Original Research Study
Acta Medica Academica 2023;52(3):195-200
DOI: 10.5644/ama2006-124.424

A Comparison of Cathelicidin Levels in the Skin of Leprosy Patients and Their Household Contacts

Fifa Argentina[1], Oki Suwarsa[2], Hendra Gunawan[2], Afiat Berbudi[3, 4]

[1]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia[*], [2]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Padjadjaran/Hasan Sadikin General Hospital, Bandung, Indonesia, [3]Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia, [4]Infectious Disease Research Center, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Correspondence: *fifaargentina.md@gmail.com*; Tel.: + 62 813 73475134

Received: 31 July 2023; **Accepted:** 29 December 2023

Abstract

Objective. This study aimed to compare cathelicidin levels in the skin of leprae patients and leprae contacts. **Patients and Methods.** This research is an analytic observational study with a cross-sectional approach. Fifty-four research subjects participated in this study. They consisted of leprae patients, household contacts, and healthy individuals. Cathelicidin levels were measured using the ELISA method. Data analysis was carried out with the help of SPSS software, and univariate and bivariate analysis was conducted. **Results.** Cathelicidin levels in the leprae group (256.8±22.9 pg/ml) were higher than in the contact group (25.9±2.7 pg/ml). Likewise, the contact group had higher cathelicidin levels than healthy controls (1.4±0.1 pg/ml). Statistically, there were differences in cathelicidin levels between groups, P<0.050. **Conclusion.** Cathelicidin levels in leprae patients were higher than those in household contacts.

Key Words: Antimicrobial Peptide ▪ Cathelicidin ▪ Enzyme-Linked Immunoabsorbent Assay ▪ Leprosy ▪ Neutrophils.

Introduction

Leprosy is a chronic infection caused by *Mycobacterium leprae* and is still a serious health problem in many countries (1). Data from WHO state that in 2022 there were 174,087 new cases of leprosy recorded, with 9554 of them accompanied by grade 2 disabilities (G2D) (2). New cases of leprosy are always present in endemic areas, and in some endemic areas, it continues to increase (3, 4). Indonesia is the third-highest country globally in terms of leprosy cases, following India and Brazil. In 2022, there were 15,052 registered cases of leprosy and 12,095 new cases, resulting in a new case detection rate of 45.16 per 1,000,000 population (5, 6). The clinical manifestations of leprosy are varied, and the mechanism of this infection is closely related to the innate immune response (7–9).

The innate response is a natural response that exists in the body for the body's defense against infection (9). Antimicrobial peptide (AMP) is part of the innate immune response (10, 11). Defensin and cathelicidin are part of the antimicrobial peptide (12). Cathelicidin is an antimicrobial protein found in neutrophils and keratinocytes (13, 14). Several studies have shown that cathelicidin deficiency is related to the severity of the infection experienced (15, 16). One study showed that cathelicidin deficiency in salivary neutrophils was associated with more severe oral infections (16). Cathelicidin in skin neutrophils is also believed to play a role in the severity of leprosy (12, 14, 15, 17). A study on *Mycobacterium tuberculosis* infection showed that AMP from neutrophils could potentially prevent the severity of pulmonary

[*] The study was conducted at the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

tuberculosis (18). The similarity of the causative genus between leprosy and tuberculosis infections suggests that the potential of cathelicidin to reduce the severity of tuberculosis could have a similar effect on leprosy cases. Cathelicidin is believed to be able to act as a marker of the presence and severity of *Mycobacterium leprae* infection (17).

As human beings, of course, leprosy patients cannot live alone. Leprosy patients often come into contact with many people, such as family members at home, friends, and neighbors (household contacts) (2). Their condition means that those who have close contact with leprosy patients have the potential to experience leprosy infection (19). However, these household contacts (HCs) do not necessarily become directly infected with leprosy. Many HCs are clinically healthy, which is believed to result from the immune response in household contacts. HCs are believed to have innate and adaptive immune responses, which are more optimal than leprosy patients (20). Ideally, leprosy patients will have lower cathelicidin levels than HCs. However, another study on the severity of tuberculosis patients and HCs using cathelicidin markers presented a different picture (21). The study stated that tuberculosis HCs have lower cathelicidin levels than tuberculosis patients.

This study compared cathelicidin levels in leprosy patients' skin and that of household contacts.

Methods

Study Design and Participants

This study is an analytic observational study with a cross-sectional approach. The study was conducted between September and December 2022. This study used primary data, where the research subjects were PB and MB type leprae patients and their families who live at home and always accompany patients for treatment at the Dermatology Polyclinic of Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia. Fifty-four research subjects participated in this study, including leprosy patients, leprae household contacts (HCs), and healthy individuals. The inclusion criteria for leprosy patients were: patients diagnosed with leprosy (WHO classification (PB and MB types) (2) by a dermatologist at Dr. Mohammad Hoesin General Hospital, aged over 18 years, who agreed to participate in this study. Exclusion criteria for leprosy patients and HCs were those suffering from skin diseases other than leprosy, and those taking antibiotics or corticosteroids. HC inclusion criteria were subjects living at home with leprosy patients for at least six months, aged over 18 years, and who agreed to participate in this study. Detailed sociodemographic data of the patients, consisting of gender, age, and daily activities were recorded. The daily activities of patients were investigated to determine their interactions with their household contacts.

Skin Scraping Method

The research participants underwent procedures for specimen collection, including skin scraping from both ear lobes and two different skin lesions (for patients), and both upper arms (for household contacts and healthy participants). The scalpel used for skin scraping was put into a tube containing 70% alcohol. Using the non-sharp edge of a scalpel, skin scrapings are collected from the skin lesions (macules) and unaffected skin of individuals with leprosy, their household contacts, and healthy individuals, in the brachii area. We conducted the dermal scraping process on leprosy patients, obtaining two samples measuring 5 cm × 5 cm from the afflicted lesion area (macules) and healthy skin located 7 cm apart from the lesion site. One hand selected and stretched the skin area, while the other hand held the incision in a manner that ensured the cut remained parallel to the skin's surface. Subsequently, the scraping procedure was performed approximately 10–20 times in a single direction, followed by three repetitions on each dull edge of the scalpel while exerting substantial force.

Cathelicidin Level Evaluation

Examination of cathelicidin levels was carried out using the enzyme-linked immunosorbent assay (ELISA) technique. The skin scraping samples of

the research subjects were homogenized and centrifuged at 5000 rpm for 10 minutes at 4°C (22). The supernatant was taken and used for ELISA examination. The ELISA procedure was carried out according to the ELISA kit cathelicidin manual (Cloud Clone*, Hangzhou, PRC).

Ethical Approval

This study received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya (Ref. No. 155/FKUNSRI/XI/2022), and informed consent was provided by each volunteer participating.

Statistical Analysis

Data were analyzed using SPSS 25.0 (SPSS Inc., Armonk, NY, United States). Univariate analysis was performed to present the data distribution for each test variable. Bivariate analysis was performed to compare cathelicidin levels between test groups, with $P<0.05$.

Results

A total of 54 research subjects participated in this study, including leprosy patients, leprae household contacts (HCs), and healthy individuals.

Table 1 shows the baseline characteristics of the research subjects. Most of the leprosy group were male, aged 21–40 years, and performed activities outside the home. Most HC groups were aged 41–60 years old and worked outside their home. The healthy group was predominantly male, aged 21–40, and most worked at home.

Table 2 shows a comparison of cathelicidin levels between groups. Cathelicidin levels in the leprosy group were higher than in the HC group. Likewise, the HC group had higher cathelicidin levels than the healthy controls. Statistically, there were significant differences in cathelicidin levels between groups (Table 3).

Table 1. Baseline Characteristics of Participants

Variables	Groups		
	Leprosy patients N (%)	Household contacts N (%)	Healthy N (%)
Gender			
Male	10 (55.6)	10 (55.6)	10 (55.6)
Female	8 (44.4)	8 (44.4)	8 (44.4)
Age (years old)			
<40	11 (61.1)	7 (38.9)	13 (72.2)
≥40	7 (38.9)	11 (61.1)	5 (27.8)
Daily activities			
Inside home	11 (61.1)	7 (38.9)	11 (61.1)
Outside home	7 (38.9)	11 (61.1)	7 (38.9)
Type of leprosy			
Paucibacillary	10 (55.6)	-	-
Multibacillary	8 (44.4)	-	-

Table 2. Comparison of Cathelicidin Levels between Groups

Variable	Groups			P-value*
	Leprosy patients	Household contacts	Healthy	
Cathelicidin levels	256.8±22.9	25.9±2.7	1.4±0.1*	0.0001

*One-way ANOVA; †pg/ml±SD.

Table 3. Pos-hoc Analysis between Groups

Groups	Leprosy patients	Household contacts	Healthy
Leprosy	--	0.0001*	0.0001*
Household contacts	0.0001*	-	0.0001*
Healthy	0.0001*	0.0001*	-

*Pos-hoc Bonferroni.

Discussion

This study showed that cathelicidin levels in leprosy patients were higher than in household contacts (HCs). Cathelicidin is part of the innate immune system, where this protein is an antimicrobial protein (AMP) produced by neutrophils to treat various infections. The higher the cathelicidin level, the more moderate the severity of *Mycobacterium* infection (11, 12). Another study showed that keratinocytes and skin cells, such as eccrine gland cells, produce and secrete AMPs, including cathelicidin (14). In our study, cathelicidin was evaluated in skin scrapings because it is synthesized by epithelial cells and provided by infiltrating immune cells, such as neutrophils and macrophages (23). The infiltrating immune cells transport cathelicidins to infected or injured skin (23).

The results of this study are inconsistent with several studies that state that cathelicidin deficiency causes *Mycobacterium tuberculosis* infection to become more severe compared to the HCs group (24, 25). There are several theories and other studies that can explain the findings of this study. Previous studies have measured cathelicidin levels in *M. tuberculosis* infection, where the primary infection site is in the lungs so that the cathelicidin levels that represent the immune system are in the serum (21, 24). In leprosy patients, the primary site of infection is in the skin, so the level of cathelicidin in infected skin scrapings represents the patient's infection status (26). Other studies state that cathelicidin levels are identical to bacterial load or how many microorganisms there are in the body (27, 28). The more bacteria or microorganisms in the body, the higher the production of cathelicidin (28, 29). This can explain why cathelicidin levels in the HC group are lower compared to leprosy patients. HCs have a lower bacterial load than leprosy patients.

The immune system is a simultaneous process triggered by antigenic stimuli that aim to destroy the stimulus triggers (30). The body's defense mechanism has three levels: the physical barrier of the skin and mucosal surfaces, the innate immune system, and the adaptive immune system (30). The physical skin barrier is essential because it protects against contact with the outside world. The skin surface is also inhabited by various microbes, viruses, and fungi, known as the skin microbiome, to strengthen the skin barrier (31). The innate immune system cooperates with the physical defenses of the skin and mucosa, enzymes, macrophages, polymorphonuclear, eosinophils, and natural-killer cells, to deal with non-specific foreign bodies or organisms.

Vitamin D and downstream receptor signaling are essential in enhancing the capabilities of macrophages and other immune cells (32). Increasing the immune cells' ability will encourage the human body's antimicrobial defense (33). Several AMPs are induced by vitamin D signaling, including cathelicidins, defensins, hepcidins, and neutrophil peptides acting as major intrinsic antibiotics. Previous studies have also suggested that vitamin D signaling is related to the transcriptional activation of AMPs, including cathelicidins and defensins (32, 33).

Cathelicidin activation-induced vitamin D, as a component of immunity in the skin, is affected by sun exposure. As a tropical country, Indonesia has sufficient sun exposure to activate vitamin D (34). Cathelicidin levels in the skin show the immunity system activity against *M. leprae* infection. Cathelicidin levels on the surface of the skin are an accumulation of the results of the synthesis of

skin epithelial cells and neutrophils that infiltrate the skin that is being infected (13). Cathelicidin has the potential to be developed into a marker to assess the bacterial load of leprosy infection. The limitation of our study is the number of samples that were only taken from one region in Indonesia. In future studies, multicentre sampling should be carried out so that the results obtained are more representative of leprosy patients and their household contacts.

Conclusion

Cathelicidin levels in leprosy patients were higher than those in household contacts and healthy individuals.

What Is Already Known on This Topic:
Cathelicidin is an antimicrobial protein found in neutrophils and keratinocytes. Cathelicidin in skin neutrophils is also believed to play a role in the severity of leprosy. Household contacts or people who live together with leprosy patients are believed to have more innate and adaptive immune responses than the patients.

What This Study Adds:
This is the first study to explore and compare cathelicidin levels in skin scrapings of leprae patients and their household contacts. Cathelicidin is believed to be able to act as a marker of the presence and severity of Mycobacterium leprae infection. In this study, we found that cathelicidin levels in leprosy patients were higher than those in household contacts.

Authors' Contributions: Conception and design: FA and OS; Acquisition, analysis and interpretation of data: FA and HG; Drafting the article: FA, HG and AB; Revising it critically for important intellectual content: HG, AB; Approved final version of the manuscript: FA, OS, HG and AB.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Chen KH, Lin CY, Su SB, Chen KT. Leprosy: A Review of Epidemiology, Clinical Diagnosis, and Management. J Trop Med. 2022;2022:8652062. doi: 10.1155/2022/8652062.
2. World Health Organization. The Global Health Observatory-Leprosy (Hansen's disease). 2023. [cited 2023 Nov 30]. Available from: https://www.who.int/data.
3. Srinivas G, Muthuvel T, Lal V, Vaikundanathan K, Schwienhorst-Stich EM, Kasang C. Risk of disability among adult leprosy cases and determinants of delay in diagnosis in five states of India: A case-control study. PLoS Negl Trop Dis. 2019;13(6):e0007495. doi: 10.1371/journal.pntd.0007495.
4. Ogunsumi DO, Lal V, Puchner KP, van Brakel W, Schwienhorst-Stich EM, Kasang C, et al. Measuring endemicity and burden of leprosy across countries and regions: A systematic review and Delphi survey. PLoS Negl Trop Dis. 2021;15(9):e0009769. doi: 10.1371/journal.pntd.0009769.
5. World Health Organization. The Global Health Observatory-Leprosy- New cases detection rate per 1,000,000 population. 2023. [cited 2023 Nov 30]. Available from: https://www.who.int/data.
6. Dharmawan Y, Korfage IJ, Abqari U, Widjanarko B, Richardus JH. Measuring leprosy case detection delay and associated factors in Indonesia: a community-based study. BMC Infect Dis. 2023;23(1):555. doi: 10.1186/s12879-023-08552-x.
7. Reinar LM, Forsetlund L, Bjørndal A, Lockwood DN. WITHDRAWN: Interventions for skin changes caused by nerve damage in leprosy. Cochrane Database Syst Rev. 2019;8(8):CD004833. doi: 10.1002/14651858.CD004833.pub4.
8. Ebenezer GJ, Scollard DM. Treatment and Evaluation Advances in Leprosy Neuropathy. Neurotherapeutics. 2021;18(4):2337-50. doi: 10.1007/s13311-021-01153-z. Epub 2021 Nov 19.
9. Pinheiro RO, Schmitz V, Silva BJA, Dias AA, de Souza BJ, de Mattos Barbosa MG, et al. Innate Immune Responses in Leprosy. Front Immunol. 2018;9:518. doi: 10.3389/fimmu.2018.00518.
10. Mi Z, Liu H, Zhang F. Advances in the Immunology and Genetics of Leprosy. Front Immunol. 2020;11:567. doi: 10.3389/fimmu.2020.00567.
11. Grossi de Oliveira AL, Chaves AT, Cardoso MS, Pinheiro GRG, Antunes DE, Grossi MAF, et al. Reduced vitamin D receptor (VDR) and cathelicidin antimicrobial peptide (CAMP) gene expression contribute to the maintenance of inflammatory immune response in leprosy patients. Microbes Infect. 2022;24(6-7):104981. doi: 10.1016/j.micinf.2022.104981. Epub 2022 Apr 21.
12. Grossi de Oliveira AL, Chaves AT, Santos Cardoso M, Gomide Pinheiro GR, Parreiras de Jesus AC, de Faria Grossi MA, et al. Hypovitaminosis D and reduced cathelicidin are strongly correlated during the multidrug therapy against leprosy. Microb Pathog. 2020;147:104373. doi: 10.1016/j.micpath.2020.104373. Epub 2020 Jul 6.
13. Kim EW, Teles RMB, Haile S, Liu PT, Modlin RL. Vitamin D status contributes to the antimicrobial activity of macrophages against Mycobacterium leprae. PLoS Negl Trop Dis. 2018;12(7):e0006608. doi: 10.1371/journal.pntd.0006608.
14. Lyrio EC, Campos-Souza IC, Corrêa LC, Lechuga GC, Verícimo M, Castro HC, et al. Interaction of Mycobacte-

rium leprae with the HaCaT human keratinocyte cell line: new frontiers in the cellular immunology of leprosy. Exp Dermatol. 2015;24(7):536-42. doi: 10.1111/exd.12714. Epub 2015 May 4.

15. Matzner M, Al Samie AR, Winkler HM, Nemeth J, Grasnek A, Indra A, et al. Low serum levels of cathelicidin LL-37 in leprosy. Acta Trop. 2011;117(1):56-9. doi: 10.1016/j.actatropica.2010.09.007. Epub 2010 Sep 29.
16. Tokajuk J, Deptuła P, Piktel E, Daniluk T, Chmielewska S, Wollny T, et al. Cathelicidin LL-37 in Health and Diseases of the Oral Cavity. Biomedicines. 2022;10(5):1086. doi: 10.3390/biomedicines10051086.
17. Luo Y, Kiriya M, Tanigawa K, Kawashima A, Nakamura Y, Ishii N, et al. Host-Related Laboratory Parameters for Leprosy Reactions. Front Med (Lausanne). 2021;8:694376. doi: 10.3389/fmed.2021.694376.
18. Zhan Y, Jiang L. Status of vitamin D, antimicrobial peptide cathelicidin and T helper-associated cytokines in patients with diabetes mellitus and pulmonary tuberculosis. Exp Ther Med. 2015;9(1):11-6. doi: 10.3892/etm.2014.2042. Epub 2014 Oct 31.
19. Krismawati H, Oktavian A, Maladan Y, Wahyuni T. Risk factor for Mycobacterium leprae detection in household contacts with leprosy patients: A study in Papua, East Indonesia. Med J Indo. 2020;29(1):64-70. doi: 10.13181/mji.oa.192962.
20. Teixeira CSS, Pescarini JM, Alves FJO, Nery JS, Sanchez MN, Teles C, et al. Incidence of and Factors Associated With Leprosy Among Household Contacts of Patients With Leprosy in Brazil. JAMA Dermatol. 2020;156(6):640-8. doi: 10.1001/jamadermatol.2020.0653.
21. Chawla S, Gupta V, Gour N, Grover K, Goel PK, Kaushal P, et al. Active case finding of tuberculosis among household contacts of newly diagnosed tuberculosis patients: A community-based study from southern Haryana. J Family Med Prim Care. 2020;9(7):3701-3706. doi: 10.4103/jfmpc.jfmpc_532_20.
22. Hidayat R, Wulandari P. Enzyme linked immunosorbant assay (ELISA) technique guideline. Bioscientia Med J Biomed Translat Res. 2021;5(5):447-53. doi: 10.32539/bsm.v5i2.228.
23. Gupta S, Winglee K, Gallo R, Bishai WR. Bacterial subversion of cAMP signalling inhibits cathelicidin expression, which is required for innate resistance to Mycobacterium tuberculosis. J Pathol. 2017;242(1):52-61. doi: 10.1002/path.4878. Epub 2017 Mar 15.
24. Park BW, Ha JM, Cho EB, Jin JK, Park EJ, Park HR, et al. A Study on Vitamin D and Cathelicidin Status in Patients with Rosacea: Serum Level and Tissue Expression. Ann Dermatol. 2018;30(2):136-142. doi: 10.5021/ad.2018.30.2.136. Epub 2018 Feb 21.
25. Chandra P, Grigsby SJ, Philips JA. Immune evasion and provocation by Mycobacterium tuberculosis. Nat Rev Microbiol. 2022;20(12):750-66. doi: 10.1038/s41579-022-00763-4. Epub 2022 Jul 25.
26. Nguyen AV, Soulika AM. The Dynamics of the Skin's Immune System. Int J Mol Sci. 2019;20(8):1811. doi: 10.3390/ijms20081811.
27. Al-Jaberi FAH, Crone CG, Lindenstrøm T, Arildsen NS, Lindeløv ES, Aagaard L, et al. Reduced vitamin D-induced cathelicidin production and killing of Mycobacterium tuberculosis in macrophages from a patient with a non-functional vitamin D receptor: A case report. Front Immunol. 2022;13:1038960. doi: 10.3389/fimmu.2022.1038960.
28. Acen EL, Kateete DP, Worodria W, Olum R, Joloba ML, Bbuye M, et al. Evaluation of circulating serum cathelicidin levels as a potential biomarker to discriminate between active and latent tuberculosis in Uganda. PLoS One. 2022;17(8):e0272788. doi: 10.1371/journal.pone.0272788.
29. Rowe-Magnus DA, Kao AY, Prieto AC, Pu M, Kao C. Cathelicidin Peptides Restrict Bacterial Growth via Membrane Perturbation and Induction of Reactive Oxygen Species. mBio. 2019;10(5):e02021-19. doi: 10.1128/mBio.02021-19.
30. Nicholson LB. The immune system. Essays Biochem. 2016;60(3):275-301. doi: 10.1042/EBC20160017.
31. Lee HJ, Kim M. Skin Barrier Function and the Microbiome. Int J Mol Sci. 2022;23(21):13071. doi: 10.3390/ijms232113071.
32. Dimitrov V, White JH. Species-specific regulation of innate immunity by vitamin D signaling. J Steroid Biochem Mol Biol. 2016;164:246-53. doi: 10.1016/j.jsbmb.2015.09.016. Epub 2015 Sep 11.
33. Chung C, Silwal P, Kim I, Modlin RL, Jo EK. Vitamin D-Cathelicidin Axis: at the Crossroads between Protective Immunity and Pathological Inflammation during Infection. Immune Netw. 2020;20(2):e12. doi: 10.4110/in.2020.20.e12.
34. Augustine LF, Nair KM, Kulkarni B. Sun exposure as a strategy for acquiring vitamin D in developing countries of tropical region: Challenges & way forward. Indian J Med Res. 2021;154(3):423-32. doi: 10.4103/ijmr.IJMR_1244_18.