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Comparative evaluation of PCR amplification of RLEP, 16S rRNA, rpoT and Sod A gene targets for detection of *M. leprae* DNA from clinical and environmental samples

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ABSTRACT

Purpose: PCR assay is a highly sensitive, specific and reliable diagnostic tool for the identification of pathogens in many infectious diseases. Genome sequencing *Mycobacterium leprae* revealed several gene targets that could be used for the detection of DNA from clinical and environmental samples. The PCR sensitivity of particular gene targets for specific clinical and environmental isolates has not yet been established. The present study was conducted to compare the sensitivity of *RLEP*, *rpoT*, *Sod A* and *16S rRNA* gene targets in the detection of *M. leprae* in slit skin smear (SSS), blood, soil samples of leprosy patients and their surroundings.

Method: Leprosy patients were classified into Paucibacillary (PB) and Multibacillary (MB) types. Ziehl–Neelsen (ZN) staining method for all the SSS samples and Bacteriological Index (BI) was calculated for all patients. Standard laboratory protocol was used for DNA extraction from SSS, blood and soil samples. PCR technique was performed for the detection of *M. leprae* DNA from all the above-mentioned samples.

Results: *RLEP* gene target was able to detect the presence of *M. leprae* in 83% of SSS, 100% of blood samples and in 36% of soil samples and was noted to be the best out of all other gene targets (*rpoT*, *Sod A* and *16S rRNA*). It was noted that the *RLEP* gene target was able to detect the highest number (53%) of BI-negative leprosy patients amongst all the gene targets used in this study.

Conclusion: Amongst all the gene targets used in this study, PCR positivity using *RLEP* gene target was the highest in all the clinical and environmental samples. Further, the *RLEP* gene target was able to detect 53% of blood samples as positive in BI-negative leprosy cases indicating its future standardization and use for diagnostic purposes.

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Introduction

Leprosy is a chronic infectious disease of humans caused by *Mycobacterium leprae* and was discovered by G.A. Hansen in 1873. *M. leprae* is a non-cultivable mycobacteria, and diagnosis of the disease is based on its clinical, histopathological characteristics and finding the bacteria in skin scrapings and in biopsies taken from the patients. Due to its long incubation period and because very early lesions often do not satisfy the cardinal signs of leprosy, it becomes difficult to diagnose the disease in the early stages. Therefore, there is an urgent need to develop a tool for diagnosing the disease early so that the patient can be covered with chemotherapy under the control program. The diagnosis of leprosy is routinely based on clinical symptoms and finding acid-fast bacilli (AFB) in smears of skin scrapings. AFB staining technique requires the presence of at least 10^4 organisms per gram of tissue for its reliable detection under the microscope [1] and thus the organisms have a very low sensitivity of detection, especially in patients with intermediate lesions and at the Tuberculoid (TT)/Borderline Tuberculoid (BT) end of the disease spectrum where AFBs are rare or absent.

Several attempts have been made in the past to establish a test for the diagnosis of early leprosy; however, none of the tests were successful in diagnosing more than 60% of early cases of leprosy [2–5] and, therefore, til today, there is no laboratory-based specific and sensitive assay for the detection of early leprosy.

Modern molecular methods like amplification by polymerase chain reaction (PCR) are more specific and sensitive for detecting bacillary DNA in clinical samples [6]. During the last 30 years, PCR methods have been developed to amplify different gene targets of *M. leprae*, but it is not known which target is most suitable for the diagnosis of leprosy. *RLEP* was found to be very sensitive and specific for *M. leprae* and detects 10 fg of purified *M. leprae* DNA [7,8]. *16S rRNA* is a gene conserved in bacteria which codes for the 16S part of the ribosome [9,10]. It has been used for RT-PCR targeting *16S rRNA* for identification of viable bacilli from patients. Martinez et al. reported that with regard to *16S rRNA* and *RLEP*, RT-PCR acts as a better target compared with *superoxide dismutase* (*Sod A*) and *RLEP* RT-PCR for viability testing of *M. leprae* [11].

Enormous numbers of leprosy bacilli are expelled into the environment from the nasal discharges of lepromatous patients [12]. There is also evidence to support the excretion of bacilli from skin lesions [13,14]. A single mouth-wash of an LL patient may discharge 1.6 million *M. leprae* [15]. The risk of transmission of leprosy in the community, therefore, is subject to the availability of leprosy cases and other related environmental factors. There are evidences to support that *M. leprae* is able to survive for many days in the environment. Soil is known to be a medium which can preserve a variety of microorganisms [16]. Although PCR has already been applied for the detection of *M. leprae* for many years, it has been used mainly in samples from biopsies and SSS of leprosy and suspected cases and not in environmental samples.

The aim of the present study is to evaluate the usefulness of the PCR method on different clinical and environmental samples using different gene targets, such as *RLEP*, *rpoT*, *sod A* and *16S rRNA* genes, and compare their efficiency in PCR positivity in clinical samples (blood and SSS) and environmental samples (soil) which were obtained from patients and from their surroundings.

Materials and methods

Ethical approval

Informed consent was obtained from all the patients, and the study was approved by the Ethical Committee of The Leprosy Mission Trust India.

Recruitment of patients

Thirty newly diagnosed AFB negative PB and 30 MB leprosy cases as per standard criteria of the World Health Organisation (WHO) were enrolled from the Outpatient Department of the TLM Hospital Shahdara, Delhi and Purulia, West Bengal. Grading of the bacterial load was determined by ZN staining of SSS. Fifty soil samples were collected in sterile plastic containers following a procedure described earlier [17] from residing areas and/or around the houses of leprosy patients staying in different villages of different blocks of the Purulia district of West Bengal (Table 1).

Sampling area

Different blocks of Purulia, West Bengal and Shahdara of Delhi were selected for sampling.

Slit skin smear sample collection

SSS samples were collected after taking four horizontal scrapes of tissue from an incision (5 mm long and 2 mm deep) made with the help of a sterile surgical scalpel blade (No. 15) on the left and right earlobes and skin lesions without any contamination with blood along the skin-slit part. The tissue material thus obtained on the blade by scraping was placed in 700 μl of 70% ethanol and mixed well in a micro-centrifuge tube. Later the SSS suspensions were kept at 4 °C for further use.

Collection of blood samples

Blood samples (2 ml each) were withdrawn from patients by antecubital venipuncture and collected in an EDTA vial;

Table 1 – SSS samples of PB and MB leprosy patients.

BI grading	Number of PB cases	Number of MB cases	Total number of cases
0	30	–	30
1+	–	6	6
2+	–	6	6
3+	–	6	6
4+	–	6	6
5+	–	6	6
Total	30	30	60

BI = Bacteriological Index; PB = Paucibacillary; MB = Multibacillary.

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