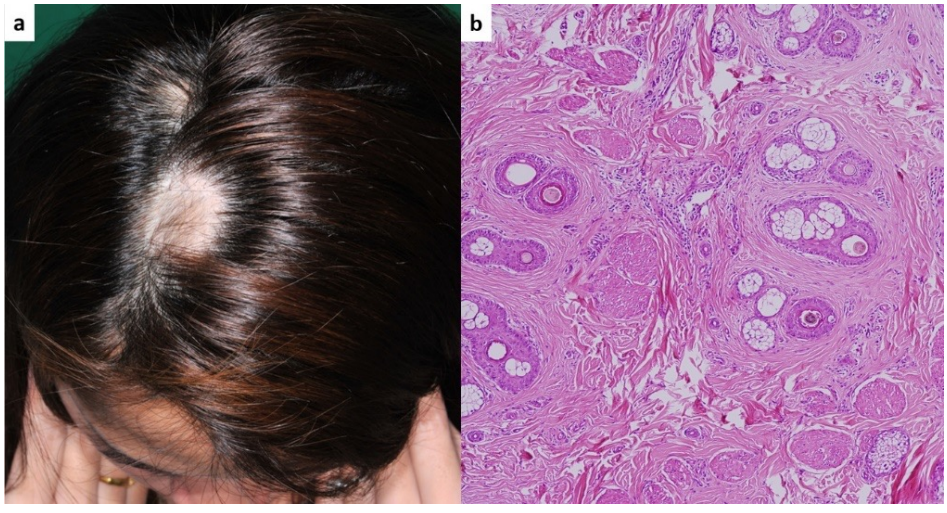


Fig 4. Unspecified scarring alopecia; **(a)** Female who had circumscribed patches of alopecia for two months, **(b)** Intense perifollicular fibrosis; HE x100 magnification

Baseline characteristics of patients with acute AA vs. patients with chronic AA

Patients' demographic data for acute and chronic AA is compared in Table 1. Among the 109 AA patients, 102 had adequate horizontal section tissue for histopathology assessment. There were 54 males and 59 females, with a mean (SD) age of 36 (±11.7) years. Clinical subtypes of AA included patchy AA (95%), alopecia totalis/universalis (3%), and acute diffuse and total AA (2%). All patients were classified by clinical onset of AA. Seventy-six patients (74.5%) were classified as having acute AA as the duration from disease onset was equal to or less than three months. Twenty-six patients (25.5%) were classified as having chronic AA as the duration of disease onset was greater than three months. There were no significant differences between patients with acute and chronic AA for mean age, gender, and alopecia clinical subtypes.

Histologic features of the patients with acute AA vs. chronic AA

Table 2 shows histologic features from horizontal sections of patients with acute AA and patients with chronic AA. The percentage of terminal telogen hairs in the acute group was significantly higher than those in the chronic group ($p = 0.016$). The median anagen to telogen ratio (%) was significantly different between the acute and chronic group (21.2%:78.8% vs. 36.0%:64.0%; $p = 0.016$). However, the median number of follicular stelae or streamers was significantly higher in the chronic group than acute group (3.7 ± 2.6 vs. 2.5 ± 2.2.; $p = 0.023$). Other parameters included the number of terminal anagen hairs, terminal telogen hairs, total terminal hairs, vellus hairs, total hairs, terminal to vellus ratio, total follicular units, lymphocyte, and eosinophilic infiltration, fibrosis, and pigmented hair casts that were not substantially

TABLE 1. Population characteristics of patients with AA.

Characteristics	All patients N = 102	Acute AA N = 76	Chronic AA N = 26	*P* value
Age, year,				
median (range)	35 (18-67)	35 (18-67)	34.5 (22-52)	0.94
mean ± SD	36.3 ± 11.7	36.3 ± 12.5	36.2 ± 9.4	
Sex, N (%)				
Female	54 (52.9)	41 (54)	13 (50)	0.82
Male	48 (47.1)	35 (46)	13 (50)	0.82
Type of AA, N (%)				
Patchy AA	97 (95.1)	73 (96.1)	24 (92.3)	0.07
Alopecia totalis, universalis	3 (2.9)	1 (1.3)	2 (7.7)	0.07
Acute, diffuse, total AA	2 (2.0)	2 (2.6)	0 (0)	0.07

TABLE 2. Comparison of histologic features between acute and chronic AA.

Histologic features	Patients (n = 102)	Acute ≤ 3 months (n = 76)	Chronic > 3 months (n = 26)	*P*- value
Age, years, mean ± SD	36.3 ± 11.7	36.3 ± 12.5	36.2 ± 9.4	0.972
Terminal anagen hairs, mean ± SD	2.0 ± 2.3	1.7 ± 2.1	2.8 ± 2.7	0.027
Terminal telogen hairs, mean ± SD	6.4 ± 4.9	6.5 ± 4.8	6.2 ± 5.3	0.814
Total Terminal hairs, mean ± SD	8.4 ± 5.2	8.2 ± 5.1	9.1 ± 5.7	0.466
Vellus hairs, mean ± SD	9.0 ± 6.1	9.3 ± 6.1	8.3 ± 6.0	0.451
Total hairs, mean ± SD	17.5 ± 5.0	17.6 ± 5.1	17.1 ± 4.7	0.617
Terminal: Vellus ratio, mean ± SD	2.3:1 ± 3.4:1	2.0:1 ± 3.0:1	3.0:1 ± 4.2:1	0.167
%Anagen: %Telogen ratio, mean ± SD	24.9:75.1 ± 26.9:26.9	21.2:78.8 ± 25.8:25.8	36.0:64.0 ± 27.4:27.4	0.016
Follicular units, mean ± SD	8.1 ± 2.0	8.1 ± 1.9	8.3 ± 2.3	0.631
Follicular stelae, mean ± SD	2.8 ± 2.3	2.5 ± 2.2	3.7 ± 2.6	0.023
Eosinophilic infiltration at stelae, upper, mean ± SD	1.5 ± 3.5	1.2 ± 2.0	2.3 ± 6.1	0.160
Nanogen hairs, lower, mean ± SD	0.4 ± 1.4	0.2 ± 0.6	0.9 ± 2.5	0.020

different between the two groups. Although the acute and chronic groups had a similar median number of vellus hairs when categorized at three months (8 and 6.5, respectively; $p = 0.27$), there was an increase of vellus hairs in the group with disease onset before six months and the group with onset after six months ($p = 0.02$) (Table 3). Nanogen hairs increased significantly in the chronic group compared to the acute group at three and six month of disease duration ($p = 0.020$ and $p = 0.007$, respectively) (Tables 2 and 3).

Hair follicles were surrounded by lymphocytes (64.7% at three-month onset and 74.8% at six-month onset) and eosinophils (20.6% at three-month onset and 41.7% at six-month onset) in the lower dermis, but there was no statistically significant difference between the acute and chronic groups ($p > 0.05$).

DISCUSSION

The diagnosis of AA is usually based on characteristics of clinical features. However, clinical assessment alone sometimes is not sufficient to attain accurate diagnosis. Therefore, histopathological assessment is essential in some specific cases. Our study objective was to determine whether clinical evaluation has sufficient specificity for diagnosis of AA. A histological examination was performed to distinguish between AA and other alopecia conditions. Both horizontal and vertical histopathological sections should provide a high diagnostic yield for alopecia.[18] We also assessed differences in histological features between acute and chronic AA.

In our study, 109 out of 113 patients (96.5%) with symptoms resembling AA were diagnosed for it by histological examination. The other four patients (3.5%) were diagnosed with scarring alopecia, such as lichen planopilaris, lupus erythematosus panniculitis, and unspecified scarring alopecia. Sometimes early stage of scarring alopecia is confused with AA when absence of follicular ostia is not noticeable. There were reported cases of lupus erythematosus panniculitis in the patient who presented non-scarring alopecic patches and linear alopecia with clinical features mimicking AA.[14,19,20]

Even though histological examinations can lead to proper diagnosis, it may be impractical to perform scalp biopsies in patients with clinically relevant AA. For clinicians, a thorough history and well-performed physical examination can increase the diagnostic accuracy of AA. In some cases, an absence of follicular ostium with any evidence of changes such as slight erythema, perifollicular scale, epidermal atrophy, and tenderness can lead to other diagnoses of alopecia rather than AA. Dermoscopy allows dermatologists to obtain additional information such as exclamation mark hairs, yellow

TABLE 3. Comparison of histologic features of AA at different onset (before and after six months).

Histologic features	Patients (n = 102)	Onset ≤ 6 months (n = 90)	Onset > 6 months (n = 12)	*P*- value
Age, years, mean ± SD	36.3 ± 11.7	35.8 ± 12.1	39.8 ± 8.0	0.264
Terminal anagen hairs, mean ± SD	2.0 ± 2.3	1.9 ± 2.2	2.7 ± 3.0	0.275
Terminal telogen hairs, mean ± SD	6.4 ± 4.9	6.3 ± 4.7	7.3 ± 6.1	0.538
Total Terminal hairs, mean ± SD	8.4 ± 5.2	8.2 ± 5.0	9.9 ± 6.7	0.296
Vellus hairs, mean ± SD	9.0 ± 6.1	9.4 ± 6.0	6.2 ± 5.9	0.079
Total hairs, mean ± SD	17.5 ± 5.0	17.7 ± 5.0	16.2 ± 5.5	0.328
Terminal: Vellus ratio, mean ± SD	2.3:1 ± 3.4:1	1.9:1 ± 2.9:1	4.7:1 ± 5.5:1	0.006
%Anagen: %Telogen ratio, mean ± SD	24.9:75.1 ± 26.9:26.9	24.3:75.7 ± 26.8:26.8	29.4:70.6 ± 28.1:28.1	0.536
Follicular units, mean ± SD	8.1 ± 2.0	8.2 ± 2.0	7.6 ± 1.6	0.332
Follicular stelae, mean ± SD	2.8 ± 2.3	2.8 ± 2.4	2.9 ± 2.1	0.898
Eosinophilic infiltration at stelae, upper, mean ± SD	1.5 ± 3.5	1.6 ± 3.7	1.1 ± 2.0	0.661
Nanogen hairs, lower, mean ± SD	0.4 ± 1.4	0.2 ± 0.7	1.3 ± 3.5	0.007

dots, black dots and circle hairs to aid in the diagnosis of AA.[21] In equivocal cases, histological examination is necessary for a definitive diagnosis.

The histological features of AA are characteristic of peribulbar infiltrations.[22] When peribulbar infiltrations are notably absent, the diagnosis of AA is difficult. Other histopathologic changes can be beneficial in the diagnosis of AA, such as an increase in catagen and telogen hair follicles, follicular miniaturization, pigment hair cast, nanogen hairs, lymphocytes, and eosinophils as well as melanin in the fibrous tract.[22-26]

A prior study confirmed that disease onset was associated with the number of follicles and degree of inflammation.[22] Peribulbar infiltrations are frequently associated with acute AA rather than chronic AA, whereas an increase of catagen and telogen hair follicles, and hair miniaturization, are associated with sub-acute and chronic AA, respectively.[22,26] This study reveals histopathologic changes of AA regarding acute and chronic stages similar to previous literature[22,26], but we evaluated the change over a specified period at three months and six months. At the three-month cut-off point, there was an increase in terminal telogen hairs in the acute group, which caused a significant decrease in the anagen to telogen ratio. On the other hand, a significant increase in the number of follicular stelae (streamers) and nanogen hairs was noticed in the chronic group, and although e vellus hairs also increased, they were not statistically significant. We can assume that the designated cut-off point at three months for acute and chronic AA may have been too early to detect differences in the number of vellus hairs since increase of vellus hairs in the chronic group was consequentially seen at the sixth-month cut-off point. When patients experience hair loss for a lengthy period of time, the anagen follicles reach the catagen phase, where hair bulbs retract upward toward the isthmus, causing the connective tissue sheath to collapse and become stelae or streamers. This explains why catagen and telogen follicles increase in acute AA, and why follicular stelae are associated with chronic AA. Furthermore, nanogen hairs and small dystrophic follicles, which are unique features of long-standing alopecia areata, also begins to appear more frequently. Peribulbar eosinophils are not seen significantly between the acute and chronic AA, similar to a previous study.[27]

Our study had several strengths. First, all patients in this study underwent both horizontal and vertical scalp biopsies. Second, the histopathological findings were carefully obtained by two independent board-certified dermatopathologists.

However, our study also had limitations. First, some of the histopathological slides of our patients were

inadequate and unreadable, and therefore, some study subjects were excluded. However, only seven patients were excluded due to missing histopathology results. Second, the study was conducted at a specialized dermatology institute in Thailand. Last but not least, generalizability may also have been an issue.

CONCLUSION

Dermatologists have relied on clinical presentation to diagnose AA, but diagnosis s from clinical signs and symptoms may not always rule out other alopecia conditions besides AA. A total of 96.5% of patients with clinical relevance for AA were diagnosed with it through histopathology. Scalp biopsies should be performed in clinically unclear cases. Patients with acute AA have more telogen hairs, whereas those with chronic AA have more follicular stelae, nanogen hairs, and hair miniaturization.

ACKNOWLEDGEMENT

We would like to thank Supaporn Siripun, M.D., for her assistance in patient enrollment and data collection.

Conflict of interest: The authors do not have any conflict of interest to declare.

REFERENCES

1. Safavi K. Prevalence of alopecia areata in the First National Health and Nutrition Examination Survey. Arch Dermatol. 1992;128(5):702.
2. Mirzoyev SA, Schrum AG, Davis MDP, Torgerson RR. Lifetime incidence risk of alopecia areata estimated at 2.1% by Rochester Epidemiology Project, 1990-2009. J Invest Dermatol. 2014; 134(4):1141-2.
3. Tan E, Tay YK, Goh CL, Chin Giam Y. The pattern and profile of alopecia areata in Singapore--a study of 219 Asians. Int J Dermatol. 2002;41(11):748-53.
4. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: a review of disease pathogenesis. Br J Dermatol. 2018;179(5):1033-48.
5. Trueb RM, Dias M. Alopecia Areata: a Comprehensive Review of Pathogenesis and Management. Clin Rev Allergy Immunol. 2018;54(1):68-87.
6. McElwee K, Freyschmidt-Paul P, Ziegler A, Happle R, Hoffmann R. Genetic susceptibility and severity of alopecia areata in human and animal models. Eur J Dermatol. 2001;11(1):11-6.
7. Kantor J, Kessler LJ, Brooks DG, Cotsarelis G. Decreased serum ferritin is associated with alopecia in women. J Invest Dermatol. 2003;121(5):985-8.
8. Seyrafi H, Akhiani M, Abbasi H, Mirpour S, Gholamrezanezhad A. Evaluation of the profile of alopecia areata and the prevalence of thyroid function test abnormalities and serum autoantibodies in Iranian patients. BMC Dermatol. 2005;5:11.
9. Chu SY, Chen YJ, Tseng WC, Lin MW, Chen TJ, Hwang CY, et al. Comorbidity profiles among patients with alopecia areata: the importance of onset age, a nationwide population-based study. J Am Acad Dermatol. 2011;65(5):949-56.
10. Aksu Cerman A, Sarikaya Solak S, Kivanc Altunay I. Vitamin D deficiency in alopecia areata. Br J Dermatol. 2014;170(6):1299-304.
11. Lee S, Lee H, Lee CH, Lee WS. Comorbidities in alopecia areata: A systematic review and meta-analysis. J Am Acad Dermatol. 2019;80(2):466-77.e16.
12. Strazzulla LC, Wang EHC, Avila L, Lo Sicco K, Brinster N, Christiano AM, et al. Alopecia areata: Disease characteristics, clinical evaluation, and new perspectives on pathogenesis. J Am Acad Dermatol. 2018;78(1):1-12.
13. Messenger AG, McKillop J, Farrant P, McDonagh AJ, Sladden M. British Association of Dermatologists' guidelines for the management of alopecia areata 2012. Br J Dermatol. 2012;166(5): 916-26.
14. Kossard S. Lupus panniculitis clinically simulating alopecia areata. Australas J Dermatol. 2002;43(3):221-3.
15. Kwong RA, Kossard S. Alopecia areata masquerading as frontal fibrosing alopecia. Australas J Dermatol. 2006;47(1):63-6.
16. Hoss DM, Grant-Kels JM. Diagnosis: alopecia areata or not? Semin Cutan Med Surg. 1999;18(1):84-90.
17. Lew BL, Shin MK, Sim WY. Acute diffuse and total alopecia: A new subtype of alopecia areata with a favorable prognosis. J Am Acad Dermatol. 2009;60(1):85-93.
18. Elston DM. Vertical vs. transverse sections: both are valuable in the evaluation of alopecia. Am J Dermatopathol. 2005;27(4): 353-6.
19. Chen YA, Hsu CK, Lee JY, Yang CC. Linear lupus panniculitis of the scalp presenting as alopecia along Blaschko's lines: a distinct variant of lupus panniculitis in East Asians? J Dermatol. 2012; 39(4):385-8.
20. Chiesa-Fuxench ZC, Kim EJ, Schaffer A, Fett N. Linear lupus panniculitis of the scalp presenting as alopecia along Blaschko's lines: a variant of lupus panniculitis not unique to East Asians. J Dermatol. 2013;40(3):231-2.
21. Miteva M, Tosti A. Hair and scalp dermatoscopy. J Am Acad Dermatol. 2012;67(5):1040-8.
22. Whiting DA. Histopathologic features of alopecia areata: a new look. Arch Dermatol. 2003;139(12):1555-9.
23. Sperling LC, Lupton GP. Histopathology of non-scarring alopecia. J Cutan Pathol. 1995;22(2):97-114.
24. Sperling LC, Cowper SE, Knopp EA. An Atlas of Hair Pathology with Clinical Correlations. 2nd ed. Informa Healthcare; 2012.
25. Elston DM, McCollough ML, Bergfeld WF, Liranzo MO, Heibel M. Eosinophils in fibrous tracts and near hair bulbs: a helpful diagnostic feature of alopecia areata. J Am Acad Dermatol. 1997; 37(1):101-6.
26. Dy LC, Whiting DA. Histopathology of alopecia areata, acute and chronic: Why is it important to the clinician? Dermatol Ther. 2011;24(3):369-74.
27. Yoon TY, Lee DY, Kim YJ, Lee JY, Kim MK. Diagnostic usefulness of a peribulbar eosinophilic infiltrate in alopecia areata. JAMA Dermatol. 2014;150(9):952-6.

Direct Immunofluorescence in Cutaneous and Systemic Lupus Erythematosus: A Literature Review

Chuda Rujitharanawong, M.D., Kanokvalai Kulthanan, M.D., Papapit Tuchinda, M.D., Samruay Pinkaew, B.Ed., M.Sc., Nattacha Chanchaemsri, B.Sc., Sasipha Nuttawong, B.Sc., Leena Chularojanamontri, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Cutaneous manifestations of lupus erythematosus (LE) have a variety of clinical phenotypes and require proper investigation for diagnosis. Knowledge of the direct immunofluorescence (DIF) technique has improved and played an important role in the diagnosis of cutaneous LE. This review explores and summarizes reported DIF findings of each cutaneous LE variant. Historically, DIF findings of cutaneous LE have revealed deposits of multiple immunoreactants at the dermo-epidermal junction with either linear or granular patterns. Immunoglobulin M is the most common immunoreactant and DIF findings of cutaneous LE variants overlap. Therefore, diagnosis of cutaneous LE requires a combination of monitoring patient history, physical examinations and laboratory studies. This review helps interpret and better understand the application of DIF studies in cutaneous LE.

Keywords: Direct immunofluorescence; Lupus erythematosus (Siriraj Med J 2023; 75: 145-166)

Abbreviations

ACLE: acute cutaneous lupus erythematosus
ANA: antinuclear antibodies
BSLE: bullous systemic lupus erythematosus
CCLE: chronic cutaneous lupus erythematosus
C: complement
CB: cytoid bodies
DEJ: dermo-epidermal junction
DIF: direct immunofluorescence study
DLE: discoid lupus erythematosus
DLP: dust-like particles
DNA: Deoxyribonucleic acid
ENS: epidermal nuclear staining
Ig: immunoglobulin
LB: lupus band
LE: lupus erythematosus
NLE: neonatal lupus erythematosus
SCLE: subacute cutaneous lupus erythematosus
SLE: systemic lupus erythematosus
TEN: toxic epidermal necrolysis

INTRODUCTION

Lupus erythematosus (LE) is an autoimmune disease involving multiple organs such as the skin, musculoskeletal system, kidneys, and hematologic involvement. It predominantly affects young women aged 20-40. Cutaneous presentations can vary and is found in 59%-85% of patients. Cutaneous LE is categorized into two groups; (i) LE-specific skin rash and (ii) LE-nonspecific skin rash.[1] There are three subsets of LE-specific skin rash known as acute cutaneous LE (ACLE), subacute cutaneous LE (SCLE), and chronic cutaneous LE (CCLE).[2] LE-nonspecific skin rashes have various presentations such as vasculitis, periungual telangiectasia, non-scarring alopecia, calcinosis cutis, urticaria, and erythromelalgia.

ACLE lesions occur as localized (malar rash) or generalized distributions (lupus maculopapular rash, photosensitive lupus dermatitis, and toxic epidermal necrolysis-like). Meanwhile, SCLE presents itself as

Corresponding author: Leena Chularojanamontri
E-mail: leenajim@gmail.com
Received 20 August 2022 Revised 3 September 2022 Accepted 12 September 2022
ORCID ID:http://orcid.org/0000-0001-6625-6445
https://doi.org/10.33192/smj.v75i2.260754

papulosquamous (psoriasiform) SCLE or annular polycyclic SCLE. CCLE is the most common form of cutaneous LE and is often found as discoid lupus erythematosus (DLE). Other variants of CCLE include tumid LE, lupus panniculitis/lupus profundus, hypertrophic/verrucous DLE, mucosal LE, oral DLE, conjunctival DLE, lichenoid DLE and chilblain LE.[3] Of the three subtypes of LE-specific sin rash, ACLE is generally associated with systemic involvement and is parallel with underlying SLE disease activity (90%), followed by SCLE (33%).[4,5] CCLE rarely progresses to systemic involvement (5%).[6]

Generally, LE can be diagnosed by studying patient history, clinical presentations (skin and other organ involvement), and serology. A skin biopsy for histopathology examination and a direct immunofluorescence (DIF) test are helpful in detecting unusual or atypical presentations. Basal layer degeneration (interface dermatitis) with mucin deposition and infiltration of mononuclear cells at the perivascular and peri-appendage are hallmark signs of LE. However, these histological findings differ among cutaneous LE subtypes and are also found in other connective tissue diseases. In those cases, DIF is helpful in providing a more precise diagnosis.[7] Thus, this article aims to review DIF findings among different LE-skin rash subtypes. The literature was searched in electronic database (PubMed) using the terms of "cutaneous lupus erythematosus", "oral lupus erythematosus", "systemic lupus erythematosus" and "direct immunofluorescence" through June 2022.

DIF tests

The immunofluorescence technique was developed in the 1940s by Coons.[8] DIF reveals tissue-bound autoantibodies in tissues or cells.[8] In 1963, the technique was introduced to dermatology, leading to the discovery of immunoreactants along the dermo-epidermal junction (DEJ), which are also known as lupus bands (LB)[9] (Fig 1A). A suitable skin biopsy site is determined by the suspected disease. In cutaneous LE, biopsies should be performed at active lesion areas, which are often exposed to the sun, because deposits of immune complex usually present in lesion skin.[10] Occasionally, DIF tests in LE patients also reveal positive LB in non-lesion and non-sun exposed areas.[11] Established lesions with longer durations (one to six months) can provide higher positive yields (80%) than lesions that are less than one month old (30%).[10,12]

In a DIF test, skin biopsy specimens should either be quick frozen or put in Michel's transport medium for subsequent quick freezing. Michel's transport medium can store skin biopsy specimens for up to four weeks at 4-8°C. During this process, a frozen tissue is inserted in a resin on a cooled metal chunk in the cryostat and each section is cut into 4-5 μm.[8] The sections are then placed and dried on a slide for staining. The immunoreactants, including immunoglobulin IgG IgM, IgA, complement C3 and/or fibrinogen are put into each slide.[8] Each slide is covered by a glass cover slide and interpreted under immunofluorescence microscopy.[8] The types of immunoreactants, sites, and patterns of depositions are evaluated and interpreted for diagnosis.

Various DIF results have been noted in LE. Generally, deposits of multiple immunoreactants along the DEJ are the most reported pattern (approximately 80%).[13,14] Among immunodeposits at the DEJ, IgM is the most common.[15] However, IgG deposit at the DEJ of involved skin are more specific than IgM. IgG deposits in the nucleus of keratinocytes known as epidermal nuclear staining (ENS) or *in vivo* antinuclear antibodies (ANA) are generally

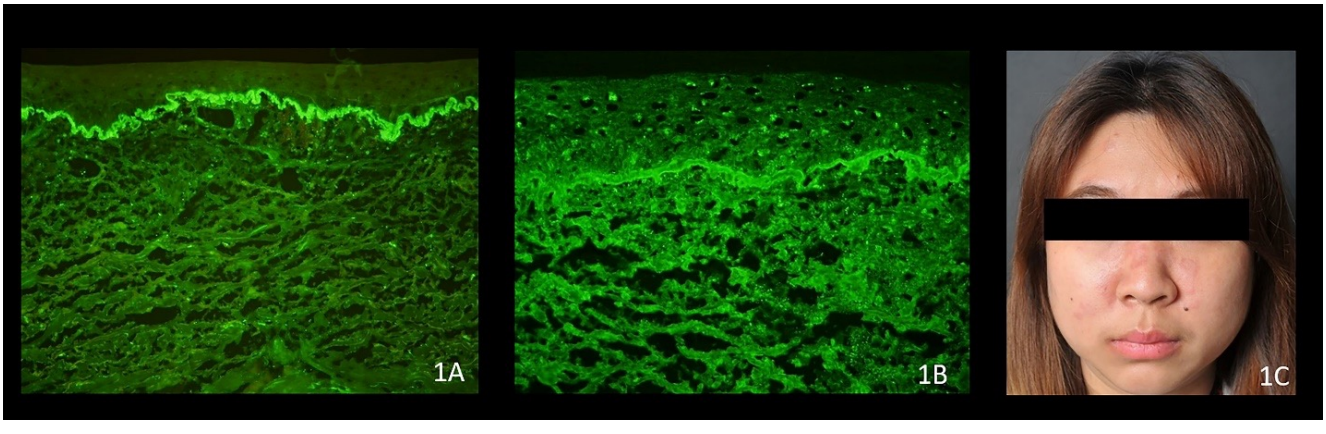


Fig 1A. Direct immunofluorescence (DIF) findings of cutaneous lupus erythematosus showing positive homogenous deposits of immunoglobulin (Ig)M at the dermo-epidermal junction (DEJ) were recognized as lupus bands. (10x magnification).
Fig 1B. DIF findings of acute cutaneous lupus erythematosus showed positive epidermal nuclear staining of IgG. (20x magnification).
Fig 1C. Clinical presentation of acute cutaneous lupus erythematosus showed ill-defined erythematous plaque localized on both cheeks and nose.

found more often than other connective tissue diseases. Dust-like particles (DLP) are defined as patchy deposits of tiny granules which appear as fine speckle patterns or technical artifacts. They can be found on the basal layer of the epidermis (intercellular and intracellular areas), DEJ, or the upper part of the dermis.[16] DLP is specific for SCLE.[16-19] Deposits of immunoreactants along blood vessels, periadnexal areas, and cytoid bodies (CB) have also been observed.[14]

DIF test of systemic lupus erythematosus (Table 1)

Based on our review, some studies investigated DIF results of cutaneous lesions in systemic lupus erythematosus (SLE) patients without identifying the type of lesion. There was a positive result in 42%-100% lesion cases, and 32%-92.9% in non-lesion cases.[7,16,20-28] The pattern of immunodeposition at the DEJ was homogenous, thready, strippled, granular, and linear. A homogenous pattern was defined as a thick, solid, well-demarcated, continued line at the DEJ. The thready pattern was defined as short, close-set threads or fibrils, sometimes with a long axis at right angles to the DEJ. Last but not least, the stippled pattern was defined as a discontinuous broken line with multiple small round points of fluorescence. The common pattern and immunodeposits at the DEJ were granular and IgM, respectively.[20,23,29]

DIF tests of LE-specific skin lesions (Table 1)

Acute cutaneous lupus erythematosus

ACLE typically presents itself as symmetrical papules confluent to plaques in photosensitive areas such as malar eminence, the forehead, V-neck, and extensor arms. (Fig 1C) ACLE lesions can persist for days to weeks. In cases of intense inflammation, some patients suffer from atypical target lesions or epidermal detachment called Stevens-Johnson syndrome or toxic epidermal necrolysis (TEN)-like pattern.[15] A positive DIF result in ACLE depends on the site of the skin biopsy. Positive results in lesion skin ranges from 60%-100% and is 25% in non-lesion skin.[13,14,13-15,25,30] Multiple immunodeposits at the DEJ was the most common DIF finding. TEN-like ACLE also exhibited immunodeposits at the DEJ.[15] ENS was positive in 47.1% of all ACLE lesions[14] (Fig 1B). Fluorescent CB in the papillary dermis, immunoreactants in the blood vessels or periadnexal areas with granular pattern were also observed.[14]

Subacute lupus erythematosus

SCLE frequently presents itself as non-scarring or non-atrophic symmetrical erythematous macules or papules. It is predominantly distributed in photosensitive areas such as the face, anterior and posterior neck or extensor arms. Other variants include drug-induced SCLE and neonatal lupus erythematosus (NLE). SCLE is associated with positive Ro/SSA antibodies.[31] The positive yields in lesion skin (34%-100%) and non-lesion skin (36%-100%) of SCLE were equal.[14,16-19,22,32-37] Deposits of various immunoreactants along the DEJ with granular pattern was common, followed by DLP staining in the epidermis, and subepidermal region.[16-19,22,25,33,35-38] As previously mentioned, DLP is associated with SCLE. Under experimental control, DLP was initially detected within two weeks after artificial light exposure.[16] A previous study showed that DLP was highly specific for SCLE, but its sensitivity was low (30%). There was no significant relation between the presence of DLP and anti-SSA/Ro antibodies, and ANA.[18] Furthermore, patterns of DIF findings were not associated with systemic involvements in SCLE.[33] IgG and IgM were detected most at the DEJ in SCLE, followed by IgA and C3.

In the case of drug-induced SCLE and NLE, DIF findings showed similar results to idiopathic SCLE.[39] Transfer of anti-RSSA/Ro and/or anti-SSB/La from the mother to fetus via the placenta also induced NLE. Annular erythematous plaques on the face and scalp are a hallmark of NLE. Data regarding DIF findings of NLE is limited as we found only one study reporting on it.[39]

Chronic cutaneous lupus erythematosus

Discoid lupus erythematosus

DLE is described by well-defined indurated erythematous coin-shaped plaques coated by adherent scales with keratotic spikes (carpet tack sign). The lesions slowly progress to an atrophic scarring center with an active erythema periphery. DLE lesions can occur in both sun-exposed areas such as the face, ears, upper chest or extensor surface of extremities and sun-protected areas such as the scalp or trunk. Scarring alopecia is usually observed in DLE (Fig 2C).

DIF tests have also revealed deposits of multiple immunoreactants along the DEJ with granular patterns that extend to the basement membrane of hair follicles or peri-appendage areas (Fig 2A & 2B). The positive yield ranges from 27.2%-100% in lesion areas and 45.5%-69.2% in non-lesion areas.[14,16,22,23,33,35,40-48] ENS has also been found, but at a lower frequency than ACLE and SCLE. DIF tests of oral DLE lesions have also revealed deposits of multiple immunoglobulins at the DEJ with either granular or homogeneous bands, similar to DLE lesions on glabrous skin.[43] Deposits of fibrinogen at the DEJ extending into the upper dermis has also been reported in oral DLE, however, the depth and thickness

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus.

Systemic lupus erythematous							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			
				Epidermis	**DEJ**		**Others**
					Pattern	**Immunoreactants**	
Tay et al.[24] (1975)	Asian (Singapore)	41%	Lesion	-	(i) Homogenous (63%)	IgM (85.5%) IgG (71.2%) C3 (57.1%) IgA (28.5%)	-
					(ii) Thready (26.8%)	Fibrinogen (14.2%) IgM (100%) IgG (70%)	
		32%	Non-lesion	-	(iii) Stippled (10%)	IgA (30%) C3 (20%) fibrinogen (10%)	-
Dantzig et al.[29] (1975)	Caucasian (United States)	13/24 (54%)	Non-lesion (Sun-protected area)	-	N/A (100%)	IgG (84.6%) IgM (84.6%) C3 (30.8%)	-
Gammon et al.[20] (1983)	Caucasian (United States)	4/4 (100%)	Lesion	-	(i) Stippled (N/A)	IgG (100%) IgA (75%) IgM (25%) C3 (25%)	-
		10/11 (90.9%)	Non-lesion (Sun-exposed area)		(ii) Granular (N/A)	IgG (90%) IgM (50%) IgA (50%) C3 (40%)	-
		13/14 (92.9%)	Non-lesion (Sun-protected area)		(iii) Homogeneous (N/A)	IgG (92.3%) IgA (53.8%) IgM (38.5%) C3 (38.5%)	-
Magro et al.[22] (1997)	Caucasian (England, Canada)	7/7 (100%)	Lesion	-	N/A (100%)	IgM (100%) IgG (70%) C3 (40%) IgA (30%)	
		4/4 (100%)	Lesion (Sun-protected area)	ENS[φ] (40%)	-	-	Positive immunoreactants at cytoplasm decoration of keratinocytes (10%)

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Systemic lupus erythematous							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			
				Epidermis	**DEJ**		**Others**
					Pattern	**Immunoreactants**	
Nyberg et al.[16] (1998)	Caucasian (Sweden)	1/1 (100%)	Lesion	-	Linear (100%)	IgM (100%)	-
Minz et al.[23] (2010)	Asian (India)	10/14 (71.4%)	Lesion	-	Granular (100%)	IgM (93.3%) IgG (67%) C3 (60%) IgA (33%)	Positive immunoreactants at BV (40%)
Brinster et al.[26] (2012)	Caucasian (United States)	2/4 (50%)	Lesion	-	Granular (100%)	IgG, IgM, C3	-
Luo et al.[21] (2013)	Asian (China)	28/28 (100%)	Lesion	-	(i) Homogenous (N/A) (ii) Granular (N/A)	IgM (86%) C3 (55.6%) IgG (25%) IgA (22%)	-
Abreu Velez et al.[7] (2016)	Caucasian (United States)	5/5 (100%)	Lesion	-	N/A (100%)	IgG, IgM, IgA, C3, C1q, fibrinogen	Positive immunoreactants at basement membranes of eccrine gland and sebaceous gland (N/A)
Elbendary et al.[28] (2016)	Caucasian (United States)	100/100 (100%)	Lesion	-	Granular (100%)	IgM (89%) IgG (76%) C3 (73%) IgA (60%)	Positive immunoreactants at stromal-epithelial junction of hair follicle and sweat gland apparatus in granular pattern (100%)
Chanprapaph et al.[27] (2019)	Asian (Thailand)	25/32 (78.1%)	Lesion	ENS (24%)	Homogenous granular (100%)	IgM (76%) C3 (48%) IgG (40%) IgA (16%)	Positive immunoreactants at follicular epithelium (100%) CB (44%), peri-eccrine area (24%) and peri-sebaceous staining (16%)

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Acute cutaneous lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Weinstein et al.[25] (1987)	- (Australia)	3/5 (60%)	Lesion (100%)	-	Granular	IgG, IgM, IgA, C3, and/or Clq	-
Ng et al.[13] (2000)	Asian (Singapore)	16/20 (80%)	Lesion	-	Homogenous Granular (100%)	C1q (94%) IgG (75%) IgM (69%) C3 (50%)	-
		1/4 (25%)	Non-lesion	-	Granular (100%)	N/A	-
Abdelmouttalib et al.[15] (2021)	Arab (Morocco)	2/2 (100%)	Lesion (TEN-like LE)	-	Granular (100%)	IgM (100%) IgG (50%) C3 (50%)	-
Roberts et al.[30] (2021)	Caucasian (England)	1/1 (100%)	Lesion	-	Linear (100%)	IgG, IgA, IgM, C3	-
Chanprapaph et al.[14] (2021)	Asian (Thailand)	17/21 (81%)	Lesion	ENS (47.1%)	Homogenous Granular (76.5%)	N/A	Positive immunoreactants at CB (70.6%), BV (35.3%), peri-follicular area (17.6%) and peri-eccrine area (5.9%)
Subacute cutaneous lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Nieboer et al.[18] (1988)	Caucasian (Netherlands)	12/35 (34%)	Lesion	DLP (100%)	DLP (100%)	IgG (100%)	Positive IgG at dermis in DLP pattern (100%)
David-Bajar et al.[33] (1992)	- (United States)	7/7 (100%)	Lesion	N/A	N/A	IgM (100%) C3b (71.4%)	-
		7/7 (100%)	Non-lesion (Sun-exposed and sun-protected area)	N/A (14.3%)	N/A (100%)	IgG (100%)	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Subacute cutaneous lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			
				Epidermis	**DEJ**		**Others**
					Pattern	**Immunoreactants**	
Valeski et al.[19] (1992)	- (United States)	32/32 (100%)	Lesion	DLP[δ] (100%)	Speckle (100%)	IgG (100%) IgM (25%) IgA (9.4%)	Positive immunoreactants at ductal and/or follicular epithelium (N/A)
Crowson et al.[32] (1997)	Caucasian (England, Canada)	6/6 (100%)	Lesion	(i) Granular (Nucleus and cytoplasm, 16.7%) (ii) Peripheral homogeneous (Nucleus, 33.3%) (iii) Granular (Nuclear, 16.7%) (iv) Peculiar (Intercellular space of epithelial cells, 16.7%)	Granular (50%)	IgM (100%)	-
Magro et al.[22] (1997)	Caucasian (England, Canada)	10/10 (100%)	Lesion	ENS (100%)	N/A (100%)	IgM (85.7%) IgG (14.3%) IgA (14.3%) C3 (14.3%)	Positive IgG at cytoplasm decoration of keratinocytes (60%)
Nyberg et al.[16] (1998)	Caucasian (Sweden)	1/2 (50%)	Lesion	-	DLP (50%)	IgG (100%)	Positive IgG at subepidermal area (50%)
			Non-lesion	-	DLP (50%)	C1q (100%)	Positive C1q at subepidermal area (50%)
Parodi et al.[36] (2000)	Caucasian (Italy)	50/58 (86%)	Lesion	DLP (6%)	N/A (100%)	IgA (52%) IgG (48%) IgM (48%)	-
		26/58 (44%)	Non-lesion (Sun-exposed area)	-	N/A (100%)	N/A	-
		21/58 (36%)	Non-lesion (Sun-protected area)	-	N/A (100%)	N/A	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Subacute cutaneous lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**Epidermis**	**DIF findings DEJ**		**Others**
					Pattern	**Immunoreactants**	
Mutasim et al.[34] (2003)	Caucasian (United States)	1/1 (100%)	Lesion	Granular[ω] (100%)	N/A (100%)	Fibrinogen (100%)	-
Suess et al.[37] (2008)	Caucasian (Germany)	2/2 (100%)	Lesion	-	Focal granular (100%)	IgM (100%) C3 (50%)	-
			Non-lesion	-	(i) Linear (100%) (ii) Intermittent granular (100%)	IgA (100%) IgG (100%) IgM (100%) C3 (100%)	-
Marzano et al.[17] (2011)	Caucasian (Italy)	5/8 (62.5%)	Lesion (Sun-exposed area)	DLP (100%)	Granular (100%)	IgG ± IgM (100%) C3 (50%)	-
Mysorekar et al.[35] (2015)	Asian (India)	4/4 (100%)	Lesion	-	Granular (100%)	IgG (100%) IgA (100%) C3 (100%) IgM (75%)	-
Chanprapaph et al.[27] (2021)	Asian (Thailand)	5/7 (71.4%)	Lesion	ENS (20%)	Homogenous granular (20%)	N/A	Positive immunoreactants at CB (20%), BV (80%), and peri-eccrine area (40%)
Neonatal lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**Epidermis**	**DIF findings DEJ**		**Others**
					Pattern	**Immunoreactants**	
Maynard et al.[39] (1991)	- (United States)	2/3 (66.7%)	Lesion	ENS (100%)	Linear (50%)	IgM (100%)	Positive fibrinogen, IgM and C3 at superficial and mid-dermal BV (100%)

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Chronic cutaneous lupus erythematosus							
Discoid lupus erythematosus							
Study	Ethnicity	Positive DIF (%)	Site of skin biopsy	DIF findings Epidermis	DEJ Pattern	DEJ Immunoreactants	Others
Schiodt et al.[43] (1981)	- (United States)	45/45 (100%)	Lesion (oral)	-	(i) Granular (N/A) (ii) Homogenous (N/A)	C3 (84%) Fibrinogen (89%) IgM (64%) IgA (27%) IgG (24%)	Positive immunoreactants at CB (N/A)
Weinstein et al.[25] (1987)	- (Australia)	5/9 (55%)	Lesion	-	Granular (100%)	IgG, IgM, IgA, C3, and/or Clq	-
David-Bajar et al.[33] (1992)	- (America)	8/11 (72.7%)	Lesion	-	Granular (100%)	C3 (100%) IgM (72.7%) IgA (36.4%) IgG (18.2%) IgG and C3 (60%)	-
		5/11 (45.5%)	Non-lesion	-	Granular (100%)	IgA (20%) IgM (20%)	-
Sugai et al.[48] (1992)	Latin America (Brazil)	47/71 (66.2%)	Lesion	-	(i) Granular (93.6%) (ii) Homogenous (70.2%) (iii) Thready (55.3%)	IgG (76.6%) IgM (61.7%) C3 (57.5%) IgA (25.5%)	-
Al-suwaid et al.[47] (1995)	Arab (Oman)	72.7%	Lesion	-	(i) Homogenous (55.5%) (ii) Granular (55.5%)	IgG (77.8%) C3 (44.4%) IgM (38.9%) IgA (22.2%)	-
Kulthanan et al.[48] (1996)	Asian (Thailand)	90/100 (90%)	Lesion	ENS (Speckle, 2%)	Granular (90%)	IgG (63%) C3 (50%) IgM (47%) IgA (22%)	Positive immunoreactants at CB (34%) and BV (15%)
Magro et al.[22] (1997)	Caucasian (England, Canada)	10/10 (100%)	Lesion	ENS (20%)	N/A (100%)	IgM (90%) IgG (70%) C3 (30%) IgA (20%)	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Chronic cutaneous lupus erythematosus							
Discoid lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Nyberg et al.[16] (1998)	Caucasian (Sweden)	9/13 (69.2%)	Lesion	-	(i) DLP (ii) Linear (Total 23.1%)	IgM (100%) C1q (83.3%) IgG (33.3%) C3 (33.3%)	Positive immunoreactants at DEJ and subepidermal area (46.2%)
			Non-lesion	-	(i) DLP (ii) Linear (Total 18.2%)	IgM (100%) IgG (75%) C1q (50%)	Positive immunoreactants at DEJ and subepidermal area (36.4%)
Badri et al.[40] (2005)	Arab (Tunisia)	1/1 (100%)	Lesion	-	Granular (100%)	IgM (100%)	-
Serpico et al.[44] (2007)	Caucasian (Italy)	1/1 (100%)	Lesion	-	Linear (100%)	IgG, IgA, fibrinogen	-
Chularojanamontri et al.[41] (2010)	Asian (Thailand)	33/61 (54.1%)	Lesion	-	N/A (100%)	IgM (82%) IgA (34.4%) C3 (31.1%) IgG (14.8%)	Positive immunoreactants at CB (100%)
Minz et al.[23] (2010)	Asian (India)	13/22 (59.1%)	Lesion	-	Granular (100%)	IgM (93.3%) IgG (67%) C3 (60%) IgA (33%)	Positive immunoreactants at BV (26.7%)
Mysorekar et al.[35] (2015)	Asian (India)	2/2 (100%)	Lesion	-	Granular (100%)	IgG (100%) IgA (100%) C3 (100%) IgM (50%)	-
Ohata et al.[46] (2016)	Asian (Japan)	-	Lesion	-	N/A	IgM (100%) C3 (88.9%) IgG (66.7%) IgA (66.7%)	-
Chanprapaph et al.[14] (2021)	Asian (Thailand)	22/81 (27.2%)	Lesion	ENS (13.6%)	Homogenous granular (68.2%)	N/A	Positive immunoreactants at CB (50%), BV (41%), peri-follicular area (27.3%) and peri-eccrine area (54.5%)

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Lupus panniculitis							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Tuffanelli et al.[51] (1971)	- (United States)	4/6 (66.7%)	Lesion	-	N/A (100%)	IgG, IgM, C3	-
Sanchez et al.[49] (1981)	- (United States)	12/17 (70.6%)	Lesion	-	N/A (100%)	IgM (100%) C3 (50%) Fibrin (16.7%) IgA (8.3%)	Positive immunoreactants at BV (25%) and CB (8.3%)
Izumi et al.[50] (1983)	- (United States)	1/1 (100%)	Lesion	-	Linear (100%)	IgG, C3	Positive immunoreactants at follicular epithelium (100%)
Lupus erythematosus tumidus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Bouzit et al.[54] (1999)	Caucasian (France)	1/1 (100%)	Lesion	-	Granular (100%)	IgM (100%)	-
Alexiades-Armenakas et al.[52] (2003)	- (United States)	5/10 (50%)	Lesion	-	(i) Linear (100%) (ii) Granular (80%)	IgM (100%) IgG (80%) IgA (20%) C3 (20%) Fibrin (20%)	-
Vieira et al.[53] (2006)	Caucasian (Spain)	4/15 (26.7%)	Lesion	-	N/A (100%)	IgG (75%) C3 (50%) IgM (25%) C1q (25%)	-
Hashimoto et al.[55] (2017)	Asian (Japan)	1/1 (100%)	Lesion	-	Linear (100%)	IgG, C3	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Study	Ethnicity	Positive DIF (%)	Site of skin biopsy	DIF findings: Epidermis	DIF findings: DEJ Pattern	DIF findings: DEJ Immunoreactants	Others
Chilblain lupus erythematosus							
Pock et al.[56] (2001)	Caucasian (Czech Republic)	1/1 (100%)	Lesion	-	(i) Granular (100%) (ii) Tatter-like and globular (100%)	IgG, IgA, IgM, C3 Fibrinogen	Positive immunoreactants at dermis (papillary dermis) in globular pattern (100%)
Patel et al.[57] (2013)	Caucasian (United Kingdom)	1/1 (100%)	Lesion	-	-	-	Positive fibrin at BV (100%)
Hypertrophic/verrucous lupus erythematosus							
Khorshid et al.[58] (1999)	Caucasian (United Kingdom)	1/1 (100%)	Lesion	ENS (Speckle,100%)	Linear (100%)	IgG, IgM and C3	Positive immunoreactants at superficial dermal BV (100%)
Non-specific lupus cutaneous manifestations							
Bullous systemic lupus erythematosus							
Olansky et al.[71] (1982)	Caucasian (United States)	2/2 (100%)	N/A	-	Linear (100%)	IgG, C3	-
Camisa et al.[65] (1983)	Caucasian (United States)	2/2 (100%)	Peri-lesion	-	Granular (100%)	IgG (100%) IgM (100%) IgA (50%)	-
Janniger et al.[68] (1991)	Caucasian (Poland)	1/1 (100%)	Peri-lesion	-	Linear and granular (100%)	IgG, IgM, C3	-
Shirahama et al.[72] (1994)	Asian (Japan)	1/1 (100%)	N/A	-	N/A (100%)	IgG, IgM, IgA	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Non-specific lupus cutaneous manifestations							
Bullous systemic lupus erythematosus							
Study	Ethnicity	Positive DIF (%)	Site of skin biopsy	DIF findings			Others
				Epidermis	DEJ		
					Pattern	Immunoreactants	
Yell et al.[59] (1995)	Caucasian (United Kingdom)	6/7 (85.7%)	Peri-lesion	-	(i) Linear (83.3%) (ii) Granular (16.7%)	IgG (83.3%) IgM (83.3%) IgA (83.3%) C3 (83.3%)	-
Yung et al.[74] (2000)	Caucasian (New Zealand)	1/1 (100%)	N/A	-	Granular (100%)	IgG, IgA, IgM, complement	-
Nitta et al.[70] (2002)	Asian (Japan)	1/1 (100%)	Peri-lesion	-	Linear and granular	IgG, IgA, IgM, C3	-
Barbosa et al.[71] (2011)	Latin America (Brazil)	1/1 (100%)	N/A	-	N/A	IgG, IgA, fibrin	-
Miziara et al.[69] (2013)	Latin America (Brazil)	1/1 (100%)	Lesion	-	Linear (100%)	IgG, IgM, IgA	-
Mysorekar et al.[35] (2015)	Asian (India)	3/3 (100%)	Peri-lesion	-	Granular (100%)	IgG, IgM, IgA, C3	-
Boddu et al.[64] (2016)	Caucasian (United States)	1/1 (100%)	N/A	-	Granular (100%)	IgG	-
Jain et al.[67] (2016)	Asian (India)	2/2 (100%)	N/A	-	(i) Granular (N/A) (ii) Linear (N/A)	IgG, IgA, IgM, C3	Positive immunoreactants at hair follicle epithelium (N/A)
Hans-Bittner et al.[66] (2017)	Latin America (Brazil)	1/1 (100%)	N/A	-	(i) Granular (N/A) (ii) Linear (N/A)	IgG, IgA, IgM, C3	-
De Risi-Pugliese et al.[62] (2018)	Caucasian (Poland)	135/138 (98%)	N/A	-	(i) Granular (N/A) (ii) Linear (N/A)	IgG (91%) IgA (72%) IgM (68%) C3 (67%)	-
Torres Saavedra et al.[73] (2020)	Latin America (Colombia)	5/5 (100%)	Peri-lesion	-	(i) Linear (75%) (ii) Granular (20%)	IgG (100%) IgM (100%) C3 (80%) IgA (60%)	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Mucosal lupus erythematosusμ							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Daniel et al.[77] (1981)	- (United States)	6/6 (100%)	Lesion	-	Granular (coarse) (100%)	C3 (100%) IgG (50%) IgM (50%) IgA (16.7%)	-
Schiodt et al.[43] (1981)	- (United States)	7/7 (100%)	Lesion	-	(i) Granular (N/A) (ii) Homogenous (N/A)	C3 (100%) IgM (86%) IgG (57%) IgA (43%)	Positive immunoreactants at CB (N/A)
Nikoo et al.[78] (2017)	Persians (Iran)	1/1 (100%)	Lesion	-	N/A	IgG (100%) IgM (100%) C3 (100%)	-
Chanprapaph et al.[14] (2021)	Asian (Thai)	5/6 (83.3%)	Lesion	-	(i) Focal granular (N/A) (ii) Homogenous granular (N/A)	IgG, IgM, IgA, C3	-
Pires et al.[79] (2021)	Latin America (Brazil)	15/15 (100%)	Lesion	-	Granular (100%)	IgG, IgM, fibrinogen	Positive immunoreactants at epidermis and dermis (loose connective tissue at epithelial ridges)
Papulonodular mucinosis							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			**Others**
				Epidermis	**DEJ**		
					Pattern	**Immunoreactants**	
Rongioletti et al.[82] (1990)	Caucasian (Italy)	2/2 (100%)	Lesion	-	(i) Granular (50%) (ii) Linear (50%)	IgM, C3	-

TABLE 1. Literature review of direct immunofluorescence findings in cutaneous lupus erythematosus. (Continued)

Papulonodular mucinosis							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			
				Epidermis	**DEJ**		**Others**
					Pattern	**Immunoreactants**	
Kanda et al.[81] (1997)	Asian (Japan)	5/6 (83.3%)	Lesion	-	(i) Linear (60%) (ii) Granular (40%)	IgG (66.7%) IgM (66.7%) IgA (66.7%) C3 (33.3%)	Positive immunoreactants at BV (20%)
		4/6 (66.7%)	Non-lesion	-	(i) Linear (75%) (ii) Granular (75%)	IgG (100%) C3 (66.7%) IgA (66.7%) IgM (33.3%) C1q (33.3%)	-
Dallo et al.[80] (2020)	Caucasian (United States)	1/1 (100%)	Lesion	-	Granular (100%)	IgA, IgM, C3	-
Nonscarring alopecia in systemic lupus erythematosus							
Study	**Ethnicity**	**Positive DIF (%)**	**Site of skin biopsy**	**DIF findings**			
				Epidermis	**DEJ**		**Others**
					Pattern	**Immunoreactants**	
Chanprapaph et al.[27] (2019)	Asian (Thailand)	25/32 (78.1%)	Lesion	ENS (24%)	Homogenous granular (100%)	IgM (76%) C3 (48%) IgG (40%) IgA (16%)	Positive immunoreactants at follicular epithelium (100%) CB (44%), peri-eccrine area (24%) and peri-sebaceous staining (16%)

Abbreviations: BV: blood vessels, C3: complement 3, CB: cytoid bodies, DEJ: dermo-epidermal junction, ENS: epidermal nuclear staining, Ig: immunoglobulin, LE: lupus erythematosus, N/A: not available, TEN: toxic epidermal necrolysis

δ This study demonstrated positive immunoreactants in the nuclear, intracellular cytoplasm and intercellular space of epithelial cells with speckle (DLP) pattern.

ω *Mutasim et al.* demonstrated positive immunoreactants including IgG, IgM, C3 and faint IgA at suprabasal and basal cells with granular pattern.

φ All studies demonstrated epidermal nuclear staining in the epidermis by IgG except *Magro et al.* which revealed C3 with IgG deposition at epidermal nuclear staining.

μ Mucosal lupus erythematosus consisted of oral manifestations of LE, SLE and DLE. The details of each type were not clarified in some articles.

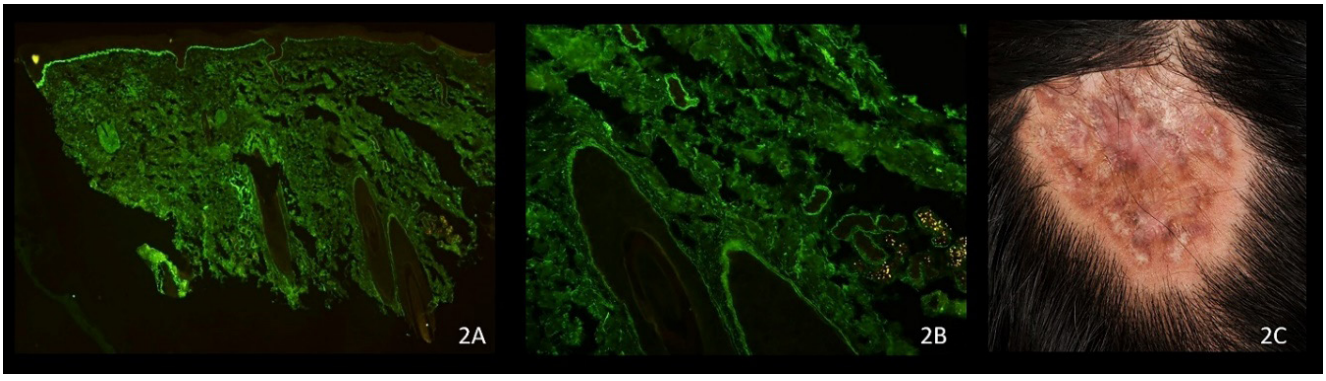


Fig 2A &2B. DIF findings of discoid lupus erythematosus showed deposits of IgM at the DEJ with granular patterns and also extended to the basement membrane of hair follicles or peri-appendage areas. (Figure 2A 4x magnification, 2B 20x magnification).
Fig 2C. Clinical presentation of scalp discoid lupus erythematosus showed well-defined erythematous atrophic plaque with scarring alopecia localized on vertex of scalp.

is less than lichen planus.[43] *Schiødt et al.* analyzed the sensitivity and specificity of DIF in oral DLE lesions and found it to be 92% and 72%, respectively.[43] A skin biopsy of oral DLE is recommended in erythematous areas.[43]

Lupus panniculitis

Lupus panniculitis is another presentation of CCLE characterized by inflammatory processes in subcutaneous fat and the deep dermis. The prevalence of lupus panniculitis is approximately 20%. Lupus panniculitis usually presents itself as erythematous, indurated, painful subcutaneous nodules localized on the face, neck, buttocks, arms and thighs (Fig 3B).[49] The positive yield of DIF tests in lesion skin was 66.7%-100%.[49-51] Although the main pathology of lupus panniculitis is located in the fat lobule, DIF findings are quite similar to other types of cutaneous LE. There were deposits of multiple immunoreactants at the DEJ, followed by positive staining in blood vessels, CB and follicular epithelium[49,50] (Fig 3A). IgM was the most reported immunoreactant in lupus panniculitis.

Lupus erythematosus tumidus

Lupus erythematosus tumidus or tumid LE presents itself as indurated erythematous, or edematous plaques localized prominently in sun-exposed areas such as the head and neck. The clinical course of tumid LE is benign and there is low incidence of systemic involvement.[52] The positive yield of tumid LE in DIF tests was 26.7%-100%.[52-55] The deposits of immunoreactants were mainly at the DEJ[52-55] (Fig 4). ENS or DLP was rarely detected in a DIF test of tumid LE.

Chilblain lupus erythematosus

Chilblain LE, a rare variant of CCLE, presents itself as pruritic purpuric papules or plaques located in areas exposed to the cold such as the ears, fingers and

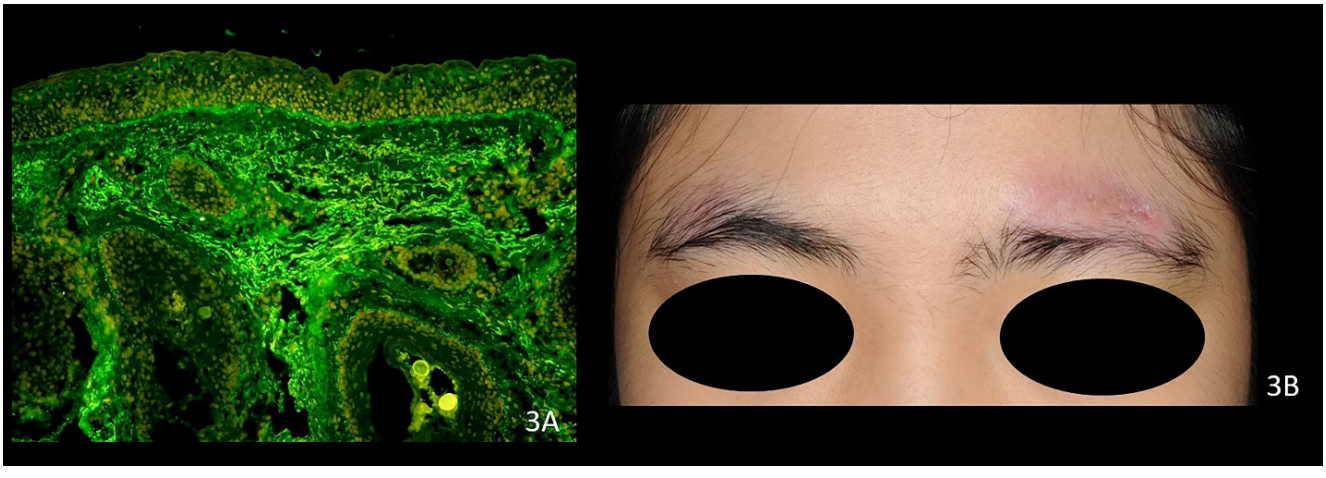


Fig 3A. DIF findings of lupus panniculitis showed granular deposits of IgM at the DEJ, followed by positive staining in follicular epithelium. (20x magnification).
Fig 3B. Clinical presentation of lupus panniculitis showed well-defined erythematous indurated plaque localized at both eyebrows.

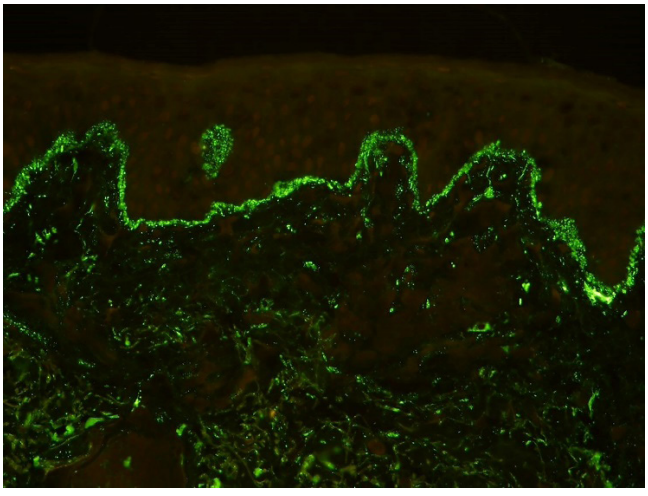


Fig 4. DIF findings of lupus erythematosus tumidus showed granular deposits of IgM at the DEJ with colloid bodies. (20x magnification).

toes. Some lesions of chilblain LE can become a painful ulceration. The prevalence of chilblain LE is around 6% of all LE cases.[56] By clinical presentation, chilblain LE is not decimated from idiopathic chilblains and lupus pernio.

DIF findings of chilblain LE are limited. *Pock et al.* revealed deposits of multiple immunoreactants at the DEJ, and both IgA and IgM staining at the papillary dermis.[56] On other hand, *Patel et al.* discovered only the staining of fibrin at dermal vessels.[57]

Hypertrophic/verrucous lupus erythematosus

Hypertrophic LE, another rare variant of CCLE, is defined by recalcitrant hyperkeratotic scaly plaques in sun-exposed areas. The clinical manifestation imitates other hyperkeratotic cutaneous diseases such as hypertrophic lichen planus and squamous cell carcinoma.

DIF findings of hypertrophic LE are in short supply. *Khorshid et al.* investigated DIF results in one patient with hypertrophic LE and revealed positive multiple immunoreactants at the DEJ in linear pattern and positive ENS by IgG in speckle pattern.[58] Moreover, staining of C3 and fibrinogen in superficial dermal blood vessels was also reported.[58]

Non-specific lupus cutaneous manifestations

Bullous systemic lupus erythematosus

Bullous systemic lupus erythematosus (BSLE) typically presents itself as an acute blister or bullae eruption over normal skin or erythematous lesions in a patient with SLE. These blisters develop commonly in areas exposed to the sun; however, they are also found in areas shielded from the sun or mucosa.[59] BSLE is the result of autoantibodies to type VII collagen which attack non-collagenous domain type 1 and 2 of type VII collagen of the DEJ.[60,61] The correlation between BSLE and systemic involvements including lupus nephritis, neuropsychiatric SLE and hematologic abnormality have been documented.[62]

DIF studies of BSLE are usually performed in the perilesional area with clinical non-lesional skin to demonstrate staining of IgG, IgM, C3 and IgA at the DEJ with linear or granular patterns[35,59,62-74] (Fig 5A & 5B). The positive yield from the DIF test was high, at approximately 85.7%-100%.[35,59,62-74] IgG was the most reported immunoreactant, while IgA was more common in BSLE than other forms of lupus.[65] Furthermore, IgA deposits were associated with disease activity.[65]

DIF findings of BSLE are hard to differentiate from other vesiculobullous diseases such as bullous pemphigoid, epidermolysis bullosa acquisita, and cicatricial pemphigoid. Direct salt-split skin immunofluorescence by 1 mol/L saline is usually performed as an additional step to provide an accurate diagnosis. Deposits of IgG at the dermal side of cleavage blisters in direct salt-split skin

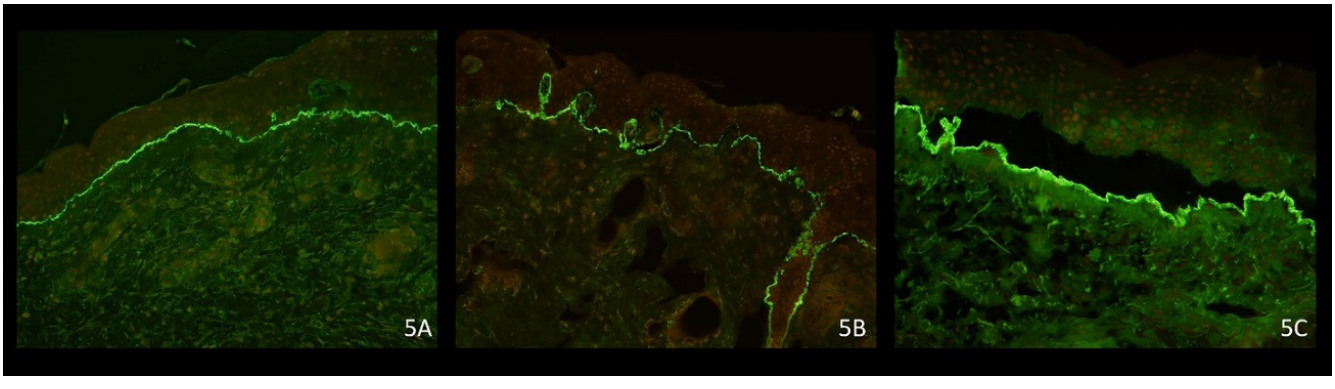


Fig 5A & 5B. DIF findings of bullous systemic lupus erythematosus (BSLE) showed immunoglobulins at the DEJ with linear or granular pattern. (10x magnification).

Fig 5C. Direct salt-split skin immunofluorescence of BSLE showed deposits of immunoglobulin at the dermal side of cleavage blister. (40x magnification)

study support the diagnosis of BSLE and epidermolysis bullosa acquisita. Generally, positive staining at the roof of clevage blisters are found in bullous pemphigoid, and not BSLE (Fig 5C).

Mucosal lupus erythematosus

Mucosal involvement is found in both of cutaneous LE and SLE. Oral mucosa is the most common site (3%-50% of patients with LE) among mucosal areas.[75] The clinical presentations of mucosal LE are generally erythematous macules, which develop into erosions or ulcers. Common locations of oral LE are the lower lip and hard palate.

DIF results of oral LE reveal deposits of multiple immunoreactants at the DEJ with granular pattern[43,77-79] (Fig 6). Moreover, deposits of multiple immunoreactants in loose connective tissue at epithelial ridges, CB and positive fibrinogen in epidermis have also been reported.[43,79] C3 is the most common immunoreactant, followed by IgM, IgG and IgA.[43,77] The positive yield of mucosal areas was around 83.3%-100%.[43,76-79] The advantage of an DIF test in this scenario is that it helps differentiate e oral LE from other oral lichenoid lesions.

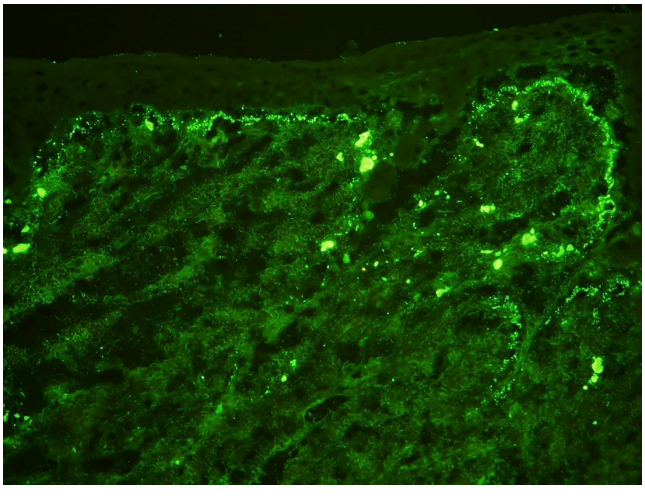


Fig 6. DIF findings of mucosal lupus erythematosus showed granular deposits of complement 3 at the DEJ with colloid bodies. (10x magnification).

Papulonodular mucinosis

Papulonodular mucinosis is a nonspecific variant of cutaneous LE which reveals mucin deposition in the dermis with minimal or no interface change by histopathology. It usually manifests as asymptomatic skin-colored papulonodular lesions on the trunks or extremities. Papulonodular mucinosis is categorized as primary cutaneous mucinosis. It cannot be distinguished from other cutaneous mucinoses such as lichen myxedematosus, scleredema and cutaneous focal mucinosis through histopathology.

The positive yield in DIF studies ranges from 83.3%-100% in areas with lesions and is 66.7% in non-lesion areas[80-82] (Table 2). Multiple immunoreactants are predominantly demonstrated at the DEJ with linear or granular patterns[80-82] (Table 1). LB is also observed in papulonodular mucinosis and staining in blood vessels has also been reported.[81] Moreover, *Kanda et al.* investigated uninvolved skin and displayed discontinuous and weak staining bands at the DEJ which was insufficient for designation as a positive LB.[81] However, the DIF findings of papulonodular mucinosis is not different from other types of CCLE.

Nonscarring alopecia in systemic lupus erythematosus

Nonscarring alopecia is also a common manifestation of nonspecific LE lesions and ranges from 17%-80%.[27] Its histopathology exhibits interface dermatitis along the DEJ or follicular epithelium according to LE specific changes. The correlation between nonscarring alopecia and disease activity of systemic involvement is known. When progressive diffuse hair loss is detected, there is an increase in reports of major organ involvement.[74]

DIF findings of nonscarring alopecia mostly revealed deposits of multiple immunoreactants at the DEJ with homogeneous granular patterns and follicular epithelium.[27] Additionally, ENS, CB and deposits in peri-eccrine and peri-sebaceous areas were also reported.[27] The positive yield in the DIF test was approximately 78.1%, with IgM being the most common immunoreactant.[27] DIF findings of several clinical manifestations of nonscarring alopecia in SLE, including mild diffuse alopecia, severe diffuse alopecia, patchy alopecia, and lupus hair are not different.[27]

DISCUSSION

Typically, DIF tests of cutaneous LE reveal deposits of multiple immunoreactants at the DEJ with linear or granular patterns, known as LB which is more common in SLE (70%) than mixed connective tissue diseases (13.5%-33%) and scleroderma (33%).[23,83] IgM is the most common immunoreactant while the least is IgA. The exact mechanism of immunoglobulin deposition at DEJ in the LE patients still needs to be elucidated. It is believed that immunoreactants are not antibodies against DEJ components but rather represent circulating immune complexes of deoxyribonucleic acid (DNA) and ANA trapped within the DEJ.[11] Furthermore, DNA released from ultraviolet-injured keratinocytes, although diffusing across the DEJ, may bind to collagen IV and serve as an antigen for circulating autoantibodies.[11] It should be noted that, sometimes, sun-damaged skin can show

TABLE 2. Characteristics of direct immunofluorescence studies in cutaneous lupus erythematosus.

Characters	SLE	Specific LE lesions								Non-specific LE lesions		
		ACLE	SCLE	DLE	Lupus panniculitis	CCLE LET	Chilblain LE	Hypertrophic LE	BSLE	Mucosal LE	Papulonodular alopecia	Nonscarring mucinosis
Positive yield	42-100% (Lesional) 32-92.2% (Non-lesional)	60-100% (Lesional) 25% (Non-lesional)	34-100% (Lesional) 36-100% (Non-lesional)	27.2-100% (Lesional) 45.5-69.2% (Non-lesional)	66.7-100% (Lesional)	26.7-100% (Lesional)	100%* (Lesional)	100%* (Lesional)	85.7-100% (Peri-lesional)	83.3-100% (Lesional)	83.3-100% (Lesional) 66.7% (Non-lesional)	78.1% (Lesional)
Sensitivity	81.8%	60%	20-45%	55-92% (Cutaneous and oral)	-	-	-	-	-	-	-	-
Specificity	95.6%	-	-	72% (Oral)	-	-	-	-	-	-	-	-
Common immunoreactants	IgM	-	IgG, IgM	-	IgM	-	-	-	IgG	C3	-	IgM

Abbreviations: ACLE: acute cutaneous lupus erythematosus, C: complement, CCLE: chronic cutaneous lupus erythematosus, CLM: cutaneous lupus mucinosis, DLE: discoid lupus erythematosus, Ig: immunoglobulin, LE: lupus erythematosus, LET: lupus erythematosus tumidus, SCLE: subacute cutaneous lupus erythematosus, SLE: systemic lupus erythematosus

* Chilblain LE and hypertrophic LE were reported as case report.

positive DIF results which are similar to cutaneous LE. The positive LB group has a reported higher incidence of systemic involvements and autoantibodies, and a poorer prognosis than the negative group.[11] Furthermore, its sensitivity for predicting active disease is higher than other laboratory parameters, including serum C3 and C4 levels, erythrocyte sedimentation rate, lymphocyte count, and anti-double stranded DNA antibodies.[21,24]

Table 2 summarizes positive yields of DIF studies among cutaneous LE subtypes. The sensitivity of non-lesion areas is reported to be lower than lesion areas.[8] Thus, to obtain higher positive yields, a skin biopsy should be performed at active lesions that are over a month old.[10,12] DIF findings among cutaneous LE subtypes also overlap. Thus, diagnosis of cutaneous LE requires a combination of monitoring patient history, physical examinations, and laboratory studies. Antibodies serology and DIF studies help confirm diagnosis and at times determine disease severity.

Conflict of interest statement: All authors have no conflicts of interest to declare relevant to the contents of this article.

Funding: This research received no specific grants from any funding agencies in the public, commercial, or not-for-profit sector.

REFERENCES

1. Gilliam JN, Sontheimer RD. Distinctive cutaneous subsets in the spectrum of lupus erythematosus. J Am Acad Dermatol 1981;4:471-5.
2. Walling HW, Sontheimer RD. Cutaneous lupus erythematosus: issues in diagnosis and treatment. Am J Clin Dermatol 2009;10: 365-81.
3. Blake SC, Daniel BS. Cutaneous lupus erythematosus: A review of the literature. Int J Womens Dermatol 2019;5:320-329.
4. Dalbeni A, Gomarasca L, Bellocchi MC, Nuvolari R, Bertagnin M, Imbalzano E, et al. A strange lupus-like malar rash with renal involvement: an angioimmunoblastic T-cell lymphoma - A case report. Clin Case Rep 2015;3:46-9.
5. Moura Filho JP, Peixoto RL, Martins LG, Melo SD, Carvalho LL, Pereira AK, et al. Lupus erythematosus: considerations about clinical, cutaneous and therapeutic aspects. An Bras Dermatol 2014;89:118-25.
6. Prystowsky SD, Gilliam JN. Discoid lupus erythematosus as part of a larger disease spectrum. Correlation of clinical features with laboratory findings in lupus erythematosus. Arch Dermatol 1975;111:1448-52.
7. Abreu Velez AM, Upegui Zapata YA, Howard MS. Periodic Acid-Schiff Staining Parallels the Immunoreactivity Seen By Direct Immunofluorescence in Autoimmune Skin Diseases. N Am J Med Sci 2016;8:151-5.
8. Chhabra S, Minz RW, Saikia B. Immunofluorescence in dermatology. Indian J Dermatol Venereol Leprol 2012;78: 677-91.
9. Burnham TK, Neblett TR, Fine G. The application of the fluorescent antibody technic to the investigation of lupus erythematosus and various dermatoses. J Invest Dermatol 1963; 41:451-6.
10. Elston DM, Stratman EJ, Miller SJ. Skin biopsy: Biopsy issues in specific diseases. J Am Acad Dermatol 2016;74:1-16; quiz 17-8.
11. Reich A, Marcinow K, Bialynicki-Birula R. The lupus band test in systemic lupus erythematosus patients. Ther Clin Risk Manag 2011;7:27-32.
12. Weigand DA. The lupus band test: a re-evaluation. J Am Acad Dermatol. 1984;11:230-4.
13. Ng PP, Tan SH, Koh ET, Tan T. Epidemiology of cutaneous lupus erythematosus in a tertiary referral centre in Singapore. Australas J Dermatol 2000;41:229-33.
14. Chanprapaph K, Tankunakorn J, Suchonwanit P, Rutnin S. Dermatologic Manifestations, Histologic Features and Disease Progression among Cutaneous Lupus Erythematosus Subtypes: A Prospective Observational Study in Asians. Dermatol Ther (Heidelb) 2021;11:131-47.
15. Abdelmouttalib A, Meziane M, Senouci K. Toxic epidermal necrolysis-like acute cutaneous lupus erythematosus: two cases report. Pan Afr Med J 2021;38:236.
16. Nyberg F, Skoglund C, Stephansson E. Early detection of epidermal dust-like particles in experimentally UV-induced lesions in patients with photosensitivity and lupus erythematosus. Acta Derm Venereol 1998;78:177-9.
17. Marzano AV, Lazzari R, Polloni I, Crosti C, Fabbri P, Cugno M. Drug-induced subacute cutaneous lupus erythematosus: evidence for differences from its idiopathic counterpart. Br J Dermatol 2011;165:335-41.
18. Nieboer C, Tak-Diamand Z, Van Leeuwen-Wallau HE. Dust-like particles: a specific direct immunofluorescence pattern in sub-acute cutaneous lupus erythematosus. Br J Dermatol 1988;118: 725-9.
19. Valeski JE, Kumar V, Forman AB, Beutner EH, Chorzelski TP. A characteristic cutaneous direct immunofluorescent pattern associated with Ro(SS-A) antibodies in subacute cutaneous lupus erythematosus. J Am Acad Dermatol 1992;27:194-8.
20. Gammon WR, Merritt CC, Henke DC, Robinson T, Henley N, DeAngelo L. Complement-activating immune deposits in systemic lupus erythematosus skin. J Invest Dermatol 1983;81: 14-20.
21. Luo YJ, Tan GZ, Yu M, Li KW, Liu YY, Guo Q, et al. Correlation of cutaneous immunoreactants in lesional skin with the serological disorders and disease activity of systemic lupus erythematosus. PLoS One 2013;8:e70983.
22. Magro CM, Crowson AN. The immunofluorescent profile of dermatomyositis: a comparative study with lupus erythematosus. J Cutan Pathol 1997;24:543-52.
23. Minz RW, Chhabra S, Singh S, Radotra BD, Kumar B. Direct immunofluorescence of skin biopsy: perspective of an immunopathologist. Indian J Dermatol Venereol Leprol 2010; 76:150-7.
24. Tay CH, Lim AL. Direct immunofluorescent study of systemic lupus erythematosus in Singapore. Australas J Dermatol 1975; 16:22-31.
25. Weinstein C, Miller MH, Axtens R, Littlejohn GO, Dorevitch AP, Buchanan R. Lupus and non-lupus cutaneous manifestations

in systemic lupus erythematosus. Aust N Z J Med 1987;17:501-6.
26. Brinster NK, Nunley J, Pariser R, Horvath B. Nonbullous neutrophilic lupus erythematosus: a newly recognized variant of cutaneous lupus erythematosus. J Am Acad Dermatol 2012;66:92-7.
27. Chanprapaph K, Udompanich S, Visessiri Y, Ngamjanyaporn P, Suchonwanit P. Nonscarring alopecia in systemic lupus erythematosus: A cross-sectional study with trichoscopic, histopathologic, and immunopathologic analyses. J Am Acad Dermatol 2019;81:1319-29.
28. Elbendary A, Zhou C, Valdebran M, Yu Y, Gad A, Kwon EJ, et al. Specificity of granular IgM deposition in folliculosebaceous units and sweat gland apparatus in direct immunofluorescence (DIF) of lupus erythematosus. J Am Acad Dermatol 2016;75:404-9.
29. Dantzig PI, Mauro J, Rayhanzadeh S, Rudofsky UH. The significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus. Br J Dermatol 1975;93:531-7.
30. Roberts EJ, Melchionda V, Saldanha G, Shaffu S, Royle J, Harman KE. Toxic epidermal necrolysis-like lupus. Clin Exp Dermatol 2021;46:1299-303.
31. Tantanate C. Anti-Ro Antibody and Its Significance. Siriraj Med J. 2006:58;687-90.
32. Crowson AN, Magro CM. Subacute cutaneous lupus erythematosus arising in the setting of calcium channel blocker therapy. Hum Pathol 1997;28:67-73.
33. David-Bajar KM, Bennion SD, DeSpain JD, Golitz LE, Lee LA. Clinical, histologic, and immunofluorescent distinctions between subacute cutaneous lupus erythematosus and discoid lupus erythematosus. J Invest Dermatol 1992;99:251-7.
34. Mutasim DF. Severe subacute cutaneous lupus erythematosus presenting with generalized erythroderma and bullae. J Am Acad Dermatol 2003;48:947-9.
35. Mysorekar VV, Sumathy TK, Shyam Prasad AL. Role of direct immunofluorescence in dermatological disorders. Indian Dermatol Online J 2015;6:172-80.
36. Parodi A, Caproni M, Cardinali C, Bernacchi E, Fuligni A, De Panfilis G, et al. Clinical, histological and immunopathological features of 58 patients with subacute cutaneous lupus erythematosus. A review by the Italian group of immunodermatology. Dermatology 2000;200:6-10.
37. Suess A, Sticherling M. Leflunomide in subacute cutaneous lupus erythematosus - two sides of a coin. Int J Dermatol 2008;47:83-6.
38. Crowson AN, Magro C. The cutaneous pathology of lupus erythematosus: a review. J Cutan Pathol 2001;28:1-23.
39. Maynard B, Leiferman KM, Peters MS. Neonatal lupus erythematosus syndrome. J Cutan Pathol 1991;18:333-8.
40. Badri T, Khaddar RK, Bouraoui S, Mokni M, Cherif F, Dhahri AB. Discoid lupus erythematosus in an infant. Dermatol Online J 2005;11:38.
41. Chularojanamontri L, Tuchinda P, Triwongwaranat D, Pinkaew S, Kulthanan K. Diagnostic significance of colloid body deposition in direct immunofluorescence. Indian J Dermatol Venereol Leprol 2010;76:373-7.
42. Kulthanan K, Roongphiboolsopit P, Chanjanakijskul S, Kullavanijaya P. Chronic discoid lupus erythematosus in Thailand: direct immunofluorescence study. Int J Dermatol 1996;35:711-4.
43. Schiødt M, Holmstrup P, Dabelsteen E, Ullman S. Deposits of immunoglobulins, complement, and fibrinogen in oral lupus erythematosus, lichen planus, and leukoplakia. Oral Surg Oral Med Oral Pathol 1981;51:603-8.
44. Serpico R, Pannone G, Santoro A, Mezza E, Piccolo S, Esposito V, et al. Report of a case of discoid lupus erythematosus localised to the oral cavity: immunofluorescence findings. Int J Immunopathol Pharmacol 2007;20:651-3.
45. Magro CM, Roberts-Barnes J, Crowson AN. Direct immunofluorescence testing in the diagnosis of immunobullous disease, collagen vascular disease, and vascular injury syndromes. Dermatol Clin 2012;30:763-98,viii.
46. Ohata C, Ohyama B, Nagata H, Furumura M, Nakama T. Comparative Study of Direct Immunofluorescence in Discoid Lupus Erythematosus and Bullous Pemphigoid. Am J Dermatopathol 2016;38:121-3.
47. al-Suwaid AR, Venkataram MN, Bhushnurmath SR. Cutaneous lupus erythematosus: comparison of direct immunofluorescence findings with histopathology. Int J Dermatol 1995;34:480-2.
48. Sugai SA, Gerbase AB, Cernea SS, Sotto MN, Oliveira ZN, Vilela MA, et al. Cutaneous lupus erythematosus: direct immunofluorescence and epidermal basal membrane study. Int J Dermatol 1992;31:260-4.
49. Sánchez NP, Peters MS, Winkelmann RK. The histopathology of lupus erythematosus panniculitis. J Am Acad Dermatol 1981;5:673-80.
50. Izumi AK, Takiguchi P. Lupus erythematosus panniculitis. Arch Dermatol 1983;119:61-4.
51. Tuffanelli DL. Lupus erythematosus panniculitis (profundus). Arch Dermatol 1971;103:231-42.
52. Alexiades-Armenakas MR, Baldassano M, Bince B, Werth V, Bystryn JC, Kamino H, et al. Tumid lupus erythematosus: criteria for classification with immunohistochemical analysis. Arthritis Rheum 2003;49:494-500.
53. Vieira V, Del Pozo J, Yebra-Pimentel MT, Martínez W, Fonseca E. Lupus erythematosus tumidus: a series of 26 cases. Int J Dermatol 2006;45:512-7.
54. Bouzit N, Grézard P, Wolf F, Balme B, Perrot H. Linear cutaneous lupus erythematosus in an adult. Dermatology 1999;199:60-2.
55. Hashimoto T, Kawakami Y, Wakabayashi H, Oda W, Hamada T, Doi H, et al. An unusual clinical presentation of lupus erythematosus tumidus localized on the thigh. Clin Exp Dermatol 2017;42:638-41.
56. Pock L, Petrovská P, Becvár R, Mandys V, Hercogová J. Verrucous form of chilblain lupus erythematosus. J Eur Acad Dermatol Venereol 2001;15:448-51.
57. Patel S, Hardo F. Chilblain lupus erythematosus. BMJ Case Rep 2013;2013:bcr2013201165.
58. Khorshid SM, Beynon HL, Rustin MH. Lupus erythematosus vegetans. Br J Dermatol 1999;141:893-6.
59. Yell JA, Allen J, Wojnarowska F, Kirtschig G, Burge SM. Bullous systemic lupus erythematosus: revised criteria for diagnosis. Br J Dermatol 1995;132:921-8.
60. Bain EE, Grover RK, Plunkett RW, Beutner EH. Detection of collagen VII autoantibodies to NC1 and NC2 domains of collagen VII by ELISA in suspected epidermolysis bullosa acquisita and bullous lupus erythematosus patients. J Dermatol Sci 2012;65:155-6.
61. Contestable JJ, Edhegard KD, Meyerle JH. Bullous systemic lupus erythematosus: a review and update to diagnosis and

treatment. Am J Clin Dermatol 2014;15:517-24.
62. de Risi-Pugliese T, Cohen Aubart F, Haroche J, Moguelet P, Grootenboer-Mignot S, Mathian A, et al. Clinical, histological, immunological presentations and outcomes of bullous systemic lupus erythematosus: 10 New cases and a literature review of 118 cases. Semin Arthritis Rheum 2018;48:83-9.
63. Barbosa WS, Rodarte CM, Guerra JG, Maciel VG, Fleury Júnior LF, Costa MB. Bullous systemic lupus erythematosus: differential diagnosis with dermatitis herpetiformis. An Bras Dermatol 2011; 86:S92-5.
64. Boddu P, Nadiri M, Malik O. Diffuse Bullous Eruptions in an Elderly Woman: Late-Onset Bullous Systemic Lupus Erythematosus. Case Rep Dermatol 2016;8:278-82.
65. Camisa C, Sharma HM. Vesiculobullous systemic lupus erythematosus. Report of two cases and a review of the literature. J Am Acad Dermatol 1983;9:924-33.
66. Hans-Bittner NR, Bittner GC, Hans G Filho, Takita LC. Bullous systemic lupus erythematosus in a 10-year-old child. An Bras Dermatol 2017;92:37-39.
67. Jain S, Basavaraj V, Vimala MG. Utility of Direct Immunofluorescence Studies in Subclassification of Autoimmune Sub-Epidermal Bullous Diseases: A 2-Year Study in a Tertiary Care Hospital. Turk Patoloji Derg 2016;32:91-8.
68. Janniger CK, Kowalewski C, Mahmood T, Lambert WC, Schwartz RA. Detection of anti-basement membrane zone antibodies in bullous systemic lupus erythematosus. J Am Acad Dermatol 1991;24:643-7.
69. Miziara ID, Mahmoud A, Chagury AA, Alves RD. Bullous Systemic Lupus Erythematosus: Case report. Int Arch Otorhinolaryngol 2013;17:344-6.
70. Nitta Y, Kawamura C, Hashimoto T. Vesiculobullous systemic lupus erythematosus: a case with circulating IgG and IgA autoantibodies to type VII collagen. J Am Acad Dermatol 2002; 47:S283-6.
71. Olansky AJ, Briggaman RA, Gammon WR, Kelly TF, Sams WM Jr. Bullous systemic lupus erythematosus. J Am Acad Dermatol 1982;7:511-20.
72. Shirahama S, Yagi H, Furukawa F, Takigawa M. A case of bullous systemic lupus erythematosus. Dermatology 1994;189: 95-6.
73. Torres Saavedra FA, Campo LR, Mendez MV, Barreneche NM, Suaza GAV, Restrepo JDR, et al. Bullous lupus as the first manifestation of systemic lupus erythematosus in the pediatric population: A diagnostic challenge in daily practice. Lupus 2020; 29:1937-42.
74. Yung A, Oakley A. Bullous systemic lupus erythematosus. Australas J Dermatol 2000;41:234-7.
75. Del Barrio-Díaz P, Reyes-Vivanco C, Cifuentes-Mutinelli M, Manríquez J, Vera-Kellet C. Association between oral lesions and disease activity in lupus erythematosus. J Eur Acad Dermatol Venereol 2020;34:349-56.
76. Chanprapaph K, Pomsoong C, Tankunakorn J, Eden C, Suchonwanit P, Rutnin S. Comparative Analyses of Clinical Features, Histopathology, and CD123 Immunohistochemistry of Oral Lupus Erythematosus, Lichen Planus, and Other Lichenoid Lesions. Dermatology 2022;238:464-75.
77. Daniels TE, Quadra-White C. Direct immunofluorescence in oral mucosal disease: a diagnostic analysis of 130 cases. Oral Surg Oral Med Oral Pathol 1981;51:38-47.
78. Nikoo A, Daneshpazhooh M, Fahim S, Ghanadan A, Mahmoudi H, Izadi Firoozabadi L. Persistent lip enlargement: An unusual presentation of lupus erythematosus. Int J Womens Dermatol 2016;3:96-9.
79. Pires JR, Nogueira MRS, Nunes AJF, Degand DRF, Pessoa LC, Damante CA, et al. Deposition of Immune Complexes in Gingival Tissues in the Presence of Periodontitis and Systemic Lupus Erythematosus. Front Immunol 2021;12:591236.
80. Dallo C, Lee K, Cragun WC, Lee M. A Case of Papulonodular Mucinosis in a Patient With Systemic Lupus Erythematosus. Am J Dermatopathol 2020;42:280-2.
81. Kanda N, Tsuchida T, Watanabe T, Tamaki K. Cutaneous lupus mucinosis: a review of our cases and the possible pathogenesis. J Cutan Pathol 1997;24:553-8.
82. Rongioletti F, Parodi A, Rebora A. Papular and nodular mucinosis as a sign of lupus erythematosus. Dermatologica 1990;180: 221-3.
83. Shibeshi D, Blaszczyk M, Jarzabek-Chorzelska M, Jabłońska S, Chorzelski T. Immunopathologic findings in systemic sclerosis patients: clinical and immunopathologic relationships. Int J Dermatol 1989;28:650-6.