

## Original Article

# Diagnosis of leprosy by PCR targeting gene encoding 36 kDa antigen of *Mycobacterium leprae*

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### Abstract

**Background & Objective:** This cross sectional study was carried out to assess the diagnostic value of PCR in different forms of leprosy. For the detection of *Mycobacterium leprae*, DNA amplification by polymerase chain reaction of a 531-bp fragment of the *Mycobacterium leprae* specific gene encoding the 36 kDa antigen.

**Methodology:** It was done on different clinical specimens (slit smear of skin, ear lobule smear and nasal smear) from 50 leprosy patients attending the Leprosy Hospital, Mohakhali, Dhaka. Patients were divided into two groups; paucibacillary (70%) group and multibacillary (30%) group. PCR showed 100% positivity in skin and ear lobule and 73.4% positivity in nasal smear of multibacillary group. PCR was positive in 40%, 25.7% and 11.4% in skin lesion, ear lobule and nasal swab in paucibacillary group respectively.

**Result:** Compared with other diagnostic procedures, PCR showed clear advantages over both modified Z-N stain and auramine-phenol stain especially in paucibacillary patients.

**Key words:** Leprosy, modified Z-N stain, polymerase chain reaction

### Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The disease mainly affects the skin, the peripheral nerves, mucosa of the upper respiratory tract and also the eyes<sup>1</sup>. Leprosy continues to be a significant health problem in certain pockets in developing countries<sup>2</sup>. A hypo pigmented patch on the skin is perhaps the most frequently noticed sign of early leprosy. Acid Fast Bacilli (AFB) in lesions of early leprosy is not easily found using histopathology techniques<sup>3</sup>.

Modified Z-N staining technique requires at least 104 organisms per gram of tissue for reliable detection<sup>4</sup>. Microscopy of slit skin smears though simple but lacks the required sensitivity and specificity to serve as effective diagnostic tool for Leprosy.

Fluorescence microscopy has got its superiority over Ziehl-Neelsen stained method due to the contrast of fluorescent microorganisms against a dark background<sup>5</sup>. It reduces observer fatigue and increases speed and accuracy<sup>6</sup>. In 1952, Gohar described the advantages of fluorescence microscopy for detecting *M. leprae* in smears. The inter-observer variance was minimal with auramine staining<sup>7</sup>.

In recent years, polymerase chain reaction (PCR) techniques are being successfully used to demonstrate the presence of AFB in small numbers in tissue. It being considered as sensitive, specific and rapid tool for the identification of *M. leprae* in clinical specimens<sup>8</sup>. PCR techniques can detect as few as 10 to 100 bacilli in skin biopsy, skin scraping or slit skin smear specimen. In the last few years, several reports have suggested the use of the polymerase chain reaction (PCR) for diagnosis of subclinical leprosy. DNA from *Mycobacterium leprae*, present in different kinds of clinical material such as lymph, blood, biopsy samples as well as in nasal secretion and in hair bulbs, can be amplified<sup>9</sup>.

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PCR can detect *M. leprae* at a higher rate than the conventional methods at all sites. There were significant differences in the detection rates of *M. leprae* DNA, nasal mucosa staining (40%) versus PCR-nose swab (65%) and ear lobe smear (55%) versus PCR-ear lobe swab (85%)<sup>10</sup>.

In Bangladesh cumulative new cases detected from 1985 to 2012 is 2, 02,776 and another study shows the new cases detected from 1993 to 2012 is 1, 67, 103. According to National Leprosy Elimination Programme on 2012 the prevalence rate of leprosy in Bangladesh is 0.223 per 10,000 people. Being a third world country, this rate is alarming.

Detection of leprosy cases is difficult because the disease is usually asymptomatic in the early stage. Incubation period is very long and varies from 3 to 20 years, so patients report late. Leprosy, particularly in case of lepromatous type infection may spread to several contacts of patients even before clinical diagnosis. Early detection of the causative microorganisms is, therefore, the key element to early identification, requiring early treatment of patients before the disease progresses and neural involvement occurs<sup>11</sup>.

For elimination of leprosy, it needs early detection and from various study we can see early detection is possible with the help of PCR. Though this technique is costly and it has got the chance of contamination even then it will give us an opportunity to detect *Mycobacterium leprae* in early leprosy. In such way we can detect leprosy in early stage and treatment can be started early.

### Materials and Methods

The present study was designed to detect *Mycobacterium leprae* from clinically diagnosed 50 (Fifty) leprosy patients. Study populations were recruited from outpatient department of Leprosy Hospital, Mohakhali, Dhaka, and they were divided into paucibacillary and multibacillary group. Laboratory works were performed in the Department of Microbiology and Immunology, BSMMU, Dhaka. Slit skin specimens from lesion and ear lobule were collected according to International Federation of Anti-Leprosy Associations (ILEP). Nasal Swabs were used to collect nasal specimens. Swabs were dipped in normal saline immediately prior to use, and introduced 2-3 cm into nostril moving the swab several times over the inferior lateral conchae. Swabs were collected, and transported at 2-8°C to the laboratory. Swabs are kept at -20°C for DNA extraction<sup>10,12</sup>.

### Polymerase chain reaction

DNA extraction from samples was performed using Qiagen DNA extraction kit following the manufacturer's instructions (QIA amp<sup>R</sup> DNA Mini and Blood Mini Handbook, April 2010). The primers S13 and S62 used for amplification were selected on the basis of the nucleotide sequence of the gene encoding the 36-kDa antigen of *M. leprae*<sup>13</sup>. The sequences of the primers

which amplify a 531-bp fragment of the *M. leprae* DNA sequence were S13 (5'-CTCCACCTGGACCGGCGAT-3') and S62 (5'-GACTAGCCTGCCAAGTCG-3').

Amplification was carried out in DNA Thermal Cycler and comprised initial denaturation and final extension. After amplification product was processed for gel documentation. The size of amplified DNA fragments was 531 bp. PCR products were identified by electrophoresis in 2% agarose gel with ethidium bromide. The gel was observed under UV transilluminator for DNA bands. The DNA bands were identified according to their molecular size. Samples showing the presence of corresponding 531 bp bands were considered positive for the presence of *Mycobacterium leprae*.

**Data analysis:** All data after collection coded and entered in data base using online MedCalc software (version-12.7.8.0). Descriptive analysis of all relevant variables were done by using proportion, central tendency and dispersion.

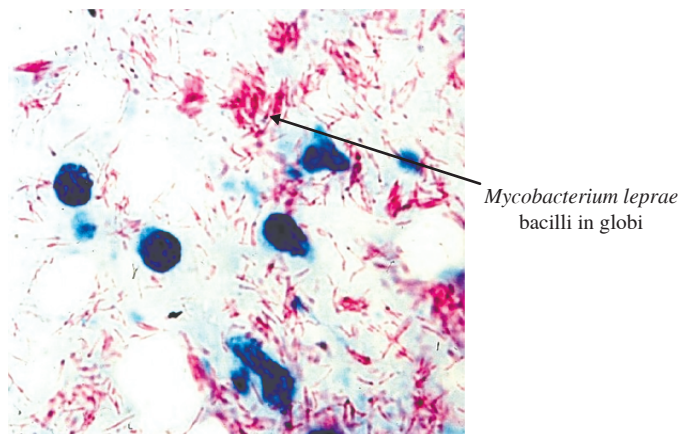
### Result

Table 1 shows result of PCR among different clinical types of leprosy in different types of samples. Among 35 paucibacillary cases 40% positive in skin lesion, 25.7% positive in ear lobule and 11.4% positive in nasal smear. Among 15 multibacillary cases 100% positive in skin lesion, 100% positive in ear lobule and 73.4% positive in nasal smear.

**Table 1 Result of PCR in different types of sample of study population (n = 50)**

Type	Positive N	Negative N
<b>Paucibacillary (35)</b>		
Skin lesion	14 (40)	21 (60)
Ear lobule	09 (25.7)	26(74.3)
Nasal smear	04 (11.4)	31 (88.6)
<b>Multibacillary (15)</b>		
Skin lesion	15 (100)	0
Ear lobule	15 (100)	0
Nasal smear	11 (73.4)	04 (26.7)

**Figures in parenthesis indicate percentage**



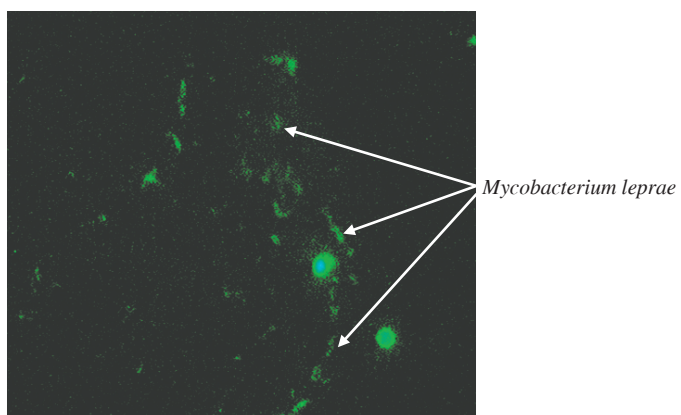
**Figure 1: Modified Z-N staining of skin smear showing *M.leprae***

In paucibacillary cases 40% skin lesion, 25.7% ear lobule swab and 11.4% nasal swab was positive by PCR, but none of the lesions were positive for microscopy by modified Z-N and auramine-phenol stain (Table 2).

**Table 2 Comparative results of PCR, Modified Z-N and Auramine -phenol stain in PB cases.**

Sites	PCR	Modified Z-N stain	Auramine phenol stain
	PB (35)	PB (35)	PB (35)
<b>Skin lesion</b>	14 (40)	0	0
<b>Ear lobule</b>	9 (25.7)	0	0
<b>Nasal smear</b>	4 (11.4)	0	0

**Figures in parenthesis indicate percentage**



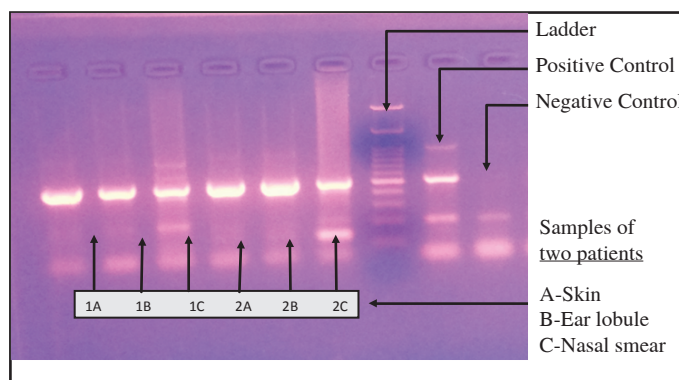
**Figure 2 Auramine-phenol staining of skin smear showing *M.leprae***

In multibacillary cases all 15 skin lesion and ear lobule swabs were positive for PCR and of nasal smear it was 73.4% but in case of modified Z-N stained microscopy in skin smear, ear lobule smear and nasal smear positivity were 100%, 86.7% and 53.3% respectively. In case of auramine-phenol positivity in skin smear, ear lobule smear and nasal smear were 100%, 100% and 66.7% respectively (Table 3).

**Table 3 Comparative results of PCR, Modified Z-N and Auramine -phenol stain in MB cases.**

Sites	PCR	Modified Z-N stain	Auramine phenol stain
	MB (15)	MB (15)	MB (15)
<b>Skin lesion</b>	15 (100)	15(100)	15(100)
<b>Ear lobule</b>	15 (100)	13(86.7)	15(100)
<b>Nasal smear</b>	11 (73.4)	08(53.3)	10(66.7)

**Figures in parenthesis indicate percentage**



**Figure 3 Gel electrophoresis of PCR showing bands of *M. leprae*. Lane: 7: 100 bp DNA ladder; Lane 8: Positive control; Lane9: Negative Control. Lane 1, 2, 3, 4, 5, 6: Positive DNA of *M. leprae* in multibacillary leprosy.**

**Discussion**

A total of 50 clinically diagnosed leprosy cases were included in this study. There were 15 (30%) multibacillary case and 35 (70%) paucibacillary case. In our study modified Z-N staining were negative by microscopy in all three types of sample in paucibacillary cases and in multibacillary cases all slit skin smear (100%) were positive by modified Z-N stain. Smear is positive in the multibacillary group, which helps establish a definite diagnosis of leprosy, but sensitivity is low in the paucibacillary group, in which smear is negative, with a limit of microscopy detection of 10<sup>4</sup> bacilli per gram of tissue<sup>4,13</sup>.

Shraddha et al. obtained in their study 32 (78%) slit skin smear positive out of 41 MB cases by PCR and 4 (44%) slit skin smear positive out of 9 PB cases by PCR. Our study showed very

much similar result to this above study. In current study all skin swabs were positive in multi bacilli cases and 14 (40%) skin swabs were positive out of 35 PB cases by PCR. Similar findings were also observed by Torres et al<sup>10</sup>. and Wichitwechkarn et al<sup>11</sup>. Our study shows the diagnostic efficacy of PCR is more efficient than microscopy in diagnosing leprosy<sup>14</sup>. PCR has got 100% detection rates in ear lobule in 15 MB patients and 9 (25.7%) in 35 paucibacilli case. PCR was positive in 11 (73.4%) nasal smears in 15 multibacillary cases and out of 35 paucibacillary cases 4 (11.4%) were positive by PCR in nasal smears. Whereas none of the lesions were positive by modified Z-N staining in paucibacillary cases. There was a study which showed 3.4% PCR positive in nasal smear in leprosy contact people<sup>15</sup>. So PCR of nasal smear in case of subclinical infection in a community could be a helpful diagnostic tool<sup>16</sup>. The PB type carries so few *M. leprae* organisms that these cases could not be detected by microscopic examination. Forty percent PCR positivity in paucibacilli type has become more meaningful and very encouraging. It is clearly showing an advantage over modified Z-N stain. Continuing efforts have to be made to address the applicability of PCR in clinical practices.

### Conclusion

PCR may be useful as a complementary tool in the diagnosis of certain doubtful cases when conventional methods are not conclusive. Since one of the most important strategies to control a disease like leprosy that has no vaccine is to detect the causative microorganism, PCR test may complement to achieve the ultimate goal of the leprosy control program.

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