Emergence of primary drug resistance to rifampicin in *Mycobacterium leprae* strains from leprosy patients in India

Mallika Lavania, Astha Nigam, Ravindra Turankar, Itu Singh, Pankaj Gupta, Senthil Kumar, Dr Utpal Sengupta, Consultant, Annamma S. John

PII: S1198-743X(15)00790-9
DOI: 10.1016/j.cmi.2015.08.004
Reference: CMI 357

To appear in: *Clinical Microbiology and Infection*

Received Date: 10 June 2015
Revised Date: 9 July 2015
Accepted Date: 10 August 2015


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Emergence of Primary Drug Resistance to Rifampicin in *Mycobacterium leprae* Strains from Leprosy Patients in India

Mallika Lavania\(^1\), Astha Nigam\(^1\), Ravindra Turankar\(^1\), Itu Singh\(^1\), Pankaj Gupta\(^2\), Senthil Kumar\(^3\), Utpal Sengupta\(^1\)# and Annamma S John\(^4\)

**Running Title:** Primary drug resistance in leprosy

\(^{1}\)Stanley Browne Laboratory, The Leprosy Mission Community Hospital, Nand Nagri, New Delhi-110093, India

\(^{2}\)The Leprosy Mission Community Hospital, Nand Nagri, New Delhi, India

\(^{3}\)The Leprosy Mission Community Hospital, Champa, Chattisgarh, India

\(^{4}\)Research and Training, The Leprosy Mission Trust India, New Delhi, India

\(#\)Corresponding Author:

Dr U Sengupta
Consultant
Stanley Browne Laboratory, The Leprosy Mission Community Hospital, Nand Nagri, New Delhi-110093, India
Ph: +91-11-22594295
Email: usengupta2002@yahoo.com, mallikalavania@gmail.com
Sir,

Although the prevalence of leprosy has significantly gone down after the introduction of WHO regimen of Multi-drug Therapy (MDT), the incidence remains a constant peril with report of approximately 215,656 cases globally in 2013. Out of these 126,913 cases were reported from India alone [1]. Out of a total number of 3196 relapse cases, India alone contributed 486 cases [1]. For most of the infectious diseases where secondary prevention is controlled by chemotherapy alone, the emergence of drug resistance ultimately becomes a concern and a threat for the intervention programmes. However, the fight against leprosy has been a great success largely due to the development of multidrug therapy (MDT) in 1981. The efficacy of MDT in curing leprosy during the last 3 decades has brought about a dramatic decline in the disease burden in all leprosy endemic countries. The annual new case detection rate (ANCDR) have also started decline in some countries. In India, the prevalence of leprosy has come down from 4.2 of 2002 to 0.68 / 10,000 population of 2014 [2]. At this stage of elimination figure of <1/10,000 any emergence of drug resistant *M. leprae* strains will greatly dampen the control programme of the country. Previous records reveal that after almost 30 years of DDS monotherapy DDS-resistant *M. leprae* was a major issue in the leprosy control programme [3]. As rifampicin is the main drug in the MDT and the only bactericidal drug, it is very important to follow the emergence of rifampicin resistance mutants in the leprosy patients. It was already indicated in the recent published literature from Brazil and India [4,5] that rifampicin resistant cases are appearing from many endemic areas of these countries. To overcome the challenge of containing the disease and to sustain the on-going declining trend of leprosy in endemic countries, it is essential to keep a watch on drug sensitivity patterns in the present settings. The Leprosy Mission Trust India is a part of sentinel centres
of WHO in surveillance of drug resistance in leprosy. The WHO sentinel surveillance was undertaken to identify leprosy cases who already completed MDT regimen and relapsed was not showing any diminishing in activity of their lesions and bacillary indices inspite of reintroduction of MDT. The cases were classified into multibacillary (MB) and paucibacillary (PB) as per WHO guidelines. Along with these cases we enrolled some new cases with high BI and who have not been administered any MDT before recruitment for detecting primary drug resistance. Primary drug resistance refer to the patients who have been never treated for leprosy with MDT.

A total of 1.27 lakh new leprosy cases were detected during the year 2013-14 from India, with an ANCDR of 9.98 per 100,000 population. Out of these approximately 5000 new cases reported to TLM hospitals for treatment.

Written informed consents were obtained from all the recruited patients and the study was approved by the Institutional Ethical Committee. A total of 215 slit- skin scrapings from relapsed leprosy and new patients were obtained between 2009 and 2014. Slit skin smears were collected into 70% ethanol in 1.5 ml Eppendorf tubes. The bacteriological index (B.I) of the slit smears varied between 1+ to 6+. DNAs of *M. leprae* were extracted from slit-skin scrapings as protocol described by Lavania et al [5]. This lysate preparations were further used for DNA sequencing followed by PCR amplification targeting *rpoB, folP1* and *gyrA* genes [5]. Sterilized distilled water was used as negative control and Reference strain of Thai 53 was used as positive control for PCR. The PCR products were confirmed by 2% agarose using gel electrophoresis, sequence data were analyzed using MEGA 5.1.

Among 215 cases, there were 200 MB and 15 PB cases. Among these 215 cases there were 184 cases of relapse, 16 new cases and 5 defaulters. Out of 16 new cases we observed mutations at already reported codon positions (424, 425, 437, 438) and a new codon
position 411 in rpoB gene region in three patients (Table 1). In rest of the specimens, we detected secondary resistance in rpoB gene at codons 410 (Glu-Val), 411 (Ala-Val), 424 (Val-Gly), 437 (Ser-Gln), 439 (Phe-Leu), 442 (Gln-His) and 455 (Leu-Pro) which has been reported in our previous study [5]. Among these 16 new cases 7 were showing primary resistant to rifampicin. Out of these 7 new cases 3 cases showed a mutation at codon 411 (Ala-Val) which was not reported earlier (Table 1). Patient 1 and patient 2 had mutations at two codon positions. All these mutations were in the rifampicin resistance determining (RRDR) region. Genotyping of these isolates were found to be of M. leprae type 1D which we reported earlier from this endemic region [6]. BI of all these new cases varied between 1+ and 6+. Resistance to DDS was noted by mutation at codon 53 (Thr-Ala) in folP in one of the new cases (Table 1).

After about 30 years of MDT, it is quite natural to expect emergence of drug resistance in M. leprae and hence during the stage of elimination it will be a major setback to the public health programme. Drug resistant leprosy infection can be caused by transmission of already resistant strains (primary resistance) or by selection of resistance conferring mutations during inadequate therapy (secondary resistance). Lavania et al [5] indicated that presence of rifampicin, dapsone and ofloxacin resistance cases in high endemic areas. M. leprae isolates resistant to single and multiple drugs have been encountered. The emergence of the drug resistance to rifampicin will reduce the efficacy of MDT and will fail to maintain the efficiency of current leprosy-control strategies around the world. Although the genome of M. leprae has been undergone massive gene decay the RRDR region of M. leprae has been stable indicating the capability of M. leprae to develop resistance under drug pressure. After 30 years of DDS monotherapy resistance to DDS emerged in 1980s and it is likely that after another 3 decades of Rifampicin based MDT emergence of Rifampicin
resistance might be a major issue in chemotherapy of leprosy. Findings from this study showed emergence resistance to rifampicin in new cases of leprosy. Emergence of new cases with resistance to rifampicin indicates that resistant strains are actively circulating in endemic regions of India from secondary resistant cases and infecting the naive population at risk. This finding suggests that there is an urgent need for establishment of a drug-resistant monitoring policy and a careful post-treatment follow-up of cured patients in order to detect relapse earlier and rapidly identify secondary resistant strains for their inclusion in a new drug regimen. This report emphasizes an urgent need for inclusion of new drugs in the multidrug regimen for treatment of such cases.

Acknowledgement:

We express our thanks to the Superintendent and staffs of TLM Hospitals for their help and assistance during the work. We also thank the people who participated during sampling.

Funding:

This work was supported by the Department of Biotechnology (Grant No:BT/Bio-CARc/08/000494/2010-11).

Transparency declarations

None to declare.
References


Table 1: Details of Primary rifampicin resistance cases

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age/Sex</th>
<th>Classification</th>
<th>Clinical presentation</th>
<th>B.I</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTD 1</td>
<td>60/M</td>
<td>LL</td>
<td>Nodules all over the body, 6 nerves involved</td>
<td>4+</td>
<td>Ala411 Val; Ala 425 Gly</td>
</tr>
<tr>
<td>PTD 2</td>
<td>36/M</td>
<td>LL</td>
<td>Infiltration &amp; Nodules all over body, Bilateral claw hand with shortening of fingers &amp; bilateral sole complete anaesthesia past 6 years</td>
<td>5+</td>
<td>Val 424 Gly; Gln 442 His</td>
</tr>
<tr>
<td>PTD 3</td>
<td>40/M</td>
<td>BL</td>
<td>Infiltration &amp; nodule all over body 1 year</td>
<td>6.0+</td>
<td>Leu 455 Pro</td>
</tr>
<tr>
<td>PTD 4</td>
<td>42/M</td>
<td>LL</td>
<td>Lt ulnar weakness, Rt ulnar paralysis with aneesthesia &amp; Rt foot weakness &amp; Lt foot drop 3-4 months</td>
<td>4.0+</td>
<td>Ala 411 Val</td>
</tr>
<tr>
<td>PTD 5</td>
<td>44/M</td>
<td>LL</td>
<td>Hypo-pigmented anesthetic patches present on Lt. thigh, sensory loss present in B/L hand and feet. never had anti-leprosy drugs before</td>
<td>1.0+</td>
<td>Ala 411 Val</td>
</tr>
<tr>
<td>PTD 6</td>
<td>48/M</td>
<td>BL</td>
<td>Presented with type 2 reaction</td>
<td>5.33+</td>
<td>Ser437Gln</td>
</tr>
<tr>
<td>PTD 7</td>
<td>37/M</td>
<td>BL</td>
<td>Presented with type 1 reaction</td>
<td>4.0+</td>
<td>Gln438Tyr</td>
</tr>
</tbody>
</table>