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RESEARCH ARTICLE

Comparative analysis of natural resistance-associated macrophage protein 1 gene expression and anti-PGL-1 antibodies in multibacillary leprosy and household contacts

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ABSTRACT

Leprosy continues to pose a significant challenge to public health, particularly in certain global regions. Accurate diagnosis and understanding of the disease's etiology are crucial for effective management and prevention. This study aimed to explore the contribution of Natural resistance-associated macrophage protein 1 (NRAMP1) and its genetic variations, as well as the levels of anti-PGL-1 antibodies, to the pathology of multibacillary leprosy in affected individuals and their household contacts. The study included 23 multibacillary leprosy patients and 28 household contacts. NRAMP1 protein expression and anti-PGL-1 IgG and IgM levels were measured using PCR and ELISA techniques, respectively. Genotypic variants of the NRAMP1 gene were also examined. Statistical analyses, including Mann-Whitney tests and univariate logistic regression, were employed to evaluate the data. Significant differences were observed in NRAMP1 protein expression and IgG and IgM levels between the patient and household contact groups. The study also highlighted the role of the NRAMP1 gene and its D543N and 3'UTR polymorphisms in leprosy susceptibility. No significant differences were observed in the genotype variants of INT4 between the two groups. These findings emphasize the potential of integrating PCR technology with serological tests to enhance diagnostic precision in leprosy. They also suggest the need for further research to clarify the role of NRAMP1 and its polymorphisms in leprosy susceptibility and resistance.

Keywords: Leprosy; NRAMP 1; leprae household contact; polymorphisms; *Mycobacterium leprae*.

INTRODUCTION

Leprosy remains a neglected disease primarily prevalent in developing countries with tropical climates. Also known as Hansen's disease, it is a chronic bacterial infection caused by *Mycobacterium leprae* (M. leprae) (Ghosh & Chaudhuri, 2015). The disease predominantly affects the skin, peripheral nerves, upper respiratory tract mucosa, and eyes, presenting with diverse symptoms and severities (Putri *et al*., 2022; WHO, 2023). Ridley and Jopling classified leprosy into five categories based on clinical and histological criteria, ranging from tuberculoid leprosy (TT) with few flat lesions and minimal nerve involvement, to lepromatous leprosy (LL) with numerous lesions and extensive nerve damage (Ridley & Jopling, 1966). The World Health Organization (WHO) further classifies leprosy as paucibacillary (five or fewer skin lesions) and multibacillary (six or more skin lesions) (Kumar *et al*., 2017; WHO, 2023).

In 2019, 202,185 new leprosy cases were reported globally to the WHO (WHO, 2020). Although the WHO's target of reducing leprosy prevalence to less than 1 per 10,000 was achieved in 2000 and by most nations by 2010, the reduction in new cases has been limited. In 2019, countries such as Brazil, India, and Indonesia reported over 10,000 new cases, with India recording 114,451 cases, followed by Brazil with 27,863 and Indonesia with 17,439 (WHO, 2020). The progression of these infections is influenced by factors including nutritional status, co-infections, environmental microbial exposure, and prior vaccinations (Adriaty *et al*., 2020). Despite WHO's efforts to improve leprosy control, the detection rates of *Mycobacterium leprae* suggest only a modest decline in the disease's prevalence (Martinez *et al*., 2014).

In Indonesia, the leprosy conditions continue to be a significant public health concern, The country consistently reports new leprosy cases, with 84.5% being Multibacillary. Of these, 8.9% are children, and 6.7% of these pediatric cases result in disabilities (Kemenkes, 2019). These cases are primarily concentrated in specific regions, especially in eastern Indonesia. East Java is particularly affected, with the highest patient count spread across 37 districts/cities. The northern coast of East Java is a major hotspot, whereas its southern coast has a reduced prevalence. Despite efforts to eliminate leprosy, the incidence of *Mycobacterium leprae* remains high. The infection rate among contacts of leprosy patients is particularly high (Ezenduka *et al*., 2012). Some specialists recommend decentralizing leprosy control through contact examination, asserting that household contact examinations are the most economical method to identify new cases (Ezenduka *et al*., 2012; Gama *et al*., 2019).

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While some individuals are susceptible to infection, others remain unaffected even after prolonged exposure to leprosy patients (Penna *et al*., 2016). Numerous household contacts residing in leprosy-endemic regions for extended periods display no clinical manifestations. These individuals, although infected, present sub-clinical infections, allowing them to recover without exhibiting overt disease symptoms. This differential susceptibility raises questions about the underlying mechanisms of resistance and immunity.

To eliminate leprosy, the role of the Natural Resistance Associated Macrophage Protein 1 (NRAMP1) gene in determining immunity and susceptibility has become a central point. This gene, also referred to as NRAMP1 or SLC11A1 encodes a membrane protein primarily located in macrophages and select leukocytes (Jabado *et al*., 2000). It plays a crucial role in intracellular defense against pathogens, including *Mycobacterium leprae*. The NRAMP1 gene functions by regulating the bacterial replication rate within macrophages, in part by controlling divalent cation concentrations. Recent genetic research highlighted the association between allele variants at the human NRAMP1 locus and susceptibility to both leprosy and tuberculosis (Hatta *et al*., 2010). This finding suggests the potential of NRAMP1 as a diagnostic marker for these diseases. Nonetheless, while gene polymorphisms can modulate the host's immune response, additional factors also contribute to susceptibility.

The complexity of the NRAMP1 protein's role becomes apparent when considering the bacterium's reproduction. If the NRAMP1 protein is not expressed upon the entry of *Mycobacterium leprae*, the bacterium proliferates (Abel *et al.*, 1998). This triggers a humoral immune response and forms antibodies against PGL-1, a component of the bacterium's outer cell wall. Conversely, NRAMP1 expression in household contacts exposed to leprosy likely inhibits the growth of *Mycobacterium leprae*, preventing the formation of these antibodies. Hatta *et al.* (2010) emphasize this point, suggesting that individuals with low NRAMP1 protein expression might be unable to counteract the exposure to incoming *Mycobacterium leprae*. Such exposure might then evolve into an adaptive immune response, marked by the presence of antibodies against PGL-1. Given these findings, understanding the role of NRAMP1's in leprosy detection and the importance of examining household contacts is crucial to combat this ancient disease.

This study aims to explore the role of NRAMP1 protein expression, D543N, 3'UTR, and INT4 genotype variants, as well as the levels of anti-PGL-1 IgG and IgM in both the household contact group and the Multibacillary leprosy patient group. Specifically, it will compare the NRAMP1 protein expression in macrophages, the D543N, 3'UTR, and INT4 genotype variants, and the levels of anti-PGL-1 IgG and IgM between the household contact group and the Multibacillary leprosy patient group. Additionally, the study aims to evaluate the correlation between the NRAMP1 protein expression, the D543N, 3'UTR, INT4 genotype variants, and the levels of anti-PGL-1 IgG and IgM to the incidence of Multibacillary leprosy cases.

MATERIALS AND METHODS

Population and Sample

The study comprised 23 Multibacillary leprosy patients (mean age: 31.9, 15 males) who visited the Leprosy Division of the Outpatient Unit of Dermatology and Venereology at Dr. Soetomo Regional General Hospital (RSUD) in Surabaya, Indonesia. Patients were categorized as multibacillary (MB) if they exhibited more than five lesions and/or tested positive in bacilloscopic, in line with the operational classification set by the World Health Organization. They were further classified as LL, BL, or BB types based on the Ridley and Jopling criteria and/or tested positive in the Acid-Fast Bacilli (BTA) examination. The inclusion criterion was that patients had not yet undergone MDTL therapy.

The control group consisted of 28 household contacts (14 males, mean age: 33.29). These contacts were defined as individuals who had resided with Multibacillary leprosy patients for a minimum of one year before the patient's diagnosis and had not been clinically or bacteriologically diagnosed with leprosy. Additionally, they were not afflicted with worm infestations, confirmed by negative results from a comprehensive fecal examination. Worm infections, including hookworms, can lead to iron deficiency. This deficiency can prompt an adaptive immune response, increasing NRAMP1 levels – a compensatory mechanism to optimize iron utilization and enhance immune defense against infections (Forbes & Gros, 2001). Additionally, all controls exhibited normal complete blood counts and urinalysis results, ensuring the reliability of our comparisons.

Concerning the selection of household contacts, patients who present for treatment at this facility are typically unaccompanied by family members or relatives residing in the same household. This logistical constraint prohibited the possibility of obtaining household contact samples from the same population cohort as the leprosy patients. Thus, the selection of household contacts for this study was focused on Sampang District, given its persistently high incidence of leprosy. Data from the Sampang District Health Office (2015) indicated 486 reported cases in 2014, which slightly decreased to 339 cases in 2015. Moreover, a longitudinal analysis over a decade no significant decline in new leprosy cases, thus justifying our targeted sample selection from this region. These control subjects were randomly selected from the Kamuning Public Health Center in Sampang District.

Exclusion criteria for both patients and household contacts included the presence of chronic or infectious diseases such as diabetes mellitus, tuberculosis, hypertension, malignant diseases, or any other condition compromising the immune system. Participation in the study was voluntary, with participants providing informed consent after receiving a detailed explanation. Ethical approval for this study was granted by the Ethical Committee of RSUD Dr. Soetomo (234/Punke/KKE/IV/2015).

Blood Sample Collection Procedure

A tourniquet is applied to the volar region of the subject's forearm for fixation. The area is disinfected using 70% alcohol. Blood is drawn from the subject using a 5cc disposable syringe. The blood sample is then transferred into tubes containing EDTA and tubes without EDTA.

Procedure ELISA analysis anti-PGL-I

Fifty microliter of coating buffer and the NT-P-BSA working solution antigen are added to the microplate, which has been divided according to a specific scheme. The microplate is then incubated for 1 hour at 37°C. After incubation, the microplate is washed three times with a washing buffer (PBST solution). 200 µl of blocking buffer is added to the microplate and incubated for another hour at 37°C. After this incubation, the blocking buffer is discarded. 50 µl of serum, which has been diluted with a dilution buffer at a ratio of 1:300, is added to the microplate. The plate is then incubated again for 1 hour at 37°C. Following this incubation, the microplate is washed three times with the washing buffer. 50 µl of the Secondary Antibody (either IgG or IgM, placed according to a specific scheme) is added to the microplate. This antibody has been diluted with a dilution buffer at a ratio of 1:2000. The microplate is then incubated for another hour at 37°C. After this incubation, the microplate is washed three more times with the washing buffer. Total of 100 µl of substrate solution is added to the microplate until a yellow/orange color develops, and the reaction time is recorded. The coloring reaction is halted after approximately 10-30 minutes (the exact time is determined based on the optimal coloring duration) by adding 100 µl of stopping solution. The absorbance value (OD) is measured using an ELISA Reader. The collected data is then saved and processed using specific Biolise software.

Procedure PCR-RFLP

DNA extraction is a crucial initial step in genetic analysis. In our study, venous blood samples served as the primary source of DNA, which was meticulously extracted using Centrifuge Eppendorf, 5424. The subsequent PCR amplification was executed in a 50 µl reaction volume. This volume encapsulated 15-60 ng of the previously extracted genomic DNA and 0.40 µM of specific primers. The thermocycling process was facilitated by PCR Applied Biosystems 2720 Thermal Cycler.

For the D543N and 3'UTR variants, thermocycling parameters were set to begin with a 5-minute incubation at 95°C. This was succeeded by 35 cycles, each comprising 45 seconds at 94°C, 45 seconds at 57°C, and 45 seconds at 72°C. The process culminated with a final extension step lasting 10 minutes at 72°C. The thermocycling parameters for the INT4 variant involved an initial 5-minute incubation at 95°C, followed by 35 cycles of 30 seconds at 94°C, 45 seconds at 59°C, and 1 minute at 72°C, concluding with a 10-minute extension at 72°C. Post-amplification, the amplicons were visualized using electrophoresis in a 1.8% agarose gel, which was subsequently stained with ethidium bromide. These amplicons were then subjected to restriction fragment length polymorphism (RFLP) analysis. The D543N, 3'UTR, and INT4 amplicons were digested using the restriction enzymes *AvaII*, *FokI*, and *ApaI*, respectively. The products of this restriction-enzyme digestion were again visualized using electrophoresis, this time in an agarose gel concentration ranging between 1.8% and 3.8%, followed by staining with ethidium bromide.

To further validate and confirm the presence of polymorphisms, direct sequencing of each band post-RCR-RFLP analysis was undertaken. A volume of 5 µl of the PCR product was treated with 2 µl of ExoSAP-IT from USB, Cleveland, Ohio, and subjected to a two-step incubation: first at 37°C for 15 minutes and then at 80°C for 15 minutes. Sequencing was facilitated using the Electrophoresis Container Mupid 2x. The sequencing reaction was performed in a 20 µl final volume, which included 7 µl of the PCR product, 3.2 pmol of the forward primer, 4 µl of Ready Reaction Mix, and 2 µl of BigDye Sequencing Buffer. The sequencing parameters were set to start with a 1-minute incubation at 96°C, followed by 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C, 1 minute at 60°C, and a concluding step of 10 minutes at 72°C, all in the PCR Applied Biosystems 2720 Thermal Cycler. The interpretation of the immunocytochemical staining results will be based on the count of positive monocytes observed using 10 fields of view at a 40X magnification, utilizing a USB PC Camera 301P.

Data Analysis

We employed the Mann-Whitney test to evaluate the differences in NRAMP1 expression and anti-PGL-1 levels between MB leprosy patients and household contacts. To determine if NRAMP1 expression and anti-PGL-1 levels are potential risk factors for leprosy development, a univariate logistic regression analysis was executed. Furthermore, we investigated the association between the likelihood of developing leprosy and specific genotype variants – namely D543N, 3'UTR, and INT4 – using the Chi-square test. The outcomes of these statistical analyses were presented along with odds ratios (OR) and their corresponding 95% confidence intervals, providing insights into the potential genetic tendency to leprosy.

RESULTS

Figures 1 and 2 show the immunocytochemical examinations on 23 PBMC (Peripheral Blood Mononuclear Cell) samples from MB leprosy patients and 23 PBMC samples from household contacts to detect NRAMP1 protein expression in the cytoplasm of monocytes, respectively.

As depicted in Table 1, a Mann-Whitney *U* test reveals a significant difference in NRAMP1 expression levels between individuals diagnosed with leprae and their household contacts ($p < 0.001$). The computed odds ratio (OR=0.345, 95% CI=0.149 –

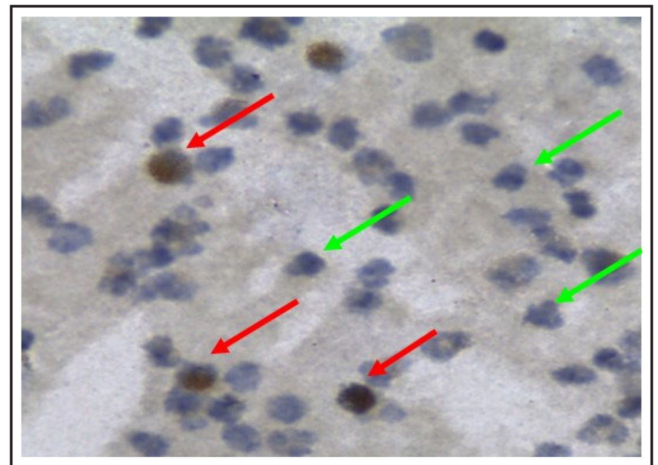

Figure 1. The Immunocytochemistry results of household contacts with *multibacillary* type using the BSA Indirect Enzyme Immuno Assay method. In the cytoplasm of positive monocytes, NRAMP1 protein is visible in a yellowish-brown color (indicated by a red arrow), while in the negative monocytes, the cytoplasm is colorless (indicated by a green arrow).

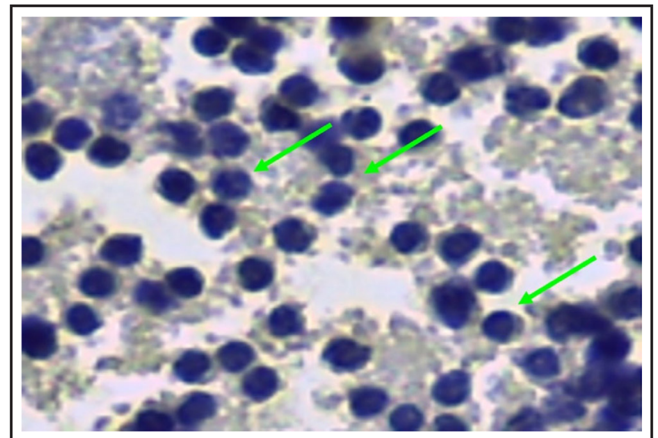

Figure 2. Immunocytochemistry results of *multibacillary* leprosy patients using the BSA Indirect Enzyme Immuno Assay method. In the negative monocytes, the cytoplasm is colorless (indicated by the green color).

Table 1. Distribution of Protein NRAMP1 expression, IgG and IgM between MB leprosy patients and household contact

Variable	Patients				Household				p-value	OR	95% CI
	Median	IQR	Min	Max	Median	IQR	Min	Max			
NRAMP1 expression	0	0.5	0	2	5.5	8.75	0	42	<0.001	0.35	0.149 – 0.799
IgG	1718.1	4765.3	46.02	29063	81.03	380.92	0	6840.1	<0.001	1	1.000 – 1.001
IgM	2299.7	1599.52	0	30896	574.73	312.5	87.49	1381.1	<0.001	1	1.001 – 1.003

Table 2. Distribution of Nramp1 genotype among between leprosy patients and household contact

Name	Genotype	Group		χ^2 (*p*-value)	OR	95% CI
		Patients	Household			
D543N	G/G (ref)	20	7			
	G/A	2	19	19.826 (<0.001)	27.143	4.997 – 147.438
	A/A	1	2	2.134 (0.144)	5.714	0.446 to 73.195
3'UTR	TG/TG (ref)	9	16			
	TG/del	0*	9			
	del/del	14	3	8.776 (0.003)	0.121	0.027 – 0.535
INT4	G/G	22	25			
	G/C	1	3	0.708 (0.400)	2.64	0.256 – 27.259

Note. As the TG/del category yielded a zero value, further statistical analysis could not be conducted.

0.799) highlights the association between the absence of NRAMP1 protein and an elevated risk of developing leprae. Individuals with diminished NRAMP1 protein expression are approximately 2.9 times more susceptible to contracting the disease.

It is interesting to note that the Odds Ratio associated with IgG and Ig-M of anti-PGL-1 is determined to be very close to 1.000, indicating a marginal increase in the odds of leprae occurrence corresponding to each unit increase in IgG or IgM.

Table 2 presents the distribution of D543N, 3'UTR, and INT4 genotype variants. A Chi-square test revealed a significant association between D543N genotypes (G/G vs G/A) and leprae occurrence (χ^2 (1) = 19.826, p<.001). The odds ratio of 27.143 (95% CI=4.997 – 147.438) explains that individuals with the G/G genotype are approximately 27 times more likely to develop leprae than those with the G/A variant. While an odds ratio of 5.714 indicates a potential increased risk of developing leprosy for individuals with the A/A genotype relative to those with the G/G genotype, this association did not reach statistical significance. Regarding the 3'UTR polymorphism, the odds of leprae disease occurrence were significantly lower in individuals with the TG/TG genotype compared to the del/del variant (OR=0.121, 95% CI=0.027 - 0.535). The TG/del genotype was not present among the sampled leprae patients; hence we could not process further calculations. Analysis of the INT4 polymorphisms revealed an odds ratio of 2.6, suggesting higher susceptibility to leprae in individuals with the G/G genotype relative to G/C carriers; however, this observation was not supported by statistical significance (95% CI: 0.256 - 27.259).

DISCUSSION

Our study revealed higher levels of NRAMP1 expression in household contacts compared to MB leprosy patients. This finding corroborates earlier research that reported increased NRAMP1 levels in pulmonary ward nurses compared to tuberculosis patients (Anggraini, 2008). This suggests a potential protective role for NRAMP1, where its reduced expression could signify increased susceptibility to leprosy. This aligns with NRAMP1's function in modulating intracellular concentrations of divalent metal ions such as Fe2+ and Mn2+, thus limiting the growth of intracellular pathogens like Mycobacterium leprae (Stocks *et al*., 2018).

NRAMP1 is a divalent metal ion transporter found in macrophages and granulocytes. It localizes to vesicles within the endosomal pathway and is recruited to the phagosomal membrane during phagocytosis. By regulating divalent metal concentrations, NRAMP1 indirectly controls the replication of intracellular pathogens. Mutations in NRAMP1 have been linked to heightened susceptibility to various diseases, including tuberculosis, leishmaniasis, and autoimmune conditions in both rats and humans (Peracino *et al*., 2013).

NRAMP1 influences microbial survival within macrophages by interacting with the pathogen's superoxide dismutase (SOD), which requires Mn2+ or Fe2+ as cofactors. The protein facilitates the transport of Fe2+ into the macrophage cytoplasm and subsequent removal from organelles post-phagosome formation. This depletion of Fe2+ impedes the production of bacterial metalloenzymes within the phagosome, constraining the pathogen's ability to generate active enzymes such as SOD. In contrast, a dysfunctional NRAMP1 transporter could elevate Fe2+ concentrations in the phagosome, fostering mycobacterial growth and enhancing host susceptibility to infection (Supek *et al*., 1997).

Anti PGL-1

Utilizing the enzyme-linked immunosorbent assay (ELISA) methodology, we observed elevated levels of higher levels of IgG and IgM in leprae patients compared to their household contacts. This finding supports previous research (Bakker *et al*., 2006) which also employed ELISA techniques to measure IgM antibodies against the phenolic glycolipid-I (PGL-I) of Mycobacterium leprae. Bakker's study emphasized the heightened risk of leprosy among household contacts, thus highlighting the importance of contact status as a significant risk factor for the disease.

Despite the elevated risk associated with close contact, household members displayed lower levels of IgG and IgM antibodies. This discrepancy in immunological response could suggest a differential activation of the immune system, potentially indicating the absence of an active infection among these contacts. This interpretation is consistent with Baker's focus on serological status, which showed varying rates of leprosy incidence among individuals who were seropositive and seronegative for anti-PGL-1 antibodies.

Our findings are also consistent with (Cabral *et al.*, 2013), who suggested that measuring serum IgG/IgM and salivary anti-PGL-1 IgA/IgM levels could serve as a monitoring tool for subclinical infection in household contacts of leprae patients. Furthermore, (Bakker *et al*., 2006) and (Penna *et al*., 2016) indicated that individuals who were anti-PGL-1 positive at baseline were three times more likely to develop leprosy compared to those who were seronegative. However, (Penna *et al*., 2016) also underline that the proportion of leprosy cases that were PGL-1 positive at baseline was always under 50%. According to these authors, while ELISA anti-PGL-1 tests are indicative of exposure to M. leprae, they are not definitive diagnostic tools for leprosy or for infection with the bacteria.

Zenha *et al*. (2009) proposed that monitoring anti-PGL-1 levels during multi-drug therapy could be a sensitive tool for evaluating treatment efficacy. This is because anti-PGL-1 levels are reflective of the total bacterial index, providing a measure of the bacterial load in the patient.

It is essential to consider the role of PGL-I in the pathogenesis of Mycobacterium leprae. The bacteria specifically bind to laminin, a glycoprotein that is a key component of the basal membrane. This interaction facilitates the bacteria's survival by disrupting lipid homeostasis within infected cells, leading to an environment that allows Mycobacterium leprae to thrive. PGL-I also downregulates the immune response, inhibiting the maturation and activation of dendritic cells, and making it easier for the bacteria to infect macrophages and Schwann cells.

Furthermore, while our study found a statistically significant relationship between IgG and IgM levels and leprosy, the clinical implications may be limited due to the narrow confidence interval which emphasizes the need for a more comprehensive examination of other potential risk factors to gain a fuller understanding of leprosy's etiology and progression. A more comprehensive approach could encompass integrated molecular and serological analyses (Gama *et al*., 2019), as well as complex segregation analysis (CSA) studies, such as 10p13, 6q25-27, and 6p21 (Cambri & Mira, 2018). Furthermore, a recent review on advancements in leprosy diagnosis summarizes the molecular biology and biotechnological technologies to validate the clinical diagnosis of leprosy (Sharma & Singh, 2022).

NRAMP Genotype

In the realm of leprosy research, various research efforts have focused on the NRAMP1 gene and its polymorphic variants. Located in the chromosome 2q35 region, the NRAMP1 gene is expressed in macrophages and encodes a protein found in lysosomal membranes. During phagocytosis, this protein is recruited to phagosome membranes containing pathogens, where it acts as a transporter of iron and other divalent ions. Iron plays a crucial role in both host immune defense and mycobacterial growth (Mazini *et al*., 2016).

Genotypic variants of the NRAMP1 gene have been studied in different populations and subtypes of leprosy. We found that in MB leprae patients, the G/G genotype was predominant, whereas household contacts mainly exhibited the G/A genotype. Additionally, the 3'UTR TGTG del/del genotype was more common among household contacts, while the TGTG/TG genotype was more frequent among patients. However, no significant differences were observed in the genotype variants of INT4 between household contacts and MB patients. This aligns with Hatta's 2010 study in South Sulawesi, Indonesia, which found an association of the INT4 polymorphism only with the paucibacillary type of leprosy and not with the MB type.

Contrastingly, a study conducted in Mali, West Africa, did not find an association with leprosy per se but did identify a link between the NRAMP13'UTR polymorphism and specific leprosy subtypes (Meisner *et al.*, 2001). Similarly, research in Thailand involving the INT4, D543N, and 3'UTR polymorphisms of NRAMP1 found no significant differences in the distribution of genotypes and allele frequencies between leprosy patients and control groups (Vejbaesya *et al*., 2007).

Given the heterogeneous findings across various populations and subtypes of leprosy, it becomes evident that while the NRAMP1 gene and its polymorphisms may play a significant role in susceptibility and resistance to leprosy, the results are not universally applicable. This suggests the need for further research to clarify these associations and their implications for leprosy diagnosis and treatment.

Limitations

This study has several limitations that warrant consideration. First, the absence of a health control group deprives us of a baseline for normal levels of IgG and IgM antibodies. This makes it challenging to determine the significance of elevated antibody levels in leprae patients and their household contacts. Second, the small sample size constrains our ability to identify the significant roles of certain variables, potentially affecting the study's statistical power and generalizability. Third, antibody levels are subject to fluctuation over time; therefore, a single measurement may not accurately capture these variations, thereby impacting the predictive value of these immunological markers. Lastly, the study design lacks a longitudinal approach or long-term follow-up, limiting our understanding of the full spectrum of disease progression and the long-term efficacy of preventive measures. Future research should consider employing a more comprehensive design that includes a health control group and longitudinal tracking to address these limitations.

While acknowledging the temporal gap since our study's completion nearly a decade ago, it is important to emphasize that the exploration of the NRAMP1 gene for early detection and the analysis of household contacts in leprosy research remain largely overlooked areas. According to a recent review (Li *et al*., 2024), there is only a solitary study from Brazil investigating the NRAMP gene's influence on leprosy susceptibility. This gap highlights the novelty aspect of our work within the Indonesian context, considering the country's unique genetic and environmental conditions. Moreover, there is an escalation of leprosy rates in Indonesia to 0.55 per 10,000 residents in 2022 (Widi, 2023). Therefore, our findings remain valuable, contributing to global leprosy understanding and guiding future research directions.

Implications

This study offers the potential use of combining PCR results with serological tests to enhance the predictive value of the PCR technology for diagnosing leprosy. This has been also proposed by (Martinez *et al*., 2014) who posited that although PCR could be a useful tool for the detection of subclinical infection, only a few investigations have consistently associated the presence of the *Mycobacterium leprae* DNA with further development of the disease among household contacts (Reis *et al*., 2014). Earlier diagnosis of leprosy is invaluable for mitigating the severity of the disease and preventing disability.

Furthermore, the study emphasizes the importance to monitor IgG and IgM anti-PGL-1 antibodies in individuals who are in close contact with leprosy patients. According to (Bakker *et al*., 2006) who study across five islands in Indonesia, individuals residing in larger households – with more than seven family members – had a 3.1 times higher risk compared to those in smaller households with 1-4 members. Those who were seropositive had a 3.8 times higher risk compared to seronegative individuals. The elevated risk associated with seropositivity further highlight the importance of serological tests in assessing leprosy risk. This could guide healthcare providers in customizing preventive measures for individuals who are at higher risk, thereby aiding in the early detection and treatment of the disease.

CONCLUSIONS

In summary, this study reveals the significant differences in NRAMP1 protein expression as well as IgG and IgM between multibacillary leprosy patients and household contacts. This study highlights the complex nature of leprosy diagnosis and management, emphasizing the potential of integrating PCR technology with serological tests for enhanced diagnostic precision. The study also emphasizes the significant role of the NRAMP1 gene and D543N and 3'UTR polymorphism in determining leprosy susceptibility. This suggests a need for further research to clarify these associations and their implications for leprosy diagnosis and treatment. Moreover, the study accentuates the importance of monitoring IgG and IgM anti-PGL-1 antibodies, particularly in individuals in close contact with leprosy patients. Notwithstanding the limitations, this study not only contributes to the growing body of literature on leprosy's etiology but also provides actionable insights that could inform public health policies. By focusing on antibody monitoring, the study

could facilitate the development of more targeted and effective preventive measures, potentially reducing the incidence of leprosy and its impact on healthcare systems.

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Conflict of interest
The author(s) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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