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

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Abstract

Objective. This study aimed to compare cathelicidin levels in the skin of leprosy patients and leprosy 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 leprosy 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 leprosy 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 leprosy 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...
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 ef-
fect 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 mem-
bers 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 opti-
mal than leprosy patients (20). Ideally, leprosy pa-
tients will have lower cathelicidin levels than HCs.
However, another study on the severity of tubercu-
losis patients and HCs using cathelicidin markers
presented a different picture (21). The study stated
that tuberculosis HCs have lower cathelicidin lev-
els than tuberculosis patients.

This study compared cathelicidin levels in lep-
rosy 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 conduct-
ed between September and December 2022. This
study used primary data, where the research sub-
jects were PB and MB type lepra patients and
their families who live at home and always accom-
pany patients for treatment at the Dermatology
Polyclinic of Dr. Mohammad Hoesin General
Hospital, Palembang, Indonesia. Fifty-four re-
search 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 lep-
rosy (WHO classification (PB and MB types) (2) by
a dermatologist at Dr. Mohammad Hoesin General
Hospital, aged over 18 years, who agreed to partici-
pate in this study. Exclusion criteria for leprosy pa-
tients and HCs were those suffering from skin dis-
eases other than leprosy, and those taking antibiotics
or corticosteroids. HC inclusion criteria were sub-
jects living at home with leprosy patients for at least
six months, aged over 18 years, and who agreed to
participate in this study. Detailed sociodemograph-
ic data of the patients, consisting of gender, age, and
daily activities were recorded. The daily activities of
patients were investigated to determine their inter-
actions 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 (mac-
ules) and unaffected skin of individuals with lepro-
sy, their household contacts, and healthy individu-
als, 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 re-
mained parallel to the skin’s surface. Subsequently,
the scraping procedure was performed approxi-
mately 10–20 times in a single direction, followed
by three repetitions on each dull edge of the scal-
pel 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.
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 tuberculosis 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

### Table 3. Pos-hoc Analysis between Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leprosy patients</th>
<th>Household contacts</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leprosy</td>
<td>-</td>
<td>0.0001*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Household contacts</td>
<td>0.0001*</td>
<td>-</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Healthy</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>-</td>
</tr>
</tbody>
</table>

*Pos-hoc Bonferroni.
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**


