Evaluation of a human immunodeficiency virus specific IgA capture enzyme immunoassay for the diagnosis of human immunodeficiency virus infection in infants

SUMMARY

A human immunodeficiency virus (HIV) specific IgA capture enzyme immunoassay (IgA-CEIA) was evaluated for the early diagnosis of infection in infants born from infected women. Sera were collected prospectively from 80 infants born from seropositive women. One hundred and sixty five specimens were available to study specific IgA, 69 from infected children and 96 from seroverted patients. All samples from non infected infants were negative by IgA-CEIA. Overall 84% (58/69) of samples from perinatally infected children were positive by IgA-CEIA. Results by age group showed that 61% of samples were positive in the first 3 months of life, 87 % in the group of age between 4 and 6 months, 91% between 7 and 18 months and 94% in older infants. Levels of total serum IgA below 5 mg/dL correlated with HIV specific IgA false negative results. Although levels of specific IgA increased according with the age of infected children, no correlation was observed between the presence of clinical manifestation of AIDS and the levels of specific IgA. According to these findings, the IgA-CEIA test would be of great value to complement other methods for the early diagnosis of infection in infants, specially in countries with limited resources as those from the developing world.

Introduction

The World Health Organization has estimated that at least 1.5 million
children have already been infected with HIV in the world, most of them living in the developing world (6,25). In 1995 it has been reported that approximately 500,000 infants were born from HIV infected mothers (1). The Joint United Nations Programme on HIV/AIDS has reported that, on average, 1000 new infections in children occurred each day during 1996 (22). As perinatal infection is the most frequent route of transmission for HIV in children (21), the enlarging number of infected young women lets anticipate an increase in the number of infected children for the coming years specially in the developing world. Early diagnosis of perinatal HIV infection is relevant for intervention using antiretroviral therapy and prophylaxis for infections (13). Maternal IgG antibodies to HIV are transferred across the placenta and may persist for 18 months (5), consequently standard serological tests are not reliable for early diagnosis of perinatal transmitted HIV infection. A number of studies (8,18) have shown that PCR and HIV culture have excellent sensitivity and specificity detecting 30-50% of infected infants at birth or during the neonatal period and 100% of them after 3-6 months. As both are expensive and require expertise and specially designed laboratory facilities, their use in the developing world is severely restricted. Detection of p24 Antigen (15) and ”in vitro” production of antibodies (IVAP)(3) have been proposed for early diagnosis in children but they are limited by their low sensitivity to detect infection during the first 3 months of life (4,9,12,14).

The detection of IgA antibodies to HIV has been described for the diagnosis of perinatal acquired infection. Reported methods either used IgA specific Western blot assay (11,17,23,24) or EIA assays (10) which require the removal of competing IgG by multiple cycles of protein G treatment making this procedure laborious and expensive. Parekh et al (16) reported a less expensive IgA capture enzyme immunoassay as an alternative to Western blot assay and proposed it as a useful tool for the diagnosis in the developing world with limited resources. In this report we evaluated the results obtained by a capture enzyme immunoassay for early diagnosis of HIV infection in infants, and its tentative application as a screening test to complement other methods used to research the presence of the virus.

Materials and methods

Patient population. Eighty children born from HIV infected women, were enrolled at the outpatient Infectious Service of the National Public Pediatric Hospital in Montevideo, Uruguay. The children were under a clinical follow up programme and blood samples were collected at periodic intervals of 3-4 months during the first year of life and every 6 to 12 months after the year. Not all the samples were available to carry out this research, due to the small volume of some of them. In every case, an oral consent was obtained from the mother or person in charge of each child before carrying out the studies. Children were considered seroreverted when, between 6 and 18 months of life, 2 or more HIV-EIA tests were non reactive, or when one EIA was non reactive after 18 months of life. Infection was defined by the presence of an EIA test reactive confirmed by Western blot assay, for those children over 18 months of life. Children less than 18 months old were considered infected when positive by polimerase chain reaction (PCR) in two successive samples. One hundred sixty five samples were available to study HIV specific IgA. IgA-CEIA assay. Nunc immunoplates II wells were coated with 150 μL of rabbit anti-Human IgA (Dako Immunoglobulins) diluted in phosphate-buffered saline (PBS) pH 7.2 at a concentration of 4.4 mg/ml. Coating was performed at 37°C for 2 hours and plates were blocked with 3% BSA in PBS for 1 hour at room temperature. After washing with PBS, 50 μL of specimens and 80 μL of sample diluent (Abbott Recombinant HIV-1/HIV-2 3’ GEN. diluent) were added to wells and the plates were incubated at 4-8°C for 18-20 hours. After washing the wells six times with PBS with 0.05% Tween 20”(PBS-T), an optimal dilution of 150 μL of recombinant antigens conjugated with horse radish peroxidase (rp24pKRR955 E.coli; rpCKS-p41 pTB319 E.coli; rp41 H1V-2 pJC104 E.coli,Abbott) was added and incubated for 1 hour at 37°C. Reaction was developed, with 150μL of 0.02% o-phenylenediamine (OPD) in citrate buffer (pH 5.0) for 30 minutes at room temperature. Optical
Fig. 1 - IgA capture enzyme immune assay results for seroreverted children

Cutoff: Negative controls O.D. mean plus 3 Standard deviations

densities (OD) values were read in a multiwell spectrophotometer at 492 nm after previous addition of 150 μL of 2N sulfuric acid. **Cutoff calculation.** Positive and negative samples were included in each run and the cutoff was calculated adding 3 Standard deviations to the mean of at least two negative controls. Positive control sera were then from infected children over 18 months old, whereas negative control sera were obtained from non-infected children (born both to infected and non-infected mothers) **Seroology.** All samples were studied by enzyme immunoassay (EIA Abbott Recombinat HIV- 1/HIV-2 3rd Generation) and reactive samples were confirmed by in house indirect immunofluorescence or Western blot (LAV blot HIV-1 Pasteur Sanofi). P24 antigen was investigated prior immune complex dissociation by the Vironostika HIV-1 Antigen Microelisa System (Organon Teknika). Reactive results were confirmed by a neutralization test. **Nested-PCR.** HIV specific DNA was amplified from blood mononuclear cells according to the protocol of J.Albert et al. (2), using JA17 and JA20 primers (pol gene) for the first amplification round and JA18 and JA19 for the second one. A commercial (Behring) radial immunodiffusion test was used for the determination of IgA serum levels. **Data analysis.** The Student t test was used to compare means.

**Results**

The results for IgA-CEIA on 96 sera from 49 seroreverted children are shown in Figure 1 (showed as OD/cutoff related to age). Optical density (OD) for the group was in a range between 0.017 and 0.077. No differences were observed as a function of age of the infants. According to the established cutoff no false positive results were detected in this group. Results comparing samples obtained before and after seroreversion (Figure 2), showed no statistical differences (p>0.05) in the OD values.
Fig 2 - IgA capture enzyme immune assay results for seroreverted children. Comparison of results in samples obtained before and after seroreversion. Results expressed in optical density (OD).

Fig. 3 - IgA capture immune assay results for the group of HIV infected children.
IgA-CEIA results for the 69 sera from 31 infected children are presented in Figure 3 (showed as OD/ cutoff related to age). Fifty eight in sixty nine (84%) of the samples showed an OD over the established cutoff level (mean of negative controls plus 3 Std. deviation). The OD for the group was in a range between 0.022 and 3.0. A statistical significant difference in OD was observed between the group of infants aged less than 18 months and those older (p<0.001). No differences in the OD means of samples obtained from symptomatic or asymptomatic children were observed (data not shown). The results for IgA-CEIA on infected infants up to 18 months old were grouped by age (Figure 4). Eleven of 18 samples obtained from infected children in the first 3 months of life were reactive (61%), 7 of 8 samples from infected infants aged between 4 to 6 months showed reactivity (87%) and 10 of 11 sera from children between 7 and 18 months were reactive (91%). Seric total IgA levels were available from 15 of the infants in study and the results were correlated with IgA-CEIA findings. The measure of total serum IgA in the group of the infected children showed concentration in between the normal ranges for the age (5-100 mg%), except for 4 cases with values below 5 mg%, all of them with false negative results by IgA-CEIA.

**DISCUSSION**

Though a number of studies (10,11,17,23,24) have reported the usefulness of IgA HIV-specific studies for the early diagnosis of HIV infection in infants born to seropositive women, the procedure has not been extensively used for that purpose specially in countries with limited resources. The necessity of a very expensive and laborious treatment for the adsorption of IgG previous to the test, counteracts its utility and has limited their massive application. The description of a simple capture test by Parekh et al. (16) established an alternative of value to the Western blot and modified EIA tests. However, up
to the present, no data from field studies in developing countries using capture tests have been published.

The obtained data suggest that the presence of maternal IgG does not interfere with the technique (Figure 2) because no false positives were observed before seroreversion. For the first 3 months of life our test recognized 61% of the infected infants, and the relative sensitivity of the procedure improves for older infants up to 87% between 4 and 6 months, and 93% for children older than 6 months. Different explanations for the lack of sensitivity of specific IgA investigation on the first months of life have been proposed. The importance of the maturation of the immune system in the course of the first years of life is probably reflected in our observation of a more consistent IgA specific response (higher OD results) according to the age of the infected children, although we can not discard an influence of the progressing infection in those findings. Follow up of one infant showed that a negative IgA-CEIA test became positive around 6 months of age simultaneously with the normalization of seric IgA levels in the absence of clinical manifestation of AIDS. A similar observation was reported by Henrad. D. et al. (7). Hypogammaglobulinemia is observed in some AIDS cases. That was the case of 2 of our patients, both with negative IgA-CEIA results and an outcome to death within the 3 months of enrollment. According to our findings the levels of circulating IgA are relevant to explain some negative results. None of the studied patients aged under 18 months with levels of IgA below 5 mg/dL were reactive to specific IgA. The observed poor sensitivity probably reflects in some cases the timing of virus transmission. Intrapartum or postpartum transmission or protected virus replication in the first few weeks of life may elicit an undetected humoral response in the first months of life. The observation that anti HIV-Env antibodies develop earlier (first 3 months of life) in infants than anti Gag or Pol antibodies (20) pointed to the importance of the use of more than one antigen to detect precociously the immune response. The observation that 76% of our infants in the first 4 months of life were detected as infected by IgA-CEIA compared with the 37% reported by Parekh (16) could be explained for the inclusion of Gag and Env antigens in the test. In some cases even in the presence of normal levels of IgA, specific HIV-IgA is not detectable. That is the case of one child 32 months old with normal level of IgA and consistently negative for specific IgA. It has been reported for HIV infected adults that the frequency and the level of specific IgA does not change during the course of the infection from the asymptomatic stage to AIDS (19). In the pediatric patients studied, no correlation was either observed between the results of HIV specific IgA and disease (AIDS). The results reported by the authors showed for the IgA capture test a sensitivity and specificity similar to that previously reported for the tests to detect specific IgA, independently of the used procedure. The authors think that according to their findings, IgA-CEIA test is of great value to complement other direct methods for the early diagnosis of infection in infants born from seropositive women, specially in countries from developing world, with limited resources and a growing number of suspected cases. Besides, even in the case of easy access to PCR or virus isolation procedures the combination of different tests increases their individual predictive value and results in a more reliable and rapid diagnosis. For infants over 6 months of age the test could be used alone as a screening test, in those circumstances of extreme shortage of resources.

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References


