Medical Journal of the
Islamic Republic of Iran

Volume 10
Number 3
Fall 1375
November 1996

A RAPID AND SIMPLE METHOD OF DNA EXTRACTION FROM CLINICAL SPECIMENS CONTAINING *MYCOBACTERIUM LEPRAE* FOR PCR ASSAYS

ABDOLNASSER RAFI, MOHAMMED H. MOBAYEN*, FRANCOIS FEVAL*, AND JOHN L. STANFORD

*From the Department of Medical Microbiology, UCL Medical School, University of London, U.K., and the *Baba Baghi Leprosy Hospital, Tabriz, Islamic Republic of Iran.*

ABSTRACT

We describe a rapid, simple, and reliable procedure for routine isolation of *Mycobacterium leprae* DNA from slit-skin swab specimens. This one-step DNA extraction method is based on the utilization of Chelex^R 100, a chelating ion exchange resin. Slit-skin swab specimens from seven untreated leprosy patients at Baba Baghi Leprosy Hospital in Iran were processed by this procedure. The polymerase chain reaction (PCR) was performed with primers for a 530-base pair (bp) fragment of the gene encoding the 36 kDa antigen of *M. leprae*. All the specimens were found to be PCR-positive, suggesting the efficiency of the present DNA extraction method.

MJIRI, Vol. 10, No. 3, 233-235, 1996.

INTRODUCTION

The polymerase chain reaction (PCR) has recently been used for the detection of *Mycobacterium leprae* in clinical specimens, and has been shown to have significant potential as a rapid, sensitive, and specific technique for diagnosing disease caused by this bacterium.[1-4] One of the important issues in the use of this assay is the extraction of DNA from clinical samples prior to performance of PCR. Although current methods for DNA extraction from clinical samples are successful, they nevertheless remain time consuming and too laborious and would be impractical for routine use in most clinical laboratories.[1,3,5] The major impediment of such methods is that they require several washes, centrifugations, and tube transfers, all of which can increase the risk of contamination during PCR analysis.

The procedure described here for DNA extraction utilizes Chelex^R 100, a chelating ion exchange resin. The alkalinity of Chelex^R suspensions and boiling to 100°C result in disruption of the cell membranes and denaturation of the DNA,[6] thus providing a DNA solution suitable for PCR assays.

The PCR assay was carried out to detect specific *M. leprae* DNA in slit-skin swab specimens from leprosy patients by a set of primers with a detection limit of approximately one bacterium.[7]

MATERIALS AND METHODS

Clinical specimens

Slit-skin swab specimens from skin lesions of seven untreated leprosy patients consisting of three multibacillary (MB) leprosy patients and four paucibacillary (PB) leprosy patients at Baba Baghi Leprosy Hospital were taken according to standard method.[8] Then the specimens were sent to London for DNA extraction and PCR analysis.

DNA extraction procedure

Each slit-skin swab specimen was placed in a tube containing 0.5 mL of sterile distilled water and vortexed for 1 min. The swab was then pressed against the tube wall and discarded. For subsequent DNA extraction, 50 µL of the above suspension was added to 200 µL of a suspension

containing 20% Chelex^R 100 (Bio-Rad) in a 0.1% Lauryl sulfate (Sigma), 1% Nonidet P40 (BDH), and 1% Tween 20 aqueous solution (Sigma), placed in a 1.5 mL screw-capped microcentrifuge tube, and briefly vortexed. The mixture was then boiled for 10 min. It was centrifuged at 12000 g for 10 min. Five µL of the supernatant was used directly for PCR examination.

Chromosomal DNA preparation

M. leprae DNA was isolated from killed *M. leprae* (1.25×10^9 AFB/mL, killed by radiation) by the method described above and was used as a positive control in PCR amplification.

PCR procedure

Briefly, 5 µL of each DNA extract was incubated in a 45 µL reaction mixture containing 100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15mM $MgCl_2$, 0.1% gelatin, 1 µM each of primers S13 (5'-CTCCACCTGGACCGGCGAT-3') and S62 (5'-GACTAGCCTGCCAAGTCG-3') (synthesized by Oswel DNA Service, Edinburgh, UK), 0.2 mM each of deoxynucleotides dATP, dCTP, dGTP and dTTP (Pharmacia), and 2.5 units of Taq polymerase (Perkin-Elmer Cetus). The reaction mixtures were covered with 40 µL of sterile mineral oil to prevent evaporation. A control tube containing no target DNA as a negative control, with another tube containing chromosomal DNA of *M. leprae* as a positive control were included with every set of tests. Precautions were taken to avoid contamination with extraneous DNA.

The reaction was performed using an automated thermal cycler and 45 amplification cycles were performed. Each cycle consisted of denaturation at 94°C for 2 min, annealing of primers at 55°C for 2 min, and primer extension at 72°C for 3 min. After the 45th cycle, the extension reaction was continued for another 12 min at 72°C.[7] The presence of the 530-bp amplification product was sought by electrophoresis of 5µL of the amplified mixture at 8.8 V/cm for 45 min on agarose gel (0.8%). The DNA was stained with ethidium bromide (0.5 µg/mL) and visualized on a 302-nm ultraviolet transilluminator.[9] The molecular size marker used was ϕ X 174 DNA Hae III digest (Sigma).

RESULTS

DNA extraction using Chelex^R 100 was done on seven slit-skin swab specimens from untreated leprosy patients in order to perform PCR analysis. All of the seven specimens were found to be PCR-positive for *M. leprae* and produced 530-bp DNA fragments. In total, promising results were obtained by the Chelex^R 100 procedure.

DISCUSSION

In this study of DNA extraction, we have described a one-step method for rapid and simple extraction of DNA from clinical specimens of leprosy without estimation of sensitivity.

By using Chelex^R 100 the time of preparation of these specimens for PCR analysis is dramatically reduced and the number of manipulation steps is minimized in order to decrease cross-contamination and contamination from extraneous DNA and, within 25 minutes, a DNA solution suitable for PCR examination is obtained. The PCR-positivity of all seven specimens indicates the efficacy of our procedure. Obtaining PCR-positive results on specimens from PB patients also indicate the efficiency of this DNA extraction method, since the number of leprosy bacilli present in such specimens are significantly less than in MB leprosy.

In view of the fact that the detection limit of PCR with primers S13 and S62 used in this study is reported to be 1 to 10 bacilli,[7] the Chelex^R 100 procedure in this PCR is seemingly a rapid and reliable method for the identification of *M. leprae* in clinical specimens.

Because *M. leprae* has a very resistant and complex cell wall, we believe that this application of Chelex^R 100 chelating resin will be helpful for DNA extraction from other bacteria, including other mycobacteria from clinical specimens as well. However, further studies with different specimens of various bacteria are necessary to more fully evaluate this procedure.

We plan to extend this study and perform different DNA extraction methods and PCR assays in order to obtain comparative results and prove whether the Chelex^R 100 procedure has an advantage compared to other DNA extraction methods, and whether or not Chelex^R 100 has a considerable advantage in increasing the efficiency of PCR.

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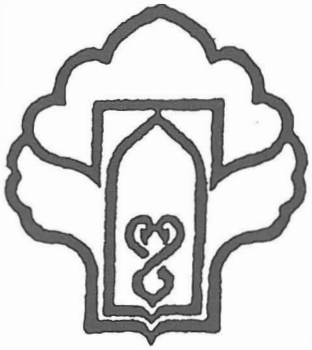

Shahid Sadoughi Yazd
University of Medical Sciences

Seventh Congress of Infertility in Iran

The 7th Congress of Infertility will be held from 24 to 27 February 1997, at the Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Abstracts of original work related to all aspects of infertility will be considered, with particular emphasis on **Assisted Reproductive Technology (ART).**

Abstracts must be received no later than 15 January, 1997.

Please contact: Dept. of International Relations, Shahid Sadoughi University of Medical Sciences, P.O. Box 734, Yazd, Iran. Fax: 098-351-49111