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Seroepidemiologic survey of the household contacts of leprosy patients

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SUMMARY

OBJECTIVE: Leprosy is a disabling infectious disease caused by *Mycobacterium leprae*. This study aimed to investigate the prevalence of leprosy among household contacts of leprosy patients.

METHODS: This study is a serological survey in household contacts of leprosy patients who had been treated or were undergoing treatment in the city of Presidente Prudente, São Paulo, Brazil, from 2006–2016, using clinical examination and screening for anti- Phenolic glycolipid-I antibodies with Mycobacterium leprae-flow serology.

RESULTS: A total of 263 index cases of leprosy were identified during the study period. Of these, 53 were approached, and among their household contacts, 108 were examined. The ML-flow test was positive in 2 (1.85%) individuals, but clinical examination revealed no signs or symptoms of leprosy in them. Therefore, they were considered to have a subclinical infection. Leprosy was not confirmed in any household contacts. In this study, a lower percentage of household contacts, when compared to that in the literature, had a positive Mycobacterium leprae-flow test result.

CONCLUSION: The use of Mycobacterium leprae-flow should be encouraged during the follow-up of at-risk populations, such as the household contacts of leprosy patients.

KEYWORDS: Hansen's disease. Leprosy. Mycobacterium leprae. Serologic tests.

INTRODUCTION

Leprosy is a chronic infectious and contagious disease caused by *Mycobacterium leprae*, which mainly affects the skin and peripheral nervous system[1]. According to the World Health Organization's (WHO) operational classification, multibacillary (MB) patients have more than five lesions or a positive bacilloscopic index, whereas paucibacillary (PB) patients have up to five lesions and a negative bacilloscopic index[1].

The main route of infection is the upper airway, with intimate and prolonged contact with the patient being the main risk factor for leprosy transmission[2]. This risk is 5–10 times higher if a family member has already presented with the disease[2].

In 2020, 127 countries reported 127,396 new cases to the WHO, the majority of which in India, with 65,147 cases[3]. Brazil ranked second, with 17,979 new cases[3]. The COVID-19 pandemic has disrupted program implementation and a reduction in new case detection by 37% in 2020 compared with 2019[3].

In Brazil, it is recommended that clinical examination of the household contacts (HHC) at the time of the diagnosis of the index case be conducted; if examination findings are normal, contacts are expected to receive the bacillus Calmette-Guérrin (BCG) vaccine[4]. However, due to its long incubation period (between 2 and 7 years), the disease may manifest later, thus requiring several years of monitoring[1].

Phenolic glycolipid-I (PGL-I), an *M. leprae*-specific membrane component, chemically comprises a trisaccharide, linked by a molecule of phenol to a chain of fatty acids[1]. It is present in the capsule of bacillus and can induce the production of antibodies, especially immunoglobulin M (IgM)[1]. Detection of these IgM antibodies in serum is suggestive of *M. leprae* infection[1]. Native and synthetic antigens are used in serological tests for leprosy diagnosis[1]. Enzyme-linked immunosorbent assay (ELISA) is the most common method used in these tests[5-8]. The rapid immunochromatographic and semi-quantitative lateral flow (ML-flow) tests have shown high sensitivity (97.4%) and specificity (90.2%) for the detection of MB leprosy, in addition to a 91% concordance rate with ELISA[9].

Clinical examination is insufficient for identifying infected individuals at an asymptomatic stage. As these individuals can disseminate *M. leprae* and have an increased risk of developing

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the disease, an active search for such individuals among the HHC of leprosy patients is required. Serological tests with PGL-I may assist with this search as they can contribute to early diagnosis[9].

Previous studies show the effectiveness of the ML-flow test in the detection of the disease among asymptomatic HHC of MB patients[9-11]. The method demonstrated concordance with the bacilloscopy, in addition to the ability to detect seropositive smear-negative patients[12].

We aimed to investigate the prevalence of leprosy among apparently healthy HHC of leprosy patients in Presidente Prudente, a city in the state of São Paulo, from 2006 to 2016, using clinical examination and the ML-flow test.

METHODS

This cross-sectional epidemiological study included HHC of leprosy patients who have been treated or are receiving treatment from 2006 to 2016 in Presidente Prudente, a city in the state of São Paulo, Brazil. It was a seroepidemiological survey in which clinical examination and the ML-flow test were applied. All procedures were approved by the Ethics Committee of the Universidade do Oeste Paulista, and the study was registered under the CAAE protocol (approval: 69516017.2.0000.5515) in October 2017.

For a simple random sampling scheme, assuming the probability of a type I error (α) of 5%, the value used in the formula to calculate the 95% confidence interval for normally distributed data was Z_{α}=1.96. Considering proportion p to be unknown, a value of 0.5 was used. Finally, the maximum margin of error allowed was ±2.5% (i.e., 5%). Thus, the ideal sample size for this study, considering a population of 263 leprosy patients, was 156.

The health department and primary and secondary care centers follow the guidelines of the Ministry of Health and WHO for the surveillance and elimination of leprosy and care of leprosy patients. Among the recommendations are tracking and monitoring HHC and conducting BCG vaccinations. Therefore, HHC were selected to participate in this study.

The HHC were defined as a person who either lives or has lived in the same house as a leprosy patient in the past 5 years before the patient's diagnosis.

Leprosy patients (index cases) were identified from the digital database of the health department. Information, such as telephone numbers, addresses, operational classification of the disease, and BCG vaccination status of the contacts, was extracted from their electronic records at the health department where the patients usually visited for their treatment and follow-up.

The researchers contacted the leprosy patients, who, in turn, identified their HHC and provided their names and contact information. All patients and their HHC were assured that their information would be kept safe, as described in the consent form they signed before study commencement. The inclusion criteria for HHC were that they were residents of Presidente Prudente and had no personal history of leprosy. Subjects were excluded if they presented with coexisting infection or any disease that could affect the peripheral nervous system, such as diabetes mellitus and alcoholism.

Mycobacterium leprae-flow Serological Test

The ML-flow test was performed during the household visit (using the blood obtained from pricking the index finger of the HHC) to detect circulating IgM antibodies against a semi-synthetic analog of PGL-I of *M. leprae* linked to bovine serum albumin (NT-P-BSA). The test was performed using a device containing a porous tape, marked at one end with the antibody (represented by the detection reagent, formed from mobile colloidal gold particles). It has a line in the center where the antigen is inserted, and a control line is marked with human IgM. Visual readings were taken by two independent readers, and a positive result was defined by visualization of both the control and test lines. The absence of the test line was considered a negative result, according to the manufacturer's specifications (IPTSP/UFG, GO, Brazil)[9].

Clinical examination

The HHC were clinically assessed at home, using a structured examination schema that contained the details of dermatological and neurological signs and symptoms of leprosy. This schema was designed to include even the nonspecific signs and symptoms of leprosy. The examination was performed by a specialist who had experience in diagnosing leprosy. The diagnosis of a case of leprosy was based on the presence of at least one of the following signs and symptoms:

1. lesion(s) and/or area(s) of the skin with altered sensation and
2. involvement of the peripheral nerve(s), with or without the thickening associated with sensory and/or motor and/or autonomic alterations[4].

Data were collected over 12 months (from November 2017 to November 2018).

The data were analyzed using Fisher's exact test to compare the frequencies between the groups. The significance level was set at $p<0.05$.

RESULTS

A total of 263 patients with leprosy were diagnosed during the study period. Of these index cases, 53 were enrolled in the study, and 210 were excluded for several reasons (Figure 1). Of the 53 participants, 108 household contacts were located.

A grouped bar chart representing the distribution of the index cases and examined HHC for each year of the study period is shown in Figure 2.

The grouped bar chart shows that the number of HHC examined in the later years was proportionally greater than that in the previous years. In contrast, the highest number of cases of leprosy was observed in the years at the beginning of the study period.

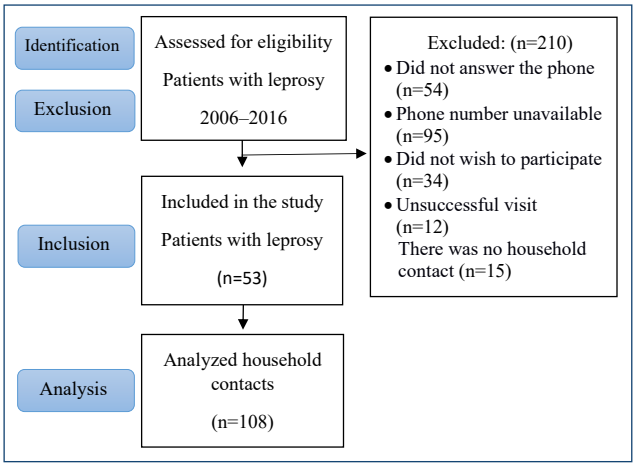

Figure 1. Flowchart of study participants.

The majority of the HHC were Caucasian women, who had been vaccinated with BCG at the time of the index case diagnosis and had a negative ML-flow test result (Table 1).

Table 1. Number and percentages of the examined contacts and the index cases according to the variables studied.

Data of the contacts examined (n=108)		
	Options	**n (%)**
Sex	Female	67 (62.04)
	Male	41 (37.96)
Age (years)	Mean±standard deviation	44.6±21.2
Ethnicity	Caucasian	65 (60.19)
	African American	6 (5.55)
	Mixed/Native	37 (34.26)
BCG vaccine	No	10 (9.26)
	Yes	98 (90.74)
ML-flow	Positive	2 (1.85)
	Negative	106 (98.15)
Case index data (n=53)		
Sex	Female	35 (66.04)
	Male	18 (33.96)
Age (years)	Mean±standard deviation	53.8±19
Ethnicity	Caucasian	34 (64.15)
	African American	2 (3.77)
	Mixed/Native	17 (32.08)
Operating classification	MB	27 (50.94)
	PB	26 (49.06)

BCG: bacillus Calmette-Guérrin; MB: multibacillary; PB: paucibacillary.

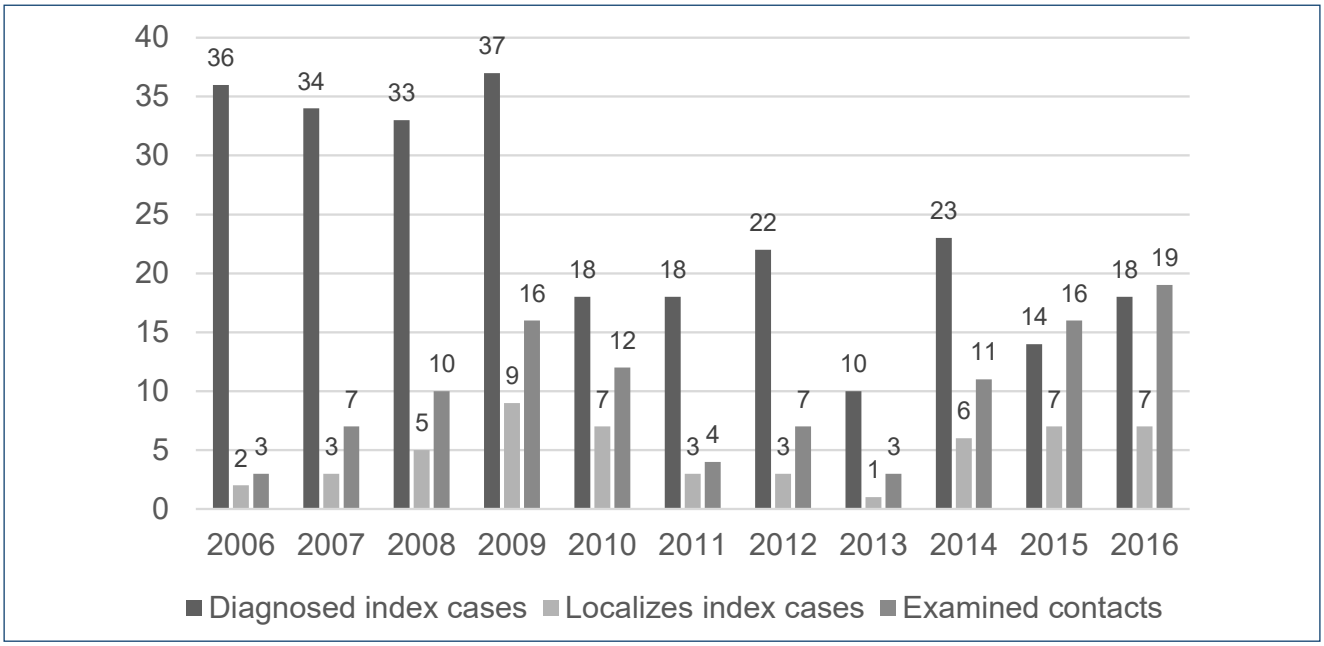

Figure 2. Distribution of the number of cases and contacts examined during the study period, from 2006–2016.

In two HHC (1.85%), the ML-flow test result was positive. One of them was a 100-year-old mixed-race woman who had received the BCG vaccine, and the index case related to her was a man with MB leprosy diagnosed in 2015. The second positive HHC was a 27-year-old mixed-race woman who had received the BCG vaccine, and the diagnosed case related to her was a man with PB leprosy diagnosed in 2009.

According to the operational classification, the HHC were almost equally related to the MB or PB leprosy cases. Of the 106 HHC who had a negative ML-flow test result, 54 (50.94%) were related to patients with MB leprosy and 52 (49.06%) to patients with PB leprosy (p=1).

Dermatological and neurological clinical examinations did not show leprosy symptoms or lesions in any of the HHC. Thus, the 2 (1.85%) HHC who had a positive ML-flow test were considered to have a subclinical infection and will be monitored annually, for 5 years, to enable early diagnosis if they eventually develop the disease. The other 106 (98.15%) HHC who had negative ML-flow test results and normal findings on clinical examination were considered normal. Thus, no new cases of leprosy were confirmed.

DISCUSSION

In this study, to improve early detection rates of patients with leprosy, anti-PGL-I antibodies were tested using ML-flow in the HHC of leprosy patients. A detection rate of 1.85% was observed, similar to that reported by Soares et al.[8] (1%). However, this rate is lower than the PGL-I seropositivity of up to 39% reported in most other studies[8,11].

Early detection of patients with leprosy is important in achieving disease control and elimination. For this, the most effective strategy is to monitor patients' HHC as a priority, especially if they have had prolonged exposure to untreated MB index cases. However, only clinical examination of HHC is recommended when the index case is diagnosed, which is not always sufficient to detect leprosy in the initial stage of infection as the diagnosis is only made when there are skin lesions and/or nerve damage. At this stage, transmission and incapacitating sequelae may have already occurred[5,7,11]. The use of other tools such as the ML-flow test may contribute to the identification of individuals with subclinical leprosy.

Positive serology in asymptomatic HHC not only means the transmission by the index case before treatment but also serves as a warning of the possibility that there are undiagnosed individuals living together with these HHC in the family nucleus or around.

Infected HHC who have good immunity against *M. leprae* or who will develop PB leprosy in the future may not have detectable anti-PGL-I antibody levels[6,9], and this may have been the case in this study. In addition, HHC of MB leprosy index cases who present with a high bacillary burden are more likely to become infected than those of PB leprosy index cases[8,9]. This could be another explanation for the low positivity in this study, as 49.06% of the index cases had PB leprosy.

Individuals who have had intimate and prolonged contact with untreated MB patients are at the greatest risk of transmitting leprosy[1]. Successful treatment of the index case will lead to cessation of *M. leprae* shedding within a few weeks of beginning multidrug therapy, reducing overall transmission and infection rates in HHC living with the case[1]. In this study, serological evaluation of the HHC was performed after the treatment of index cases; therefore, they were no longer exposed to the bacillus, and the period of exposure to the index case in the contagious phase may have been insufficient for disease transmission. Furthermore, in cases where an infection has occurred, the anti-PGL-I titer would likely be low-to-negative, particularly for those in the earlier years.

A vast majority of HHC received a prophylactic dose of the BCG vaccine at the time of the diagnosis of the index case. The BCG vaccine activates T-cell clones that recognize specific epitopes of *M. leprae*, conferring a protective effect against disease progression and leading to negative results for PGL-I tests that were previously positive[10,13].

This study has several limitations. The sample size was smaller than the calculated (ideal) size, which should have been 156, but 53 patients were included. Although we used strategies (including home visits, especially for the oldest cases) to reduce barriers and difficulties associated with accessing the index cases, we experienced difficulty in recruiting the participants because they either refused to participate or could not be located. Nevertheless, all registration data from the health sector database were considered for the initial screening of HHC, which probably preserved the sample representativeness. We also did not adopt any discriminatory criteria related to race, sex, and/or socioeconomic factors, which could have resulted in selection bias.

CONCLUSIONS

Our findings demonstrated no significant relationship between the studied variables. However, as only two HHC with a positive ML-flow test were observed, there was insufficient evidence to identify a relationship if any. Thus, future studies with larger sample sizes should be conducted to either rectify or ratify these

results. Because the ML-flow test is a quick, easy-to-perform, low-cost test that does not require laboratory structure, it can be easily used by health workers in field conditions and at different levels of care. Thus, it should be employed in screening at-risk populations, such as HHC, constituting an auxiliary tool for identifying an undiagnosed case, to sustain the elimination of leprosy in regions where this has already been achieved, as is the case in the region studied here.

AUTHORS' CONTRIBUTIONS

AMB: Conceptualization, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing. **SUS:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **ACCGT:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MAMMA:** Conceptualization, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing.

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