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CASE REPORTS ON LEPROSY – A SHARED PRACTICE

Quantitative PCR diagnostic value in monitoring a patient with leprosy: case report

Valor diagnóstico da PCR quantitativa em acompanhamento de um paciente com hanseníase: relato de caso

Valor diagnóstico de la PCR cuantitativa en el seguimiento de un paciente con lepra: reporte de un caso

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ABSTRACT

Introduction: leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae*. **Objective:** the study aims to report the follow-up of a patient with leprosy, presenting a positive quantitative polymerase chain reaction (qPCR). **Case description:** the patient is a 53-year-old male residing in the rural area of São Geraldo da Piedade, Minas Gerais State, Brazil. In 2015, the patient sought care at the Governador Valadares Municipal Central Polyclinic and was referred to the Dr. Alexandre Castelo Branco Reference Center for Endemic Diseases

and Special Programs (CREDEN-PES) to initiate treatment. However, he did not adhere to the therapy, citing difficulty accessing the center. In 2017, he returned to CREDEN-PES, where he was examined and collected material for bacilloscopy and qPCR. The bacilloscopy index (BI) result was zero and positive for qPCR. The patient was referred for treatment in his hometown and adhered to two doses of multibacillary multidrug therapy (MB-MDT). In 2021, he sought CREDEN-PES for new evaluations, resulting in BI = 1.5 and decreased plantar sensitivity. He began a uniform treatment regimen for leprosy (U-MDT). In 2023, he moved to Paraná State and recently reported his condition: regular health, altered balance, frequent falls, loss of strength in the knees, dry skin, and swelling behind the ear. The positivity of the qPCR reaction prompted the team to follow up with this patient, who initially did not adhere to the treatment. **Conclusion:** it is believed that using laboratory tools to assist and reinforce the diagnosis and treatment has contributed to more effective leprosy control.

Keywords: *Leprosy. Diagnosis. qPCR. Active Search.*

RESUMO

Introdução: a hanseníase é uma doença granulomatosa crônica causada pelo *Mycobacterium leprae*. Ela representa um problema de saúde pública relevante no país e impacta na população de maneira geral. **Objetivo:** relatar o acompanhamento de um paciente com hanseníase que apresenta reação em cadeia polimerase quantitativa (qPCR) positiva. **Descrição do caso:** paciente do sexo masculino, 53 anos, residente na zona rural de São Geraldo da Piedade, Minas Gerais, Brasil. Em 2015, o paciente procurou a Policlínica Central Municipal de Governador Valadares e foi encaminhado ao Centro de Referência em Doenças Endêmicas e Programas Especiais Dr. Alexandre Castelo Branco (CREDEN-PES) para dar início ao tratamento, porém ele não aderiu à terapêutica alegando dificuldade de acesso ao Centro de tratamento. Em 2017, o indivíduo retornou ao CREDEN-PES, onde não só foi examinado, mas também foi coletado material para baciloscopia e para qPCR. Por conseguinte, o resultado do índice baciloscópico (IB) foi zero e positivo para qPCR. Dessa forma, o paciente foi encaminhado para tratamento no município de origem e aderiu a duas doses de poliquimioterapia multibacilar (PQT-MB). No ano de 2021, voltou ao Centro de Referência para novas avaliações e essas resultaram em IB igual a 1,5 e diminuição da sensibilidade plantar. Em seguida, iniciou o uso do esquema único de tratamento para hanseníase (MDT-U). Em 2023, mudou-se para o Paraná e recentemente

relatou o quadro subsequente: estado de saúde regular, alteração de equilíbrio, quedas frequentes, perda de força nos joelhos, pele seca e edema atrás da orelha. A positividade da reação qPCR motivou a equipe a procurar e a acompanhar esse paciente, que inicialmente não aderiu ao tratamento. **Conclusão:** acredita-se que o uso de ferramentas laboratoriais para auxiliar e para reforçar o diagnóstico, assim como o tratamento, tem contribuído para um controle mais eficaz da hanseníase.

Palavras-chave: *Hanseníase. Diagnóstico. qPCR. Busca Ativa.*

RESUMEN

Introducción: la lepra es una enfermedad granulomatosa crónica causada por *Mycobacterium leprae*. Es un importante problema de salud pública en Brasil y tiene impacto en la población en general. **Objetivo:** relatar el seguimiento de un paciente con lepra con reacción en cadena de la polimerasa cuantitativa (qPCR) positiva. **Descripción del caso:** paciente masculino, de 53 años de edad, residente en la zona rural de São Geraldo da Piedade, Minas Gerais, Brasil. En 2015, el paciente acudió a la Policlínica Central Municipal de Governador Valadares y fue remetido al Centro de Referencia de Enfermedades Endémicas y Programas Especiales Dr. Alexandre Castelo Branco (CREDEN-PES) para iniciar tratamiento, pero no cumplió la terapia alegando dificultad de acceso al centro de tratamiento. En 2017, el individuo volvió al CREDEN-PES, donde no solo fue examinado, sino que también se le recogió material para baciloscopia y qPCR. El resultado del índice baciloscópico (IB) fue cero y positivo para la qPCR. Como consecuencia, el paciente fue remitido para tratamiento en el municipio de origen y cumplió dos dosis de terapia multimedicamentosa multibacilar (MDT-MB). En 2021, volvió al Centro de Referencia para nuevas evaluaciones, que resultaron en un IB de 1,5 y una disminución de la sensibilidad plantar. Entonces empezó a utilizar el régimen de tratamiento único para la lepra (MDT-U). En 2023, se mudó para Paraná y recientemente relató lo siguiente: estado de salud regular, alteración del equilibrio, caídas frecuentes, pérdida de fuerza en las rodillas, piel seca y edema detrás de la oreja. La positividad de la reacción de la qPCR motivó al equipo a buscar y seguir a este paciente, que inicialmente no adhirió al tratamiento. **Conclusión:** se cree que el uso de herramientas de laboratorio para ayudar y reforzar el diagnóstico, así como el tratamiento, ha contribuido a un control más eficaz de la lepra.

Palabras clave: *Lepra. Diagnóstico. qPCR. Búsqueda Activa.*

INTRODUCTION

It is well established that leprosy primarily affects the peripheral nerves, and the clinical manifestations of the disease result from the immune response pattern triggered by the infection[1].

Considering the epidemiological data on leprosy in Brazil and, more specifically, in the state of Minas Gerais, it is evident that, despite a reduction in the number of new cases, transmission remains active, as indicated by the incidence of cases in children under 15 years of age[2,3]. This highlights the importance of developing more sensitive and specific diagnostic methods for detecting subclinical infection.

Using molecular techniques, such as deoxyribonucleic acid (DNA) amplification, to identify the bacillus in various clinical samples offers high potential for early diagnosing leprosy. The quantitative polymerase chain reaction (qPCR) technique has, therefore, emerged as an alternative to traditional diagnostic methods[4,5].

This case report thus reinforces the applicability of qPCR for identifying *Mycobacterium leprae* (*M. leprae*) DNA in dermal scrapings, serving as an epidemiological marker for leprosy screening.

CASE REPORT AND DISCUSSION

A 53-year-old male patient residing in a rural area of São Geraldo da Piedade in Minas Gerais State, Brazil.

In 2015, the patient sought care at the dermatology department of the Central Municipal Clinic in Governador Valadares. He was subsequently referred for treatment at the Dr. Alexandre Castelo Branco Reference Center for Endemic Diseases and Special Programs (CREDEN-PES) in Governador Valadares, Minas Gerais. At CREDEN-PES, a slit skin smear was performed, yielding a bacilloscopic index (BI) of 0. A histopathological examination was also conducted, with results suggesting tuberculoid leprosy (TT). These procedures were complemented by biochemical tests, which revealed the following values: glucose at 317 mg/dL, triglycerides at 236 mg/dL, ALT (TGP) at 57 U/L, and the presence of calcium oxalate crystals in the urine. However, the patient did not adhere to the prescribed treatment for leprosy.

In 2017, the patient returned to CREDEN-PES, where he underwent a clinical examination, and samples were collected for slit skin smear (from the right earlobe, left earlobe, right elbow, and a lesion) as well as qPCR analysis. The BI was 0, but the qPCR for the Specific Repetitive Element gene (RLEP) was positive, with a cycle threshold (Ct) of 24.7. Following the diagnosis of leprosy, the patient was referred for treatment in his

municipality of origin but adhered to only two doses of the multibacillary multidrug therapy (MDT-MB).

In 2021, the patient returned to CREDEN-PES for further evaluation. At that time, the BI was 1.5, and decreased sensitivity to purple nylon filament (monofilament esthesiometer) was observed in both plantar regions. Consequently, he began treatment with uniform multidrug therapy (MDT-U).

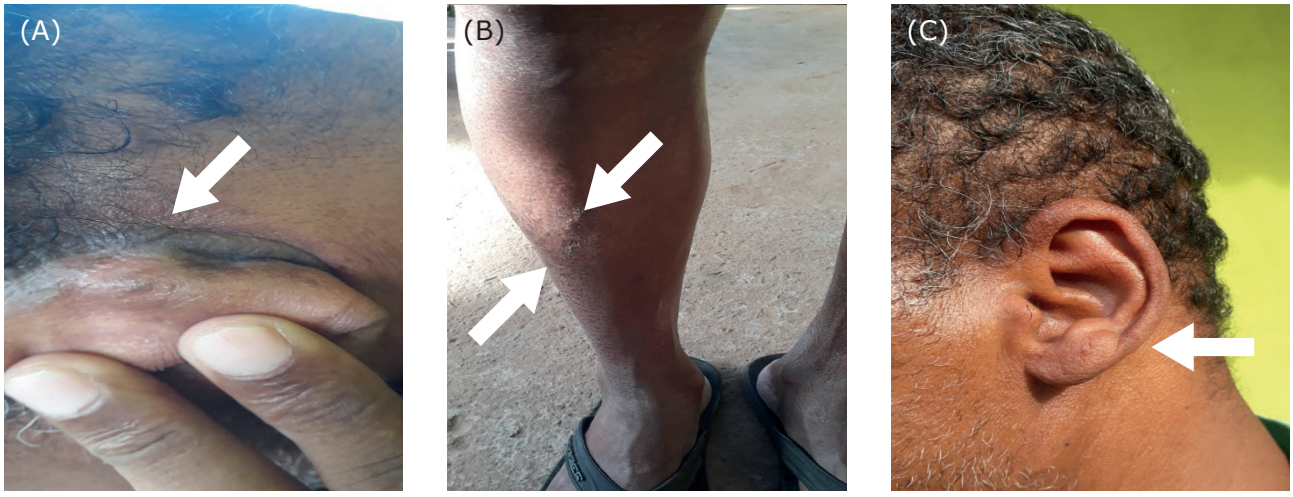
This case was selected in 2023 as part of a quality control initiative to reevaluate certain patients. Upon contact, it was found that the patient had moved to Paraná. During the follow-up, the patient reported the following symptoms: regular health, altered balance, frequent falls, loss of strength in the knees, dry skin, and swelling behind the earlobe.

Figures 1A, 1B, and 1C show images of the patient's current condition, focusing on the areas where the lesions are most prominent.

The follow-up of this patient highlighted the importance of incorporating qPCR into leprosy diagnostic tests. The fact that the slit skin smear yielded negative results in 2015 and 2017 (during the early stages of the disease) contrasted with the positive qPCR reaction in the dermal scraping sample in 2017. This finding is consistent with studies published by the Leprosy Research Center (NuPqHans) at the Federal University of Juiz de Fora[6-8] and other authors[9-11]. It is noteworthy that 25% of paucibacillary (PB) patients had *M. leprae* DNA detected in dermal scrapings or blood samples. Meanwhile, in the multibacillary (MB) group, qPCR was positive in 69.6% of patients, and slit skin smear was positive in 56.5%. It was found that 48.8% of all the samples investigated were positive for *M. leprae* DNA, in contrast to only 30.2% of positive results from slit skin smear[6].

Notably, in this case report, the BI of the patient in a slit skin smear showed a result of 1.5 only in 2021, thus confirming multibacillary leprosy.

Figure 1 – A) Altered appearance of the auricular region. B) Dry skin and scarring from nodules. C) Visible infiltrate in the earlobe.



Source: Created by the authors.

CONCLUSION

The use of the qPCR reaction in active case finding for the early diagnosis of leprosy has highlighted the importance of this tool, particularly in the context of an endemic disease in the region. In this regard, it reinforces a key attribute of primary health care by bridging the gap between surveillance efforts and timely interventions.

Additionally, it is important to note that this test can support health professionals working in remote units far from reference centers, enabling a conclusive diagnosis of leprosy – even in cases where the BI has not yet become positive.

Identifying a multibacillary (MB) patient with a positive qPCR result prompted the healthcare team to locate and monitor him throughout treatment. This case supports the idea that using laboratory tools enhances early diagnosis of leprosy, thus contributing to the "Zero Leprosy" goal established by the World Health Organization.

ETHICAL APPROVAL AND INFORMED CONSENT: *the study was approved by the Research Ethics Committee (CEP) of the Federal University of Juiz de Fora, CAAE: 67528922.6.1001.5147.*

CONFLICTS OF INTEREST: *the authors declare no conflicts of interest related to this article.*

AUTHOR CONTRIBUTIONS: ***Pinheiro MDS****,* ***Oliveira LBP****, and* ***Fraga LAO*** *contributed to the study's conception and design.* ***Pinheiro MDS****,* ***Vieira MP****,* ***Santos DCM****,* ***Oliveira LBP****, and* ***Fraga LAO*** *contributed to sample collection, experimental procedures, data acquisition, and analysis.* ***Pinheiro MDS****,* ***Vieira MP****,* ***Santos DCM****,* ***Oliveira LBP****,* ***Fraga LAO****, and* ***Castelo Branco A*** *contributed to the writing and revision of the manuscript.* ***Fraga LAO*** *was responsible for funding acquisition.* ***Castelo Branco A*** *performed the medical evaluation of the study subject. All authors critically reviewed the manuscript.*

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REFERENCES

1. Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. Clinical Microbiology Reviews. 2006;19(2):338-81. doi: https://doi.org/10.1128/cmr.19.2.338-381.2006.

2. Leite MLC, Miranda MCR, Pugedo AC, Grossi MAF, Ramalho KC, Costa JV. Situação epidemiológica da hanseníase Minas Gerais – municípios silenciosos – 2009-2018. 10º Simpósio Brasileiro de Hansenologia. Hansen Int. 2019;44(Suppl.1):e-2684. doi: https://doi.org/10.47878/hansenologia.2019.v44.34050.

3. Minas Gerais (Estado). Plano Estadual de Saúde 2020-2023. Belo Horizonte: Secretaria de Estado de Saúde de Minas Gerais; 2020. [citado em 20 fev. 2023]. Disponível em: https://www.saude.mg.gov.br/images/1_noticias/09_2021/01_jan-fev-marc/08-02-Plano-Estadual-de-Saude%20-de-Minas-Gerais%20%202020-2023.pdf.

4. Kang TJ, Kim SK, Lee SB, Chae GT, Kim JP. Comparison of two different PCR amplification products (the 18-kDa protein gene vs. RLEP repetitive sequence) in the diagnosis of *Mycobacterium leprae*: PCR for diagnosis of *M. leprae*. Clinical and Experimental Dermatology. 2003;28(4):420-4. doi: https://doi.org/10.1046/j.1365-2230.2003.01300.x.

5. Goulart IMB, Goulart LR. Leprosy: diagnostic and control challenges for a worldwide disease. Archives of Dermatological Research. 2008;300(6):269-90. doi: https://doi.org/10.1007/s00403-008-0857-y.

6. Gama RS, Gomides TAR, Gama CFM, Moreira SJM, Manta FSN, Oliveira LBP, et al. The high frequency of *M. leprae* DNA detection is due to asymptomatic household contacts. BMC Infectious Diseases. 2018;18(153):1-6. doi: https://doi.org/10.1186/s12879-018-3056-2.

7. Gama RS, Souza MLM, Sarno EN, Moraes MO, Gonçalves A, Stefani MMA, et al. A novel integrated molecular and serological analysis method to predict new cases of leprosy amongst household contacts. PLoS. Negl. Trop. Dis. 2019;13(6):e0007400. doi: https://doi.org/10.1371/journal. pntd.0007400.

8. Pinheiro MDS, Oliveira NG, Santos DCM, Leite HM, Baptista IMFD, Fairley JK, et al. Impact of qPCR on the diagnostic evaluation of individuals with suspected leprosy. Research, Society, and Development. 2023;12(3):e13312340549. doi: https://doi.org/10.33448/rsd-v12i3.40549.

9. Martinez AN, Britto CFPC, Nery JAC, Sampaio EP, Jardim MR, Sarno EN, et al. Evaluation of real-time and conventional PCR targeting complex 85 genes for detecting *Mycobacterium leprae* DNA in skin biopsy samples from patients diagnosed with leprosy. Journal of Clinical Microbiology. 2006;44(9):3154-9. doi: https://doi.org/10.1128/jcm.02250-05.

10. Martinez AN, Lahiri R, Pittman TL, Scollard D, Truman R, Moraes MO, et al. Molecular determination of *Mycobacterium leprae* viability by use of real-time PCR. Journal of Clinical Microbiology. 2009;47(7):2124-30. doi: https://doi.org/10.1128/jcm.00512-09.

11. FIOCRUZ [Internet]. Rio de Janeiro: Fiocruz; 2021. [atualizado em 21 set. 2021; citado em 30 dez. 2023]. Menezes M. Fiocruz cria teste molecular para hanseníase inédito no Brasil. [aproximadamente 1 página]. Disponível em: https://portal.fiocruz.br/noticia/fiocruz-cria-teste-molecular-para-hanseníase-inedito-no-brasil.