Cohort study of the seasonal effect on nasal carriage and the presence of Mycobacterium leprae in an endemic area in the general population

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Abstract

Leprosy continues to be a significant health problem in certain pockets in developing countries. Better understanding of the transmission and source of the infection would help to decipher the transmission link, leading to control of the spread of the disease. The nose is considered to be a portal of entry, suggesting an aerial route for transmission through droplet infection. The evidence suggests that many individuals from endemic countries carry Mycobacterium leprae in their nasal cavities without having obvious symptoms of leprosy. The objective of the present study was to assess the presence of M. leprae on the nasal mucosa in the general population from a leprosy-endemic pocket. M. leprae detection was carried out using PCR targeting RLEP. Four hundred subjects from an area highly endemic for leprosy were included in the study and followed up during three different seasons—winter, summer, and monsoon—for evidence of nasal exposure to M. leprae. PCR positivity for M. leprae was observed in 29%, 21% and 31% of the samples collected in winter, summer and the monsoon season, respectively. Twenty-six individuals from the cohort showed amplification for M. leprae for all seasons. Our results are consistent with reports in the literature showing widespread exposure to M. leprae in the endemic community. The results also suggest possible association of the environmental conditions (climate) with the transmission pattern and levels of exposure to M. leprae. However, the present study indicated that the population from highly endemic pockets will have exposure to M. leprae irrespective of season.

Keywords: Endemic, humidity, Mycobacterium leprae, PCR, seasonal

Introduction

Members of the genus Mycobacterium are typically found in the soil and water. The majority of mycobacteria are non-pathogenic. However, Mycobacterium leprae, the causative agent of leprosy (Hansen’s disease), has affected human beings from Biblical times in the Middle East, and has been recognized in India since Vedic times. Leprosy is a chronic, infectious disease that is believed to be of low contagiousness [1]. There are many reports on the levels of exposure to the bacillus in endemic countries, but its significance in transmission and disease outcome is yet to be established. The disease primarily affects the superficial parts of the body, especially the peripheral nerves and appendages of the skin, such as sweat and sebaceous glands and mucous membranes.

According to the WHO, the global registered total number of leprosy cases from 130 countries and territories at the beginning of 2011 was 228,474, whereas the number of new cases detected during 2010 was 192,246 (excluding the small number of cases in Europe) [2]. In India, the year 2010–2011 started with a total prevalence rate (PR) of 0.69/10,000 on 1 April 2010. Up to then, out of 35 States/Union Territories, 32 had achieved leprosy elimination. A total of 510 districts
(80.6%) of a total of 633 districts also achieved elimination by March 2010 [3] (http://www.nlep.nic.in/Progress%20report%20%202010-11.pdf).

The risk of transmission is related to the presence of infectious cases and, perhaps, their surrounding environmental factors. It has been shown that humidity favours the survival of *M. leprae* in the environment [4]. Although *M. leprae* primarily spreads through infectious human sources, there is evidence from the published literature indicating the presence of possible non-human sources of the organism [5–7]. One of the reports shows that ‘naturally’ infected armadillos or monkeys could be a source of *M. leprae* infection [8]. Inanimate objects or fomites, such as articles used by infectious patients, can theoretically spread infection. Furthermore, nasal secretions [9,10], discharged into the atmosphere by coughing, sneezing, etc. by an infected person, have been shown to disperse the bacilli in dust particles and through airborne droplets falling in soil [5,11–13] and in water [14] which may act as source of infection. Experimental evidence of *M. leprae* infection through exposure to *M. leprae* aerosol in experimental susceptible mice proved beyond doubt that infection can be transmitted by the nasal route [15]. Recently, amoebae have been shown to favour the growth of *M. leprae* in vitro culture [16].

Smith et al. [17] reported that 1.6% of 2552 nasal swabs from normal healthy individuals in an endemic population had evidence of the presence of *M. leprae* as demonstrated by PCR, and 68% of saliva samples of such a population were positive for *M. leprae*-reactive IgA [18–21]. The further study of endemic populations indicated that households with leprosy patients have higher attack rates of leprosy than those without such exposure. The attack rates were also higher when the index cases had a higher bacterial load [22]. Some other studies have also reported that the nasal route is a major route of exit and entry for *M. leprae* [23,24].

In the present study, to determine the role of the atmospheric environment, we investigated, by PCR, the nasal mucosa of a cohort population from a highly endemic area (Baligara, Purulia) for the presence of *M. leprae* in the peak times of three different seasons: monsoon, summer, and winter. Baligara is a small village situated 3 miles from The Leprosy Mission Community Hospital and Simonpur Leprosy Colony–Purulia, a southern district in the state of West Bengal in India. The population of the village is 2451 (2011 Census), living in approximately 426 households. A total of 56 new leprosy cases were reported from Baligara in 2010. Fifteen (26.78%) of these 56 cases were children, with an age range of 6–13 years. The PR is c 2.2/10 000.

### Materials and Methods

#### Ethical approval

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

#### Sample details

The subjects were randomly selected from the village population, and there was no known bias in the selection process. From the population, 16.31% (*n* = 400) was chosen as the sample (Table 1). Eight (2%) old treated cases were randomly included in the study, six of whom were treated with paucibacillary multidrug therapy, and two of whom were treated with multibacillary multidrug therapy.

#### Collection of nasal samples

Pernasal swabs (Medical Wire and Equipment, Corsham, Wiltshire, England) were used to collect nasal specimens. Swabs were dipped in normal saline immediately prior to use, and passed through the base of the turbinate until the posterior wall of the nasopharynx was encountered. The nasal swabs were obtained separately from both nostrils of the healthy individuals. Swabs were collected, chilled, maintained at 4°C, and transported at 2–8°C to the laboratory. The collected nasal swabs were kept at −20°C for further analysis.

Nasal swabs were collected from a cohort of 400 subjects in three different seasons (monsoon, summer, and winter) from Baligara, a village from an area highly endemic for leprosy in the Purulia district of West Bengal. Positive controls and negative controls were added as quality controls for the authenticity of the data, and to rule out false positives and false negatives. We used known *M. leprae* DNA as a positive control, and plain swabs without any material as negative controls for sample processing and PCR. We analysed some of the samples randomly by using 16S rRNA gene region primers to cross-check the results.

#### Extraction of *M. leprae* DNA and PCR for RLEP sequences

These nasal swabs were processed for DNA extraction with a method described by Jadhav et al. [25]. Swabs from both nostrils of an individual were lysed together in one tube. PCR

### TABLE 1. Demographic details of the sample

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults, n (%)</td>
<td>160 (40)</td>
<td>214 (53.50)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>19–70</td>
<td>14 (3.5)</td>
</tr>
<tr>
<td>Children, n (%)</td>
<td>12 (3)</td>
<td>14 (3.5)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>5–18</td>
<td>14 (3.5)</td>
</tr>
</tbody>
</table>
targeting the RLEP gene sequence in *M. leprae* was performed as described by Donoghue et al. [26], with 5′-TGCAATGTCAAGGCTTTGAGG-3′ as a forward primer and 3′-CACCGATAACGGCGGCACAA-5′ as a reverse primer, in order to amplify a fragment of 129 bp. Amplification was confirmed by 2% agarose gel electrophoresis.

Statistics
Graphpad software was used to analyse the data. Comparisons were made within and between all of the seasons by using the Fischer exact test. All comparisons were considered to be significant at *p* ≤ 0.05.

Results

Detection of *M. leprae* by PCR targeting RLEP
PCR targeting RLEP gene sequences in *M. leprae* was performed for a cohort of 400 subjects in three different seasons. Samples were collected in each season from the same group of 400 subjects, and PCR was performed.

Out of 400 subjects, 124 were positive in the monsoon, 84 were positive in summer, and 117 were positive in winter. The RLEP was amplified and confirmed by 2% agarose gel electrophoresis (Fig. 1). We identified statistically significant differences in PCR positivity between monsoon and summer (31% vs. 21%, *p* < 0.05) and between summer and winter (21% vs. 29%, *p* < 0.05), indicating that exposure to *M. leprae* is greater in the monsoon than in summer and winter (Table 2). We analysed 26 samples that were consistently positive in all three seasons by 16S rRNA, and all were found to be positive (data not shown).

Effect of humidity on PCR positivity of *M. leprae* from the nasal mucosa
We compared the seasonal variation in the exposure levels in the cohort study as indicated by PCR positivity. By applying the *t*-test, we found that PCR positivity for the presence of *M. leprae* in the nasal cavity was highest during the monsoon, when humidity was highest, and significant differences were found between all three seasons (Table 2). Similar findings were also made in our previous study in an area with relatively low endemicity for leprosy, where the exposure levels were low but the seasonal variations were similar to those reported here (data not shown).

Discussion

The transmission patterns of an infectious disease may depend on environmental factors that directly facilitate the pathogen contacting the host for the propagation of disease, or the acquisition of protective immunity by the host. Alternatively, they may reflect non-environmental factors, such as socio-economic conditions, behaviour, or nutritional status, each of which may vary within and between populations, and thereby produce variations in host susceptibility to such environmental exposure.

Understanding the natural history of a disease, its geographical distribution and host-pathogen interactions is important in order to establish a successful control programme. It is necessary to mention that, in spite of its success, the WHO leprosy elimination campaign, which is mainly based on new cases, and the treatment and follow-up of household contacts, has not reduced the incidence of leprosy in certain endemic areas of the world. Increased detection of hidden cases cannot explain the new occurrence of leprosy among young children, which is an indicator of ongoing active transmission [27].

**TABLE 2. Comparative PCR positivity in different seasons**

<table>
<thead>
<tr>
<th>Number</th>
<th>Season</th>
<th>No. of samples collected</th>
<th>No. PCR-positive</th>
<th>% PCR positivity</th>
<th>p-Value for difference in PCR positivity in different seasons</th>
<th>Average relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer</td>
<td>400</td>
<td>84</td>
<td>21</td>
<td>Summer vs. monsoon (0.01), significant</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>Winter</td>
<td>400</td>
<td>117</td>
<td>29</td>
<td>Summer vs. winter (0.04), significant</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>Monsoon</td>
<td>400</td>
<td>124</td>
<td>31</td>
<td>Winter vs. monsoon (0.45), not-significant</td>
<td>92</td>
</tr>
</tbody>
</table>
Although several countries those have reached the elimination target still report the existence of some pockets with clusters of leprosy cases. Leprosy must be eliminated in these high-endemicity regions, in order to achieve a sustained low level of transmission and reduced incidence rates. India also has such small pockets of areas highly endemic for leprosy in several parts of the country.

*M. leprae* has been shown to be capable of tolerating adverse environmental conditions. In the available literature, hot and humid weather, wet soil and water have all been proposed as factors that favour survival of the bacilli for few months (Tuberculosis and Leprosy Control of Ethiopia 10th Annual Review Meeting, 2002) [28]. Sterne et al. [29] reported that, in Karonga Districts of Malawi, the leprosy incidence was more than twice as high in the northern district as in the southern district. The most obvious environmental difference between these regions is the north’s higher rainfall and more fertile soil. In the south, rates are similar between southern hills and the southern lake shore, with slightly lower rates in the semi-urban area around the district capital. This may suggest that the geographical variation in leprosy incidence rates is dependent on environmental factors. Transmission of *M. leprae* may be more frequent in humid conditions when the secretions from the nose are more abundant [30,31]. An environmental source [11,12,32] will determine the exposure which in turn can result in infection of susceptible humans. Our study indicates that leprosy transmission may be influenced by environmental changes. The present study also shows that there is seasonal variation in exposure to *M. leprae*. When the temperature is low and humidity is high in the monsoon, PCR positivity in nasal swabs increases, and during summer (dry and hot weather) it declines. Argaw et al. [27] proposed that leprosy occurs most frequently when a suitable microenvironment, such as moist soil, coexists with other known or unknown predisposing factors. Future studies, including field-collected validation data (temperature, rivers, water sources, humidity, etc.), may shed more light on the precise factors associated with the environmental risk of leprosy. More studies, such as investigations of different types of environmental samples and patient samples, are required to establish the extent of the role of viable *M. leprae* in the environment or different environmental reservoirs in the transmission dynamics for leprosy. To achieve leprosy eradication, a strategy that is effective in preventing the disease is required. This can be achieved by a better understanding of the modes of transmission and potential sources of the pathogen. Control of transmission may be feasible through the identification and treatment of individuals within infection clusters, allowing progress towards the eradication of leprosy. Future studies should focus on the development of methods and tools for studying the transmission of *M. leprae* and on identifying direct methods for testing innovative interventions.

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**Transparency Declaration**

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**References**