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<th>Prevalence and levels of filaria-specific urinary IgG4 among children less than five years of age and the association of antibody positivity between children and their mothers.</th>
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PREVALENCE AND LEVELS OF FILARIA-SPECIFIC URINARY IgG4 AMONG CHILDREN LESS THAN FIVE YEARS OF AGE AND THE ASSOCIATION OF ANTIBODY POSITIVITY BETWEEN CHILDREN AND THEIR MOTHERS

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Abstract. An enzyme-linked immunosorbent assay (ELISA) to detect filaria-specific urinary IgG4 was tested in samples from 203 children less than five years old and their parents (165 mothers and 127 fathers) in Sri Lanka. There were four IgG4-positive children within 58 days after birth, suggesting the transfer of the antibody from mothers. No positive children were found between days 65 and 417. After day 1,000, the number of the positive individuals and the level of IgG4 increased quickly. The children of urinary IgG4-positive parents showed a higher IgG4 positive rate than those of negative parents. The children of positive mothers had a higher prevalence than those of negative mothers, whereas, the positivity of the fathers was not associated with that of their children. Collecting urine samples was easy to perform and well accepted because of its non-invasiveness. The ELISA will be useful for monitoring filarial infections in very young children, who are a sentinel population for evaluating the intensity of filariasis transmission and effectiveness of control measures.

INTRODUCTION

In filariasis epidemiology, only a limited number of studies have been carried out focusing on very young children. The low prevalence of microfilaremia and few clinical cases among them are some of the reasons.1,2 Recent application of highly sensitive antigen assays have detected more infections among children than recognized previously, and the importance of early infection in relation to future clinical manifestations was suggested.3 In addition, infections of childhood have been recognized as a valuable indicator of recent transmission levels, which can be used to evaluate effects of control measures.4–6 However, all methods used so far to detect filarial infection require blood, and this hinders sample collection in the field, particularly with pediatric subjects.

We reported a sensitive and specific enzyme-linked immunosorbent assay (ELISA) that detects filarial-specific IgG4 in urine. The ELISA had a sensitivity of 95.6% when tested on 91 Sri Lankan Wuchereria bancrofti microfilaria-positive and/or antigen-positive individuals, and a specificity of 99.0% with 298 controls from non-endemic areas from Thailand, Laos, and Japan.7 In the present study, taking advantage of the ease in collecting samples, we measured urinary IgG4 levels in pediatric subjects less than five years of age and their parents. The purpose of this study was to determine the prevalence and levels of IgG4 and to study the relationship between the antibody positivity of parents and their children. The usefulness of the ELISA in the evaluation of filarial transmission levels and control measures is discussed.

MATERIALS AND METHODS

The study was conducted in Walgama village, Matara District, Sri Lanka, where periodic type of W. bancrofti transmitted by Culex quinquefasciatus is endemic. A previous survey carried out in the same village covering all ages reported a microfilarial rate of 5.7%,2 and another study with the immunochromatographic card test (ICT) showed a prevalence of 31.1%.8 A house-to-house visit was made to collect urine samples from 203 children less than five years old and their parents (165 mothers and 127 fathers). An effort was made to include children of all ages (0–4 years old) as evenly as possible. If one of the parents was absent, he or she was not included in the study. A special plastic sampling bag with an adhesive collar (ATOM Pediatric Urine Collector; ATOM Medical, Tokyo, Japan) was used for collection of urine in babies (0–1.5 years old). It was attached to the skin around the opening of urethra and urine was collected a few hours later. Sodium azide was added to each sample (15 mL) at the final concentration of 0.1%. The samples were transferred to Japan and kept at 4°C until used. Samples can be stored at least for 14 months without deterioration.

The technical details of the ELISA have been previously reported.7 Briefly, crude antigen from Brugia pahangi females was isolated and resuspended at a concentration of 5 µg/mL in phosphate-buffered saline. Urine samples (100 µL) were incubated overnight at 25°C in antigen-coated wells of an ELISA plate. Samples were washed and peroxidase-conjugated mouse monoclonal antibody to human IgG4 (Caltag Laboratories Inc., San Francisco, CA) diluted 1:1,000, was added. ABTS (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) was used as the substrate and absorbance measured at 415 nm. The level of urinary IgG4 was expressed as an antibody unit that was calculated from a standard curve constructed for each ELISA plate using diluted standard sera obtained from confirmed cases of Bancroftian filariasis. The units in this system range from 0 to 7,290. Samples with units above this range were regarded as 7,290 units. The cut-off value (54.7 units) was computed as the geometric mean (unit + 1) plus three standard deviations of samples from 256 Thai and Laotian people living in areas without filariasis.

This study was reviewed and approved by the Ethical Committee of the University of Ruhuna (Galle, Sri Lanka). All parents were given an explanation of the purposes, methods, merits, and risks of the study for them and their children, and their consent was then obtained. For statistical analysis, a P value < 0.05 was considered significant.

RESULTS

The prevalence of IgG4-positive individuals according to age and sex is shown in Table 1. Children ≥ 3 years of age
(combined groups) had a significantly higher rate (24.1%) than the younger children (6.5%) \( (P < 0.001, \text{by chi-square test}) \). There was no difference among children when compared by sex, but fathers (62.2%) had a higher rate than mothers (49.1%) \( (P < 0.03, \text{by chi-square test}) \).

Urinary levels of filaria-specific IgG4 (antibody units) of 203 children and their parents (total = 292) according to age are shown in Figure 1. Age was based on the number of days after birth. One year was regarded as 365 days. There were four positive individuals within 58 days after birth. They had relatively low levels of antibody units (geometric mean = 162.7 units). No positive individuals were found between days 65 and 417, and only three positive individuals were found between days 423 and 1,000. The number of positive individuals increased after day 1,000 (2.7 years). The geometric mean of antibody units in 3–4-year-old IgG4-positive individuals was 308.1 units, which was not different from that of their mothers (293.7 units) \( (P > 0.8, \text{by } t\)-test) or fathers (587.9 units) \( (P > 0.07, \text{by } t\)-test). The average antibody unit value was higher in the fathers than in the mothers \( (P < 0.002, \text{by } t\)-test).

To study the effect of IgG4 positivity of parents on their children’s positivity, 122 pairs of parents were categorized into four groups (both parents positive; mother positive, father negative; mother negative, father positive; and both parents negative). A comparison of the rates of IgG4 positivity between these parental groups and their 145 children is shown in Table 2. When both parents were IgG4 positive, their children had a higher positive rate (26.5%) than those of the negative parents (3.1%) \( (P = 0.005, \text{by Fisher’s exact test}) \) and than those of the parents in which only the father was positive (5.1%) \( (P = 0.007, \text{by Fisher’s exact test}) \). The difference was not significant when compared with the parents in which only the mother was positive (16.0%) \( (P = 0.237, \text{by Fisher’s exact test}) \). When all mothers, including those not paired with a father, were dichotomized into positive and negative groups, the children of the positive mothers showed a higher rate (21.1%) than those of the negative mothers (5.9%) \( (P < 0.002, \text{by chi-square test}) \). Similarly dichotomized fathers did not show a significant difference (17.4% versus 8.5%; \( P > 0.1, \text{by chi-square test}) \).

To study the effect of antibody levels of the parents on those of their children, antibody units of mothers or fathers were correlated with those of children in two age groups: <3 years old and the 3–4 years old. In this computation, the antibody unit of a mother or father with two children was used twice in pairs with her or his children’s units. All units were transformed into log (antibody unit + 1) to accommodate zero

![Figure 1](image_url)

**FIGURE 1.** Urinary IgG4 levels of children less than five years old and their parents according to age. **A,** Children and their parents. **B,** Children only. The urinary IgG4 level is expressed as log (antibody unit + 1). The dotted horizontal lines indicate the cut-off values.

<table>
<thead>
<tr>
<th>Category</th>
<th>No. in category</th>
<th>No. examined</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo (+) and Fa (+)</td>
<td>43</td>
<td>49</td>
<td>13</td>
<td>26.5</td>
</tr>
<tr>
<td>Mo (+) and Fa (−)</td>
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<td>25</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>Mo (−) and Fa (+)</td>
<td>33</td>
<td>39</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Mo (−) and Fa (−)</td>
<td>25</td>
<td>32</td>
<td>1</td>
<td>3.1</td>
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<tr>
<td>All mothers/fathers</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo (+)</td>
<td>81</td>
<td>95</td>
<td>20</td>
<td>21.1</td>
</tr>
<tr>
<td>Mo (−)</td>
<td>84</td>
<td>102</td>
<td>6</td>
<td>5.9</td>
</tr>
<tr>
<td>Fa (+)</td>
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<td>92</td>
<td>16</td>
<td>17.4</td>
</tr>
<tr>
<td>Fa (−)</td>
<td>48</td>
<td>59</td>
<td>5</td>
<td>8.5</td>
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*Mo = mother; Fa = father; + = positive; − = negative.*
units. The only significant positive correlation was between IgG4 levels of mothers and children 3–4 years old (r = 0.34, P < 0.005).

**DISCUSSION**

When the IgG4 ELISA was tested with urine samples, it confirmed previously reported epidemiologic findings based on parasitologic methods, i.e., higher prevalences in older age groups and in adult males. Prevalence did not differ by sex in those less than five years of age, suggesting similar exposure to infective bites and more homogenous biologic and behavioral conditions in children as a host compared with adults. The IgG4 response in children occurred quickly, and the mean antibody unit among those positive reached the level of adults at 3–4 years of age. In Tanzania, the highest serum IgG4 level was reported in 1−9-year-old children with microfilaremia. In several studies based on microfilaria or filarial antigens, higher positivity rates were reported in children of positive parents, especially mothers, than in those of negative parents. The present study with urinary IgG4 confirmed that the children of positive parents had a higher positive rate than those of negative parents or of only fathers who were positive. However, there was no difference when these positive rates were compared with those of only mothers who were positive. Also, the children of positive mothers had a higher rate than those of negative mothers, whereas positivity of the father did not influence that of the children. The strong influence of mothers is often attributed to in utero sensitization of a fetus to parasite antigens. A recent study showed that a positive correlation of anti-filarial IgG4 levels between mothers and their 0–2-year-old children supports this idea. Our result, which showed a similar correlation between mothers and children 3–4 years old and no correlation between fathers and children, is consistent with this observation. Close mother-child contact in daily life, together with the peridomestic habitat of the mosquito vector *C. quinquefasciatus*, could also enhance the influence of the mother. From an epidemiologic standpoint, IgG4-positive mothers would be an important target group for treatment. Thus, the ELISA using urine has definite advantages in the field. In the village where many people do not agree to blood sampling, urine collection was well accepted or even welcomed. Babies did not show any sign of fear or irritation during sampling with a collection bag. The inclusion of young children as a sentinel population could provide a valuable indicator for evaluating the intensity of filarial transmission, and its change after control measures. In addition, purified and easy-to-supply antigens will result in further improvement of the ELISA.

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**REFERENCES**

2. Weerasooriya MV, Weerasooriya TR, Gunawardena NK, Sama-


