Revista da Sociedade Brasileira de Medicina Tropical 49(1):83-89, Jan-Feb, 2016
http://dx.doi.org/10.1590/0037-8682-0325-2015

Major Article

Factors associated with seropositivity for APGL-I among household contacts of leprosy patients

Carlos Gustavo Wambier[1],[2], *Sarah Perillo de Farias Wambier*[2], *Renata Bazan Furini*[1], *João Carlos Lopes Simão*[1], *Marco Andrey Cipriani Frade*[1] *and Norma Tiraboschi Foss*[1]

[1]. Divisão de Dermatologia, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil. [2]. Departamento de Medicina, Universidade Estadual de Ponta Grossa, Ponta Grossa, Paraná, Brasil.

ABSTRACT

Introduction: Leprosy is mainly transmitted among family members who share genetic and ambient factors. The clinical form of leprosy in the index case and kinship could be risk factors for leprosy transmission. High antibody levels in household contacts (HC) in the absence of neural or skin lesions may characterize latent infection. This study aimed to evaluate the association between seropositivity for anti-phenolic glycolipid-I immunoglobulin M antibodies (APGL-I) in HC and the clinical classification of the index case and to analyze the association between APGL-I positivity with other factors such as age, kinship, and gender. **Methods:** We performed a survey among 320 HC of 120 leprosy patients who were evaluated and followed-up in a leprosy outpatient clinic of a university hospital. All HC underwent complete skin examination, peripheral nerve palpation, skin sensory tests, and serologic tests for the detection and quantification of APGL-I. **Results:** The overall seropositivity rate was 20%, and was greatly affected by kinship. APGL-I seropositivity was higher in siblings (41%), followed by parents (28%), spouses (26%), other (19%), and offspring (14%). Independent risk factors for seropositivity were being siblings (OR 3.3) and being a HC of an index case with indeterminate leprosy (OR 5.3). APGL-I seropositivity was associated with index cases with a bacillary index of 4 (88%; p<.001). Seropositivity among HC was not significantly associated with their gender and age. There was no statistical difference in the seropositivity rates of HC of index patients with paucibacillary and multibacillary leprosy. **Conclusions:** Strict evaluation and follow-up of HC with positive results for APGL-I is recommended. Special attention should be paid during the screening of siblings of the index cases, HC of patients with a high bacillary index, and HC of patients with indeterminate leprosy.

Keywords: Leprosy. Household contacts. Serology. Antibodies.

INTRODUCTION

Leprosy is a chronic infection by *Mycobacterium leprae* that causes a spectrum of clinical manifestations attributed to individual cellular immune reactivity[1,2]. In cases of tuberculoid leprosy (TT), the immune system is capable of destroying the causative pathogen through the cellular immune response, resulting in well-defined granulomatous skin lesions, with no detectable bacilli[1], and low levels of anti-phenolic glycolipid-I immunoglobulin M (APGL-I), specific antibodies against *Mycobacterium leprae*[1,3]. On the other end of the spectrum, cases of lepromatous leprosy (LL) are characterized by an individual's inability to develop an effective cellular immune response, leading to mycobacterial survival and multiplication, and dissemination of skin lesions with a high bacillary load. These LL patients, however, develop a strong, yet ineffective, humoral immune response with high levels of APGL-I[1,4].

Corresponding author: Prof. Carlos Gustavo Wambier.
e-mail: cwambier@usp.br
Received 9 October 2015
Accepted 15 February 2016

Most patients with multibacillary leprosy (MB) present with high levels of APGL-I that correlated with the bacillary load[3,4]. Studies have reported that 40-95% of MB patients present with high levels of APGL-I[3,5,6,7,8], whereas 15-28% of paucibacillary leprosy (PB) patients present with low levels of APGL-I[6,8], similar to that noted in the normal control population[1]. Therefore, APGL-I quantification could be used as an indirect parameter of mycobacterial load and the possible transmission of bacilli. After specific treatment with standard regimens recommended by the World Health Organization (WHO)[9], individuals present with a steep decrease in APGL-I levels[3,4,5,6].

Transmission of *M. leprae* is favored by the high resistance of the bacillus outside the human body, where it may remain viable for up to 9 days[10], and by long and close contact with a MB patient, a.k.a. the index case. These factors are characteristic of household contacts (HC), as they live in the same house for many years, and are exposed insidiously to the bacilli. The bacillary viability outside the human body and repeated exposure for long periods act synergistically with the characteristic high infect ability of *M. leprae*. However, as a regulatory mechanism, this pathogen has a very slow replication rate and a very long incubation time. Owing to these complex interactions with the infected host, pathogenicity is low[1].

In endemic areas, special attention must be given to the possibility of household transmission. Health professionals in these regions must remain alert to the clinical classification of the index case and the kinship with the exposed HC, as these constitute risk factors for the disease[11]. A previous study reported that HC have a high risk of leprosy[12]. A previous study reported a higher incidence of APGL-I positivity among HC than among the general population[6]. High levels of APGL-I in the absence of neural or skin lesions in HC may characterize a subclinical infection[13]. Subclinical leprosy infection remains controversial, but it could be defined as a latent infection when patients present with evidence of increasing antibody levels in the absence of neural or cutaneous lesions. This *latent* infection could spontaneously resolve with a steady decrease in antibody levels or progress by presenting obvious cardinal signs of leprosy. One may imagine that a case of indeterminate leprosy, which is considered an early form of infective presentation, may remain as an occult subclinical infection for years before a skin lesion or symptom is noticed.

In healthy individuals, the seropositivity to APGL-I is associated with a higher risk for leprosy. In a study involving 60 healthy, APGL-I-positive HC, 10 (17%) developed signs of leprosy after a 4-year follow-up, resulting in a 8.65 times higher risk of leprosy for APGL-I-positive HC than that for the other APGL-I negative HC[14].

Susceptibility of HC to infection can be inferred based on the APGL-I levels, which may also suggest, in cases of infection, whether the individual is prone to the development of MB or PB leprosy. In a prospective study, healthy HC with leprosy were followed-up for 12 years. The authors found that APGL-I-positive individuals had 7.65 times higher risk of exhibiting leprosy signs than the APGL-I-negative individuals, and they had a much higher risk for developing MB leprosy (34.4 times) than that for developing PB leprosy (3.52 times)[15]. These results indicate that in the eventual initiation of chemoprophylaxis for HC by the World Health Organization (WHO)[16], APGL-I-positive HC would be the key focus for reducing leprosy transmission rates.

Although the relevance of detecting APGL-I in HC is well known, the relationship between the levels of this antibody and the clinical classification of the index case to which the HC is exposed is still unknown. This study aimed to evaluate the association between the results of tests for detecting APGL-I in HC and the clinical classification of the index case and to analyze the association between APGL-I positivity with other factors such as age, kinship, and gender.

METHODS

A cross-sectional survey included HC of all newly diagnosed leprosy patients (index cases) who were treated from 2000 to 2007 at an outpatient clinic of a national reference center for leprosy treatment in the State of São Paulo, Brazil, during the study period.

Index cases were classified according to the WHO International Classification of Diseases, 10th revision (ICD-10)[17] based on the clinical manifestations, histological examination results, and results of skin smear tests as follows: TT, borderline-tuberculoid (BT), borderline (BB), borderline-lepromatous (BL), LL, and indeterminate leprosy[2][18]. They were also operationally classified as MB (6 or more skin patches or more than one nerve involvement) or PB (1-5 skin patches or only one nerve involvement)[9], which was used to guide the prescription of multidrug therapy (MDT) recommended by the WHO.

All HC underwent complete skin examination, peripheral nerve palpation, skin sensory tests, and serologic tests for the detection and quantification of APGL-I antibodies.

Exclusion criteria were as follows: current residence in another state, debilitating chronic diseases, autoimmune or inflammatory diseases, and other systemic infections. All individuals participated in this study voluntarily. After obtaining oral consent, the contacts or legal responsible adults (if age <18 years) read and signed the informed consent form. The present study was approved by the Hospital of Clinics of Ribeirão Preto Ethics Committee under #12838/2006.

Enzyme-linked immunosorbent assay (ELISA) was used for detection and quantification of APGL-I. A sample of 5-10mL of peripheral venous blood was drawn into a covered tube (BD Vacutainer® Serum Tube, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) without any anticoagulant. Blood was allowed to clot at room temperature before centrifugation at 3,500 rotations per minute for 10-15 minutes (approximate relative centrifugal force of 1,240G). The serum samples were separated into aliquot tubes using a transfer pipette and were immediately stored at -20°C. Frozen aliquots were thawed by acclimatization in room temperature for approximately 10 minutes followed by incubation in a humid chamber at 37°C, with gentle swirls every 10 minutes until the serum was completely thawed. Ninety-six-well polystyrene plates (PolySorp, Corning Costar, NY, USA) were coated with 2µg/mL of a semi-synthetic analogue of phenolic glycolipid-I, natural disaccharide-octyl-bovine serum albumin, (ND-O-BSA); Colorado State University, USA, in sodium carbonate buffer, pH 9.6, and stored at 4°C overnight until use. Serum samples from each patient were diluted 1:100 in 15mM Tris-Tween (20mM Tris, 150mM NaCl and 0.1% Tween) buffer containing 5% sheep serum; 10µL was added to each well and the plate was incubated in a humid chamber for 1h at 37°C. The samples were then washed with 15mM Tris-Tween buffer and anti-human IgM β-galactosidase conjugate (Sigma-Aldrich, USA) diluted 1:600 in 15mM Tris-Tween buffer containing 5% sheep serum was added. The plates were then incubated at 37°C for 1h. Ten microliters of 4-methylumbelliferyl β-D-galactopyranoside (Sigma-Aldrich, USA), a fluorogenic substrate, were then added to the samples and was incubated at 37°C for 30 min. The optical density (OD) of each plate was read at 450nm with a MultiSkan™ Microplate photometer (Thermo Scientific, USA). Each serum sample was tested in duplicate; the mean absorbance of the duplicates was used to calculate the antibody titers, expressed by the ELISA index for APGL-I, which was calculated by the ratio of the mean OD of the sample duplicates to the cut-off OD. The cut-off OD was calculated by adding three standard deviations to the mean absorbance of 35 healthy control subjects. APGL-I≥1.0 was considered positive.

Fisher's exact test was used to evaluate the significance of the association between categorical variables. Logistic regression was used to determine the strength of the association (*odds ratio*) between seropositivity of HC and their gender, age group, kinship, and classification of leprosy of the index case. P-values <.05 were considered significant. Statistical analysis was performed with Stata™ SE 12.0 for Mac (StataCorp LP, College Station, TX, USA).

RESULTS

Of a total of 120 index cases, 320 HC were included in the study. Of these, 17% were spouses (mean age 48.4 years), 35% were offspring (mean age 20.6 years), 5% were siblings (mean age 32.5 years), 6% were parents (mean age 55.7 years), and 38% were other (mean age 19.1 years). The age of HC ranged from 1 to 82 years (mean 27.3±19.5 years). HC were divided into 3 groups based on their age: <14 years (31.3%), 14-34 years (35.9%), and >34 years (32.8%). Most (59.1%) HC were female. One (20%) in every five HC had a positive serological test result.

Table 1 shows the epidemiologic and serologic characteristics of the HC evaluated in this study. The frequency of APGL-I positivity was similar among the three age groups: <14 years (18%), 14-34 years (21.7%), and >34 years (21%). seropositivity for APGL-I was noted in 23.2% of female HC and 16.2% of male HC (p=.157). When the clinical form of the index case

TABLE 1 - Epidemiological characteristics and anti-phenolic glycolipid-I immunoglobulin M seropositivity of 320 household contacts of leprosy cases evaluated from 2000 to 2007.

	Individuals		APGL-I ≥1	
	n	%	n	%
Age group (years)				
<14	100	31.0	18	18.0
14-34	115	36.0	25	22.0
>34	105	33.0	22	21.0
Gender				
male	131	41.0	21	16.0
female	189	59.0	44	23.0
Clinical classification of the index case				
indeterminate leprosy	23	7.0	7	30.0
tuberculoid leprosy	34	11.0	3	9.0
borderline-tuberculoid leprosy	51	16.0	12	24.0
borderline leprosy	43	13.0	5	12.0
borderline-lepromatous leprosy	66	21.0	16	24.0
lepromatous leprosy	103	32.0	22	21.0
Kinship				
spouse	54	17.0	14	26.0
offspring	111	35.0	16	14.0
siblings	17	5.0	7	41.0
parents	18	6.0	5	28.0
other	120	38.0	23	19.0
WHO classification of the index case				
multibacillary	215	67.0	43	20.0
paucibacillary	105	33.0	22	21.0
Bacillary index of the index case				
0	96	30.0	18	18.0
1	37	12.0	3	8.0
2	32	10.0	4	13.0
3	87	27.0	22	25.0
4	8	3.0	7	88.0
Total	320	100.0	65	20.0

APGL-I: anti-phenolic glycolipid-I immunoglobulin M; **WHO:** World Health Organization.

was analyzed, APGL-I seropositivity rates among HC was as follows: indeterminate leprosy, 30%; BT, 24%; BL, 24%; LL, 21%; BB, 12%; and TT, 9%. When kinship was analyzed, APGL-I seropositivity among siblings was extremely common (41%), followed by parents (28%), spouses (26%), other (19%), and offspring (14%). Bacillary index (BI) among index cases ranged from zero to four. APGL-I seropositivity rates among HC of index cases with BI of 0, 1, 2, 3, and 4 was 18%, 8%, 13%, 25%, and 88%, respectively. A BI of 4 was associated with APGL-I seropositivity (p<.001). The frequency of seropositivity among HC of index cases with MB (20%) did not differ significantly from that among HC of index cases with PB (21.0%; p=.88).

Multivariate logistic regression (**Table 2**) showed that HC seropositivity is associated with the classification of leprosy of the index case. Index cases with indeterminate leprosy presented *odds ratio* of 5.3, index cases with BT presented odds ratio of 3.5, index cases with BL presented odds ratio of 3.1, and index cases with LL presented odds ratio of 2.7. With respect to results of the kinship analysis, siblings had an *odds ratio* of 3.3, and parents had an *odds ratio* of 2.0. There was statistical significance (p<.05) for index cases with indeterminate leprosy (p=.03) and siblings (p=.04).

Figure 1 illustrates a *box-plot* distribution of the quantification of APGL-I antibodies among HC by the clinical classification of leprosy of the index case. This plotting method also depicts the number of HC whose APGL-I levels were much higher than the levels expected for that distribution, the outliners, which are probably individuals with the greatest risk of MB leprosy. MB forms presented the highest number of outliners, which occurred mostly in LL and BL. In contrast, TT presented no outliners.

DISCUSSION

The results of this study showed a high (20%) seropositivity rate among the HC, probably because of the high prevalence of LL, BL, BB, and BT in this outpatient clinic of a national reference center for leprosy treatment. A previous study in Rio de Janeiro involving 2,135 HC revealed seropositivity rate of 16%[19], while a study in hyperendemic municipalities in the Brazilian Amazon Region involving 1,592 school children revealed a seropositivity of 48.8%, and of 256 HC, 41.8% were seropositive for APGL-I[20]. In the endemic areas of Brazil, the expected APGL-I seropositivity rate is 7% among non-contacts and 10% among HC[14]. In a study of healthy blood donors in Ribeirão Preto, only 3% (10/324) were seropositive[7]. Thus, seropositivity is directly related to genetic susceptibility and frequent exposure to *M. leprae*. This may be the reason why seropositivity was extremely high (41%) among siblings, since siblings who reside in the same house are exposed to similar mycobacterial load, and may have similar levels of susceptibility owing to similar genetic backgrounds.

TABLE 2 - Association of anti-phenolic glycolipid-I immunoglobulin M seropositivity with characteristics of the 320 household contacts evaluated from 2000 to 2007: clinical classification of the index case, age group, kinship, and age group.

	Odds ratio	p	95% confidence interval
Index case			
indeterminate leprosy	5.3	*0.03	[1.2–24.2]
tuberculoid leprosy	1	-	-
borderline-tuberculoid leprosy	3.5	0.08	[0.9–13.8]
borderline leprosy	1.3	0.71	[0.3–6.3]
borderline-lepromatous leprosy	3.1	0.10	[0.8–11.9]
lepromatous leprosy	2.7	0.14	[0.7–9.7]
Age group (years)			
<14	0.9	0.73	[0.4–1.8]
14-34	1	-	-
>34	0.5	0.13	[0.2–1.2]
Kinship			
offspring	0.6	0.16	[0.3–1.2]
spouse	1.8	0.25	[0.7–5.0]
sibling	3.3	*0.04	[1.0–10.5]
parents	2.0	0.30	[0.5–7.7]
other	1	-	-
Gender			
male	1	-	-
female	1.5	0.19	[0.8–2.8]

*p<.05

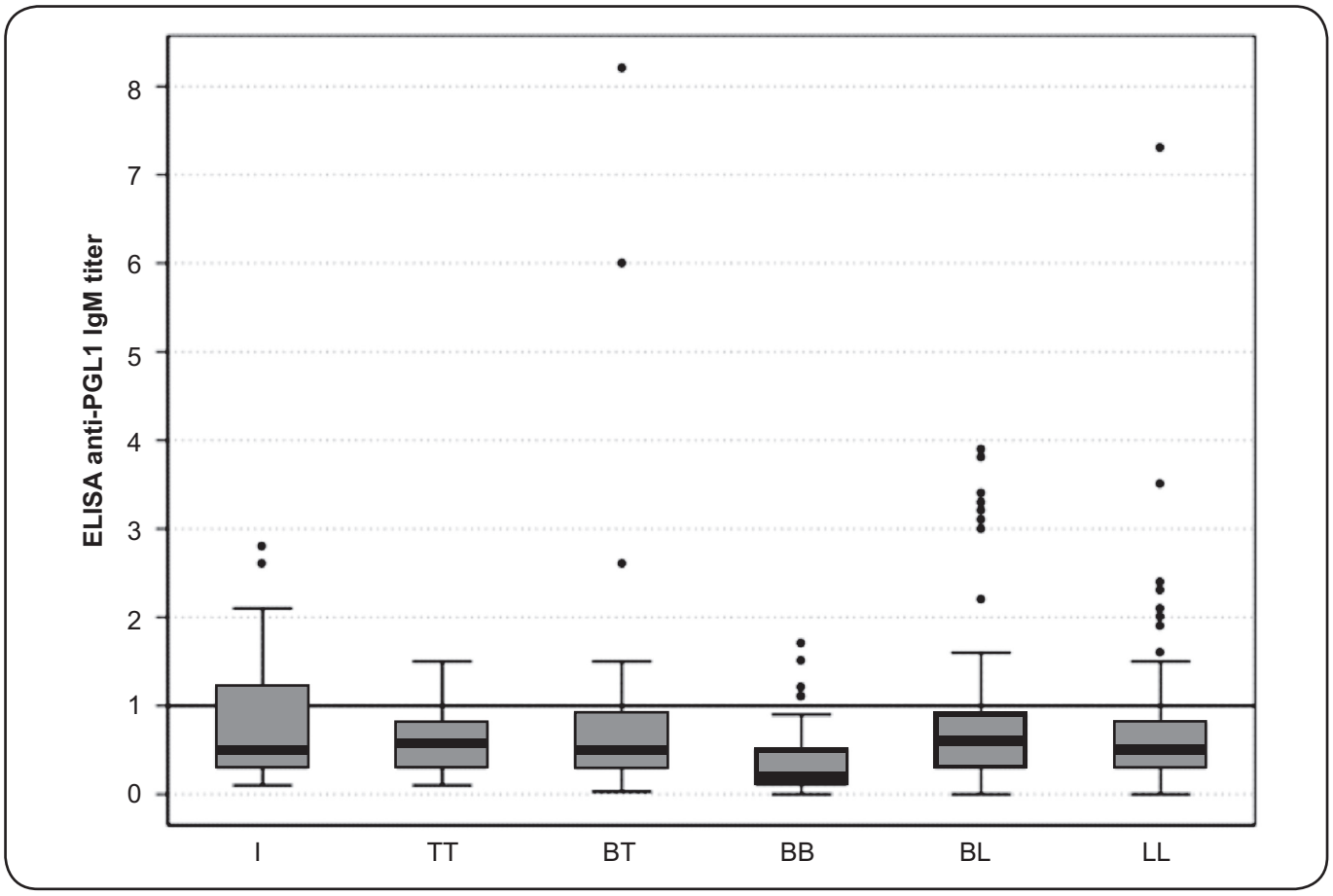

FIGURE 1 - Serologic test results (ELISA) regarding anti-phenolic glycolipid-I immunoglobulin M of 320 household contacts evaluated from 2000 to 2007 according to theclinical classification of the index case. I: indeterminate leprosy; **TT:** tuberculoid leprosy; **BT:** borderline-tuberculoid leprosy; **BB:** borderline leprosy; **BL:** borderline-lepromatous leprosy; **LL:** lepromatous leprosy; **ELISA:** enzyme-linked immunosorbent assay; **APGL-I:** anti-phenolic glycolipid-I immunoglobulin M.

When clinical classification of the index cases was analyzed, results showed a surprisingly high seropositivity among HC of index cases with indeterminate leprosy (30%) with an elevated odds ratio of 5.3, followed by high odds ratio of 3.5 for BT, of 3.1 for BL, and 2.7 for LL. Although the small sample size is one of the limitations of this study, this controversial result suggests that we must reconsider the role of indeterminate leprosy in the early detection of leprosy in HC. As suggested in the literature, indeterminate leprosy is the initial clinical manifestation of a recent infection, when an immune-cellular specific response against *M. leprae* is dubious. These results suggest that some of these cases could evolve into MB forms, thus favoring household transmission, as described by Martinez et al.[21]. Conversely, there is a possibility of other occult MB cases in the same house, leading to exposure to the bacilli and a high probability of subclinical infection among these HC. If more than one family member has a subclinical infection, the one with preserved cellular immunity is expected to present inflammatory symptoms first, which probably presents as indeterminate leprosy or as the PB form. Among those with strong cellular immunity, the subclinical infection is spontaneously cured before the development of skin or neural symptoms.

A recent study that evaluated the impact of APGL-I seropositivity on the protective effect of Bacillus Calmette-Guérin (BCG) vaccination among HC[19] showed that after the follow-up, the prevalence of leprosy was 2.2% among APGL-I-negative HC and 5.6% among APGL-I-positive HC. Most MB cases were diagnosed in unvaccinated APGL-I positive HC. Vaccination increased the rate of developing clinical manifestations of PB in APGL-I-positive HC[19]. BCG vaccination increases tumor necrosis factor alpha (TNF-α) production in MB patients, and interferon gamma (IFN-γ and interleukin-17 (IL-17) production in PB patients[22].

In general, HC are individuals who share genetic and ambient factors. The HC of MB index cases are more frequently exposed to bacilli from the index case, and there is a tendency to develop clinical forms similar to that of the index case, as depicted by the outliners in **Figure 1**. Owing to the long incubation period of leprosy, HC with high APGL-I titers need to be rigorously followed-up for years, with careful evaluation for subtle manifestations of leprosy, by esthesiometry, electroneuromyography, and attentive palpation of peripheral nerves, even if they do not present with leprosy manifestations

during the first visits. Our study results reveal that attention should be paid to the evaluation of HC of index cases with indeterminate leprosy, as well as MB cases, and their siblings for the high APGL-I seropositivity and in some cases, high APGL-I levels, which could represent subclinical leprosy.

Thus, other than being used for serological monitoring in the therapeutic follow-up of MB cases, serological tests for APGL-I can also be used for detecting cases of subclinical leprosy, especially among HC of index cases with a high BI, because they have the potential to develop MB leprosy, increasing transmission rates. The follow-up of these HC is of extreme importance, since an increase or maintenance of APGL-I levels indicates active subclinical infection[19] [20] [23]. Individual immunologic status could occult clinically detectable signs of leprosy for many years[24]. It is important to reemphasize that none of the HC included in this study presented with leprosy during the course of the present study. In 2017, an ongoing study will be able to present the results of a 10-year follow-up of these HC, including data obtained by clinical and serological assessments.

In 2015, Araujo et al.[25] presented the results of a 10-year follow-up of 2,992 HC. Approximately 1.6% of these HC developed clinical manifestations for the diagnosis of leprosy during the study period. APGL-I-positive HC had a relative risk of 5.688 for a diagnosis of leprosy; the majority of HC diagnosed with leprosy were seropositive for APGL-I (55.3%). Conversely, the majority of healthy HC were seronegative (88.2%). Among the 47 HC diagnosed with leprosy, most HC were diagnosed in the first year. None of the 24 HC of index cases with indeterminate leprosy were diagnosed with leprosy, and the study did not specifically provide detailed data regarding the APGL-I seropositivity rate according to each index case classification.

This study presented detailed characterization of the HC profile more susceptible to be infected by leprosy, however, a larger sample size and current data on the percentage of later diagnosis of leprosy would certainly improve the insights of this study.

Additional studies are needed for the introduction of chemoprophylaxis or vaccination among HC, to prevent the development of leprosy in healthy individuals[16]; rifampicin along with another antibiotic agent can be administered, especially to seropositive HC, who may have a higher risk for developing MB leprosy throughout the following years[19]. Currently, rifampicin resistance does not seem to be a significant factor for relapse of leprosy[16]. Nevertheless, drug resistance should be considered when making decisions regarding chemoprophylaxis for leprosy patients[16]. In the absence of effective chemoprophylaxis or vaccines, early detection and treatment of all cases before the development of disabilities remains the key strategy for reducing the disease burden[26].

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Foss NT. Hanseniase: aspectos clinicos, imunológicos e terapêuticos. An Bras Dermatol 1999; 74:113-119.
2. Souza CS. Hanseníase: formas clínicas e diagnóstico diferencial. Med (Ribeirão Preto Online) 1997; 30:325.
3. Zenha EMR, Ferreira MN, Foss NT. Use of anti-PGL-1 antibodies to monitor therapy regimes in leprosy patients. Braz J Med Biol Res 2009; 42:968-972.
4. Cho SN, Cellona RV, Fajardo TT, Abalos RM, dela Cruz EC, Walsh GP, et al. Detection of phenolic glycolipid-I antigen and antibody in sera from new and relapsed lepromatous patients treated with various drug regimens. Int J Lepr Other Mycobact Dis 1991; 59:25-31.
5. Roche PW, Britton WJ, Failbus SS, Neupane KD, Theuvenet WJ. Serological monitoring of the response to chemotherapy in leprosy patients. Int J Lepr Other Mycobact Dis 1993; 61:35-43.
6. Cellona RV, Walsh GP, Fajardo Jr TT, Abalos RM, dela Cruz EC, Guido-Villahermosa L, et al. Cross-sectional assessment of ELISA reactivity in leprosy patients, contacts, and normal population using the semisynthetic antigen natural disaccharide octyl bovine serum albumin (ND-O-BSA) *in Cebu,* The Philippines. Int J Lepr Other Mycobact Dis Off Organ Int Lepr Assoc 1993; 61:192-198.
7. Foss NT, Callera F, Alberto FL. Anti-PGL1 levels in leprosy patients and their contacts. Brazilian J Med Biol Res 1993; 26:43-51.
8. Dhandayuthapani S, Anandan D, Bhatia VN. ELISA & lepromin skin tests in household contacts of leprosy patients. Indian J Med Res 1990; 91:431-436.
9. World Health Organization. Leprosy Elimination Advisory Group. Guide to eliminate leprosy as a public health problem: WHO standard multidrug therapy (MDT) cures leprosy, stops transmission and prevents disabilities. Geneva: 2000.
10. Desikan KV. Viability of *Mycobacterium leprae* outside the human body. Lepr Rev 1977; 48:231-235.
11. Moura RS, Calado KL, Oliveira MLW, Bührer-Sékula S. Leprosy serology using PGL-I: a systematic review. Rev Soc Bras Med Trop 2008; 41 (supl II):11-18.
12. van Beers SM, de Wit MY, Klatser PR. The epidemiology of *Mycobacterium leprae*: recent insight. FEMS Microbiol Lett 1996; 136:221-230.
13. Baumgart KW, Britton WJ, Mullins RJ, Basten A, Barnetson RS. Subclinical infection with *Mycobacterium leprae*-a problem for leprosy control strategies. Trans R Soc Trop Med Hyg 1993; 87:412-415.
14. Brasil MTLR, Oliveira LR, Rímoli NS, Cavallari FS, Gonçalves O, Lessa Z, et al. Sorologia Anti PGL-1 e risco de ocorrência de hanseníase em área de alta endemicidade do Estado de São Paulo: quatro anos de seguimento. Rev Bras Epidemiol 2003; 6:262-271.
15. Douglas JT, Cellona RV, Fajardo TT, Abalos RM, Balagon MVF, Klatser PR. Prospective study of serological conversion as a risk factor for development of leprosy among household contacts. Clin Diagn Lab Immunol 2004; 11:897-900.
16. World Health Organization. Global leprosy update, 2013; reducing disease burden. Heal Sect Secr Leag Nations 2014; 89:389-400.
17. World Health Organization. WHO | International Classification of Diseases (ICD) [Internet]. Int. Classif. Dis. 2015 [cited 2015 Dec 28]; Available from: http://apps.who.int/classifications/icd10/browse/2016/en#/A30

18. Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. Int J Lepr Other Mycobact Dis 1966; 34:255-273.

19. Düppre NC, Camacho LAB, Sales AM, Illarramendi X, Nery JAC, Sampaio EP, et al. Impact of PGL-I Seropositivity on the Protective Effect of BCG Vaccination among Leprosy Contacts: A Cohort Study. PLoS Negl Trop Dis 2012; 6:e1711.

20. Barreto JG, Guimarães LS, Frade MAC, Rosa PS, Salgado CG. High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region. Mem Inst Oswaldo Cruz 2012; 107 (supl 1):60-67.

21. Martinez TS, Figueira MMNR, Costa AV, Gonçalves MA, Goulart LR, Goulart IMB. Oral mucosa as a source of *Mycobacterium leprae* infection and transmission, and implications of bacterial DNA detection and the immunological status. Clin Microbiol Infect 2011; 17:1653-1658.

22. Zenha EMR, Wambier CG, Novelino AL, Andrade TAM, Ferreira MAN, Frade MAC, et al. Clinical and immunological evaluation after BCG-id vaccine in leprosy patients in a 5-year follow-up study. J Inflamm Res 2012; 5:125-135.

23. Frota CC, Freitas MVC, Foss NT, Lima LNC, Rodrigues LC, Barreto ML, et al. Seropositivity to anti-phenolic glycolipid-I in leprosy cases, contacts and no known contacts of leprosy in an endemic and a non-endemic area in northeast Brazil. Trans R Soc Trop Med Hyg 2010; 104:490-495.

24. Saad MH, Medeiros MA, Gallo ME, Fonseca LS. Use of the anti-PGL-I antibody ELISA and the Mitsuda reaction in early diagnosis of leprosy. Brazilian J Med Biol Res 1991; 24:801-805.

25. Araujo S, Rezende MMF, Sousa DCR, Rosa MR, Santos DC, Goulart LR, et al. Risk-benefit assessment of Bacillus Calmette-Guérin vaccination, anti-phenolic glycolipid I serology, and Mitsuda test response: 10-year follow-up of household contacts of leprosy patients. Rev Soc Bras Med Trop 2015; 48:739-745.

26. World Health Organization. Global leprosy update, 2014: need for early case detection. Relev épidémiologique Hebd/Sect d'hygiène du Secrétariat la Société des Nations. Heal Sect Secr Leag Nations 2015; 90:461-474.