Cohort Study of Hepatitis B Virus (HBV) Vaccine-Mediated Immunity in South African Children

Anna McNaughton[1*], Louise Hattingh[2*], Emily Adland[3], Samantha Daniels[2], Anriette Van Zyl[2], Connie S Akiror[4], Susan Wareing[5], Katie Jeffery[5], Paul Klenerman[1,5], Philip J R Goulder[3], Pieter Jooste[2], Philippa C Matthews[1,5*]

[1]Nuffield Department of Medicine, Peter Medawar Building for Pathogen Research, South Parks Road, Oxford OX1 3SY, UK; [2]Department of Paediatrics, Kimberley Hospital, Kimberley, 8300, South Africa; [3]Department of Paediatrics, Peter Medawar Building for Pathogen Research, South Parks Road, Oxford OX1 3SY, UK; [4]Global Healthcare Public Foundation, Makindu Lane, Kololo, Kampala, Uganda; [5]Department of Infectious Diseases and Microbiology, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK

Introduction

HBV infection is endemic in South Africa; the estimated prevalence among adults is 6-11% (1). In a recent study in Kimberley (Fig 1), we demonstrated cases of HBV infection among children with HIV, despite the inclusion of the HBV vaccination in routine childhood immunisation programmes in South Africa since 1995 (2).

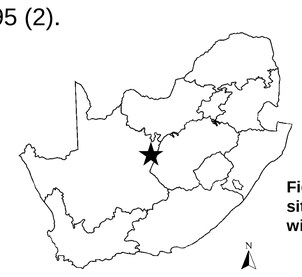

Figure 1. Map of South Africa; Kimberley, situated in the Northern Cape, is marked with a star.

The vaccine, a safe and affordable recombinant surface antigen protein, has been available for over two decades (3), and is highly efficacious, generating immunity to HBV in >90% of immunocompetent individuals after three doses (3). Since the mid-1990s, the WHO has encouraged universal coverage of the HBV vaccine through its Expanded Programme on Immunisation (EPI).

EPI guidelines recommend the primary HBV vaccine dose in the first day of life (4). However, in South Africa, the first dose is conventionally delayed until age 6 weeks, leaving a window during which vertical transmission can occur peripartum or in the early weeks of life. Estimates for coverage of the third vaccine dose range from 56-97% (2).

South Africa's high HIV prevalence poses a further challenge to the success of national HBV initiatives, as being HIV positive can increase the risk of peripartum transmission of HBV, and the HBV vaccine has been demonstrated to have reduced efficacy in HIV positive individuals (5).

Aims

•In order to investigate HBV vaccine coverage and vaccine-mediated immunity in South Africa, we set out to investigate HBV infection and sero-epidemiology in HIV-positive and HIV-negative children.
•The aim is to provide a detailed picture of the impact of existing prevention measures on HBV prevalence, and to highlight some of the remaining challenges for HBV elimination.

Materials and Methods

Study cohort

Children were recruited as part of the Co-infection in South-African Children (COSAC) study, in Kimberley, South Africa (6). Subjects were recruited between the ages of 6 - 60 months. These children were recruited from two sources:

•**HIV-negative participants (n=174):** recruited after admission to hospital with a clinical diagnosis of respiratory tract infection between July 2014 and August 2016.

•**HIV-positive children (n=136):** recruited from HIV outpatient clinics between September 2009 and July 2016. Additional data were recorded on commencement of ART (anti-retroviral therapy), CD4+ T cell count, CD4+ T cell percentage, and HIV RNA viral load, when available.

In order to study the influence of age on vaccine-mediated responses, we also collected data from a third group of older HIV-positive children (age >60 months, n=92).

Table 1: Characteristics of three paediatric study cohorts, comprising 402 children, recruited from Kimberley Hospital, South Africa.
KReC = Kimberley Respiratory Cohort. IQR = interquartile range.

Cohort	HIV negative; (age ≤60 months)	HIV positive (age ≤60 months)	HIV positive (age >60 months)
Number of subjects	174	136	92
Age range in months	8-58	6-60	64-193
Median age in months (IQR)	18 (12-26)	29 (18-40)	137 (122-154)
Sex (% male)	55.4	44.9	45.6

HBsAg testing was carried out in Kimberley Hospital, South Africa and, anti-HBs and anti-HBc testing were carried out by the Oxford University Hospitals Laboratory, UK. Limit of detection of the anti-HBs assay was 10 mIU/ml.

Vaccine mediated immunity

Due to the varying use of different thresholds for Anti-HBs, we have presented our results pertaining to both thresholds of ≥10 mIU/ml and ≥100 mIU/ml.

Ethics

Ethics approval for the study was obtained from the Ethics Committee of the Faculty of Health Science, University of the Free State, Bloemfontein, South Africa (HIV Study Ref: ETOVS Nr 08/09 and COSAC Study Ref: ECUFS NR 80/2014). Written consent for enrolment into the study was obtained from the child's parent or guardian.

Results

HBV prevalence

Evidence of current infection with HBV, determined by the detection of HBsAg, was observed in 0.8% of the cohort (Table 2). Anti-HBc was detected in 0.8% subjects (n=3), one of whom was also HBsAg positive.

Table 2: Demographic and serological profiles of five subjects, with serological evidence of current or previous infection with HBV (based on positive HBsAg (n=3) or anti-HBc (n=2))
[a]ART indicates the participant was receiving anti-retroviral therapy to treat HIV infection; [b]Hepatitis B surface antigen test; [c]Hepatitis B core antibody test; [d]Hepatitis B envelope antigen test; [e]Hepatitis B surface antibody test (vaccine mediated response). KReC = Kimberley Respiratory Cohort. n/a = not applicable.

Subject	K306	K405	KReC51	KReC151	K093
Cohort	HIV-positive age ≤60 months	HIV positive age ≤60 months	HIV negative	HIV negative	HIV positive age >60 months
Sex	F	F	F	M	F
Age (months) at time of sampling	18	37	20	15	118
HIV infection	Positive	Positive	Negative	Negative	Positive
ART[a] (if HIV positive)	Yes	Yes	n/a	n/a	No
Number of doses of HBV vaccine	NK	NK	NK	3	NK
HBsAg result[b]	Detected	Detected	Detected	Not detected	Not detected
Anti-HBc result[c]	Not detected	Not detected	Detected	Detected	Detected
HBeAg result[d]	Not done	Not done	Detected	Not done	Not done
Anti-HBs result[e]	Not detected	Not detected	Not detected	Detected	Not detected
Interpretation	Active infection	Active infection	Active infection	Immunised, infected and cleared	Infected and cleared

Vaccine response and HIV status

Across the whole cohort age 6-60 months, 238/310 children (77%) had an anti-HBs titre ≥10 mIU/ml suggesting some degree of vaccine-mediated immunity. The median anti-HBs titre in HIV-negative participants was significantly higher than among HIV-positive children (196 mIU/ml, vs. 11 mIU/ml, respectively, p<0.0001) (Fig 2A).

Irrespective of the antibody titre used as a threshold for immunity, anti-HBs was higher in HIV-negative compared to HIV-positive children (Fig 2B, C). We found no significant difference in the anti-HBs titres between male and female participants, either with or without HIV infection.

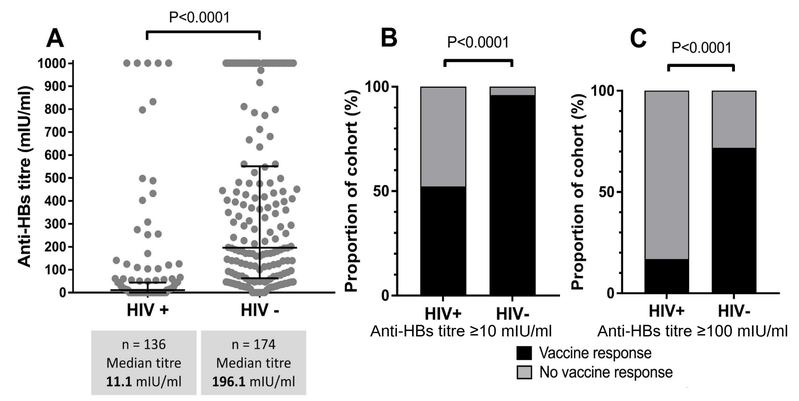

Figure 2: Hepatitis B surface antibody (anti-HBs) titres mediated by vaccination in HIV-positive (HIV+) and HIV-negative (HIV-) children aged 6-60 months.
A: Scatter plot representing vaccine-mediated antibody titres, indicating median and interquartile ranges, for HIV-positive and HIV-negative children (p-value by Mann Whitney U test). B: Proportion of HIV-positive and HIV-negative children with anti-HBs ≥10 mIU/ml (p-value by Fisher's Exact Test). C: Proportion of HIV-positive and HIV-negative children with anti-HBs ≥100 mIU/ml (p-value by Fisher's Exact Test).

Vaccine response according to age and HIV status

Among HIV-positive children, those with anti-HBs ≥100mIU/ml were significantly younger than those with lower antibody titres (Fig 3A). No such difference was observed within the HIV-negative group (Fig 3B). Using a threshold of ≥10mIU/ml, no significant differences were observed.

To expand our view of the HIV-positive group, we also added analysis of an older cohort (aged >60 months), and demonstrated that mean anti-HBs titres were significantly lower in this older group (p<0.0001), with only 1/92 subjects achieving a detectable anti-HBs titre of ≥100mIU/ml (Fig 3C).

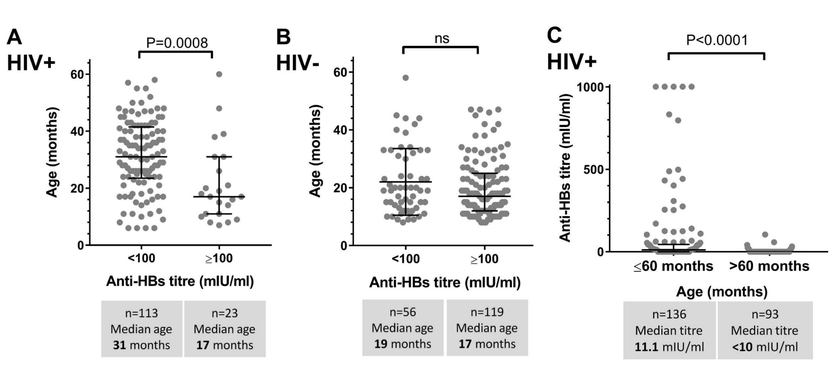

Figure 3: Relationship between age and vaccine-mediated Hepatitis B surface antibody (anti-HBs) titres in HIV-positive and HIV-negative children
Ages of children attaining anti-HBs titres ≥100 mIU/ml for HIV-positive (A) and HIV-negative children (B) age 6-60 months. Median ages, interquartile ranges and p-values by Mann-Whitney U test are indicated. C: Relationship between age and vaccine-mediated Ab titre among HIV-positive children including those age 6-60 months and an older cohort age >60 months (range 64-193 months; see Table 4).

Vaccine response and ART

Study subjects had been treated with ART for varying lengths of time (median 20 months; IQR 6-33 months). We compared anti-HBs titres of subjects being treated with ART compared to those not currently receiving ART (Fig 4) and found no significant difference.

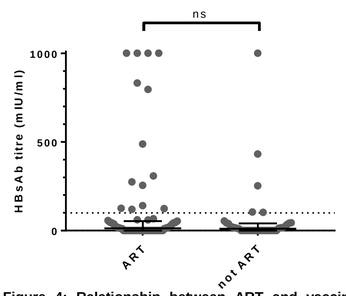

Figure 4: Relationship between ART and vaccine-mediated Hepatitis B surface antibody (anti-HBs) titres in HIV-positive children

Factors predicting a protective vaccine response

•Being HIV-positive was associated with reduced odds of developing protective anti-HBs titres, based on titres of both ≥10 mIU/ml (OR 26.2, 95% CI 11.2-58.6) and ≥100 mIU/ml (OR 11.6, 95% CI 6.7-20.4) (Fig 5).
•Younger age (<24 months) increased the odds of having an anti-HBs titre of ≥10 mIU/ml (OR 0.3, 95% CI 0.2-0.5) or ≥100 mIU/ml (OR 0.3, 95% CI 0.2-0.4).
•Among the HIV-positive subjects only, age <24 months only elevated the odds for developing an anti-HBs response of ≥100 mIU/ml (OR 0.1, 95% CI 0.06-0.4) (Fig 5B).
•Gender, ART, CD4+ count, CD4+ ratio and HIV viral load were not significantly predictive of anti-HBs titres.

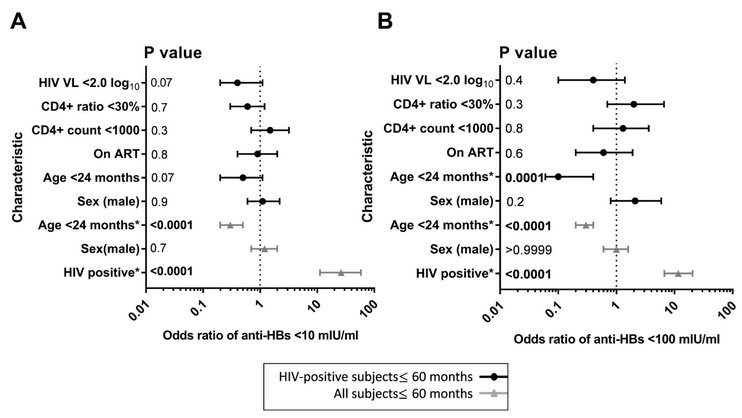

Figure 5. Odds ratios for protective response to HBV vaccination in children age 6-60 months
Odds ratios are shown for anti-HBs titre <10mIU/ml (A) and <100mIU/ml (B) in the whole cohort (grey) and in HIV-positive children (black).
Statistically significant OR are denoted * and significant p-values are indicated in bold.

Discussion

HBV vaccination schedules in South Africa leave a potential window for perinatal HBV transmission (2). This is illustrated by ongoing cases of paediatric infection. Systematic efforts to deliver the first vaccine dose soon after birth and introduce antenatal screening could reduce such transmission events.

Vaccine-mediated immunity to HBV

Anti-HBs titres were significantly lower in HIV-positive participants when compared to HIV-negative subjects, in keeping with previous reports (5). We also found that vaccine responses in HIV-positive subjects wane rapidly after immunisation (7).

There was no difference in anti-HBs titres according to ART, although there are previous reports correlating ART with improved vaccine responses (5). Our findings suggest that ART may not completely reconstitute immune responses to vaccination, and that alternative strategies, such as booster vaccinations after immune reconstitution, which has been documented to take up to 5 years, may be of benefit.

Conclusion

These data reflect the positive impact of HBV immunisation, illustrating a low frequency of HBV infection (<1% prevalence) in an endemic setting. However, the high burden of HIV infection in South Africa remains a challenge to ongoing HBV elimination, and sustained efforts are required in funding, research, education, diagnostic screening, prevention of mother to child transmission, and ultimately the ongoing quest for a cure.

Find out more

This work will feature in an oral presentation (P. Matthews, Sat 2nd Dec 8am) and is available in full on BioRxiv, together with a model to simulate transmission and prevention of HBV.
https://doi.org/10.1101/162594

Research website: https://www.expmednmd.ox.ac.uk/hepatitis-b-virus

Email: philippa.matthews@ndm.ox.ac.uk

References

(1) Schweitzer A, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. The Lancet. 2015;386:1546–55
(2) Jooste P, et al. Screening, characterisation and prevention of Hepatitis B virus (HBV) co-infection in HIV-positive children in South Africa. Journal of Clinical Virology. 2016;85:71–4
(3) McMahon BJ, et al. Frequency of adverse reactions to hepatitis B vaccine in 43,618 persons. The American Journal of Medicine. 1992 Mar;92(3):254–6
(4) World Health Organization. Preventing Perinatal Hepatitis B Virus Transmission : A Guide for Introducing and Strengthening Hepatitis B Birth Dose Vaccination. 2015; http://apps.who.int/iris/bitstream/10665/208278/1/.
(5) Pippi F, et al. Serological response to hepatitis B virus vaccine in HIV-infected children in Tanzania. HIV Medicine. 2008;9(7):519–25
(6) Jooste P, van Zyl A, Adland E, Daniels S, Hattingh L, Brits A, et al. Screening, characterisation and prevention of Hepatitis B virus (HBV) co-infection in HIV-positive children in South Africa. Journal of Clinical Virology. 2016;85:71–4.
(7) Chaouch H, Hachfi W, Fodha I, Kallala O, Saadi S, Bousaadia A, et al. Impact and long-term protection of hepatitis B vaccination: 17 years after universal hepatitis B vaccination in Tunisia. Epidemiology and Infection. 2016;144(16):3365–75.

OxSA-HEP
Oxford-South Africa-Hepatitis Research Project