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ORIGINAL RESEARCH

Gene Expression of Human Beta-Defensin-3 and Cathelicidin in the Skin of Leprosy Patients, Household Contacts, and Healthy Individuals from Indonesia

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Background: Leprosy, a chronic infectious peripheral neuropathy, is caused by *Mycobacterium leprae*. This bacterium produces triacylated lipopeptides that can induce the immune system via the Toll-like receptor 2/1 (TLR 2/1) complex. Activation of TLR 2/1 produces proinflammatory cytokines and antimicrobial peptides (AMPs), including human beta-defensin-3 (HBD-3) and cathelicidin.

Purpose: To analyze differences in gene expression of HBD-3 and cathelicidin in the skin of leprosy patients, household contacts, and healthy individuals.

Patients and Methods: An analytic observational study was conducted at the Outpatient Clinic of Dermatology and Venereology of Dr Mohammad Hoesin General Hospital, Palembang, Indonesia, from January 2021 to June 2022. In each group of 18 subjects, 72 samples were collected, including skin lesion in leprosy patients, normal skin in leprosy patients, household contacts, and healthy individuals. A comparison of HBD-3 and cathelicidin gene expression between the four groups was analyzed using Pearson Chi Square, Kruskal–Wallis, and Mann–Whitney Test.

Results: The median value of HBD-3 gene expression on skin lesion in leprosy patients was 260.61 (0.19–3734.10); normal skin in leprosy patients was 1.91 (0.01–151.17); household contacts skin was 7.93 (0.27–121.10); and healthy individuals' skin was 1.00 (1.00–1.00) is highly significant difference ($p < 0.0001$). The median value of cathelicidin gene expression on skin lesion in leprosy patients was 38.72 (0.28–1852.17); normal skin in leprosy patients was 0.48 (0.01–15.83); household contacts skin was 9.8 (0.04–128.0); and healthy individual skin was 1.00 (1.00–1.00), also highly significant difference ($p < 0.0001$).

Conclusion: Gene expression of HBD-3 and cathelicidin increased in skin lesions of leprosy patients and household contacts.

Keywords: cathelicidin, HBD-3, household contacts, leprosy

Introduction

Leprosy is a chronic granulomatous infectious disease caused by *Mycobacterium leprae* (*M. leprae*), and *M. lepromatous* which mainly attacks the skin, peripheral nerves, mucosa of the upper respiratory tract, eyes, muscles, bones, and testis.[1–3] Indonesia is the third-highest country in the world after India and Brazil for new leprosy cases.[4–6] Household contacts are people who have been in close contact with leprosy patients and are 2–8 times more likely to suffer from leprosy than non-contacts or general population.[5,7,8] Frequent exposure of contacts to *M. leprae* results in a persistently active innate immune system.[5] The World Health Organization (WHO) classifies leprosy into two types based on cardinal signs: paucibacillary (PB) and multibacillary (MB) leprosy.[1,3,6]

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M. leprae produce triacylated lipopeptides that can induce the immune system through the Toll-like receptor 2/1 (TLR 2/1) complex. TLR2/1 activation results in clearance of pathogens by macrophages, neutrophils, and epithelial cells. Some of the consequences of TLR 2/1 activation are the production of proinflammatory cytokines and antimicrobial peptides (AMPs).[9] AMPs are important elements of the innate immune system and are involved in modulating the adaptive immune system. Stimulation of TLR-2/1 leads to the induction of human beta defensin (HBD)-3 and cathelicidin.[9,10]

HBD-3 and cathelicidin are two types of AMPs found on the mucosal surface that are primarily produced by keratinocytes.[10–13] These AMPs are important factors of intrinsic immunity that function in the immunological response to various viral, bacterial, and fungal infections that can cause membrane depolarization and cell lysis due to its highly cationic nature.[13–15] The aim of this study was to analyze differences in gene expression of HBD-3 and cathelicidin in the skin of leprosy patients, household contacts, and healthy individuals.

Materials and Methods

Study Population and Inclusion Criteria

This was a cross-sectional analytic observational study conducted at the Outpatient Clinic of Dermatology and Venereology at Dr Mohammad Hoesin Hospital Palembang in Indonesia. A study was performed from June 2021 to June 2022. In each group of 18 subjects, 72 samples were collected, including skin lesion in leprosy patients, normal skin in leprosy patients, household contacts, and healthy individuals. Each participant gave written informed consent prior to enrollment.

The inclusion criteria for the leprosy group were patients aged 18 years or older, diagnosed with leprosy based on the cardinal signs of leprosy. Patients who used moisturizer less than 24 hours before enrolling in the study, patients who used topical antibiotics or topical corticosteroids on skin lesions seven days prior to enrolling in the study, and patients with co-morbidities other than leprosy, such as atopic dermatitis or skin infection, were excluded from the study. Household contacts must be older than 18 and have lived with leprosy patients for at least six months. Household contacts with other skin diseases, those who had taken topical or oral antibiotics or corticosteroids during the prior seven days, or those who had regularly applied moisturizer during that time were excluded from the study. Healthy individuals were the same age and gender as leprosy patients, and the exclusion criteria were having a previous skin disease and a history of taking medications, including corticosteroids, topical antibiotics, and emollients.

The study was approved by the Institutional Review Board of the Health Research Ethics Committee in Universitas Padjadjaran, Bandung, West Java, Indonesia, number 357/UN6.KEP/2021 and conducted in accordance with the latest version of the Declaration of Helsinki.

Sample Collection

Skin swabs from skin lesions and normal skin of leprosy patients, household contacts, and healthy individuals on the brachii area were collected using a sterile cotton-tipped swab after soaked in wetting solution of DNA/RNA Shield™ collection tube w/swab (catalog number R1107, Zymo Research, USA). A swabbing procedure in leprosy skin was employed, wherein two samples (5 cm x 5 cm) were collected from an affected lesional area and a healthy skin (7 cm from lesional site). Skin areas were selected and stretched with one hand, and the swab in the other hand was held in such a manner that the swab shaft was parallel to the skin surface. Following that, the swab was moved fifty times in one direction, three times facing each side of the swab and applying firm pressure.[16] The collected swab samples were stored in –20°C until further processing. Genomic RNA from the samples was extracted using the Quick-RNA™ MiniPrep Plus (catalog number R1058, Zymo Research, USA). Examination of gene expression of HBD-3 and cathelicidin was carried out using the real-time polymerase-chain reaction technique. The primers used for HBD-3 were 5'-AAAGTGACCAAGCACACC-3' (forward), 5'-TCCTCCATGACCTGGAAC-3' (reverse). The primers used for human LL-37/cathelicidin were 5'-CAGGCCCACGAT GGATG -3' (forward), 5'- CGTCCTTCTTGAAGTCACAAT C-3' (reverse). The primers used for human glyceraldehyde-3-phosphate dehydrogenase (GADPH) as housekeeping gene were 5'-CATCAGCAATGCCTCCTGC-3' (forward), 5'-ATGGACTGTGGTCATGAGTCC-3' (reverse). Primer

annealing is then done under temperatures of 25°C for 10-min reverse transcription with temperatures of 42°C for 15 min, and inactivation under temperatures of 85°C for 5 min, then hold at 4°C. The relative expression ratio was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

The comparison of gene expression of HBD-3 and cathelicidin between groups was analyzed with the Pearson Chi Square, Kruskal–Wallis Tests, and Mann–Whitney test used GraphPad Prism Software ver.9.

Result

Demographics of Study Participants

A total of 54 participants were included in this study, divided into three groups: the leprosy group (n = 18), household contacts (n = 18), and healthy individuals (n = 18). There were 55.6% males and 44.4% females in the group. Most of the participants worked inside occupations and were between the ages of 21 and 40. In this study, there was no significant statistical analysis in gender (p = 0.799), age category (p = 0.172), or occupation (p = 0.067) between leprosy patients, household contacts, and healthy individuals (Table 1).

Gene Expression of HBD-3

The results showed that the median value of HBD-3 gene expression on skin lesions in leprosy patients was 260.61 (0.19–3734.10); normal skin in leprosy patients was 1.91 (0.01–151.17); household contacts' skin was 7.93 (0.27–121.10); and healthy individuals' skin was 1.00 (1.00–1.00), with the Kruskal–Wallis test, p < 0.0001, indicating a highly significant difference (Table 2). Mann Whitney Test analysis showed, there was a significant difference between HBD-3 gene expression in the healthy individual's skin and skin lesions from leprosy patients (p < 0.0001****). There was a significant difference between healthy individuals and household contacts (p = 0.0001****). In leprosy, there was a significant difference between skin lesions and normal skin (p < 0.0001****). The difference between the healthy individual's skin and normal leprosy skin was not significant (p = 0.068) (Figure 1).

Gene Expression of Cathelicidin

The median value of cathelicidin gene expression on skin lesions in leprosy patients was 38.72 (0.28–1852.17); normal skin in leprosy patients was 0.48 (0.01–15.83); household contacts' skin was 9.8 (0.04–128.0); and healthy individuals' skin was 1.00 (1.00–1.00), with the Kruskal–Wallis test, p < 0.0001, indicating a highly significant difference (Table 2).

There was a significant difference in cathelicidin gene expression between healthy skin and leprosy skin lesions (p = 0.0001***). Mann–Whitney Test showed that there was a significant difference between healthy individuals and

Table 1 Demographics Characteristics of Subjects

Variable	Group			P value
	Leprosy n (%)	Household Contact n (%)	Healthy Individual n (%)	
Gender				
Male	11 (61.1)	9 (50.0)	10 (55.6)	0.799[a]
Female	7 (38.9)	9 (50.0)	8 (44.4)	
Age Category				
18–20 years old	3 (16.7)	2 (11.1)	1 (5.6)	0.172[a]
21–40 years old	8 (44.4)	5 (27.8)	12 (66.7)	
41–60 years old	7 (38.9)	11 (61.1)	5 (27.8)	
Occupation				
Indoor	12 (66.7)	6 (33.3)	12 (66.7)	0.067[a]
Outdoor	6 (33.3)	12 (66.7)	6 (33.3)	

Note: [a]Pearson Chi Square Test.

Table 2 Differences of HBD-3 and Cathelicidin Between Healthy Individual, Skin Lesion in Leprosy, Normal Skin in Leprosy, and Household Contacts

Gene Expression	Healthy Individual (n=18)	Leprosy		Household (n=18)	*p* value
		Lesion Skin (n=18)	Normal Skin (n=18)		
HBD-3					
Average±SD	1.000±0.000	523.1±876.3	10.45±35.19	21.17±30.41	
Median	1.00	260.61	1.91	7.93	< 0.0001****
Min–Max	1.00–1.00	0.19–3734.10	0.01–151.17	0.27–121.10	
Cathelicidin					
Average±SD	1.000±0.000	209.6±453.7	1.84±2.86	27.53±39.66	
Median	1.00	38.72	0.48	9.8	< 0.0001****
Min –Max	1.00–1.00	0.28–1852.17	0.01–15.83	0.04–128.0	

Notes: Kruskal–Wallis Test, ****$p < 0.0001$.

household contacts (p = 0.0001***). In leprosy, there was a statistically significant difference between skin lesions and normal skin (p < 0.0001****). However, the median value of cathelicidin gene expression between healthy individuals and the normal skin of leprosy was not significant (p = 0.0691) (Figure 2).

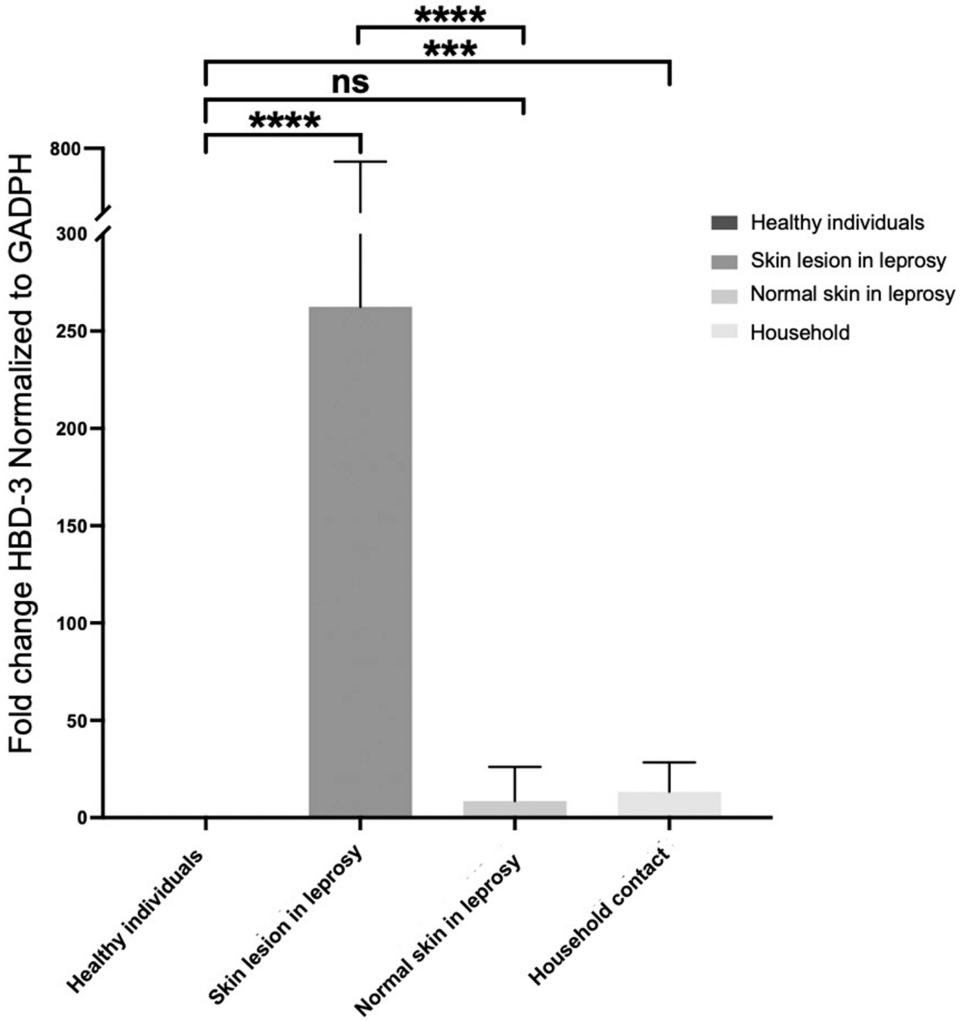

Figure 1 HBD-3 gene expression between healthy individual, skin lesion in leprosy, normal skin in leprosy, and household contacts. Mann Whitney test ***p = 0.0001; ****p < 0.0001.

Abbreviation: ns, non-significant.

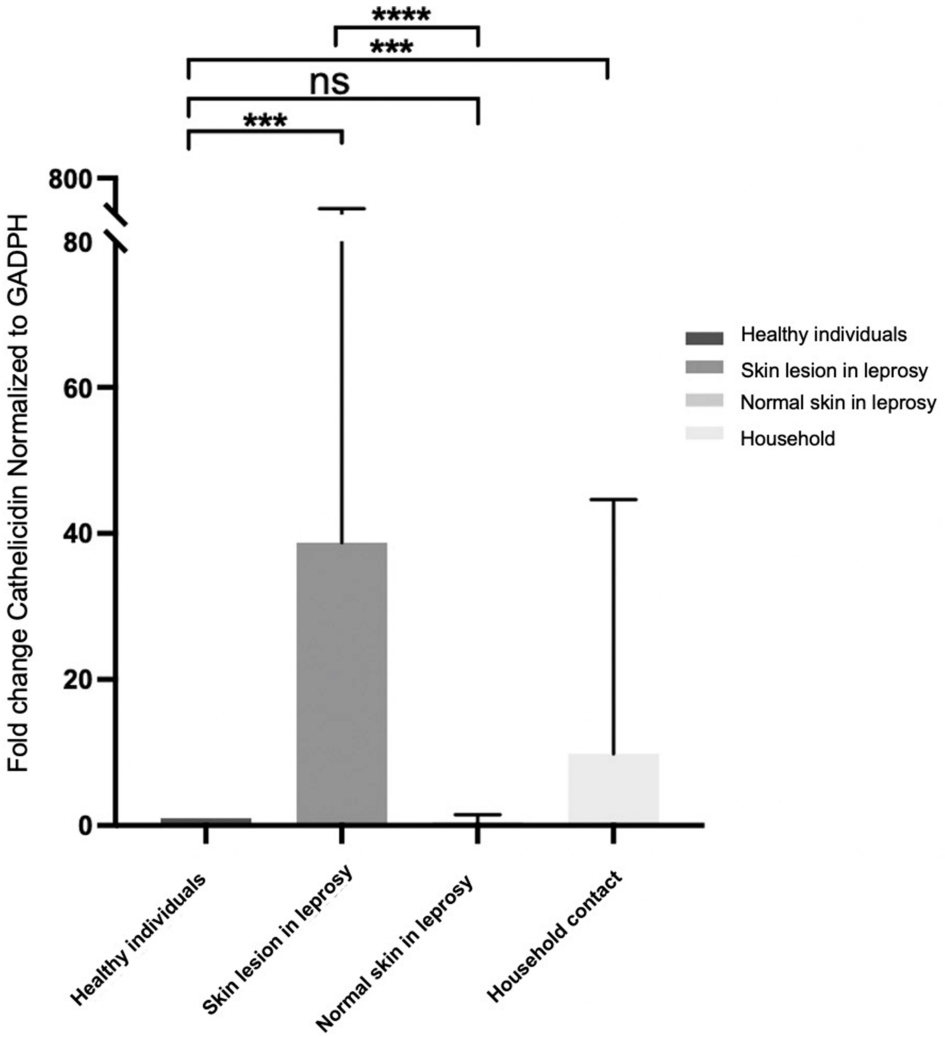

Figure 2 Cathelicidin gene expression between healthy individual, skin lesion in leprosy, normal skin in leprosy, and household contacts. Mann Whitney test ***p = 0.0001; ****p < 0.0001.
Abbreviation: ns, non-significant.

Discussion

Leprosy, a chronic infectious peripheral neuropathy, is caused by *M. leprae*[1,6] Patients exposed to *M. leprae* can experience subclinical infection, which then recovers, or become leprosy patients with a varied clinical spectrum, depending on the immune system.[17,18] People who have lived at the same household as a leprosy patients are 2–8 times more likely to develop the disease than the general population.[5,7,8] Doull et al found the risk of leprosy contact with leprosy type PB was 2 times, while the risk of contact with MB patients was 8 times.[7] Frequent exposure to *M. leprae* results in a continuously active innate immune system. This allows changes to the protein components in the body of the contact person. In addition, according to the study by Hooij et al, there were similarities in the innate immune systems of PB patients and household contacts.[19]

The innate immune system is a collaboration between the physical defenses of the skin and mucosa, enzymes, macrophages, PMNs, eosinophils, and NK cells to deal with foreign objects or organisms that enter non-specifically.[20–22] In the innate immune system, macrophages are an important determinant of the course of *M. leprae* infection and the clinical outcome. Activation of innate immunity in leprosy begins after the interaction between pathogen-associated molecular patterns (PAMPs) found in *M. leprae* and pattern recognition receptors (PRRs) found in host cells, such as TLR2/1.[9] PRRs can also recognize endogenous molecules originating from damaged cells, known as damage-associated

molecular patterns (DAMPs). The interaction between PAMP and/or DAMP with PRR will cause the secretion of AMPs, such as HBD-3 and cathelicidin, cytokines, and chemokines. Several studies have demonstrated that defensins act as natural antibiotics and as signaling molecules that can activate host cells to play a role in body defense.[23] The mechanism of action of AMPs is by binding to membranes, which ultimately cause cells to undergo lysis.[22–24]

In this study, the gene expression of HBD-3 and cathelicidin in the three groups was assessed and then compared. Previously, the sample characteristics of each group were first compared. There were no differences in gender, age, and occupation between leprosy patients, household contacts, and healthy individuals, which means that the differences in the gene expression of HBD-3 and cathelicidin obtained were not influenced by these three factors. Furthermore, Wittersheim et al discovered no connection between sex-related and age-influenced HBD-3 gene expression.[25]

Defensins are peptides consisting of 28–44 amino acids with three disulfide bonds,[26] which are classified into three types based on their structures: alpha, beta, and theta defensins.[23,27] Human defensins have a broad antibacterial spectrum and high antibacterial activity. Therefore, it acts as an important effector molecule on mucosal, skin, and epithelial surfaces.[28] In humans, there are four types of beta-defensins, such as HBD-1, HBD-2, HBD-3, and HBD 4.[21,23] In this study, we assessed the HBD-3 subtype, which is primarily present in the skin.[9] The results showed that there were differences in HBD-3 gene expression between skin lesions in leprosy patients, normal skin of leprosy patients, household contacts, and healthy individuals. HBD-3 gene expression was higher in skin lesions than in normal skin of leprosy patients. A triggered innate immune system results in increased expression of the HBD-3 gene. HBD-3 functions as a natural antibiotic as well as a signaling molecule, activating host cells to participate in the immune system.[23]

Cathelicidin is an important factor in reducing *M. leprae* infection, and vitamin D was identified as the main regulator of cathelicidin expression. A new strategy for treating *M. leprae* is targeted therapy with cathelicidin modulation. Cathelicidin is highly increased in keratinocytes during an infection.[29] In this study, the results showed that there were differences in cathelicidin expression between leprosy skin lesions, normal skin of leprosy, and household contacts with healthy individuals. The expression of the cathelicidin gene in leprosy skin lesions was found to be the highest compared with other groups. Leprosy patients had lower blood serum levels of cathelicidin before and after receiving MDT than healthy individuals, according to a study by Matzner et al, which indicates that lower serum cathelicidin levels are related to leprosy. However, Matzner's study examined at the blood serum's protein levels.[12] In contrast to this study, mRNA samples from skin swabs were used to analyze the cathelicidin gene expression. According to a study by Maier et al, mRNA and protein levels of gene expression had a notoriously poor correlation. It is likely that high mRNA amounts of gene expression were present despite limited protein synthesis.[30]

Household contacts of leprosy patients are at a higher risk of getting the disease, especially if the patients have high bacillary loads. Favorably, a large majority of people who have been exposed are naturally immune to *M. leprae* infection. Frequent *M. leprae* exposure in household contacts results in a constantly modified innate immune response. Pathogens and pathogen-infected cells are successfully removed if the innate immune response is sufficient. Prolonged intense activation, can result in an immune response directed against the host.[19] The gene expression of HBD-3 and cathelicidin in household contacts was increased in this study, suggesting that M. leprae exposure to household contacts plays important to increase AMPs at the mRNA level.

Conclusion

HBD-3 and cathelicidin are believed to contribute to the pathogenesis of leprosy. Gene expression of HBD-3 and cathelicidin increased in skin lesions of leprosy patients and household contacts. Therefore, based on this study, it may be possible to modulate the production of HBD-3 and cathelicidin as an additional target therapy for leprosy.

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Disclosure

The authors report no conflicts of interest in this work.

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