The qRT-PCR in A Nasal Swab Leprosy Patient's at Dr. M. Djamil Padang Hospital

Tutty Ariani[1], Ennesta Asri[2], Andani Eka Putra[3]
[1]Department of Dermatology and Venereology of Dr. M. Djamil General Hospital, Padang, Indonesia
[2]Faculty of Medicine, Andalas University, Padang, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Andalas University, Padang, Indonesia
Email: tuttyariani@med.unand.ac.id

Abstract

Leprosy, caused by *Mycobacterium leprae*, in 2016, Indonesia reported 16,826 new leprosy cases, with a prevalence rate of 0.71 per 10,000 individuals and a new case detection rate of 6.5 per 10,000 individuals. This study describes the result of a quantitative real-time polymerase chain reaction (qRT-PCR) in the nasal mucosa smear of a leprosy patient at the Dermatology and Venereology Polyclinic of Dr. M. Djamil Padang Hospital. This study adopts an observational approach with a width cut design, specifically employing a cross-sectional study methodology on leprosy patient at the Dermatology and Venereology Polyclinic of Dr. M. Djamil Padang Hospital, Indonesia between February 2023 until February 2024. The qRT-PCR quantity value of nasal mucous discharge is analyzed by a real-time PCR machine. The provided data presents characteristics of a sample population (N = 36) with leprosy. The majority of the leprosy-affected individuals in this study are between 8 and 68 years old, with a median age of 34. According to the World Health Organization (WHO) classification indicates that 83.33% of cases are multibacillary (MB). A combination RLEP/ qRT-PCR assay was developed to enable laboratory-based care and follow-up of leprosy patients attending our outpatient polyclinic in M. Djamil, Padang. The assay offers a sensitive and precise method for assessing the bacterial load and viability of *M. leprae* from nasal swab samples. It can be used for treatment response monitoring and early diagnosis.

Keywords: *Nasal Mucosal Smear, Mycobacterium Leprae, qRT-PCR.*

– – – – – – – – – – ◆ – – – – – – – – – –

A. INTRODUCTION

Leprosy, caused by *Mycobacterium leprae*, is characterized by its acid-fast bacillus morphology and resistance to in vitro culture. The bacteria primarily infect various tissues, including the reticuloendothelial system, epidermis, oral mucosa, upper respiratory tract, eyes, muscles, and testes (Siswanto et al., 2020). Transmission occurs through respiratory droplets or direct cutaneous contact. Despite being a curable disease, challenges in eradicating leprosy persist due to deficiencies in diagnostic tools, prevention and treatment strategies, and control and monitoring of high-risk populations (Farah et al., 2017).

In 2016, Indonesia reported 16,826 new leprosy cases, with a prevalence rate of 0.71 per 10,000 individuals and a new case detection rate of 6.5 per 10,000 individuals. 83% of cases were multibacillary (MB), grade 2 disabilities, or pediatric cases. Eleven provinces still exceed a prevalence of one case per 10,000 inhabitants, with 139 districts and cities surpassing the same threshold. Provincial-level

elimination efforts will benefit from district- and city-level initiatives (Republik Indonesia, 2020).

Despite achieving leprosy eradication in West Sumatra with a prevalence rate of 0.12 cases per 10,000 population, 19 districts in the region reported new cases in 2021, indicating ongoing disease transmission and delayed case detection (Dinas Kesehatan Padang Pariaman, 2021).

Mycobacterium leprae exhibits minimal invasiveness and pathogenicity, typically entering the body through the respiratory tract. The bacteria migrate to nerve tissues, penetrating Schwann cells, and can also be found in macrophages, muscle cells, and endothelial cells. The state of the bacteria within Schwann cells or macrophages depends on the individual's resistance (Jadhav et al., 2005).

Infections stimulate the immune system, involving lymphocytes and histiocytes, attacking infected tissues. Clinical manifestations may include nerve involvement with decreased sensibility. The patient's immune response strength determines the further progression of the condition if not detected and managed promptly. Cellular Immune Systems (CIS) provide protection, resolving lesions or progressing to Paucibacillary (PB) or Multibacillary (MB) leprosy. Chronic phases may exhibit sudden immune response escalation, resulting in inflammation—a leprosy reaction (types 1 and 2) (Nath et al, 2015).

Diagnostic challenges arise due to clinical manifestations resembling other skin diseases, necessitating additional procedures (Alferdi, 2022). At Dr. M. Djamil Padang Hospital, leprosy diagnosis relies on three cardinal signs: acid-fast bacteria in skin smears, nerve thickening, and anesthetic or hypoesthetic erythematous or hypopigmented patches (RI, 2018). Although the sensitivity of acid-fast bacteria in skin smears is low, especially in paucibacillary cases.

Alternative immunological methods, like T-cell immune response quantification via Interferon-gamma (IFNγ) production, have been consistent in various leprosy patients. However, challenges arise due to similar IFNγ secretion patterns in household contacts and PB cases. Current serological and Cell Mediated Immunity (CMI) based assays for early leprosy detection are unsatisfactory, necessitating alternative methods such as PCR (Jiang et al., 2022).

The PCR, a precise molecular technique, is used to identify and amplify nucleic acids (Reis et al., 2014). The implementation qRT-PCR has significantly improved *M. leprae* detection (Manta et al., 2019). Researchers use Quantitative PCR (qPCR) as an alternative method for DNA or RNA quantification (Tatipally et al., 2018)(Viljoen et al., 2005).

The qRT-PCR method, targeting the RLEP gene sequence in the *M. leprae* genome, is crucial for identifying *M. leprae* DNA because RLEP have 19-37 copied gene that can make this gene specific to *M. leprae*. (Goldsmith et al., 2019)(Wirayasa, 2022). Efforts to detect *M. leprae* DNA in diverse clinical specimens have shown sensitivity ranging from 50-70% (Morgado de Abreu et al., 2014). In one study, RLEP-based PCR detected 73% of patients with a zero bacterial index (Adhin, 2020)(Jadhav et al., 2005).

The study aims to evaluate the performance of the qRT-PCR on nasal mucosa smears from leprosy patients at the Dermatology and Venereology Polyclinic Dr. M. Djamil Padang Hospital. Progress in qRT-PCR-based methods, focusing on the RLEP gene, is essential for more effective identification of *M. leprae* in nasal mucosa smears from contacts of leprosy patients in West Sumatera (Adhin, 2020).

B. METHOD

This study adopts an observational approach with a width cut design, specifically employing a cross-sectional study methodology. The target population for this study encompasses all patients diagnosed with Pausibasiler type and Multibasiler type. The Inclusion Criteria are: all patients, including new patients, those undergoing Multidrug Therapy (MDT) treatment, or under surveillance, are eligible for inclusion and participants must express their willingness to participate in the study by providing informed consent with exclusion criteria: individuals unwilling to participate and serve as respondents are excluded from the study.

The examination and sampling procedures were conducted at the Dermatology and Venereology Polyclinic Dr. M. Djamil Padang Hospital. The collected samples were subsequently sent to the Biomedical Laboratory of Medicine Faculty, Unand between early February 2023 and February 2024. The qRT-PCR quantity value of nasal mucous discharge is analyzed by real-time PCR machine.

C. RESULTS AND DISCUSSION

The provided data presents characteristics of a sample population (N = 36) with leprosy. The majority of the leprosy-affected individuals in this study are between 8 and 68 years old, with a median age of 34. A balanced gender distribution is noted, with 44.4% being male. Education backgrounds vary, with the majority having at least a high school education. Housewives constitute a significant occupational group. Bacterial index results: negative: 14 (38.89%), 1+: 3 (8.33%), 2+: 5 (13.89%), 3+: 4 (11.11%), 4+: 7 (19.44%), 5+: 3 (8.33%). According to the World Health Organization (WHO) classification of leprosy, 83.33% of cases are multibacillary (MB). Various Ridley Jopling classifications further emphasize the diverse clinical manifestations of leprosy with the most are included to borderline lepromatous (BL) and borderline tuberculoid (BT). Leprosy reactions are present in a considerable proportion, with 16.67% experiencing Reversal Reaction and 25% presenting with Erythema Nodosum Leprosum (ENL). PCR examinations reveal high positivity rates in both nasopharynx (94.44%) and lesions (88.89%), suggesting a prevalent *mycobacterial* presence.

Table 1. Demographic and Clinical Characteristic of Leprosy Patient

	n (%) (N = 36)
Age (years), median (min-max)	34 (8-68)
Sex	
Male	16 (44,4)
Female	20 (55,6)

Education Background	
No Formal Education	7 (19,4)
Primary-Junior High School	10 (27,3)
Senior High School	17 (47,2)
College	2 (5,6)
Occupation	
Housewife	17 (42,5)
Employer	2 (5)
Labourer	1 (2,5)
Student	4 (10)
Farmer	5 12,5)
Self-employer	7 (17,5)
Bacterial Index	
Negative	14 (38,89)
1+	3 (8,33)
2+	5 (13,89)
3+	4 (11,11)
4+	7 (19,44)
5+	3 (8,33)
WHO Classification	
Multibacillary (MB)	30 (83,33)
Pausibacillary (PB)	6 (16,67)
Ridley Jopling Classification	
Tuberlucoid (TT)	2 (5,56)
Borderline Tuberculoid (BT)	12 (33,33)
Mid Bordeline (BB)	2 (5,56)
Borderline Lepromatous (BL)	13 (36,11)
Lepromatous (LL)	7 (19,44)
Leprosy Reaction	
Reversal Reaction	6 (16,67)
Erythema Nodosum Leprosum (ENL)	9 (25)
Polymerase Chain Reaction Examination	
Nasopharyinx positive, (mean of CT value)	34 (94,44)
Lesion positive, (mean of CT Value)	32 (88,89)

The prevalence of leprosy cases in Indonesia is quite significant. According to the Indonesian Ministry of Health, in 2022 there will still be 15,052 registered cases of leprosy with a prevalence of 0.55 per 10,000 population. In 2021, there will still be 13,487 active leprosy cases recorded with 7,146 new cases discovered. According to the Ministry of Health, the prevalence of leprosy in West Sumatera is the lowest in Indonesia, namely 0.1 per 10,000 population. Number of leprosy patients in the Dermatology and Venereology Polyclinic of the Hospital. Dr. M. Djamil Padang in 2023 there will be 36 cases with an average age of 34 years. This is following research

by Meki, et al. (Semarang, 2022) which shows that the age range of leprosy patients is predominantly 33-55 years old (50%). These results indicate a high level of productivity and mobility (Pranata et al., 2022).

There were 16 (44.4%) males and 20 (55.5%) females. This is in line with research by Ronald et al (Brazil, 2016) but is inversely proportional to research conducted by Nia, et al (Serang, 2022) which states that male sufferers are more dominant. The number of respondents were more female than male, because the mobility of men is higher. After all, they have to earn a living outside the home, so few come to the Dermatology and Venereology Polyclinic. The results of the Indian Association of Leprologists (IAL) report found that in various countries in the world, including Indonesia, men suffer from leprosy more than women with a ratio of 2:1. The low incidence of leprosy in women is likely due to environmental and socio-cultural factors (Rengasamy et al., 2020) (Kurniatillah et al., 2022).

In this study, the most common patient education category was high school. This is in line with research conducted by Ronald, et al (Brazil, 2016). Meanwhile, the undergraduate education level in the sample was only 2 out of 36 people. This can be related to the theory that the spread of leprosy is also related to economic status, nutrition, and hygiene conditions, where these three things are also related to a person's level of education (Martins et al., 2016).

Based on the occupational group of 36 leprosy patients, the largest occupational group was housewives, this was associated with the gender dominance in the sample being female. This is inversely proportional to research conducted by Meki et al., 2022, where the highest number of occupational groups were civil servants. This could be because, in the research conducted by Meki et al., the most common gender was male (Pranata et al., 2022).

For the bacterial index, the most negative results were obtained (38.89%), this is in contrast to the research of Jasmin et al in 2022 who conducted research on 30 leprosy patients and obtained results that 24 of them had a bacterial index of >3+ (Jasani et al., 2022). The bacterial index is associated with the number of leprosy patients. Quantity of bacterial bacilli seen from a microscope in a particular sample, the higher the bacterial index, the greater the bacterial load, which also determines the severity of the disease and future therapy plans. The differences in bacterial index results can be attributed to technical errors in performing the Slit Skin Smear, sampling techniques, calculations on the microscope, observer variations in clinical assessment, and various other factors that can cause differences (Jha et al., 2016).

Based on the WHO classification, there were more patients in the MB group (83.33%) while for the Ridley Jopling classification, the largest group was BL (36.11%). This is different from the research of Ruchi et al (India, 2012) who conducted research on 100 leprosy patients and obtained the results that 60 of them fell into the PB group (Gupta et al., 2012). As well as research conducted by SM Jha et al (Nepal, 2016), the results of their research classified leprosy based on Ridley Jopling found that the largest group was in the BT group (Jha et al., 2016). This difference can occur depending on when the patient arrives for treatment. The later

the patient arrives for treatment, the more severe the patient's disease.

Leprosy reactions occurred in 15 patients with 9 people experiencing ENL reactions and 6 people experiencing type 1 reactions, in contrast to the research of Rabia et al. (Pakistan, 2021), the results obtained were more in type 1 reactions (60%) and ENL reactions (40 %). The leprosy reaction is triggered by the body's immune system response to the presence of the leprosy bacteria. So because of this, there may be differences in results, because the immune reaction depends on each patient's body (Muddebihal et al., 2023).

The PCR results were compared between nasopharyngeal and lesion samples, and a higher positive rate was obtained in the nasopharynx (94.44%). Lucas et al. in 2005 also carried out a PCR examination which was used to strengthen the DNA sequence of *M. leprae* in nasal mucosal biopsies and showed that the nasal also plays a role in transmitting this bacillus, it can detect the early stages of leprosy even before the lesions are visible on the skin, nerves or other parts of the body (Suneetha, Arunthathi, Kurian, & Chacko, n, 2000)(Patrocínio et al., 2005). In his research, the results showed that PCR could detect DNA from *M. leprae* in 36 (69.2%) cases, a figure higher than that found in skin smears (10–50%). Martinez et al who tested RNA-based viability for *M. leprae* in nasal swabs found a sensitivity of 97.8% with a specificity of 73.3%. The RNA test combined with quantification with RLEP qPCR was successfully tested as a diagnostic test in leprosy patients. This study also concluded that this provides evidence that the mucosa can be a secondary site of transmission and infection of *M. Leprae* (WHO, 2023).

However, it should be emphasized that laboratory examinations are not sufficient to diagnose or classify leprosy. Its nature is as a tool to facilitate the diagnosis of a case. The finding of detection of *M. leprae* bacilli in the nasal mucosa using qPCR in leprosy cases strengthens the fact that leprosy transmission may originate from the nasal cavity. In addition, the results of this study reaffirm the important role of contacts in the epidemiological chain and emphasize the need for close monitoring of these individuals.

D. CONCLUSION

A combination RLEP/ qRT-PCR assay was developed to enable laboratory-based care and follow-up of leprosy patients attending our outpatient polyclinic in Dr. M. Djamil Padang hospital. The assay offers a sensitive and precise method for assessing the bacterial load and viability of *M. leprae* from nasal swab samples. It can be used for treatment response monitoring, early diagnosis, and research into the potential role of *M. leprae* nasal carriage in aerosol infection-mediated human-to-human transmission. While thankfully neither budgetary nor logistical constraints prevented the assay from being used on individual patients in our own context, these assays are often more appropriate for epidemiological research problems than for tailored therapy.

However, further clinical studies are still needed regarding the application of qRT-PCR from nasal mucose in diagnosing leprosy to prove its efficacy. Apart from

that, there needs to be cooperation between health workers and government so that this method can be applied widely in Indonesia, it is hoped that leprosy in this country can be eradicated.

ACKNOWLEDGEMENTS

The authors would like to thank the Dr. M. Djamil General Hospital, Padang, West Sumatera, Indonesia to complete the whole process of data collection.

REFERENCES

1. Adhin, A. (2020). Desain Sekuen Primer Dan Probe Quantitative Polymerase Chain Reaction Gen Spesifik RLEP Mycobacterium Leprae. *Jurnal Analis Medika Biosains (JAMBS)*, *7*(1), 1. https://doi.org/10.32807/jambs.v7i1.159
2. Alferdi, A. (2022). Resiliensi Hospitality Yesus terhadap Orang Kusta dan Implementasinya bagi Orang Percaya di Masa Pandemi Covid-19. *KENOSIS: Jurnal Kajian Teologi*, *8*(1), 41–54. https://doi.org/10.37196/kenosis.v8i1.384
3. Dinas Kesehatan Padang Pariaman. (2021). *Laporan Tahunan Puskesmas Pauh Kambar*.
4. Goldsmith, L. A., Katz, S. I., & Gilchrest, B. A. (2019). *Fitzpatrick_s Dermatology in General Medicine*. 2288.
5. Gupta, R., Kar, H. K., & Bharadwaj, M. (2012). Revalidation of various clinical criteria for the classification of leprosy--a clinic-pathological study. *Leprosy Review*, *83*(4), 354–362.
6. Jadhav, R. S., Kamble, R. R., Shinde, V. S., Edward, S., & Edward, V. K. (2005). Use of reverse transcription polymerase chain reaction for the detection of Mycobacterium leprae in the slit-skin smears of leprosy patients. *Indian Journal of Leprosy*, *77*(2), 116–127.
7. Jasani, J., & Maru, A. (2022). A clinico-histopathological analysis and bacillary index in a study of skin biopsy of leprosy. *International Journal of Health Sciences*, *6*(June), 11014–11023. https://doi.org/10.53730/ijhs.v6ns5.11273
8. Jha, S., Dangol, A., Shakya, S., & Jha, B. (2016). Clinico-bacterial correlation of bacterial index in Hansen's disease. *Journal of Pathology of Nepal*, *6*(12), 998–1000. https://doi.org/10.3126/jpn.v6i12.16285
9. Jiang, H., Shi, Y., Chokkakula, S., Zhang, W., Long, S., Wang, Z., Kong, W., Long, H., Wu, L., Hu, L., Yao, Q., & Wang, H. (2022). Utility of Multi-target Nested PCR and ELISPOT Assays for the Detection of Paucibacillary Leprosy: A Possible Conclusion of Clinical Laboratory Misdiagnosis. *Frontiers in Cellular and Infection Microbiology*, *12*(April). https://doi.org/10.3389/fcimb.2022.814413
10. Kurniatillah, N., Hayat, F., & Lusinto, W. H. (2022). Situasi Penyakit Kusta Di Kabupaten Serang Tahun 2020. *Journal of Baja Health Science*, *2*(02), 148–157. https://doi.org/10.47080/joubahs.v2i02.2194
11. Manta, F. S. N., Barbieri, R. R., Moreira, S. J. M., Santos, P. T. S., Nery, J. A. C., Duppre, N. C., Sales, A. M., Pacheco, A. G., Hacker, M. A., Machado, A. M., Sarno, E. N., & Moraes, M. O. (2019). Quantitative PCR for leprosy diagnosis and

monitoring in household contacts: A follow-up study, 2011–2018. *Scientific Reports*, *9*(1), 1–8. https://doi.org/10.1038/s41598-019-52640-5

12. Martins, R. J., Carloni, M. E. O. G., Moimaz, S. A. S., Garbin, C. A. S., & Garbin, A. J. Í. (2016). Sociodemographic and epidemiological profile of leprosy patients in an endemic region in Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, *49*(6), 777–780. https://doi.org/10.1590/0037-8682-0069-2016
13. Morgado de Abreu, M. A. M., Roselino, A. M., Enokihara, M., Nonogaki, S., Prestes-Carneiro, L. E., Weckx, L. L. M., & Alchorne, M. M. A. (2014). Mycobacterium leprae is identified in the oral mucosa from paucibacillary and multibacillary leprosy patients. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *20*(1), 59–64. https://doi.org/10.1111/1469-0691.12190
14. Muddebihal, A., Sardana, K., Khurana, A., Sachdeva, S., Singh, I., Ahuja, M., & Sharma, R. (2023). A prospective case control study of resistance to rifampicin, dapsone and ofloxacin in Type 1 and Type 2 leprosy reactions and the therapeutic impact of modified treatment regimen on reactions. In *Journal of the European Academy of Dermatology and Venereology : JEADV* (Vol. 37, Issue 2, pp. e149–e151). https://doi.org/10.1111/jdv.18709
15. Naaz, Farah; Mohanty, Partha Sarathi; Bansal, Avi Kumar; Kumar, Dilip; Gupta, U. D. (2017). Challenges Beyond Elimination in Leprosy. *International Journal of Mycobacteriology*, *6*(3), 222–228. https://doi.org/10.4103/ijmy.ijmy_70_17
16. Nath, I., Saini, C., & Valluri, V. L. (2015). Immunology of leprosy and diagnostic challenges. *Clinics in Dermatology*, *33*(1), 90–98. https://doi.org/10.1016/j.clindermatol.2014.07.005
17. Patrocínio, L. G., Goulart, I. M. B., Goulart, L. R., Patrocínio, J. A., Ferreira, F. R., & Fleury, R. N. (2005). Detection of Mycobacterium leprae in nasal mucosa biopsies by the polymerase chain reaction. *FEMS Immunology and Medical Microbiology*, *44*(3), 311–316. https://doi.org/10.1016/j.femsim.2005.01.002
18. Pranata, M., Nugrahaini, A. R. D. N., & Fajariah, N. (2022). Characteristics and Therapy in Leprosy Patients at The Semarang City of Tugurejo Hospital. *Medical Sains*, *7*(4), 943–950.
19. Reis, E. M., Araujo, S., Lobato, J., Neves, A. F., Costa, A. V, Gonçalves, M. A., Goulart, L. R., & Goulart, I. M. B. (2014). Mycobacterium leprae DNA in peripheral blood may indicate a bacilli migration route and high-risk for leprosy onset. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *20*(5), 447–452. https://doi.org/10.1111/1469-0691.12349
20. Republik Indonesia, K. kesehatan. (2020). *Buku Pedoman Pelayanan Kusta*.
21. RI, K. K. (2018). *Pusat data dan Informasi Kementerian Kesehatan RI. Jakarta.*
22. Siswanto, T. Asrianti, and D. M. (2020). Neglected Tropical Disease Kusta (Epidemiologi Aplikatif). *Mulawarman Univ. Press*, 1–65.
23. Suneetha, S., Arunthathi, S., Kurian, N., & Chacko, C. J. (n.d.). Histological changes in the nerve, skin and nasal mucosa of patients with primary neuritic

leprosy. *Acta Leprologica*, *12*(1), 11–18.
24. Tatipally, S., Srikantam, A., & Kasetty, S. (2018). Polymerase Chain Reaction (PCR) as a Potential Point of Care Laboratory Test for Leprosy Diagnosis-A Systematic Review. *Tropical Medicine and Infectious Disease*, *3*(4). https://doi.org/10.3390/tropicalmed3040107
25. Viljoen, G., Nel, L., & Crowther, J. (2005). *Molecular Diagnostic PCR Handbook*.
26. WHO. (2023). *Leprosy*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/leprosy
27. Wirayasa, A. (2022). Nasal Swab Multiplex PCR (MPCR) sebagai Metode Diagnosis Dini pada Narakontak Serumah Penyandang Lepra. *Jurnal Sosial Dan Sains*, *2*(5), 638–647. https://doi.org/10.59188/jurnalsosains.v2i5.398