Antibody responses against vaccine-preventable infectious diseases in HIV-exposed and unexposed Malawian infants

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Research Article
Abstract

Background

The evaluation of immunological status with respect to vaccine-preventable infectious diseases allows to identify populations with suboptimal protection. HIV-exposed infants, even if not infected with HIV, have higher morbidity and mortality in comparison to their unexposed counterparts, and even if the underlying mechanisms have not been clearly elucidated, dysfunctional immune responses might be involved. The aim of this study was to evaluate post-vaccination immune responses in two groups of infants (HIV-exposed and HIV-unexposed) living in the area of Blantyre, Malawi, measuring IgG levels against *Haemophilus Influenzae type B* (HiB), Hepatitis B (HBV), and *Streptococcus pneumoniae* (PCP).

Methods

Sixty-two infants, 49 HIV-exposed, uninfected (HEU), born to women living with HIV, and 13 HIV-unexposed, uninfected (HUU), born to HIV-negative mothers, were included in the study. The infants were visited monthly, from birth to 12 months, and blood samples were collected at 6 months. Anti-HiB, -HBV and -PCP vaccines are administered in Malawi at 6, 10, and 14 weeks of life. The antibody responses to the vaccines were determined by ELISA tests.

Results

The geometric mean concentrations (GMCs) of anti-HiB, anti-HBs and anti-PCP antibodies were not different between the two groups. The proportion of infants with protective levels (> 0.15 mg/l) versus HiB was lower (although not significantly) in HEU in comparison to HUU infants (81.6% vs 100%, p = 0.095). There was no significant difference between the two groups in the proportion of infants with protective antibody levels against HBV or PCP, although this proportion was lower than expected in both groups, varying from 81.6–84%. Overall, only 61.3% of the infants showed protective levels against all three vaccine antigens.

Conclusions

The humoral response after vaccination was similar in HEU and HUU infants. A disturbingly high proportion of infants without protective antibody levels against HBV and PCP in both groups of infants and against HiB in HEU infants was observed.

Background

Malawi is a sub-Saharan country with a high HIV/AIDS prevalence [1] and an estimated rate of under-five mortality of 38.6 per 1000 live births in 2020, mostly caused by malaria, diarrhea, and pneumonia [2].
national HIV prevalence among childbearing women has been estimated at around 10.3% [3], but the early adoption in 2011 of the Option B+ strategy for the prevention of mother-to-child transmission (PMTCT) [4, 5] has limited the rate of acquisition of perinatal HIV infections, which has declined in the past years to levels between 4 and 10% [6, 7]. At the same time, the population of children exposed to HIV and antiretroviral drugs (HIV Exposed Uninfected, HEU) significantly increased, exceeding 7.0% of the general child population in 2018 [8]. Although not infected with HIV, the exposed children have been reported to have a higher vulnerability to viral and bacterial respiratory tract infections, invasive pneumococcal and group B Streptococcal infections, and gastrointestinal disease [9], and to have a two or three times higher risk of hospitalization in comparison to unexposed (HIV Unexposed Uninfected, HUU) children [10–11]. The immunological abnormalities in HEU infants have been attributed to an impaired maternal IgG transplacental passage [12] and to an incomplete T cell response [13], which can influence the ability to respond to vaccinations [14].

To determine if inadequate response to vaccinations could be responsible for the higher vulnerability of the HEU population, with the present study we aimed to analyze the antibody responses to vaccines against *Haemophilus influenzae type B* (HiB), Hepatitis B virus (HBV), and *Streptococcus pneumoniae* (PCP) after the vaccination schedule (6, 10, and 14 weeks of life) was completed in two contemporary cohorts of HEU and HUU infants in Malawi. Assessing the actual level of protection is important, because, although Malawian official reports indicate an overall vaccine coverage in the country of around 90–95% [15], field studies revealed that in the last years the deterioration of immunization services and the high dropout rate have increased the number of children with an incomplete vaccination schedule and not fully protected from preventable diseases [16, 17]. Most of these studies were also based on interviews or analysis of documentation, and specific immunological studies are lacking.

**Methods**

**Population characteristics**

The present study is part of a larger study conducted between January 2019 and June 2021, aimed to investigate the impact of maternal HIV infection and antiretroviral therapy (ART) on exposed infants under Option B+, and to assess the factors influencing maternal retention in care. The study was conducted within the structures of the DREAM (Disease Relief through Excellent and Advanced Means) Program of the Community of S. Egidio, an Italian faith-based non-governmental organization, offering health services for HIV, NCDs, and other health concerns in several African countries [18–20]. Three clinical sites, all located in the Blantyre district, were involved: the urban DREAM Center, in Mandala, Blantyre, and the semi-urban sites of Chileka and Machinjiri. Women (> 18 years of age) were enrolled during pregnancy, between 32 and 36 weeks of gestation, when their HIV positive (or negative) test documentation was confirmed and demographic, clinical, and socioeconomic information were collected. Mother/child pairs of both groups were followed with monthly visits until 12 months from delivery. Adherence was measured as the number of missed visits. The vaccinations (anti-HiB, -HBV and -PCP, at 6, 10, and 14 weeks of life) were not performed in the same clinical health centers where mothers/infants
were visited. The HiB and HBV antigens are part of the same vaccine preparation (PENTA), while the PCP vaccine administration is scheduled at the same time but using a different preparation. The information about the infant's vaccination status was collected from the mothers attending the monthly visits and recorded.

At 6 months a blood sample was collected from the infants by locally trained people. Plasma was separated and stored frozen locally and subsequently shipped in dry ice to the Laboratory of the Istituto Superiore di Sanità in Rome where the samples were stored at -80° until the analyses were performed. All the available samples from 6-month old infants were analyzed in the present serological study.

**Laboratory evaluations**

The IgG levels against *H. influenzae* and *Streptococcus pneumoniae* were evaluated using commercial ELISA kits (VaccZyme™ Haemophilus influenzae type B IgG kit, and VaccZyme™ anti-PCP IgG Enzyme immune Assay, Binding Site, Birmingham, UK) according to the manufacturer's instructions. The range of detection for anti-HiB IgG was 0.11–9.0 mg/l. An antibody level greater than 0.15 mg/l is considered the minimum protective level, but 1.0 mg/l is indicated as the optimal IgG level for long-term protection [21].

The range of detection for anti-PCP IgG was 3.3–270 mg/l. There is no international agreement on anti-PCP antibody protective level; the surrogate level of protection of ≥ 50 mg/l is generally accepted [22–24] on the basis of the results from previous studies (using the same immunoassays on healthy unvaccinated subjects). To verify that the antibody protective level, extrapolated from European patients, could be applied to an African infant population, we assessed, using the same commercial assay, the anti-PCP antibody levels in 69 samples obtained from 6-month old HEU Malawian infants enrolled in a previous study conducted between 2008 and 2010 (before the introduction of the PCP vaccination program) [25]. In view of the mean antibody level of negative samples = 6.95 mg/l and the value of 3SD = 11.4 (final value of 41.1 mg/l), the protection level for anti-PCP IgG of 50 mg/l was considered reasonable.

Anti-HBs IgG levels were evaluated using the Monolisa Anti-HBs Plus EIA kits (Bio-Rad Laboratories, Marnes La Coquette, France). The range of detection was between 2.0 and 1000 mIU/ml. Levels > 10 mIU/ml are considered protective [26], and levels > 100 mIU/ml are considered necessary for long-term protection against infection [27].

The lower and the upper levels of the range of detection of the different ELISA kits were used to categorize undetectable or “above the curve” results for the statistical analysis.

**Statistical analysis**

The SPSS software, version 27 (IBM, Somers, NY, USA), was used for the statistical analyses. Socio-demographic data are presented as medians with interquartile range (IQR) and percentages. Geometric mean and 95% CI were used for antibody levels. Differences between groups were evaluated using the χ² test or Fisher's exact test when appropriate for categorical variables, and by the Mann-Whitney U test for
quantitative variables. Spearman's correlation coefficient was used to evaluate correlations between quantitative variables. Differences were considered statistically significant when \( p < 0.05 \).

**Results**

**Population characteristics**

Sixty (13 HIV-negative, and 47 living with HIV) pregnant women and their infants were included in this study. The median age of the women of both groups was 30.0 years. The median duration of ART at enrolment for HIV-positive women was 1.6 years (IQR: 0.4–7.3). The proportion of women living in a rural/semi-rural setting was similar in the two groups (women living with HIV: 75.0%; HIV-negative women: 92.3%, \( p = 0.176 \)). No differences in the other socioeconomic parameters were observed between the two groups (Table 1). Delivery occurred by vaginal route in most of the cases, with no difference between groups (women living with HIV: 83.7%, HIV-negative women: 84.6%, \( p = 0.942 \)). There were 2 twin births. A total of 62 infants (49 HEU and 13 HUU) were studied. Median weight at postbirth visit (within 15 days from delivery) was similar in the two infant groups (HEU: 3.50 Kg; HUU: 3.45 Kg, \( p = 0.651 \)). All birth weights were > 2.5 Kg. The growth rate did not differ between infants born to HIV-negative mothers and those born to mothers living with HIV, showing a similar weight gain during the first year of life (Table 1). At the monthly visits mothers reported full compliance with the vaccination schedule.
Table 1
Characteristic of mothers and infants included in the study. Values are reported as medians with IQR or percentages.

<table>
<thead>
<tr>
<th>Mothers</th>
<th>Living with HIV</th>
<th>HIV negative</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. mothers</td>
<td>47</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.0 (23.3–33.8)</td>
<td>30.0 (25.5–32.5)</td>
<td>0.994</td>
</tr>
<tr>
<td>Body Mass Index (kg/m$^2$)</td>
<td>25.0 (22.5–28.8)</td>
<td>26.3 (22.5–28.8)</td>
<td>0.958</td>
</tr>
<tr>
<td>Duration of ART (years)</td>
<td>1.6 (0.4–7.3)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Electricity at home</td>
<td>18 (37.5%)</td>
<td>4 (30.8%)</td>
<td>0.654</td>
</tr>
<tr>
<td>Water at home</td>
<td>28 (58.3%)</td>
<td>10 (76.9%)</td>
<td>0.220</td>
</tr>
<tr>
<td>Residency</td>
<td>0.176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural/semirural</td>
<td>36 (75.0.1%)</td>
<td>12 (92.3%)</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>12 (24.5%)</td>
<td>1 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td>0.835</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary or none</td>
<td>28 (58.3%)</td>
<td>8 (61.5%)</td>
<td></td>
</tr>
<tr>
<td>Secondary or above</td>
<td>20 (40.8%)</td>
<td>5 (38.5%)</td>
<td></td>
</tr>
<tr>
<td>N. of missing visits</td>
<td>1 (0–2)</td>
<td>1 (0-1.5)</td>
<td>0.720</td>
</tr>
<tr>
<td>Infants</td>
<td>HEU</td>
<td>HUU</td>
<td></td>
</tr>
<tr>
<td>N. infants</td>
<td>49</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Sex M/F n, %</td>
<td>30/19 (61.2/38.8%)</td>
<td>4/9 (30.8/69.2)</td>
<td>0.065</td>
</tr>
<tr>
<td>Weight at postbirth visit (Kg)*</td>
<td>3.50 (3.1–4.0)</td>
<td>3.45 (3.0–3.8)</td>
<td>0.651</td>
</tr>
<tr>
<td>Weight gain between 1–6 months (kg)</td>
<td>2.95 (2.48–3.43)</td>
<td>2.80 (2.40–3.70)</td>
<td>0.775</td>
</tr>
</tbody>
</table>

*Within 15 days of birth

Responses to vaccines

**a) Haemophilus influenzae type B**

The geometric mean concentrations (GMCs) of anti-HiB IgG were 1.39 mg/l in HEU and 2.54 mg/l in HUU, $p = 0.194$ (Table 2, Fig. 1). The proportion of infants with protective levels ($\geq 0.15$ mg/l) was 81.6% (40/49) in HEU and 100% (13/13) in HUU infants ($p = 0.095$). The proportion of infants with anti HiB levels $> 1.0$ mg/l, did not differ in the two groups: 65.3% in HEU and 76.9% in HUU infants ($p = 0.153$).
Table 2
Antibody levels against *Haemophilus influenzae* type B (HiB), Hepatitis B (HBs), *Streptococcus pneumoniae* (PCP) measured at 6 months of age in 49 HIV-exposed uninfected (HEU) and 13 HIV-unexposed uninfected (HUU) infants. Values are expressed as geometric means and 95% CI. The proportion of infants with protective levels is reported in brackets.

<table>
<thead>
<tr>
<th></th>
<th>HIV exposed uninfected (HEU)</th>
<th>HIV unexposed uninfected (HUU)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. infants</td>
<td>49</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Anti-HiB IgG (mg/l)</td>
<td>1.39 (0.75–1.21)</td>
<td>2.54 (2.19–2.90)</td>
<td>0.194</td>
</tr>
<tr>
<td>Infants with anti-HiB IgG (n, %) &gt;0.15mg/ml</td>
<td>40 (81.6%)</td>
<td>13 (100%)</td>
<td>0.095</td>
</tr>
<tr>
<td>&gt;1.0 mg/ml</td>
<td>32 (65.3%)</td>
<td>10 (76.9%)</td>
<td>0.426</td>
</tr>
<tr>
<td>Anti-HBs IgG (mIU/l)</td>
<td>62.5 (57.02–58.4)</td>
<td>74.7 (74.2–75.2)</td>
<td>0.949</td>
</tr>
<tr>
<td>Infants with anti-HBs IgG (n, %) &gt;10 mIU/l</td>
<td>40 (81.6%)</td>
<td>11 (84.6%)</td>
<td>0.802</td>
</tr>
<tr>
<td>&gt;100 mIU/l</td>
<td>26 (53.1%)</td>
<td>8 (61.5%)</td>
<td>0.756</td>
</tr>
<tr>
<td>Anti-PCP IgG (mg/l)</td>
<td>83.7 (71.6–73.8)</td>
<td>90.9 (89.5–92.4)</td>
<td>0.809</td>
</tr>
<tr>
<td>Infants with anti-PcP IgG &gt;50 mg/l (n, %)</td>
<td>38 (80.9%)</td>
<td>11 (84.6%)</td>
<td>0.576</td>
</tr>
</tbody>
</table>

b) Hepatitis B

Anti-HBs IgG levels did not significantly differ in the two groups of infants (HEU: 62.5 mIU/ml, HUU: 74.7 mIU/ml, p = 0.949, Table 2, Fig. 1). Overall, 81.6% of HEU and 84.6% of HUU infants reached anti HBs-IgG protective (≥ 10 mUI/ml) levels, without significant differences between groups (p = 0.802). Only 53.1% of HEU and 61.5% of HUU infants (p = 0.756) had anti-HBs IgG levels higher than 100 mIU/ml.

c) Streptococcus pneumoniae

No significant differences were observed in anti-PCP IgG levels between HEU and HUU infants (83.7 mg/l vs 90.9 mg/l respectively, p = 0.809, Table 2, Fig. 1). The proportion of infants with a level of anti-PCP-IgG >50mg/l did not differ between HEU and HUU infants ( HEU: 80.7%, HUU: 90.9%, p = 0.719).

Analyses of vaccine responses

Protective levels against all three vaccine antigens were observed only in 61.3% (38/62) of the infants (Fig. 2) without significant differences between groups. All 13 HUU infants had an adequate response to at least 2 out of 3 vaccine antigens, and 69.2% (9/13) responded to all vaccines. In the HEU group, only 59.2% of infants (29/49) showed protective levels against all three vaccine antigens. Two (4.1%) HEU infants did not respond to any vaccine, and 7 (14.3%) only to 1 vaccine antigen out of 3.
The antibody concentrations against vaccine antigens were not correlated to maternal age (HiB: $r = 0.240$, $p = 0.854$; HBV: $r = 0.23$, $p = 0.861$; PCP: $r = 0.123$, $p = 0.353$), nor to the ART duration of mothers living with HIV (Hib: $r = 0.110$, $p = 0.468$; HBV: $r = 0.230$, $p = 0.833$; PCP: $r = 0.124$, $p = 0.411$). No sex-related differences or correlations between the magnitude of Ig response and infants' weight were detected (data not shown). The analysis of socio-demographic parameters (education level, employment, residence), did not show any significant association with the antibody concentrations (data not shown).

The study timeline overlapped the spread of COVID-19 at the global level. Among the 62 infants, most of the infants (79.0%, HEU:39, HUU:9) completed the vaccination schedule in the pre-COVID era, and 14 infants (22.6%, HEU:10, HUU:4) started the vaccination schedule after the promulgation of the containment health strategies. Overall, the rate of infants with protective levels to all three vaccine antigens was similar in the pre- and post-COVID-19 periods (60.4% vs 64.3%, $p = 0.381$), without differences between groups.

**Discussion**

The results of this study, in terms of assessment of antibody concentrations, were reassuring regarding the ability of HEU infants to mount an immune response to vaccines against preventable diseases similar to that of their unexposed counterparts. However, the analysis of the serological responses showed an alarming rate of 6 month-old infants without antibody protective levels against three relevant infectious diseases.

The vaccines against *Haemophilus influenzae* type B and Hepatitis B have been introduced in the Malawian vaccine program in 2002, and the one against *Streptococcus pneumoniae* in 2011. Although all three vaccine antigens are administrated at the same time (6, 10, and 14 weeks), and 2 out of 3 in the same vaccine preparation (HiB, and HBV), antibody levels in infants varied for each pathogen [28]. For Hib, all HUU infants and 81.6% of HEU infants reached IgG protective levels. Although this difference is not statistically significant, this finding could be suggestive of a selective disturbance in vaccine response to *Haemophilus influenzae* in HEU infants as recently reported in a study on both humoral and cellular response to vaccines in a cohort of Malawian HEU infants [14]. The responses to the other two vaccines did not differ between groups in our study, with proportions of infants with antibody protective levels varying from 80.6 to 84.6% against Hepatitis B and *S. pneumoniae*. However, using the threshold of 1.0 mg/l for HiB and of 100 mIU/l for HBV, we found that only a low proportion of HEU (HiB: 65.3%, HBV: 53.1%) and of HUU (HiB: 76.9%, HBV: 61.3%) infants developed antibodies levels predictive of a long-term protection [29].

Independently from HIV exposition, the low proportion of infants with protective IgG levels (and especially with levels predictive of long-term protection) are the most disturbing data of this study. Considering that the serological screening was preformed only three months after completing the vaccine cycle, our results are indicative of a suboptimal response to vaccine; 9/49 (18.4%) of HEU infants did not develop any
response or responded only to 1 vaccine antigen out of 3, and more than 30% of HUU infants had adequate responses only to 2 out of 3 antigens.

In 2021 WHO/UNICEF have estimated an immunization coverage of 93% in Malawian infants [30], which is far above the prevalence of protective responses that we found in our study. The data that we presented have relevant clinical implications, suggesting a suboptimal adherence to the National vaccine program. No correlations were found with the mothers’ age, education, or socioeconomic level, which in similar studies have been identified as significant determinants of missed vaccination [31, 32].

It is important to note that the study period mostly overlapped with the first pandemic wave of COVID-19. The health policies for COVID-19 containment in Malawi activated at the end of March 2020 [33] could have worsened the rate of missing vaccinations. Nevertheless, the temporal analysis we performed did not show significant differences in the proportion of infants with protective levels between the pre-and post-pandemic periods. Our findings are in agreement with recent reports regarding the decline at the global level of the vaccination rate against preventable diseases in the last decade [17].

One of the main limitations of the study is the lack of official documentation on infant vaccine attendance, which could have provided more solid and direct information on the causes of the low protection. For our cohort the services providing immunization were distinct from the health facilities where the study was carried out, and the registration of vaccinations in the children's health passports, although officially planned, is not common in real life. Other studies in the field underlined overwhelming difficulties in the determination of the real prevalence of non- and under-vaccinated children in Malawi only based on the available documentation [31, 34].

Another important limitation of this study is the limited sample size, in particular for the HUU infant group, which has reduced the statistical power to detect differences in vaccine responses between the study groups, and to identify predictors of low vaccine responses. In a previous analysis of the same cohort we have reported a high drop out rate in HIV-negative women compared to HIV-positive women [35]. We have hypothesized that while the mothers living with HIV were more motivated to maintain a tighter contact with healthcare services in order to receive regular HIV treatment [36, 37], this was not true for the HIV-negative women and their HUU infants.

Nevertheless, we believe that this study has the merit to offer a direct representation of the serological responses to infants' vaccinations against preventable diseases in the area of Blantyre.

**Conclusions**

In conclusion, our study provides reassuring data on the immunological response of HEU infants, indicating limited immunological differences between HIV-exposed and unexposed infants. On the other hand, the study reveals disturbingly high rates of HEU and HUU infants without protective levels against vaccine-preventable diseases. Future serological studies should be carried out in combination with
documentary investigations (documented records of the child vaccination), in the attempt to identify the main barriers contributing to the suboptimal protection level observed in Malawi.

**Abbreviations**

**HIV:** Human Immunodeficiency Virus; **HEU:** HIV Exposed Uninfected; **HUU:** HIV Unexposed Uninfected  
**HiB:** *Haemophilus Influenzae* type *B*; **HBV:** Hepatitis B virus; **PCP:** *Streptococcus pneumoniae*;

**Declarations**

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**Authors’ contribution**

SB and MG were responsible for the design of the study, and wrote the manuscript. SB was responsible for statistical analysis. CMG and MFP designed and supervised the laboratory procedures. SO supervised the implementation of the project. RM, TK and RL were responsible for data and sample collection at the clinical sites, RA was involved in the laboratory assays. MF and MA contributed to the data acquisition and to the interpretation of data. FC, PS and MCM contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The study has been conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the National Health Research Committee in Malawi (approval number 2085) and written informed consent was obtained from all women participating in the study and from all the parents and/or legal guardians of the infants included in the study.

**Competing interests**
The authors report there are no competing interests to declare.

Consent for publication

Consent was not applicable for publication.

References


Figures

Figure 1

Antibody levels in 49 HEU and 13 HUU infants against *Haemophilus influenzae* type B (anti-HiB IgG), Hepatitis B (anti-HBs IgG), and *Streptococcus pneumoniae* (anti-PCP IgG). The shadows indicate the threshold of protective levels: HiB ≥ 0.15 mg/l; HBV ≥ 10 mIU/ml PCP ≥ 50 mg/l; the dotted lines represent levels predictive of long-term protection. Black bars represent geometric means.
Figure 2

Rate of infants with protective levels of antibodies to all three vaccine antigens (HiB, HBV and S. pneumoniae) in HEU and HUU infants.