HIV-1 Specific Immunoglobulin A Antibodies as an Effective Marker of Perinatal Infection in Developing Countries

by D. Moodley,* H. M. Coovadia,* R. A. Bobat* and J. L. Sullivan**

*Department of Paediatrics and Child Health, Faculty of Medicine, University of Natal, Durban, South Africa
**Department of Paediatrics, University of Massachusetts, Worcester, USA

Summary

HIV-1 specific IgA antibody testing using commercially available reagents was evaluated at birth to 15 months in a group of infants born to HIV-seropositive South African women. Following IgG depletion of serum samples, 33/35 (94 per cent) of the infected infants and 3/99 (3 per cent) of the uninfected infants showed positive IgA reactivity. Sensitivity at birth was 24 per cent and improved with age; 82 per cent at 3 months, 87 per cent at 6 months and 94 per cent at 12 months. The overall positive and negative predictive values were 92 and 98 per cent, respectively. An evaluation of IgA and PCR in a subsample of infants indicated a better sensitivity of PCR within 3 months of birth, but IgA detection offered a higher overall sensitivity (87 v. 83 per cent) and specificity (91 v. 85 per cent). No significant difference in IgA level was observed between transmitting mothers and non-transmitting mothers. A moderate correlation existed between IgA level in the infant and the cumulative morbidity score, however a stronger association was observed between high IgA levels in the infected infant and rapid disease progression. The viral specific IgA assay is a simple, reliable and cost-effective diagnostic and prognostic test for perinatal HIV infection in developing countries.

Introduction

Diagnosis of HIV-1 vertical transmission is hampered by the presence of maternal immunoglobulin G (IgG) antibodies in the baby which confound results obtained through conventional screening by enzyme immuno-assay (EIA). Advancement in technology such as viral culture and polymerase chain reaction (PCR) in more developed countries has improved early diagnosis in the infant from 1 month of age, although sensitivity at birth is as low as 30 per cent. However, due to the lack of appropriate funds, technology, and expertise in developing countries the persistence or clearance of HIV-1 specific IgG antibodies in peripheral blood at 18 months remain the only definitive laboratory criteria for distinguishing between HIV-1 infected and uninfected infants in less developed countries.

An initial report of the presence of anti-HIV IgA antibodies by enzyme immuno-assay (EIA) as an indication of a more specific immune response has been widely investigated as a diagnostic marker of perinatal HIV-1 infection in more developed countries. The sensitivity and specificity of this assay has improved since the first report of its use in the early diagnosis of perinatal HIV-1 infection, and due to the low cost and simplicity it has been recommended for less developed countries. However, the reliability and predictive value of this assay among infants in less developed countries have not been fully assessed. We therefore compared the HIV-1 IgA EIA to PCR performed on serum and peripheral blood lymphocytes, respectively, from South African Black children born to HIV-1 seropositive mothers. We report the sensitivity, specificity, predictive values, and cost of both tests at periodic time intervals.

Patients and Methods

A total number of 745 stored serum samples obtained from 172 children born to HIV-1 seropositive women at King Edward VIII Hospital, Durban, South Africa, were tested for HIV-1 specific IgA antibodies. Blood samples were collected at birth and at 3-monthly intervals thereafter until 18 months of age. Among infants surviving to at least 15 months of age, the diagnosis of HIV infection was made on the basis of a HIV antibody test (Abbot HIV-1/2 ELISA, N Chicago, IL). Infants who had lost maternal antibody were diagnosed as uninfected. Among children who died younger than age 15 months, diagnosis of HIV infection was made on the basis of clinical
criteria described at a mother-to-child transmission meeting in Ghent. Serum samples were centrifuged and stored at -20°C until testing. Peripheral blood lymphocytes were isolated and cryopreserved for PCR.

Serum pre-treatment and HIV IgA EIA
Briefly, a commercially available indirect enzyme immuno-assay (Genelavia Mixt EIA, Sanofi Diagnostics, Pasteur and Genetic Systems Corporation, USA) was modified to detect HIV-1 specific IgA antibodies in human sera or plasma. HIV-1 specific IgA antibodies reactive to specific purified antigens (gp160 recombinant proteins and peptides mimicking the immunodominant epitopes of the HIV-1 envelope glycoproteins) were detectable with the substitution of the EIA kit conjugate (peroxidase-labelled anti-human IgG and IgM goat antibodies) with a peroxidase-labelled rabbit anti-human IgA conjugate diluted 1:500 (Natal Institute of Immunology, Durban, South Africa). Serum samples were pre-treated for the removal of IgG class antibodies by precipitation (Whittaker Bioproducts, Walkersville, USA) according to the manufacturer's instructions except for the increased incubation period (1 h). Samples were tested for HIV-1 specific IgA antibodies at a final dilution of 1:100.

Calculation and interpretation of results
The presence or absence of antibodies to HIV-1 was determined by comparing the optical density measured for each sample to that of the calculated cut-off. A pre-determined cut-off for this population was assessed as the mean HIV-1 specific IgA optical density +3 SD observed in 100 uninfected children born of HIV-1 seropositive women. For each assay specification, the cut-off was assessed as the sum of the predetermined cut-off and the mean optical density of the duplicately tested negative control serum samples. Samples with optical density less than this cut-off were considered negative for HIV-1 specific IgA antibodies, and samples with optical density above this cut-off were positive for HIV-1 specific IgA antibodies.

PCR
Viral DNA amplification by PCR (Roche Amplicor) was conducted on peripheral blood mononuclear cells from 18 HIV-infected and 34 uninfected children at the same time as IgA testing.

Clinical investigation
Clinical examination was undertaken at each clinic visit by a senior paediatrician (RB). Morbidity was scored for each child as a cumulative assessment of clinical findings at each visit. Briefly, episodes of diarrhoea, respiratory tract infections, fever, and thrush were each given scores according to severity and frequency. The mean morbidity score was determined as the total score during the study period divided by the number of clinic visits.

Statistical analysis
Sensitivity, specificity, and predictive values were calculated at different ages for IgA and PCR. The level of HIV-1 specific IgA antibodies as a predictive marker of disease progression was further related to the clinical outcome in HIV-1 infected children by the Spearman correlation coefficient test.

Results
The HIV-1 infection status of 134 children was determined 18 months following recruitment, of which 35 (26 per cent) were considered infected and 99 (74 per cent) uninfected. The infection status of 38 children remains unknown, due to loss to follow-up or death before 15 months of age without clinical signs of infection. Seven of the 35 infected children died before 12 months of age with clinical signs of HIV-1 infection (Ghent Classification).

Serum HIV-1 specific IgA antibodies were detectable in thirty three of the 35 (sensitivity 94 per cent) infected children. At birth, eight of the 33 (24 per cent) infected children had IgA levels above the cut-off for uninfected children. Sensitivity improved with age, 82 per cent (18/22) at 3 months, 87 per cent (13/15) at 6 months, and 93 per cent at 12 months (Fig. 1). The overall positive and negative predictive values were 92 and 98 per cent, respectively. Ninety-six of the 99 (specificity 97 per cent) of the uninfected children had no HIV-1 specific IgA antibodies. Serial HIV-1 IgA levels in the infected children gradually increased from birth to 9 months of age and subsequently dropped until 18 months, but still remaining above the cut-off.

Of the 15 samples tested positive for IgA antibodies and viral amplification (PCR), three were positive for IgA by 1 month of age and seven positive...
Age-related sensitivity of IgA antibody testing and PCR in HIV-1 infected children

<table>
<thead>
<tr>
<th>Total Number tested</th>
<th>Birth</th>
<th>1 month</th>
<th>3 months</th>
<th>≥ 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>15</td>
<td>4 (38.5%)</td>
<td>3 (23.1%)</td>
<td>5 (30.8%)</td>
</tr>
<tr>
<td>IgA</td>
<td>14</td>
<td>3 (21.4%)</td>
<td>0</td>
<td>8 (57.1%)</td>
</tr>
</tbody>
</table>

Spearman correlation coefficient test in the infected group showed a moderate association \( r = 0.52 \) between IgA antibody level at 3 months and the objective morbidity score \( (P = 0.05) \). The mean IgA antibody level and the age at which samples were first tested positive in the infants who died differed significantly from the infected infants who were alive at 15 months (Table 3). Furthermore, six of the seven infants who died from AIDS or HIV-related symptoms within 6 months of birth showed IgA levels at birth twice that of the cut-off or higher (range 0.68–1.29).

IgA antibody levels did not differ significantly between 13 transmitting and 41 non-transmitting mothers \( (1.01 \text{ vs. } 0.89; \ P = 0.4) \).

Discussion

We describe a simple cost-effective and early diagnostic technique for vertically transmitted HIV infection, a moderate association between HIV-1 specific IgA antibody level and poor clinical outcome, and the evidently stronger association between high IgA level and rapid disease progression.

Early studies that explored the diagnostic use of IgA antibody detection in HIV infected infants report sensitivity ranging from 66 to 98 per cent with limited use before 3 months of age. Improvement included the removal of IgG antibodies by precipitation for immuno-assays, the development of immunobots and fluoro-immuno-assays.\(^{5,8,9}\)

In this study, we report the sensitivity and specificity of an IgA antibody test employing standard commercially available reagents comparable to other similar studies conducted in developed countries.\(^{5,9}\) The use of more sophisticated tests such as p24 antigen testing, viral culture, and PCR in developed countries showed sensitivities of 65, 92 and 97 per cent, respectively.\(^{10}\) Furthermore, culture and PCR offer a better sensitivity in the first 3 months of life as reported in this study (PCR 80 per cent, IgA 67 per cent) and that of others.\(^{1,2}\) However, in an overall evaluation the IgA assay identified more positive samples (IgA 87 per cent, PCR 83 per cent) and fewer false positives (IgA 9 per cent, PCR 15 per cent). False positivity with PCR has been known to occur through contamination by PCR product from previous reaction commonly referred to as 'carryover'.\(^{11}\) This is particularly true of false positive samples tested sequentially in the last batch in this study. Furthermore, the relatively lower sensitivity of PCR in this study as compared to other studies in developed countries, could probably be due to primer-template mismatches as a result of inherent genetic variability in the virus.\(^{12}\) Viral heterogeneity has previously been reported between European and African strains\(^{13}\) and in the population being studied.\(^{14}\)

The presence of IgA antibodies, which do not cross the placenta is indicative of a more specific immune response in the infant. Both PCR and IgA testing failed to identify at least 50 per cent of the infected infants at birth. It is possible that samples that tested positive for PCR and IgA were of infants who were infected in utero. Therefore, most of the infants

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall efficiency of IgA antibody testing and PCR in South African children born to HIV-1 seropositive mothers</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>IgA</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>83.3% (15/18)</td>
<td>86.7% (13/15)</td>
<td>1.00</td>
</tr>
<tr>
<td>Specificity</td>
<td>85.3% (29/34)</td>
<td>91.2% (31/34)</td>
<td>0.70</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>75.0%</td>
<td>81.3%</td>
<td>0.70</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>90.6%</td>
<td>93.9%</td>
<td>0.67</td>
</tr>
</tbody>
</table>
(60 per cent) could have been infected during delivery or through breastfeeding as reported previously in this cohort and in other studies. A finding which also supports late transmission is the increasing sensitivity of other direct tests such as p24 antigen detection and viral culture.

The level of IgA antibodies has been previously identified as a powerful predictor of progression to disease in intravenous drug users and homosexuals. IgA levels in this study were distinctly higher in infants who progressed more rapidly to their death classified as HIV-related or AIDS.

Although Re et al. (1992) reported the predictive role of maternal IgA antibody level predictive of transmissibility, our results failed to show any association between vertical transmission and maternal antibody level; therefore, implicating a non-protective role of this subclass of antibodies.

The IgA assay used in this study is simple, reliable, cost effective and useful in developing countries in the absence of suitably equipped laboratories and technical expertise.

### References