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Brief communication

Salivary anti-PGL-1 IgM may indicate active transmission of *Mycobacterium leprae* among young people under 16 years of age

Alexandre Casimiro de Macedo[a], *José Evandro Cunha Jr.*[a], *Juliana Navarro Ueda Yaochite*[a], *Clodis Maria Tavares*[b], *Aparecida Tiemi Nagao-Dias*[a,*]

[a] *Universidade Federal do Ceará (UFC), Faculdade de Farmácia, Departamento de Análises Clínicas e Toxicológicas, Fortaleza, CE, Brazil*
[b] *Universidade Federal de Alagoas, Faculdade de Enfermagem e Farmácia (ESENFAR), Maceio, AL, Brazil*

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ABSTRACT

Considering that the main route of *Mycobacterium leprae* transmission is the upper respiratory tract, detection of salivary antibodies can be a useful tool for diagnosing early infection. The study aimed to analyze salivary anti-PGL-1 IgA and IgM antibodies in 169 children aged 4–16 years old, who lived nearby or inside the house of multibacillary or paucibacillary leprosy patients in two endemic cities in Alagoas State – Brazil. Salivary anti-PGL-1 antibodies were quantified by modified ELISA method. The frequency of contact and clinical form of the index case were significantly associated with salivary antibody levels. High frequency of IgM positivity strongly suggests active transmission of *M. leprae* in these communities. We suggest in the present work that salivary anti-PGL IgA and IgM are important biomarkers to be used for identifying communities with probable active transmission of *M. leprae*.

Introduction

Brazil is the second country with the highest incidence of leprosy in the world. In 2015, the country presented a detection rate of 14.06 cases per 100,000 inhabitants.[1] Although the number of new cases seems to decrease, it may not represent the reality. For instance, the high incidence of the disease among children means that an active transmission occurs in the community.[2] In 2015, the detection rates of leprosy among people under 15 years old in Santana do Ipanema and Rio Largo, two Brazilian cities located in Alagoas State, were 13.77 and 32.81 per 100,000 inhabitants, respectively.[3]

As the bacteria are not cultivable, secretory antibodies can be a useful tool to detect early infection. The nasopharynx is the main portal of entry for *Mycobacterium leprae* (*M. leprae*), and the nasal epithelial cells are an important reservoir of the bacteria.[4] As mucosal immune organs and tissues compose an integrated system, saliva is frequently considered to be representative of mucosal humoral immune response. The purpose of the present work was to evaluate salivary anti-phenolic

* *Corresponding author.*
E-mail address: anagaodias@gmail.com (A.T. Nagao-Dias).
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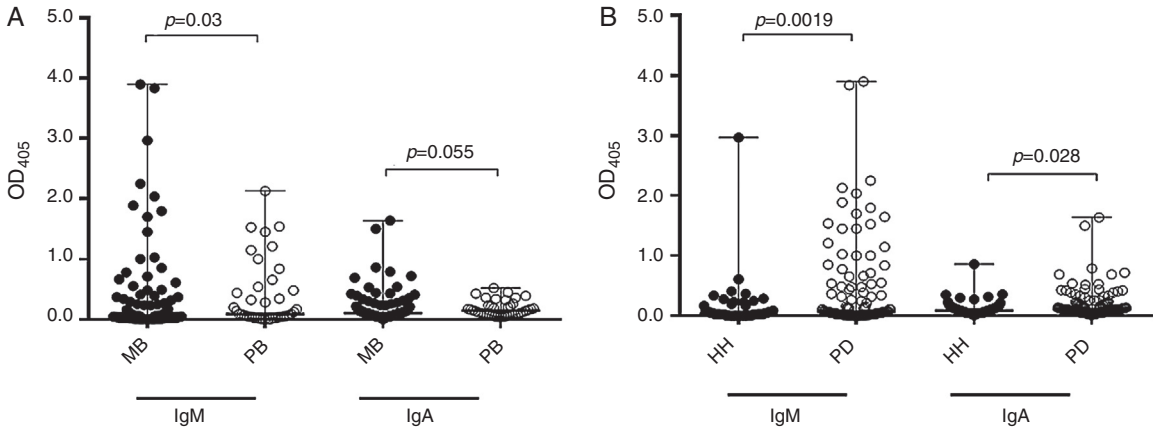

Fig. 1 – Levels of salivary anti-PGL-1 antibodies in 169 young contacts of leprosy patients. (A) Median and range of salivary anti-PGL1 IgM and IgA in contacts of multibacillary (MB contacts, *n* = 115) and paucibacillary (PB contacts, *n* = 40) leprosy patients. (B) Median and range of salivary anti-PGL1 IgM and IgA levels in household (HH, *n* = 57) and peridomiciliar (PD, *n* = 112) contacts. Salivary anti-PGL-1 antibodies were detected by modified ELISA method.

glycolipid 1 antigen (PGL-1) IgA and IgM isotypes among 169 leprosy contacts aged 4–16 years living in the municipalities of Santana do Ipanema and Rio Largo (Alagoas state, Brazil).

Methods

Subjects and sample collection

The contacts ($n = 169$) included in the study were classified as paucibacillary (PB contacts, $n = 40$) or multibacillary (MB contacts, $n = 115$) contacts, according to clinical form of the index case. Fourteen contacts were not classified because the information was not available in the patients' medical records. The participants were also classified as household contacts (HH, $n = 57$) or peridomiciliar contacts (PD, $n = 112$). Peridomiciliar contacts were those who were relatives of the index case but did not live in the same house or those who lived close to the index case's house (up to five houses apart). The project was approved by the National Committee for Ethics in Research. Unstimulated saliva samples were collected into tubes, which were transported with ice packs to the laboratory, where they were kept at −20 °C until testing (up to three weeks after collection). The presence of lesions and nerve enlargement were investigated at the moment of sample collection. Cases suspected of having the disease were referred to a doctor and excluded from the study.

Detection of salivary anti-PGL-1 antibodies

Microplates were coated with native PGL-1 at 5 mg/L in absolute alcohol for 2 h at 37 °C (protocol modified from Brito e Cabral et al., 2013).[5] After blocking with 1% fetal bovine serum (FBS, LGC Bio, Brazil)-Tris solution for 2 h at 37 °C, the wells were incubated with previously cenrifuged saliva samples (diluted to 1:50 with 1% FBS-Tris). After 18 h at 4 °C and washing with 0.05% FBS-Tris solution, anti-human IgA or anti-IgM alkaline phosphatase antibodies (Sigma, USA, 1:1000 in 1% FBS-Tris) were left on the plates for 2 h at 37 °C. After new incubation for 2 h at 37 °C, and washing, the substrate solution (1 mg/mL p-nitrophenyl phosphate in 10% diethanolamine containing 0.5 mM $MgCl_2$, pH 9.8) was added to the wells. After 100 min at room temperature, absorbance readings were recorded at 405 nm using an ELISA microplate reader. The results were expressed as the OD mean of the values (minus blank). The cut-off was based on the 97th percentile of normal controls.[6] Results 30% above the cut-off value were considered to be positive.

Analysis of data

The data were analyzed using nonparametric tests as the data did not follow a Gaussian distribution (Kolgomorov–Smirnov test). All statistical analysis was performed using GraphPad Prism version 5.0. The level of statistical significance was 5% ($p < 0.05$).

Results

Salivary anti-PGL-1 IgM presented good correlation to salivary IgA titers (Spearman correlation, $r = 0.71$, $p < 0.0001$). No statistical significance was found regarding the age range, either for IgM or IgA (Kruskall–Wallis test, $p = 0.149$ and $p = 0.312$, respectively, Table 1). No significant differences were either found in IgM or IgA titers in respect to the degree of relationship with the index case ($p = 0.325$ and $p = 0.590$, respectively, Table 1). Contacts who reported having weekly contact with the index case had higher IgM antibody titers than those with daily contact ($p = 0.04$, Table 1). MB leprosy contacts presented higher levels of salivary anti-PGL-1 IgM and IgA (Mann–Whitney test, $p = 0.03$ and $p = 0.05$, respectively) than PB leprosy contacts (Fig. 1A). Interestingly, PD contacts had higher levels of salivary IgM and IgA (Mann–Whitney test, $p = 0.019$ and $p = 0.028$, respectively) than the HH contacts (Fig. 1B).

Discussion

With the advent of multidrug therapy the report of new cases of leprosy had a sharp decrease. However, this decline has