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Natural history of chronic hepatitis B virus infection in West Africa: a longitudinal population-based study from The Gambia

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Complete List of Authors:	Shimakawa, Yusuke; London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health Lemoine, Maud; Imperial College London, ; MRC Unit, The Gambia, Njai, Harr Freeya; MRC Unit, The Gambia, Bottomley, Christian; London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health Ndow, Gibril; MRC Unit, The Gambia, ; IARC, The Gambia Hepatitis Intervention Study Goldin, Robert; Imperial College London, Department of Histopathology Jatta, Abdoulie; MRC Unit, The Gambia, Jeng-Barry, Adam; MRC Unit, The Gambia, Wegmuller, Rita; MRC International Nutrition Group, MRC Keneba Moore, Sophie; London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health; MRC International Nutrition Group, MRC Keneba Baldeh, Ignatius; Ministry of Health and Social Welfare, Taal, Makie; Ministry of Health and Social Welfare, D'Alessandro, Umberto; MRC Unit, The Gambia, ; London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health Whittle, Hilton; London School of Hygiene and Tropical Medicine, Faculty of Infectious and Tropical Diseases Njie, Ramou; MRC Unit, The Gambia, ; IARC, The Gambia Hepatitis Intervention Study Thursz, Mark; Imperial College, Department of Academic Medicine Mendy, Maimuna; IARC,
Keywords:	HEPATITIS B, EPIDEMIOLOGY, HEPATOCELLULAR CARCINOMA

Title

Natural history of chronic hepatitis B virus infection in West Africa: a longitudinal population-based study from The Gambia

Short Title

Natural history of chronic hepatitis B in West Africa

Authors

Yusuke Shimakawa, PhD,[1,2,3,*] Maud Lemoine, PhD,[1,4,*] Harr Freeya Njai, PhD,[1] Christian Bottomley, PhD,[2] Gibril Ndow, MD,[1,5] Robert D Goldin, MD,[4] Abdoulie Jatta,[1] Adam Jeng-Barry,[1] Rita Wegmuller, PhD,[6] Sophie Moore, PhD,[2,6] Ignatius Baldeh, MSc,[7] Makie Taal, PhD,[7] Umberto D'Alessandro, PhD,[1,2] Hilton Whittle, FMedSci,[8] Ramou Njie, PhD,[1,5] Mark Thursz, MD,[4] Maimuna Mendy, PhD[9]

* Equally contributed

[1] Medical Research Council (MRC) Unit, The Gambia. Banjul, The Gambia.

[2] Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine. London, UK.

[3] Unité d'Épidémiologie des Maladies Émergentes, Institut Pasteur. Paris, France

[4] Department of Hepatology, Imperial College London. London, UK.

[5] The Gambia Hepatitis Intervention Study, IARC, c/o MRC Unit, The Gambia. Banjul, The Gambia.

[6] MRC International Nutrition Group, MRC Keneba. West Kiang, The Gambia.

[7] Ministry of Health and Social Welfare. Banjul, The Gambia.

[8] Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine. London, UK.

[9] International Agency for Research on Cancer (IARC). Lyon, France.

Correspondence

Prof Mark Thursz

Department of Hepatology, Imperial College London, Norfolk Place, London, W2 1NY, UK

Email: m.thursz@imperial.ac.uk

Phone +44-(0)2033121903. Fax +44-(0)2077069161.

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Abbreviations

ALT	Alanine transaminase
APRI	Aspartate transaminase (AST)-to-Platelet Ratio Index
AST	Aspartate transaminase
EASL	European Association for the Study of the Liver
EIA	Enzyme immunoassay
EPI	Expanded Program on Immunization
ESLD	End-stage liver disease
GAVI	Global Alliance for Vaccines and Immunization
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma

HCV	Hepatitis C virus
HDV	Hepatitis D virus
IARC	International Agency for Research on Cancer
MRC	Medical Research Council
OR	Odds ratio
PROLIFICA	Prevention of Liver Fibrosis and Cancer in Africa
SSA	Sub-Saharan Africa
WHO	World Health Organization

Abstract

Background

The natural history of chronic hepatitis B virus (HBV) infection in sub-Saharan Africa is unknown. Data is required to inform WHO guidelines which are currently based on studies in Europe and Asia.

Methods

Between 1974 and 2008, sero-surveys were repeated in two Gambian villages, and an open cohort of treatment-naïve chronic HBV carriers was recruited. Participants were followed to estimate the rates of hepatitis B e (HBeAg) and surface antigen (HBsAg) clearance and incidence of hepatocellular carcinoma (HCC). In 2012-2013, a comprehensive liver assessment was conducted to estimate the prevalence of severe liver disease.

Results

405 chronic carriers (95% genotype E), recruited at a median age of 10.8 years, were followed for a median length of 28.4 years. Annually, 7.4% (95% CI: 6.3-8.8%) cleared HBeAg and 1.0% (0.8-1.2%) cleared HBsAg. The incidence of HCC was 55.5/100,000 carrier-years (95%

CI: 24.9-123.5). In the 2012-2013 survey (n=301), 5.5% (95% CI: 3.4-9.0%) had significant liver fibrosis. HBV genotype A (versus E), chronic aflatoxin B1 exposure, and an HBsAg-positive mother, a proxy for mother-to-infant transmission, were risk factors for liver fibrosis. A small proportion (16.0%) of chronic carriers were infected via mother-to-infant transmission, however, this population represented a large proportion (63.0%) of the cases requiring antiviral therapy.

Conclusions

The incidence of HCC amongst chronic HBV carriers in West Africa was higher than that in Europe but lower than rates in East Asia. High risk of severe liver disease amongst the few who are infected by their mothers underlines the importance of interrupting perinatal transmission in sub-Saharan Africa.

Summary Box

What is already known about this subject?

- Chronic hepatitis B virus infection is a common cause of liver disease in sub-Saharan Africa.

- Although the WHO recently published its first HBV treatment guidelines with a main focus on resource-limited countries, their recommendations are based on Western and Asian studies, since there have been no natural history data from sub-Saharan Africa.
- Mother-to-infant transmission is a risk factor for chronic HBV infection, however, it is unclear whether this mode of transmission further increases the risk of severe liver disease in chronic carriers.

What are the new findings?

- The incidence rate of hepatocellular carcinoma (HCC) in treatment-naïve male chronic HBV carriers in The Gambia was higher than Europe but lower than in East Asia.
- Mother-to-infant transmission was a risk factor for persistent viral replication, elevated transaminase, significant fibrosis and HCC.
- The majority (63.0%) of cases requiring antiviral therapy were attributable to maternal transmission.
- Among chronic HBV carriers, genotype A (versus E) and chronic exposure to aflatoxin B1 were associated with an elevated risk of significant liver fibrosis.

How might it impact on clinical practice in foreseeable future?

- The disproportionate risk of severe liver disease amongst people who acquired HBV from their mothers emphasizes the importance of interrupting perinatal transmission in sub-Saharan Africa.

INTRODUCTION

In sub-Saharan Africa (SSA) chronic hepatitis B virus (HBV) infection is a major public health problem, which causes an estimated 61,000 deaths due to cirrhosis or hepatocellular carcinoma (HCC) each year [1]. Before the introduction of hepatitis B vaccine, >70% of African children were exposed to HBV at birth or during childhood and 10-20% became chronic HBV carriers [2]. Currently, all African countries have integrated hepatitis B vaccine into their Expanded Program on Immunization (EPI).

Despite its efficacy in preventing chronic HBV infection, vaccination has several limitations as a control strategy. First, a large number of people who were infected prior to the vaccination programs are left with chronic HBV infection [3]. Second, hepatitis B vaccine does not always prevent mother-to-infant transmission [4], especially when the vaccine is not given at birth [5]. Though this mode of transmission is less frequent than horizontal transmission in SSA [6], the risk of HCC may be higher in vertically-transmitted chronic infections [7–9].

To overcome these limitations, antiviral therapy can be used to prevent HBV-related disease in cases of chronic HBV infection and also to prevent vertical HBV transmission. In March 2015, the World Health Organization (WHO) issued its first guidelines on chronic HBV infection to improve access to antiviral therapy in low- and middle-income countries. However, their recommendations are based on the findings from Asia, Europe and North America, since there have been no natural history data from SSA [3]. Understanding the natural history of chronic HBV infection is essential to inform decisions about who to treat and when to treat [3].

The UK Medical Research Council (MRC), the International Agency for Research on Cancer (IARC/WHO) and the Gambia Government have been supporting studies on HBV infection in The Gambia since the 1980's, and have established a population-based open cohort of treatment-naïve chronic HBV carriers. We used this cohort to describe the natural history of chronic HBV infection: i) the sero-clearance rates of hepatitis B e antigen (HBeAg) and surface antigen (HBsAg); ii) the incidence of HCC, end-stage liver disease (ESLD) and all-cause mortality; iii) longitudinal changes in serum HBV DNA and alanine transaminase (ALT) levels; and iv) the prevalence of significant liver fibrosis and chronic liver disease requiring antiviral therapy according to the European Association for the Study of the Liver (EASL) [10] or the WHO guidelines [3]. We also estimated the HBV-related disease burden attributable to the mother-to-infant transmission in SSA by examining the associations between these outcomes and maternal HBsAg, a proxy for mother-to-infant HBV transmission [8].

METHODS

Participants

The cohort of chronic HBV carriers was recruited from Keneba and Manduar, two neighboring villages in West Kiang District. They are typical of many African rural communities where Mandinka and Jola people live in mud or lath-and-plaster houses roofed with thatch or corrugated iron with subsistence agriculture [11]. Primary health care has been available free of charge at the MRC Keneba Clinic. Baseline HBV sero-surveys were undertaken in 1974 and in 1980. In the first survey the entire population was surveyed (n=1,317) and 13.2% were found to carry HBsAg [11] while the second survey was limited to children aged <15 years and their mothers (n=802) [12]. Following the third sero-survey in 1984 [13], all non-immune children in Keneba/Manduar were invited to participate in an HBV vaccine trial [14]. Hepatitis B vaccination was introduced in the EPI in 1990 with a vaccine schedule starting at birth. Hepatitis B immunoglobulin has been unavailable. Between 1985 and 2008, sero-surveys to measure the vaccine efficacy were repeated every 4-5 years [4,14–17]. In parallel, those who had been tested HBsAg-positive were followed for HBV sero-markers in 1985, 1989, 1992, 1993, 1998, 2003, and 2008 (supplementary table 1). Survey participation was 92-100% and 50-85% in those aged 0-9 and 10-19 years, respectively [12–15].

Liver assessment in 2012-2013

Following community approval, people with chronic HBV infection in the cohort were invited to a liver assessment as part of the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project [18]. Chronic infection was defined as serum HBsAg positivity at two visits at least six months apart. In individuals aged ≥13 years, HBsAg positivity at only one visit was considered as chronic infection because, in the pre-vaccination era, 90% of children in Keneba/Manduar acquired the infection by the age of 13 years and new infections were uncommon beyond this age [13]. After written informed consent, participants, who had fasted overnight, underwent a standardized clinical examination that involved blood collection, abdominal ultrasound and liver stiffness measurement using transient elastography (Fibroscan, Echosens, France). Those with serum HBV DNA ≥2,000 IU/ml or liver stiffness ≥6.5 kPa or ALT ≥40 IU/L, were invited for liver biopsy. Histopathologists in UK scored liver fibrosis using Metavir system [19]. The study was approved by the Gambia Government/MRC Joint Ethics Committee and conducted according to the guidelines of the Declaration of Helsinki.

Laboratory assays

HBsAg was detected by radioimmunoassay (Ausria-I, Abbott, USA) in 1974 [12], reverse passive hemagglutination assay (Wellcotest, Wellcome Diagnostics, UK) in 1980-1998 [15], immunochromatography (Determine, Abbott) in 2003-2008 [20], and chemiluminescent microparticle immunoassay (Architect, Abbott) in 2012-2013 [21]. HBsAg-positive samples were tested for HBeAg by radioimmunoassay in 1980-1998 [15] and later by enzyme immunoassay (EIA) (Diasorin, Biomedica, Italy) [20]. The serological tests were strongly correlated with one another [20,21]. HBV DNA levels were measured at the end of the study in stored samples collected in 1984, 1989, 1993, 2003, 2008, and 2012-2013, using in-house quantitative real-time polymerase chain reaction (detection limit: 50 IU/ml), calibrated against an international standard [22]. As previously reported, samples collected in 2003 were examined for HBV genotype and an AGG→AGT mutation at codon 249 of p53 tumor suppressor gene (p53R249S) in cell-free DNA, a biomarker of chronic aflatoxin B1 exposure [23]. Samples collected in 2012-2013 were tested for alpha-fetoprotein and antibodies to Hepatitis C virus (HCV) using microparticle EIA (AxSYM, Abbott), antibodies to Hepatitis D virus (HDV) using EIA (ETI-AB-DELTAK-2, Diasorin), and antibodies to HIV-1/2 and p24 antigen using EIA (Genscreen-ULTRA, Bio-Rad, USA). Schistosoma mansoni infection is rare in The Gambia [24] and therefore was not investigated.

Ascertainment of liver disease and death

Significant liver fibrosis, severe fibrosis and cirrhosis was defined as ≥F2, ≥F3 and F4 (Metavir) for those who had liver histopathology and liver stiffness ≥7.9, ≥8.2 and ≥9.5 kPa for those without biopsy. These cut-offs were determined by our validation study in The

Gambia, where the sensitivity of Fibroscan to predict ≥F2 was 81% and the specificity was 81% [25]. The EASL criteria for antiviral therapy are: i) viral load ≥2,000 IU/ml and significant fibrosis, or ii) viral load ≥2,000 IU/ml and moderate/severe active necroinflammation (≥A2 by Metavir activity grade), or iii) viral load ≥20,000 IU/ml and ALT ≥80 IU/L, or iv) detectable viral load and cirrhosis [10]. The WHO criteria are: i) clinically diagnosed cirrhosis, or ii) aspartate transaminase (AST)-to-platelet ratio index (APRI) >2.0, or iii) ≥30 years old and abnormal ALT and HBV DNA >20,000 IU/ml [3]. The phases of the natural history of chronic HBV infection were described [10,26] for the baseline and 2012-2013 survey (supplementary table 2).

HCC cases were identified through a follow-up examination, review of medical records in the MRC Keneba Clinic, or by data linkage with the Gambia National Cancer Registry [27]. The diagnosis was based on the identification of a focal hepatic lesion consistent with HCC on the ultrasound and elevated serum alpha-fetoprotein (≥200 ng/ml). ESLD includes HCC and non-malignant ESLD. The latter was defined as cirrhosis without HCC and the presence of ascites, hepatic encephalopathy, or hematemesis. The date of death was ascertained through a review of the medical chart in the MRC or data linkage with the West Kiang Demographic Surveillance System [28].

Statistical analyses

The person-years of follow-up for HBeAg/HBsAg clearance, HCC, ESLD, or death were calculated from the date they were identified as HBsAg-positive to the date of endpoint or last follow-up, whichever came first. The date of sero-clearance was defined as the midpoint between the last positive and the first negative result. The cumulative incidence was estimated as a function of age using the Kaplan-Meier Method. Age was used rather than time since entry into the study because most infections occur during early childhood [13], and therefore age approximates the duration of HBV infection. The associations between maternal HBsAg, as recorded at the recruitment of the child, and the HBeAg/HBsAg loss were examined using Poisson regression with robust standard error to account for clustering in children that share the same mother. The models included current age, calendar year, sex, and birthplace as covariates. The effect of maternal HBsAg on ALT and HBV DNA (log_{10} transformed) was quantified using a linear mixed model with random intercept and random slope to account for the multiple measurements made on the same individuals over time. The detection limit of the assay was assigned to samples with undetectable viral load. The effect of maternal HBsAg on significant fibrosis and meeting antiviral treatment criteria was estimated using logistic regression to control for age, sex, and birthplace (partial model), and additionally for HBV genotype and p53R249S (full model).

Population attributable fractions were calculated [29] for the effects of maternal sero-status on chronic HBV infection and HBV-related liver disease (significant fibrosis and meeting the EASL treatment criteria). This analysis included all the survey participants (1974-2008) with available maternal sero-status who did not receive hepatitis B vaccine. It was not restricted to chronic carriers so that the twofold effect of mother-to-infant transmission could be estimated, i.e., the increased risk of both chronic infection [30], and of liver disease progression in those with established chronic infection [8,9]. All analyses were performed using STATA 11.0 (Stata Corporation, USA).

RESULTS

Baseline characteristics

Between 1974 and 2008, 551 villagers tested positive for HBsAg at least once in the Keneba/Manduar sero-surveys. None had HCC at enrolment. Twenty-nine HBsAg-positive villagers did not participate in any subsequent sero-surveys. These individuals did not differ from the rest of HBsAg-positive individuals in sex, age, HBeAg, HBV DNA and ALT levels at baseline. Finally, there were 405 chronic carriers (figure 1). The median length of follow-up was 28.4 years (IQR: 17.7-32.7) with the median number of six sero-surveys (IQR: 3-8). The median age at recruitment was 10.8 years (IQR: 4.6-21.8). Half were male, and 65.2%, 26.1%, and 8.7% had a mother who was HBsAg-negative, HBsAg-positive/HBeAg-negative, and HBsAg-positive/HBeAg-positive, respectively (table 1). The children of positive mothers had high viral load (p=0.04) and abnormal ALT levels (p=0.05) at baseline. Thirty became chronic carriers despite having been fully vaccinated against HBV; median age at the first vaccine was 34 days and none received within three days of birth, and the majority (60.9%, 14/23) had HBsAg-positive mothers. In the 2003 sero-survey, 95.1% (97/102) had genotype E and the rest genotype A; 44.2% (100/226) had the p53R249S mutation [23].

Table 1. Baseline characteristics of people with chronic HBV infection by maternal HBsAg status (N=405)

Variables		All (N=405)	Unknown maternal sero-status (n=152)	With HBsAg(+) mother (n=88)	With HBsAg(-) mother (n=165)	p-value[1]
Sex	Male	204 (50%)	63 (41%)	48 (55%)	93 (56%)	0.8
	Female	201 (50%)	89 (59%)	40 (45%)	72 (44%)	
Age group (years)	<5	109 (27%)	4 (3%)	42 (48%)	63 (38%)	0.9[2]
	5 – 9	83 (20%)	9 (6%)	22 (25%)	52 (32%)	
	10 – 14	56 (14%)	16 (10%)	8 (9%)	32 (19%)	
	15 – 19	39 (10%)	23 (15%)	5 (6%)	11 (7%)	
	≥20	118 (29%)	100 (66%)	11 (12%)	7 (4%)	
Birth place	Keneba	233 (58%)	106 (70%)	39 (44%)	88 (53%)	0.4
	Manduar	172 (42%)	46 (30%)	49 (56%)	77 (47%)	
Hepatitis B vaccine	Never	375 (93%)	145 (95%)	74 (84%)	156 (95%)	0.02
	Ever	30 (7%)	7 (5%)	14 (16%)	9 (5%)	
HBeAg	Negative	213 (55%)	118 (86%)	30 (34%)	65 (40%)	0.4
	Positive	173 (45%)	19 (14%)	58 (66%)	96 (60%)	
HBV DNA (IU/ml)	<2,000	222 (57%)	121 (83%)	30 (35%)	71 (45%)	0.04[2]
	2,000-10^8	90 (23%)	20 (14%)	19 (22%)	51 (32%)	
	≥10^8	79 (20%)	5 (3%)	37 (43%)	37 (23%)	
ALT (IU/L)	<40	367 (94%)	134 (92%)	77 (91%)	156 (97%)	0.05
	≥40	25 (6%)	12 (8%)	8 (9%)	5 (3%)	
Phase of natural history	Immune tolerant	116 (29%)	8 (5%)	42 (48%)	66 (40%)	0.1
	HBeAg(+) chronic hepatitis	14 (3%)	5 (3%)	7 (8%)	2 (1%)	
	HBeAg(-) chronic hepatitis	11 (3%)	7 (5%)	1 (1%)	3 (2%)	
	Inactive carrier	190 (47%)	117 (77%)	22 (25%)	51 (31%)	
	Unclassified	74 (18%)	15 (10%)	16 (18%)	43 (26%)	
HBV genotype[3]	Genotype A	5 (5%)	1 (3%)	2 (8%)	2 (5%)	0.6
	Genotype E	97 (95%)	33 (97%)	24 (92%)	40 (95%)	
p53R249S mutation[3]	Negative	126 (56%)	50 (63%)	23 (44%)	53 (56%)	0.1
	Positive	100 (44%)	30 (37%)	29 (56%)	41 (44%)	
Median no. of follow-up sero-surveys (IQR)		6 (3, 8)	4 (3, 6)	6 (4, 8)	7 (5, 8)	0.1
Median years of follow-up (IQR)		28.4 (17.7, 32.7)	24.4 (10.2, 37.9)	28.6 (16.0, 32.0)	28.7 (23.8, 32.1)	0.2

[1] Comparison was made between participants with HBsAg-positive mothers and HBsAg-negative mothers. P-value and 95% CI were obtained by Wald test with robust standard error.

[2] Linear test for trend

[3] Determined in a subset of participants in 2003

HBeAg sero-clearance

At the enrolment, 213 (52.6%) chronic carriers had already lost HBeAg, The age-specific prevalence of HBeAg at baseline decreased with increasing age (supplementary figure 1). Of the 173 HBeAg-positive carriers at baseline, 82.1% lost HBeAg and the clearance rate was 7.4%/year (95% CI: 6.3-8.8) (table 2, figure 2). Fifteen experienced HBeAg reversion, nine of whom eventually lost HBeAg whilst six continued to carry HBeAg until the last follow-up. After adjusting for sex, current age, calendar year and birthplace, the sero-clearance rate was slower in carriers with high HBV DNA levels ($\geq 10^8$ IU/ml) at baseline (supplementary table 3). Carriers with HBsAg-positive mothers tend to clear HBeAg slowly, although this did not reach statistical significance (supplementary figure 2-A).

Table 2. Incidence rates of HBeAg and HBsAg sero-clearance, HCC, ESLD and all-cause mortality in people with chronic HBV infection by gender

Event	No. of subjects	Person-years at risk	No. of events	Rate	95% CI
HBeAg clearance	173	1912	142	7.4 / 100	6.3 – 8.8
Male	109	1231	86	7.0	5.7 – 8.6
Female	64	681	56	8.2	6.3 – 10.7
HBsAg clearance	405	8502	85	1.00 / 100	0.81 – 1.24
Male	204	4076	32	0.79	0.56 – 1.11
Female	201	4426	53	1.20	0.91 – 1.57
HCC	405	10815	6	55.5 / 100,000	24.9 – 123.5
Male	204	5200	6	115.4	51.8 – 256.8
Boys (<20 y.o.)		1930	0	0.0	N/A
Adult men (≥20 y.o.)		3270	6	183.5	82.4 – 408.5
Female	201	5615	0	0.0	N/A
ESLD (including HCC)	405	10815	8	74.0 / 100,000	37.0 – 147.9
Male	204	5200	7	134.6	64.2 – 282.4
Female	201	5615	1	17.8	2.5 – 126.4
All-cause mortality	405	10815	43	397.6 / 100,000	294.9 – 536.1
Male	204	5200	25	480.8	324.9 – 711.5
Female	201	5615	18	320.6	202.0 – 508.8

HBsAg sero-clearance

The rate of HBsAg sero-clearance was 1.0%/year (95% CI: 0.8-1.2) (table 2) with half clearing by 57 years old (figure 2). Younger age and high HBV DNA levels at baseline were associated with delayed HBsAg sero-clearance (supplementary table 4). The sero-clearance rate was slower in carriers with HbsAg-positive mothers, but this was not statistically significant (supplementary figure 2-B).

HCC, ESLD, and mortality

Of the 405 chronic carriers, 43 died; the all-cause mortality rate was 397.6/100,000 person-years (95% CI: 294.9-536.1). The most common cause of death was HCC (24.0%) in men and bacterial infection (22.2%) in women. All patients with ESLD (including HCC (n=6) and non-malignant ESLD (n=2)) died within one year of diagnosis. Incidence rates of HCC and ESLD were 55.5 (95% CI: 24.9-123.5) and 74.0 (95% CI: 37.0-147.9) per 100,000 person-years, respectively (table 2). All HCC patients were men, all but one was HBeAg-negative at enrolment, and their age at diagnosis ranged between 38 and 67 years (supplementary table 5). The HCC incidence in men ≥20 years was 183.5 (95% CI: 82.4-408.5) per 100,000 person-years. Maternal sero-status was available in three ESLD patients, and all had HBsAg-positive mothers. Crude incidence rates of HCC in carriers with HBsAg-positive mothers was 89.2/100,000 (95% CI: 22.3-356.8) while those with negative mothers was 0/100,000 (unadjusted $p<0.001$).

Mean HBV DNA and ALT over time

The trajectories of HBV DNA and ALT levels by maternal HBsAg are presented in figure 3. Viral load decreased with increasing age at measurement whilst ALT increased. Both viral load and ALT were higher in men than women (supplementary table 6). After adjusting for confounders, the geometric mean viral load was 4.7 times higher (95% CI: 2.0-11.1, p<0.001) and mean ALT was 4.0 IU/L higher (95% CI: 1.2-6.8, p=0.005) in carriers with HBsAg-positive mothers than in those with HBsAg-negative mothers.

Prevalence of chronic liver disease in 2012-2013

After excluding those who died, 83.1% (301/362) of chronic HBV carriers participated in the liver assessment in 2012-2013 (figure 1). Participation was lower in men than women, in younger than in older age groups and in carriers with positive HBeAg at baseline compared with those HBeAg-negative. Table 3 presents the characteristics of the participants. None had ever received antiviral or immunosuppressive therapy. The number co-infected with HIV, HCV, and HDV was three, one, and one, respectively. None had alcohol intake >20 g/day based on the standardized questionnaire. Between the baseline and 2012-2013 survey, the proportion of carriers in the immune tolerant phase decreased from 28.6% to 2.3% whilst the proportion in the inactive phase increased from 46.9% to 64.5% (tables 1 and 3, supplementary figure 3). Only 6.3% were in HBeAg-negative chronic hepatitis in 2012-2013. Thirty participants had a liver biopsy and 269 had a valid measurement using transient elastography. No liver specimen had steatosis. Fifteen carriers (5.5%, 95% CI: 3.4-9.0%) had significant fibrosis, including nine with severe fibrosis and one with cirrhosis. After controlling for confounders, male gender, genotype A, p53R249S mutation, persistence of HBeAg, high viral load, and ALT were risk factors for significant fibrosis (table 4). After adjusting for sex, age, birthplace, HBV genotype and p53R249S, the odds ratio (OR) for the effect of maternal HBsAg on significant fibrosis was 15.8 (95% CI: 1.4-174.1, p=0.02). Eleven participants (3.7%, 95% CI: 2.0-6.5%) met the EASL treatment criteria. Carriers with an HBsAg-positive mother, HBeAg persistence, frequent high viral load, and abnormal ALT were more likely to require antiviral therapy (table 4). Only five participants (1.7%, 95% CI: 0.7-3.9%) fulfilled the WHO treatment criteria.

Table 3. Characteristics of people with chronic HBV infection who participated in the liver assessment 2012-2013 by maternal HBsAg status (N=301)

Variables		All (N=301)	With HBsAg(+) mother (n=66)	With HBsAg(-) mother (n=123)	p-value[1]
Sex	Male	130 (43%)	32 (48%)	59 (48%)	0.9
	Female	171 (57%)	34 (52%)	64 (52%)	
Current age group (years)	<30	46 (15%)	17 (26%)	18 (14%)	0.8[2]
	30 – 39	117 (39%)	30 (45%)	66 (54%)	
	40 – 49	57 (19%)	8 (12%)	28 (23%)	
	≥50	81 (27%)	11 (17%)	11 (9%)	
Birth place	Keneba	178 (59%)	27 (41%)	65 (53%)	0.3
	Manduar	123 (41%)	39 (59%)	58 (47%)	
ALT in 2012/2013	<40 IU/L	268 (91%)	54 (84%)	110 (93%)	0.08
	≥40 IU/L	25 (9%)	10 (16%)	8 (7%)	
HBV marker in 2012/2013	HBsAg(+), HBeAg(+)	14 (5%)	6 (9%)	6 (5%)	0.3[2]
	HBsAg(+), HBeAg(-)	227 (75%)	53 (80%)	100 (81%)	
	HBsAg(-)	60 (20%)	7 (11%)	17 (14%)	
HBV DNA (IU/ml) in 2012/2013	Undetectable	135 (47%)	23 (35%)	59 (50%)	0.02[2]
	50-200	65 (22%)	16 (24%)	26 (22%)	
	200-2,000	57 (20%)	13 (20%)	23 (19%)	
	2,000-20,000	11 (4%)	4 (6%)	4 (3%)	
	≥20,000	20 (7%)	10 (15%)	7 (6%)	
Phase of natural history in 2012/2013	Immune tolerant	7 (2%)	2 (3%)	4 (3%)	0.8
	HBeAg(+) chronic hepatitis	4 (1%)	4 (6%)	0 (0%)	
	HBeAg(-) chronic hepatitis	19 (6%)	6 (9%)	7 (6%)	
	Inactive carrier	194 (65%)	41 (62%)	88 (71%)	
	Occult HBV	12 (4%)	2 (3%)	5 (4%)	
	Resolved hepatitis B	48 (16%)	5 (8%)	12 (10%)	
	Unclassified	17 (6%)	6 (9%)	7 (6%)	

[1] p-value from Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Linear test for trend

Table 4. Factors associated with significant liver fibrosis (n=271)[1] and condition fulfilling the EASL treatment criteria (n=301) among people with chronic HBV infection who participated in the liver assessment 2012-13

Variables		Significant liver fibrosis (n=271)					Meeting the EASL treatment criteria (n=301)				
		Proportion (%)	Crude OR		Adjusted OR[3]		Proportion (%)	Crude OR		Adjusted OR[3]	
			OR (95% CI)[2]	P	OR (95% CI)[2]	P		OR (95% CI)[2]	P	OR (95% CI)[2]	P
Sex	Male	12/120 (10)	1.0 (ref)	0.01	1.0 (ref)	<0.01	5/130 (4)	1.0 (ref)	0.9	1.0 (ref)	0.9
	Female	3/151 (2)	0.2 (0.1-0.7)		0.2 (0.1-0.6)		6/171 (4)	0.9 (0.3-3.0)		1.0 (0.3-3.3)	
Current age group (years)[4]	<30	3/43 (7)	1.0 (ref)	0.6	1.0 (ref)	0.9	3/46 (7)	1.0 (ref)	0.2	1.0 (ref)	0.2
	30 – 39	6/107 (6)	0.8 (0.2-2.9)		1.1 (0.3-4.8)		5/117 (4)	0.6 (0.1-2.8)		0.7 (0.1-3.0)	
	40 – 49	3/50 (6)	0.9 (0.2-4.5)		1.1 (0.2-6.4)		1/57 (2)	0.3 (0.1-2.6)		0.3 (0.1-2.8)	
	≥50	3/71 (4)	0.6 (0.1-2.9)		1.1 (0.2-6.2)		2/81 (2)	0.4 (0.1-2.3)		0.4 (0.1-2.2)	
Maternal HBsAg	Negative	4/112 (4)	1.0 (ref)	0.01	1.0 (ref)	<0.01	2/123 (2)	1.0 (ref)	0.03	1.0 (ref)	0.03
	Positive	9/61 (15)	4.7 (1.4-15.9)		5.0 (1.6-15.4)		6/66 (9)	6.1 (1.2-30.1)		5.5 (1.2-24.4)	
HBV genotype	Genotype E	8/92 (9)	1.0 (ref)	0.02	1.0 (ref)	0.04	8/101 (8)	1.0 (ref)	N/A	1.0 (ref)	N/A
	Genotype A	2/3 (67)	21.0 (1.7-266.1)		20.7 (1.2-368.1)		0/5 (0)	N/A		N/A	
R249S mutation	Negative	3/96 (3)	1.0 (ref)	0.06	1.0 (ref)	0.03	0/111 (0)	1.0 (ref)	N/A	1.0 (ref)	N/A
	Positive	9/79 (11)	4.0 (1.0-16.4)		5.1 (1.1-23.3)		8/86 (9)	N/A		N/A	
Persistence of HBeAg[4]	Negative at baseline	3/158 (2)	1.0 (ref)	<0.01	1.0 (ref)	<0.01	2/178 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	Cleared during F/U	8/101 (8)	4.4 (1.2-16.7)		12.0 (1.1-134.1)		5/109 (5)	4.2 (0.8-22.0)		9.4 (0.5-165.9)	
	Still positive	4/12 (33)	25.8 (5.4-123.8)		125.5 (9.5-1650.9)		4/14 (29)	35.2 (6.0-205.1)		111.9 (5.9-2138.1)	
% samples with HBV DNA ≥2,000	Never	2/109 (2)	1.0 (ref)	<0.01	1.0 (ref)	0.02	1/129 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	<50%	5/83 (6)	3.4 (0.7-17.9)		4.9 (0.7-36.2)		1/88 (1)	1.4 (0.1-24.1)		3.2 (0.3-37.3)	
	≥50%	8/48 (17)	10.7 (2.2-52.0)		15.5 (1.5-164.1)		9/53 (17)	26.2 (3.3-209.9)		123.9 (10.5-1461.4)	

IU/ml[4,5]											
% samples with ALT ≥40 IU/L[4,5]	Never	5/208 (2)	1.0 (ref)	<0.01	1.0 (ref)	<0.01	3/233 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	<50%	3/14 (21)	11.1 (2.3-52.5)		7.7 (1.6-36.8)		2/14 (14)	12.8 (1.9-84.9)		13.6 (1.7-106.5)	
	≥50%	5/20 (25)	13.5 (3.6-50.7)		17.2 (2.5-118.6)		5/23 (22)	21.3 (4.6-99.3)		27.6 (3.8-200.1)	

[1] Excluding participants who did not have a liver biopsy and who had invalid measurements with transient elastography.

[2] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[3] OR adjusted for sex, current age and birthplace.

[4] Test for linear trend.

[5] This only includes subjects who had at least two measurements during the follow-up.

Population attributable fractions

Maternal sero-status was recorded in 977 unvaccinated participants in Keneba/Manduar between 1974 and 2008, among whom 230 became chronic HBV carriers. The mother was HBsAg-positive in 32.2% of all the chronic carriers, 64.3% of carriers with significant fibrosis, and 71.4% of carriers requiring antiviral treatment according to the EASL guidelines. After controlling for age and sex, having an HBsAg-positive mother was associated with chronic carriage (OR: 2.0, 95% CI: 1.3-3.1), significant fibrosis (OR: 6.4, 2.1-19.8), and requiring antiviral treatment (OR: 8.5, 1.8-40.9). Consequently, the population attributable fraction, that is the proportion of chronic carriers attributable to having an HBsAg-positive mother was 16.0% (95% CI: 8.6-22.9%), and the population attributable fractions for HBV-related significant fibrosis and cases requiring antiviral treatment were 54.3% (41.5-64.3%) and 63.0% (47.0-74.1%), respectively.

DISCUSSION

This is the first long-term follow-up of a population-based cohort of chronic HBV carriers in SSA [3,31,32]. We confirmed that the age-standardized rate of HCC in the chronic carriers in this study (67.3/100,000) was much higher than in the general population in The Gambia (22.1/100,000) [27], which highlights the importance of controlling chronic HBV infection to prevent HCC. Of note, only 3.7% and 1.7% of chronic carriers assessed in 2012-2013 met the EASL and WHO criteria for antiviral treatment, respectively, making HBV a tractable health problem. The PROLIFICA project, the first treatment program for HBV mono-infected individuals in SSA, will assess the effectiveness of HBV screening and antiviral therapy in reducing HCC in The Gambia and Senegal.

The incidence rate of HCC in adult men with chronic HBV infection differs considerably by geographical location: 34/100,000 carrier-years in Europe [33], 230/100,000 in Alaska [34], 327/100,000 in New Zealand Maori [35] and 530-880/100,000 in East Asia [36,37]. In SSA, the recorded rates in adult male lie between Europe and Asia (68.3/100,000 in Senegalese army [36] and 183.5/100,000 in our population-based cohort). These variations in HCC incidence might be partly explained by a difference in the natural history of chronic HBV infection as is discussed below.

It is well established that persistence of high HBV viral load [37,38] or HBeAg [39] increases the risk of HCC, and the current study also confirmed an elevated risk of significant fibrosis in carriers with these conditions. In contrast to East Asia where about half of carrier children remain HBeAg-positive into their twenties [40], in SSA, decay of viral replication occurs much faster. We found that half of chronic carriers lost HBeAg by the age of puberty, and amongst those who cleared, the majority became inactive carriers with low or undetectable HBV DNA, and few developed HCC or HBeAg-negative chronic hepatitis.

Another question is what determines the difference in trajectory of viral replication between Asia and SSA. Evans *et al.* argued that the difference can be explained by the major mode of HBV transmission [36]: in East Asia 40% of chronic carriers were infected vertically compared with only 10% in SSA before the introduction of hepatitis B vaccine [6]. In our study we estimated that 16% of chronic infection attributable to mother-to-infant transmission.

We found that having an HBsAg-positive mother, which is a proxy for mother-to-infant transmission that occurs perinatally or during early childhood, was a risk factor for maintenance of viremia in The Gambia. Moreover, maternal HBsAg was also associated with high ALT, higher prevalence of significant fibrosis and treatment eligibility, and higher HCC incidence among chronic carriers. By restricting to chronic carriers, our analysis suggests that maternal transmission not only increases the risk of chronic infection [30] but may also further increase the risk of persistent viral replication and severe liver disease [8]. These findings are consistent with previous Asian studies that assessed the effect of maternal HBV status [7,8]. Persistent HBV replication may be facilitated in infants because they have an immature immune system [32].

In the pre-vaccine era, horizontal transmission during childhood was more common than perinatal maternal transmission in SSA, and our data support this (16.0% of chronic infection attributable to mother-to-infant transmission). However, we also found that only 3.7% of chronic carriers required antiviral therapy, and most of these cases (63.0%) were attributable to mother-to-infant transmission. This population attributable fraction may even be higher in the post-vaccine era, because the first dose of hepatitis B vaccine is usually delayed for more than one week and therefore perinatal maternal transmission is not well prevented in The Gambia [4,41,42]. Indeed, in our cohort, 60.9% of children who became chronic carriers despite having been fully vaccinated had HBsAg-positive mothers and none received the first vaccine at birth, implying that they were already infected from their mothers before the vaccination.

These findings suggest the importance of interrupting mother-to-infant transmission to reduce the HBV-related disease burden in SSA. Although the WHO recommends a timely administration of hepatitis B vaccine within 24 hours of birth to prevent perinatal and early horizontal transmission [3,5], only 11% of newborns currently receive a birth dose in SSA [43]. This is partly because birth dose is difficult to implement in population where many births take place at home, but also because the Global Alliance for Vaccines and Immunization (GAVI) only provides the pentavalent vaccine (DTP-HepB-Hib), which cannot be used at birth. The feasibility and cost-effectiveness of a timely birth dose vaccine or other strategy (e.g., antiviral therapy for infectious pregnant women) needs to be investigated in SSA [44].

The study is also the first longitudinal cohort to show the association between p53R249S, a marker of chronic aflatoxin exposure, and liver fibrosis. Moreover, we also found a differential risk in liver disease between genotypes A and E, although the number infected with genotype A was small. In West and Central Africa, genotype E is predominant followed by A, whereas in Asia genotype C is common [45]. The latter is associated with delayed HBeAg loss compared with genotypes A, B, D, and F [46], and this may explain why persistent viral replication is more common in East Asia than SSA. Unfortunately, a direct comparison of clinical outcomes between genotype C and E is difficult because their geographical distributions do not overlap.

The American Guidelines for chronic HBV infection recommend starting the screening for HCC in African HBV carriers at an early age (≥20 years old) [26]. This is based on several African case-series where a young median age at HCC diagnosis was reported [9,47]. However, of six HCC cases in this study only one (17%) was <40 years old. This needs to be further studied as this recommendation is costly.

Our study has several limitations. First, the interval between follow-up sero-surveys (4-5 years) was longer than other longitudinal studies [34,35,48] which might have affected the estimates of HBeAg/HBsAg sero-clearance. Nonetheless, the rates are within a range that has been previously reported (HBeAg clearance: 6-9%/year, HBsAg clearance: 0.5-1.6%/year) [34,35,48]. Second, ideally, we would have used maternal HBeAg status at the birth of the child as a proxy for mother-to-infant transmission, since maternal HBeAg positivity is a stronger predictor of maternal transmission than HBsAg. However, maternal sero-status was determined when the child entered the cohort, and by this time maternal HBeAg is likely to have been lost [8]. Third, the phases of the natural history of chronic HBV infection might have been incorrectly classified as they were determined on a single assessment rather than longitudinal monitoring. Fourth, HBV DNA was measured in historical samples, and its levels might have been affected by a prolonged storage and multiple freeze-thaw cycles. Nevertheless, the effect of freeze-thaw cycles is reported to be minimal for HBV DNA assays [49]. Finally, the HCC cases were ascertained through linkage with the cancer registry database, which is estimated to record only 50% of cases [50]. We attempted to mitigate this bias by also reviewing medical records at the local clinic.

In conclusion, compared to East Asia, the natural history of chronic HBV infection in West Africa is characterized by a shorter duration of viremia and lower incidence of HCC, which is probably due to the lower frequency of mother-to-infant transmission in SSA. Among those who develop severe liver disease in The Gambia the majority are infected by their mothers, emphasizing the importance of interrupting perinatal transmission in SSA.

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COMPETING INTERESTS

We declare that we have no conflict of interest.

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AUTHOR CONTRIBUTIONS

YS drafted the manuscript, and all the authors reviewed and approved it. HW initiated and MM maintained the cohort. YS, ML, RN, and MTh were responsible for the design of the liver assessment 2012-2013; YS and AJ for fieldwork; ML, GN, and RN for clinical work; HFN and AJB for laboratory assays; RDG for histopathological analysis; YS and CB for statistical analysis. RW, SM, IB, MTa, and UDA supported the conduct of the study.

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FIGURE LEGENDS

Figure 1. Flow diagram of study participants

Figure 2. Proportion of chronic HBV carriers who cleared HBeAg and HBsAg as a function of age*

* The number at risk is smaller at 5 and 15 years than at 25 years in the figure for HBsAg because the median age of recruitment was 10.8 years.

Figure 3. Changes with age in serum HBV DNA (A) and ALT levels (B) by maternal HBsAg status (- and + denote negative and positive maternal HBsAg, respectively) amongst chronic HBV carriers*

* Two outliers (ALT: 166 and 351 IU/L) in positive maternal HBsAg group are not presented in the figure 3-B.

Title

Natural history of chronic hepatitis B virus infection in West Africa: a longitudinal population-based study from The Gambia

Short Title

Natural history of chronic hepatitis B in West Africa

Authors

Yusuke Shimakawa, PhD,[1,2,3,*] Maud Lemoine, PhD,[1,4,*] Harr Freeya Njai, PhD,[1] Christian Bottomley, PhD,[2] Gibril Ndow, MD,[1,5] Robert D Goldin, MD,[4] Abdoulie Jatta,[1] Adam Jeng-Barry,[1] Rita Wegmuller, PhD,[6] Sophie Moore, PhD,[2,6] Ignatius Baldeh, MSc,[7] Makie Taal, PhD,[7] Umberto D'Alessandro, PhD,[1,2] Hilton Whittle, FMedSci,[8] Ramou Njie, PhD,[1,5] Mark Thursz, MD,[4] Maimuna Mendy, PhD[9]

* Equally contributed

[1] Medical Research Council (MRC) Unit, The Gambia. Banjul, The Gambia.

[2] Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine. London, UK.

[3] Unité d'Épidémiologie des Maladies Émergentes, Institut Pasteur. Paris, France

[4] Department of Hepatology, Imperial College London. London, UK.

[5] The Gambia Hepatitis Intervention Study, IARC, c/o MRC Unit, The Gambia. Banjul, The Gambia.

[6] MRC International Nutrition Group, MRC Keneba. West Kiang, The Gambia.

[7] Ministry of Health and Social Welfare. Banjul, The Gambia.

[8] Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine. London, UK.

[9] International Agency for Research on Cancer (IARC). Lyon, France.

Correspondence

Prof Mark Thursz

Department of Hepatology, Imperial College London, Norfolk Place, London, W2 1NY, UK

Email: m.thursz@imperial.ac.uk

Phone +44-(0)2033121903. Fax +44-(0)2077069161.

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Abbreviations

ALT	Alanine transaminase
APRI	Aspartate transaminase (AST)-to-Platelet Ratio Index
AST	Aspartate transaminase
EASL	European Association for the Study of the Liver
EIA	Enzyme immunoassay
EPI	Expanded Program on Immunization
ESLD	End-stage liver disease
GAVI	Global Alliance for Vaccines and Immunization
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma

HCV	Hepatitis C virus
HDV	Hepatitis D virus
IARC	International Agency for Research on Cancer
MRC	Medical Research Council
OR	Odds ratio
PROLIFICA	Prevention of Liver Fibrosis and Cancer in Africa
SSA	Sub-Saharan Africa
WHO	World Health Organization

Abstract

Background

The natural history of chronic hepatitis B virus (HBV) infection in sub-Saharan Africa is unknown. Data is required to inform WHO guidelines which are currently based on studies in Europe and Asia.

Methods

Between 1974 and 2008, sero-surveys were repeated in two Gambian villages, and an open cohort of treatment-naïve chronic HBV carriers was recruited. Participants were followed to estimate the rates of hepatitis B e (HBeAg) and surface antigen (HBsAg) clearance and incidence of hepatocellular carcinoma (HCC). In 2012-2013, a comprehensive liver assessment was conducted to estimate the prevalence of severe liver disease.

Results

405 chronic carriers (95% genotype E), recruited at a median age of 10.8 years, were followed for a median length of 28.4 years. Annually, 7.4% (95% CI: 6.3-8.8%) cleared HBeAg and 1.0% (0.8-1.2%) cleared HBsAg. The incidence of HCC was 55.5/100,000 carrier-years (95%

CI: 24.9-123.5). In the 2012-2013 survey (n=301), 5.5% (95% CI: 3.4-9.0%) had significant liver fibrosis. HBV genotype A (versus E), chronic aflatoxin B1 exposure, and an HBsAg-positive mother, a proxy for mother-to-infant transmission, were risk factors for liver fibrosis. A small proportion (16.0%) of chronic carriers were infected via mother-to-infant transmission, however, this population represented a large proportion (63.0%) of the cases requiring antiviral therapy.

Conclusions

The incidence of HCC amongst chronic HBV carriers in West Africa was higher than that in Europe but lower than rates in East Asia. High risk of severe liver disease amongst the few who are infected by their mothers underlines the importance of interrupting perinatal transmission in sub-Saharan Africa.

Summary Box

What is already known about this subject?

- Chronic hepatitis B virus infection is a common cause of liver disease in sub-Saharan Africa.

- Although the WHO recently published its first HBV treatment guidelines with a main focus on resource-limited countries, their recommendations are based on Western and Asian studies, since there have been no natural history data from sub-Saharan Africa.
- Mother-to-infant transmission is a risk factor for chronic HBV infection, however, it is unclear whether this mode of transmission further increases the risk of severe liver disease in chronic carriers.

What are the new findings?

- The incidence rate of hepatocellular carcinoma (HCC) in treatment-naïve male chronic HBV carriers in The Gambia was higher than Europe but lower than in East Asia.
- Mother-to-infant transmission was a risk factor for persistent viral replication, elevated transaminase, significant fibrosis and HCC.
- The majority (63.0%) of cases requiring antiviral therapy were attributable to maternal transmission.
- Among chronic HBV carriers, genotype A (versus E) and chronic exposure to aflatoxin B1 were associated with an elevated risk of significant liver fibrosis.

How might it impact on clinical practice in foreseeable future?

- The disproportionate risk of severe liver disease amongst people who acquired HBV from their mothers emphasizes the importance of interrupting perinatal transmission in sub-Saharan Africa.

INTRODUCTION

In sub-Saharan Africa (SSA) chronic hepatitis B virus (HBV) infection is a major public health problem, which causes an estimated 61,000 deaths due to cirrhosis or hepatocellular carcinoma (HCC) each year [1]. Before the introduction of hepatitis B vaccine, >70% of African children were exposed to HBV at birth or during childhood and 10-20% became chronic HBV carriers [2]. Currently, all African countries have integrated hepatitis B vaccine into their Expanded Program on Immunization (EPI).

Despite its efficacy in preventing chronic HBV infection, vaccination has several limitations as a control strategy. First, a large number of people who were infected prior to the vaccination programs are left with chronic HBV infection [3]. Second, hepatitis B vaccine does not always prevent mother-to-infant transmission [4], especially when the vaccine is not given at birth [5]. Though this mode of transmission is less frequent than horizontal transmission in SSA [6], the risk of HCC may be higher in vertically-transmitted chronic infections [7–9].

To overcome these limitations, antiviral therapy can be used to prevent HBV-related disease in cases of chronic HBV infection and also to prevent vertical HBV transmission. In March 2015, the World Health Organization (WHO) issued its first guidelines on chronic HBV infection to improve access to antiviral therapy in low- and middle-income countries. However, their recommendations are based on the findings from Asia, Europe and North America, since there have been no natural history data from SSA [3]. Understanding the natural history of chronic HBV infection is essential to inform decisions about who to treat and when to treat [3].

The UK Medical Research Council (MRC), the International Agency for Research on Cancer (IARC/WHO) and the Gambia Government have been supporting studies on HBV infection in The Gambia since the 1980's, and have established a population-based open cohort of treatment-naïve chronic HBV carriers. We used this cohort to describe the natural history of chronic HBV infection: i) the sero-clearance rates of hepatitis B e antigen (HBeAg) and surface antigen (HBsAg); ii) the incidence of HCC, end-stage liver disease (ESLD) and all-cause mortality; iii) longitudinal changes in serum HBV DNA and alanine transaminase (ALT) levels; and iv) the prevalence of significant liver fibrosis and chronic liver disease requiring antiviral therapy according to the European Association for the Study of the Liver (EASL) [10] or the WHO guidelines [3]. We also estimated the HBV-related disease burden attributable to the mother-to-infant transmission in SSA by examining the associations between these outcomes and maternal HBsAg, a proxy for mother-to-infant HBV transmission [8].

METHODS

Participants

The cohort of chronic HBV carriers was recruited from Keneba and Manduar, two neighboring villages in West Kiang District. They are typical of many African rural communities where Mandinka and Jola people live in mud or lath-and-plaster houses roofed with thatch or corrugated iron with subsistence agriculture [11]. Primary health care has been available free of charge at the MRC Keneba Clinic. Baseline HBV sero-surveys were undertaken in 1974 and in 1980. In the first survey the entire population was surveyed (n=1,317) and 13.2% were found to carry HBsAg [11] while the second survey was limited to children aged <15 years and their mothers (n=802) [12]. Following the third sero-survey in 1984 [13], all non-immune children in Keneba/Manduar were invited to participate in an HBV vaccine trial [14]. Hepatitis B vaccination was introduced in the EPI in 1990 with a vaccine schedule starting at birth. Hepatitis B immunoglobulin has been unavailable. Between 1985 and 2008, sero-surveys to measure the vaccine efficacy were repeated every 4-5 years [4,14–17]. In parallel, those who had been tested HBsAg-positive were followed for HBV sero-markers in 1985, 1989, 1992, 1993, 1998, 2003, and 2008 (supplementary table 1). Survey participation was 92-100% and 50-85% in those aged 0-9 and 10-19 years, respectively [12–15].

Liver assessment in 2012-2013

Following community approval, people with chronic HBV infection in the cohort were invited to a liver assessment as part of the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project [18]. Chronic infection was defined as serum HBsAg positivity at two visits at least six months apart. In individuals aged ≥13 years, HBsAg positivity at only one visit was considered as chronic infection because, in the pre-vaccination era, 90% of children in Keneba/Manduar acquired the infection by the age of 13 years and new infections were uncommon beyond this age [13]. After written informed consent, participants, who had fasted overnight, underwent a standardized clinical examination that involved blood collection, abdominal ultrasound and liver stiffness measurement using transient elastography (Fibroscan, Echosens, France). Those with serum HBV DNA ≥2,000 IU/ml or liver stiffness ≥6.5 kPa or ALT ≥40 IU/L, were invited for liver biopsy. Histopathologists in UK scored liver fibrosis using Metavir system [19]. The study was approved by the Gambia Government/MRC Joint Ethics Committee and conducted according to the guidelines of the Declaration of Helsinki.

Laboratory assays

HBsAg was detected by radioimmunoassay (Ausria-I, Abbott, USA) in 1974 [12], reverse passive hemagglutination assay (Wellcotest, Wellcome Diagnostics, UK) in 1980-1998 [15], immunochromatography (Determine, Abbott) in 2003-2008 [20], and chemiluminescent microparticle immunoassay (Architect, Abbott) in 2012-2013 [21]. HBsAg-positive samples were tested for HBeAg by radioimmunoassay in 1980-1998 [15] and later by enzyme immunoassay (EIA) (Diasorin, Biomedica, Italy) [20]. The serological tests were strongly correlated with one another [20,21]. HBV DNA levels were measured at the end of the study in stored samples collected in 1984, 1989, 1993, 2003, 2008, and 2012-2013, using in-house quantitative real-time polymerase chain reaction (detection limit: 50 IU/ml), calibrated against an international standard [22]. As previously reported, samples collected in 2003 were examined for HBV genotype and an AGG→AGT mutation at codon 249 of p53 tumor suppressor gene (p53R249S) in cell-free DNA, a biomarker of chronic aflatoxin B1 exposure [23]. Samples collected in 2012-2013 were tested for alpha-fetoprotein and antibodies to Hepatitis C virus (HCV) using microparticle EIA (AxSYM, Abbott), antibodies to Hepatitis D virus (HDV) using EIA (ETI-AB-DELTAK-2, Diasorin), and antibodies to HIV-1/2 and p24 antigen using EIA (Genscreen-ULTRA, Bio-Rad, USA). Schistosoma mansoni infection is rare in The Gambia [24] and therefore was not investigated.

Ascertainment of liver disease and death

Significant liver fibrosis, severe fibrosis and cirrhosis was defined as ≥F2, ≥F3 and F4 (Metavir) for those who had liver histopathology and liver stiffness ≥7.9, ≥8.2 and ≥9.5 kPa for those without biopsy. These cut-offs were determined by our validation study in The

Gambia, where the sensitivity of Fibroscan to predict ≥F2 was 81% and the specificity was 81% [25]. The EASL criteria for antiviral therapy are: i) viral load ≥2,000 IU/ml and significant fibrosis, or ii) viral load ≥2,000 IU/ml and moderate/severe active necroinflammation (≥A2 by Metavir activity grade), or iii) viral load ≥20,000 IU/ml and ALT ≥80 IU/L, or iv) detectable viral load and cirrhosis [10]. The WHO criteria are: i) clinically diagnosed cirrhosis, or ii) aspartate transaminase (AST)-to-platelet ratio index (APRI) >2.0, or iii) ≥30 years old and abnormal ALT and HBV DNA >20,000 IU/ml [3]. The phases of the natural history of chronic HBV infection were described [10,26] for the baseline and 2012-2013 survey (supplementary table 2).

HCC cases were identified through a follow-up examination, review of medical records in the MRC Keneba Clinic, or by data linkage with the Gambia National Cancer Registry [27]. The diagnosis was based on the identification of a focal hepatic lesion consistent with HCC on the ultrasound and elevated serum alpha-fetoprotein (≥200 ng/ml). ESLD includes HCC and non-malignant ESLD. The latter was defined as cirrhosis without HCC and the presence of ascites, hepatic encephalopathy, or hematemesis. The date of death was ascertained through a review of the medical chart in the MRC or data linkage with the West Kiang Demographic Surveillance System [28].

Statistical analyses

The person-years of follow-up for HBeAg/HBsAg clearance, HCC, ESLD, or death were calculated from the date they were identified as HBsAg-positive to the date of endpoint or last follow-up, whichever came first. The date of sero-clearance was defined as the midpoint between the last positive and the first negative result. The cumulative incidence was estimated as a function of age using the Kaplan-Meier Method. Age was used rather than time since entry into the study because most infections occur during early childhood [13], and therefore age approximates the duration of HBV infection. The associations between maternal HBsAg, as recorded at the recruitment of the child, and the HBeAg/HBsAg loss were examined using Poisson regression with robust standard error to account for clustering in children that share the same mother. The models included current age, calendar year, sex, and birthplace as covariates. The effect of maternal HBsAg on ALT and HBV DNA (log_{10} transformed) was quantified using a linear mixed model with random intercept and random slope to account for the multiple measurements made on the same individuals over time. The detection limit of the assay was assigned to samples with undetectable viral load. The effect of maternal HBsAg on significant fibrosis and meeting antiviral treatment criteria was estimated using logistic regression to control for age, sex, and birthplace (partial model), and additionally for HBV genotype and p53R249S (full model).

Population attributable fractions were calculated [29] for the effects of maternal sero-status on chronic HBV infection and HBV-related liver disease (significant fibrosis and meeting the EASL treatment criteria). This analysis included all the survey participants (1974-2008) with available maternal sero-status who did not receive hepatitis B vaccine. It was not restricted to chronic carriers so that the twofold effect of mother-to-infant transmission could be estimated, i.e., the increased risk of both chronic infection [30], and of liver disease progression in those with established chronic infection [8,9]. All analyses were performed using STATA 11.0 (Stata Corporation, USA).

RESULTS

Baseline characteristics

Between 1974 and 2008, 551 villagers tested positive for HBsAg at least once in the Keneba/Manduar sero-surveys. None had HCC at enrolment. Twenty-nine HBsAg-positive villagers did not participate in any subsequent sero-surveys. These individuals did not differ from the rest of HBsAg-positive individuals in sex, age, HBeAg, HBV DNA and ALT levels at baseline. Finally, there were 405 chronic carriers (figure 1). The median length of follow-up was 28.4 years (IQR: 17.7-32.7) with the median number of six sero-surveys (IQR: 3-8). The median age at recruitment was 10.8 years (IQR: 4.6-21.8). Half were male, and 65.2%, 26.1%, and 8.7% had a mother who was HBsAg-negative, HBsAg-positive/HBeAg-negative, and HBsAg-positive/HBeAg-positive, respectively (table 1). The children of positive mothers had high viral load (p=0.04) and abnormal ALT levels (p=0.05) at baseline. Thirty became chronic carriers despite having been fully vaccinated against HBV; median age at the first vaccine was 34 days and none received within three days of birth, and the majority (60.9%, 14/23) had HBsAg-positive mothers. In the 2003 sero-survey, 95.1% (97/102) had genotype E and the rest genotype A; 44.2% (100/226) had the p53R249S mutation [23].

Table 1. Baseline characteristics of people with chronic HBV infection by maternal HBsAg status (N=405)

Variables		All (N=405)	Unknown maternal sero-status (n=152)	With HBsAg(+) mother (n=88)	With HBsAg(-) mother (n=165)	p-value[1]
Sex	Male	204 (50%)	63 (41%)	48 (55%)	93 (56%)	0.8
	Female	201 (50%)	89 (59%)	40 (45%)	72 (44%)	
Age group (years)	<5	109 (27%)	4 (3%)	42 (48%)	63 (38%)	0.9[2]
	5 – 9	83 (20%)	9 (6%)	22 (25%)	52 (32%)	
	10 – 14	56 (14%)	16 (10%)	8 (9%)	32 (19%)	
	15 – 19	39 (10%)	23 (15%)	5 (6%)	11 (7%)	
	≥20	118 (29%)	100 (66%)	11 (12%)	7 (4%)	
Birth place	Keneba	233 (58%)	106 (70%)	39 (44%)	88 (53%)	0.4
	Manduar	172 (42%)	46 (30%)	49 (56%)	77 (47%)	
Hepatitis B vaccine	Never	375 (93%)	145 (95%)	74 (84%)	156 (95%)	0.02
	Ever	30 (7%)	7 (5%)	14 (16%)	9 (5%)	
HBeAg	Negative	213 (55%)	118 (86%)	30 (34%)	65 (40%)	0.4
	Positive	173 (45%)	19 (14%)	58 (66%)	96 (60%)	
HBV DNA (IU/ml)	<2,000	222 (57%)	121 (83%)	30 (35%)	71 (45%)	0.04[2]
	2,000-10^8	90 (23%)	20 (14%)	19 (22%)	51 (32%)	
	$\geq 10^8$	79 (20%)	5 (3%)	37 (43%)	37 (23%)	
ALT (IU/L)	<40	367 (94%)	134 (92%)	77 (91%)	156 (97%)	0.05
	≥40	25 (6%)	12 (8%)	8 (9%)	5 (3%)	
Phase of natural history	Immune tolerant	116 (29%)	8 (5%)	42 (48%)	66 (40%)	0.1
	HBeAg(+) chronic hepatitis	14 (3%)	5 (3%)	7 (8%)	2 (1%)	
	HBeAg(-) chronic hepatitis	11 (3%)	7 (5%)	1 (1%)	3 (2%)	
	Inactive carrier	190 (47%)	117 (77%)	22 (25%)	51 (31%)	
	Unclassified	74 (18%)	15 (10%)	16 (18%)	43 (26%)	
HBV genotype[3]	Genotype A	5 (5%)	1 (3%)	2 (8%)	2 (5%)	0.6
	Genotype E	97 (95%)	33 (97%)	24 (92%)	40 (95%)	
p53R249S mutation[3]	Negative	126 (56%)	50 (63%)	23 (44%)	53 (56%)	0.1
	Positive	100 (44%)	30 (37%)	29 (56%)	41 (44%)	
Median no. of follow-up sero-surveys (IQR)		6 (3, 8)	4 (3, 6)	6 (4, 8)	7 (5, 8)	0.1
Median years of follow-up (IQR)		28.4 (17.7, 32.7)	24.4 (10.2, 37.9)	28.6 (16.0, 32.0)	28.7 (23.8, 32.1)	0.2

[1] Comparison was made between participants with HBsAg-positive mothers and HBsAg-negative mothers. P-value and 95% CI were obtained by Wald test with robust standard error.
[2] Linear test for trend
[3] Determined in a subset of participants in 2003

HBeAg sero-clearance

At the enrolment, 213 (52.6%) chronic carriers had already lost HBeAg, The age-specific prevalence of HBeAg at baseline decreased with increasing age (supplementary figure 1). Of the 173 HBeAg-positive carriers at baseline, 82.1% lost HBeAg and the clearance rate was 7.4%/year (95% CI: 6.3-8.8) (table 2, figure 2). Fifteen experienced HBeAg reversion, nine of whom eventually lost HBeAg whilst six continued to carry HBeAg until the last follow-up. After adjusting for sex, current age, calendar year and birthplace, the sero-clearance rate was slower in carriers with high HBV DNA levels ($\geq 10^8$ IU/ml) at baseline (supplementary table 3). Carriers with HBsAg-positive mothers tend to clear HBeAg slowly, although this did not reach statistical significance (supplementary figure 2-A).

Table 2. Incidence rates of HBeAg and HBsAg sero-clearance, HCC, ESLD and all-cause mortality in people with chronic HBV infection by gender

Event	No. of subjects	Person-years at risk	No. of events	Rate	95% CI
HBeAg clearance	173	1912	142	7.4 / 100	6.3 – 8.8
Male	109	1231	86	7.0	5.7 – 8.6
Female	64	681	56	8.2	6.3 – 10.7
HBsAg clearance	405	8502	85	1.00 / 100	0.81 – 1.24
Male	204	4076	32	0.79	0.56 – 1.11
Female	201	4426	53	1.20	0.91 – 1.57
HCC	405	10815	6	55.5 / 100,000	24.9 – 123.5
Male	204	5200	6	115.4	51.8 – 256.8
Boys (<20 y.o.)		1930	0	0.0	N/A
Adult men (≥20 y.o.)		3270	6	183.5	82.4 – 408.5
Female	201	5615	0	0.0	N/A
ESLD (including HCC)	405	10815	8	74.0 / 100,000	37.0 – 147.9
Male	204	5200	7	134.6	64.2 – 282.4
Female	201	5615	1	17.8	2.5 – 126.4
All-cause mortality	405	10815	43	397.6 / 100,000	294.9 – 536.1
Male	204	5200	25	480.8	324.9 – 711.5
Female	201	5615	18	320.6	202.0 – 508.8

HBsAg sero-clearance

The rate of HBsAg sero-clearance was 1.0%/year (95% CI: 0.8-1.2) (table 2) with half clearing by 57 years old (figure 2). Younger age and high HBV DNA levels at baseline were associated with delayed HBsAg sero-clearance (supplementary table 4). The sero-clearance rate was slower in carriers with HbsAg-positive mothers, but this was not statistically significant (supplementary figure 2-B).

HCC, ESLD, and mortality

Of the 405 chronic carriers, 43 died; the all-cause mortality rate was 397.6/100,000 person-years (95% CI: 294.9-536.1). The most common cause of death was HCC (24.0%) in men and bacterial infection (22.2%) in women. All patients with ESLD (including HCC (n=6) and non-malignant ESLD (n=2)) died within one year of diagnosis. Incidence rates of HCC and ESLD were 55.5 (95% CI: 24.9-123.5) and 74.0 (95% CI: 37.0-147.9) per 100,000 person-years, respectively (table 2). All HCC patients were men, all but one was HBeAg-negative at enrolment, and their age at diagnosis ranged between 38 and 67 years (supplementary table 5). The HCC incidence in men ≥20 years was 183.5 (95% CI: 82.4-408.5) per 100,000 person-years. Maternal sero-status was available in three ESLD patients, and all had HBsAg-positive mothers. Crude incidence rates of HCC in carriers with HBsAg-positive mothers was 89.2/100,000 (95% CI: 22.3-356.8) while those with negative mothers was 0/100,000 (unadjusted $p<0.001$).

Mean HBV DNA and ALT over time

The trajectories of HBV DNA and ALT levels by maternal HBsAg are presented in figure 3. Viral load decreased with increasing age at measurement whilst ALT increased. Both viral load and ALT were higher in men than women (supplementary table 6). After adjusting for confounders, the geometric mean viral load was 4.7 times higher (95% CI: 2.0-11.1, p<0.001) and mean ALT was 4.0 IU/L higher (95% CI: 1.2-6.8, p=0.005) in carriers with HBsAg-positive mothers than in those with HBsAg-negative mothers.

Prevalence of chronic liver disease in 2012-2013

After excluding those who died, 83.1% (301/362) of chronic HBV carriers participated in the liver assessment in 2012-2013 (figure 1). Participation was lower in men than women, in younger than in older age groups and in carriers with positive HBeAg at baseline compared with those HBeAg-negative. Table 3 presents the characteristics of the participants. None had ever received antiviral or immunosuppressive therapy. The number co-infected with HIV, HCV, and HDV was three, one, and one, respectively. None had alcohol intake >20 g/day based on the standardized questionnaire. Between the baseline and 2012-2013 survey, the proportion of carriers in the immune tolerant phase decreased from 28.6% to 2.3% whilst the proportion in the inactive phase increased from 46.9% to 64.5% (tables 1 and 3, supplementary figure 3). Only 6.3% were in HBeAg-negative chronic hepatitis in 2012-2013. Thirty participants had a liver biopsy and 269 had a valid measurement using transient elastography. No liver specimen had steatosis. Fifteen carriers (5.5%, 95% CI: 3.4-9.0%) had significant fibrosis, including nine with severe fibrosis and one with cirrhosis. After controlling for confounders, male gender, genotype A, p53R249S mutation, persistence of HBeAg, high viral load, and ALT were risk factors for significant fibrosis (table 4). After adjusting for sex, age, birthplace, HBV genotype and p53R249S, the odds ratio (OR) for the effect of maternal HBsAg on significant fibrosis was 15.8 (95% CI: 1.4-174.1, p=0.02). Eleven participants (3.7%, 95% CI: 2.0-6.5%) met the EASL treatment criteria. Carriers with an HBsAg-positive mother, HBeAg persistence, frequent high viral load, and abnormal ALT were more likely to require antiviral therapy (table 4). Only five participants (1.7%, 95% CI: 0.7-3.9%) fulfilled the WHO treatment criteria.

Table 3. Characteristics of people with chronic HBV infection who participated in the liver assessment 2012-2013 by maternal HBsAg status (N=301)

Variables		All (N=301)	With HBsAg(+) mother (n=66)	With HBsAg(-) mother (n=123)	p-value[1]
Sex	Male	130 (43%)	32 (48%)	59 (48%)	0.9
	Female	171 (57%)	34 (52%)	64 (52%)	
Current age group (years)	<30	46 (15%)	17 (26%)	18 (14%)	0.8[2]
	30 – 39	117 (39%)	30 (45%)	66 (54%)	
	40 – 49	57 (19%)	8 (12%)	28 (23%)	
	≥50	81 (27%)	11 (17%)	11 (9%)	
Birth place	Keneba	178 (59%)	27 (41%)	65 (53%)	0.3
	Manduar	123 (41%)	39 (59%)	58 (47%)	
ALT in 2012/2013	<40 IU/L	268 (91%)	54 (84%)	110 (93%)	0.08
	≥40 IU/L	25 (9%)	10 (16%)	8 (7%)	
HBV marker in 2012/2013	HBsAg(+), HBeAg(+)	14 (5%)	6 (9%)	6 (5%)	0.3[2]
	HBsAg(+), HBeAg(-)	227 (75%)	53 (80%)	100 (81%)	
	HBsAg(-)	60 (20%)	7 (11%)	17 (14%)	
HBV DNA (IU/ml) in 2012/2013	Undetectable	135 (47%)	23 (35%)	59 (50%)	0.02[2]
	50-200	65 (22%)	16 (24%)	26 (22%)	
	200-2,000	57 (20%)	13 (20%)	23 (19%)	
	2,000-20,000	11 (4%)	4 (6%)	4 (3%)	
	≥20,000	20 (7%)	10 (15%)	7 (6%)	
Phase of natural history in 2012/2013	Immune tolerant	7 (2%)	2 (3%)	4 (3%)	0.8
	HBeAg(+) chronic hepatitis	4 (1%)	4 (6%)	0 (0%)	
	HBeAg(-) chronic hepatitis	19 (6%)	6 (9%)	7 (6%)	
	Inactive carrier	194 (65%)	41 (62%)	88 (71%)	
	Occult HBV	12 (4%)	2 (3%)	5 (4%)	
	Resolved hepatitis B	48 (16%)	5 (8%)	12 (10%)	
	Unclassified	17 (6%)	6 (9%)	7 (6%)	

[1] p-value from Wald test with robust standard error to take account of clustering among individuals who share the same mother.
[2] Linear test for trend

Table 4. Factors associated with significant liver fibrosis (n=271)[1] and condition fulfilling the EASL treatment criteria (n=301) among people with chronic HBV infection who participated in the liver assessment 2012-13

Variables		Significant liver fibrosis (n=271)					Meeting the EASL treatment criteria (n=301)				
		Proportion (%)	Crude OR		Adjusted OR[3]		Proportion (%)	Crude OR		Adjusted OR[3]	
			OR (95% CI)[2]	P	OR (95% CI)[2]	P		OR (95% CI)[2]	P	OR (95% CI)[2]	P
Sex	Male	12/120 (10)	1.0 (ref)	0.01	1.0 (ref)	<0.01	5/130 (4)	1.0 (ref)	0.9	1.0 (ref)	0.9
	Female	3/151 (2)	0.2 (0.1-0.7)		0.2 (0.1-0.6)		6/171 (4)	0.9 (0.3-3.0)		1.0 (0.3-3.3)	
Current age group (years)[4]	<30	3/43 (7)	1.0 (ref)	0.6	1.0 (ref)	0.9	3/46 (7)	1.0 (ref)	0.2	1.0 (ref)	0.2
	30 – 39	6/107 (6)	0.8 (0.2-2.9)		1.1 (0.3-4.8)		5/117 (4)	0.6 (0.1-2.8)		0.7 (0.1-3.0)	
	40 – 49	3/50 (6)	0.9 (0.2-4.5)		1.1 (0.2-6.4)		1/57 (2)	0.3 (0.1-2.6)		0.3 (0.1-2.8)	
	≥50	3/71 (4)	0.6 (0.1-2.9)		1.1 (0.2-6.2)		2/81 (2)	0.4 (0.1-2.3)		0.4 (0.1-2.2)	
Maternal HBsAg	Negative	4/112 (4)	1.0 (ref)	0.01	1.0 (ref)	<0.01	2/123 (2)	1.0 (ref)	0.03	1.0 (ref)	0.03
	Positive	9/61 (15)	4.7 (1.4-15.9)		5.0 (1.6-15.4)		6/66 (9)	6.1 (1.2-30.1)		5.5 (1.2-24.4)	
HBV genotype	Genotype E	8/92 (9)	1.0 (ref)	0.02	1.0 (ref)	0.04	8/101 (8)	1.0 (ref)	N/A	1.0 (ref)	N/A
	Genotype A	2/3 (67)	21.0 (1.7-266.1)		20.7 (1.2-368.1)		0/5 (0)	N/A		N/A	
R249S mutation	Negative	3/96 (3)	1.0 (ref)	0.06	1.0 (ref)	0.03	0/111 (0)	1.0 (ref)	N/A	1.0 (ref)	N/A
	Positive	9/79 (11)	4.0 (1.0-16.4)		5.1 (1.1-23.3)		8/86 (9)	N/A		N/A	
Persistence of HBeAg[4]	Negative at baseline	3/158 (2)	1.0 (ref)	<0.01	1.0 (ref)	<0.01	2/178 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	Cleared during F/U	8/101 (8)	4.4 (1.2-16.7)		12.0 (1.1-134.1)		5/109 (5)	4.2 (0.8-22.0)		9.4 (0.5-165.9)	
	Still positive	4/12 (33)	25.8 (5.4-123.8)		125.5 (9.5-1650.9)		4/14 (29)	35.2 (6.0-205.1)		111.9 (5.9-2138.1)	
% samples with HBV DNA ≥2,000	Never	2/109 (2)	1.0 (ref)	<0.01	1.0 (ref)	0.02	1/129 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	<50%	5/83 (6)	3.4 (0.7-17.9)		4.9 (0.7-36.2)		1/88 (1)	1.4 (0.1-24.1)		3.2 (0.3-37.3)	
	≥50%	8/48 (17)	10.7 (2.2-52.0)		15.5 (1.5-164.1)		9/53 (17)	26.2 (3.3-209.9)		123.9 (10.5-1461.4)	

IU/ml[4,5]											
% samples with ALT ≥40 IU/L[4,5]	Never	5/208 (2)	1.0 (ref)	<0.01	1.0 (ref)	<0.01	3/233 (1)	1.0 (ref)	<0.01	1.0 (ref)	<0.01
	<50%	3/14 (21)	11.1 (2.3-52.5)		7.7 (1.6-36.8)		2/14 (14)	12.8 (1.9-84.9)		13.6 (1.7-106.5)	
	≥50%	5/20 (25)	13.5 (3.6-50.7)		17.2 (2.5-118.6)		5/23 (22)	21.3 (4.6-99.3)		27.6 (3.8-200.1)	

[1] Excluding participants who did not have a liver biopsy and who had invalid measurements with transient elastography.
[2] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.
[3] OR adjusted for sex, current age and birthplace.
[4] Test for linear trend.
[5] This only includes subjects who had at least two measurements during the follow-up.

Population attributable fractions

Maternal sero-status was recorded in 977 unvaccinated participants in Keneba/Manduar between 1974 and 2008, among whom 230 became chronic HBV carriers. The mother was HBsAg-positive in 32.2% of all the chronic carriers, 64.3% of carriers with significant fibrosis, and 71.4% of carriers requiring antiviral treatment according to the EASL guidelines. After controlling for age and sex, having an HBsAg-positive mother was associated with chronic carriage (OR: 2.0, 95% CI: 1.3-3.1), significant fibrosis (OR: 6.4, 2.1-19.8), and requiring antiviral treatment (OR: 8.5, 1.8-40.9). Consequently, the population attributable fraction, that is the proportion of chronic carriers attributable to having an HBsAg-positive mother was 16.0% (95% CI: 8.6-22.9%), and the population attributable fractions for HBV-related significant fibrosis and cases requiring antiviral treatment were 54.3% (41.5-64.3%) and 63.0% (47.0-74.1%), respectively.

DISCUSSION

This is the first long-term follow-up of a population-based cohort of chronic HBV carriers in SSA [3,31,32]. We confirmed that the age-standardized rate of HCC in the chronic carriers in this study (67.3/100,000) was much higher than in the general population in The Gambia (22.1/100,000) [27], which highlights the importance of controlling chronic HBV infection to prevent HCC. Of note, only 3.7% and 1.7% of chronic carriers assessed in 2012-2013 met the EASL and WHO criteria for antiviral treatment, respectively, making HBV a tractable health problem. The PROLIFICA project, the first treatment program for HBV mono-infected individuals in SSA, will assess the effectiveness of HBV screening and antiviral therapy in reducing HCC in The Gambia and Senegal.

The incidence rate of HCC in adult men with chronic HBV infection differs considerably by geographical location: 34/100,000 carrier-years in Europe [33], 230/100,000 in Alaska [34], 327/100,000 in New Zealand Maori [35] and 530-880/100,000 in East Asia [36,37]. In SSA, the recorded rates in adult male lie between Europe and Asia (68.3/100,000 in Senegalese army [36] and 183.5/100,000 in our population-based cohort). These variations in HCC incidence might be partly explained by a difference in the natural history of chronic HBV infection as is discussed below.

It is well established that persistence of high HBV viral load [37,38] or HBeAg [39] increases the risk of HCC, and the current study also confirmed an elevated risk of significant fibrosis in carriers with these conditions. In contrast to East Asia where about half of carrier children remain HBeAg-positive into their twenties [40], in SSA, decay of viral replication occurs much faster. We found that half of chronic carriers lost HBeAg by the age of puberty, and amongst those who cleared, the majority became inactive carriers with low or undetectable HBV DNA, and few developed HCC or HBeAg-negative chronic hepatitis.

Another question is what determines the difference in trajectory of viral replication between Asia and SSA. Evans *et al.* argued that the difference can be explained by the major mode of HBV transmission [36]: in East Asia 40% of chronic carriers were infected vertically compared with only 10% in SSA before the introduction of hepatitis B vaccine [6]. In our study we estimated that 16% of chronic infection attributable to mother-to-infant transmission.

We found that having an HBsAg-positive mother, which is a proxy for mother-to-infant transmission that occurs perinatally or during early childhood, was a risk factor for maintenance of viremia in The Gambia. Moreover, maternal HBsAg was also associated with high ALT, higher prevalence of significant fibrosis and treatment eligibility, and higher HCC incidence among chronic carriers. By restricting to chronic carriers, our analysis suggests that maternal transmission not only increases the risk of chronic infection [30] but may also further increase the risk of persistent viral replication and severe liver disease [8]. These findings are consistent with previous Asian studies that assessed the effect of maternal HBV status [7,8]. Persistent HBV replication may be facilitated in infants because they have an immature immune system [32].

In the pre-vaccine era, horizontal transmission during childhood was more common than perinatal maternal transmission in SSA, and our data support this (16.0% of chronic infection attributable to mother-to-infant transmission). However, we also found that only 3.7% of chronic carriers required antiviral therapy, and most of these cases (63.0%) were attributable to mother-to-infant transmission. This population attributable fraction may even be higher in the post-vaccine era, because the first dose of hepatitis B vaccine is usually delayed for more than one week and therefore perinatal maternal transmission is not well prevented in The Gambia [4,41,42]. Indeed, in our cohort, 60.9% of children who became chronic carriers despite having been fully vaccinated had HBsAg-positive mothers and none received the first vaccine at birth, implying that they were already infected from their mothers before the vaccination.

These findings suggest the importance of interrupting mother-to-infant transmission to reduce the HBV-related disease burden in SSA. Although the WHO recommends a timely administration of hepatitis B vaccine within 24 hours of birth to prevent perinatal and early horizontal transmission [3,5], only 11% of newborns currently receive a birth dose in SSA [43]. This is partly because birth dose is difficult to implement in population where many births take place at home, but also because the Global Alliance for Vaccines and Immunization (GAVI) only provides the pentavalent vaccine (DTP-HepB-Hib), which cannot be used at birth. The feasibility and cost-effectiveness of a timely birth dose vaccine or other strategy (e.g., antiviral therapy for infectious pregnant women) needs to be investigated in SSA [44].

The study is also the first longitudinal cohort to show the association between p53R249S, a marker of chronic aflatoxin exposure, and liver fibrosis. Moreover, we also found a differential risk in liver disease between genotypes A and E, although the number infected with genotype A was small. In West and Central Africa, genotype E is predominant followed by A, whereas in Asia genotype C is common [45]. The latter is associated with delayed HBeAg loss compared with genotypes A, B, D, and F [46], and this may explain why persistent viral replication is more common in East Asia than SSA. Unfortunately, a direct comparison of clinical outcomes between genotype C and E is difficult because their geographical distributions do not overlap.

The American Guidelines for chronic HBV infection recommend starting the screening for HCC in African HBV carriers at an early age (≥20 years old) [26]. This is based on several African case-series where a young median age at HCC diagnosis was reported [9,47]. However, of six HCC cases in this study only one (17%) was <40 years old. This needs to be further studied as this recommendation is costly.

Our study has several limitations. First, the interval between follow-up sero-surveys (4-5 years) was longer than other longitudinal studies [34,35,48] which might have affected the estimates of HBeAg/HBsAg sero-clearance. Nonetheless, the rates are within a range that has been previously reported (HBeAg clearance: 6-9%/year, HBsAg clearance: 0.5-1.6%/year) [34,35,48]. Second, ideally, we would have used maternal HBeAg status at the birth of the child as a proxy for mother-to-infant transmission, since maternal HBeAg positivity is a stronger predictor of maternal transmission than HBsAg. However, maternal sero-status was determined when the child entered the cohort, and by this time maternal HBeAg is likely to have been lost [8]. Third, the phases of the natural history of chronic HBV infection might have been incorrectly classified as they were determined on a single assessment rather than longitudinal monitoring. Fourth, HBV DNA was measured in historical samples, and its levels might have been affected by a prolonged storage and multiple freeze-thaw cycles. Nevertheless, the effect of freeze-thaw cycles is reported to be minimal for HBV DNA assays [49]. Finally, the HCC cases were ascertained through linkage with the cancer registry database, which is estimated to record only 50% of cases [50]. We attempted to mitigate this bias by also reviewing medical records at the local clinic.

In conclusion, compared to East Asia, the natural history of chronic HBV infection in West Africa is characterized by a shorter duration of viremia and lower incidence of HCC, which is probably due to the lower frequency of mother-to-infant transmission in SSA. Among those who develop severe liver disease in The Gambia the majority are infected by their mothers, emphasizing the importance of interrupting perinatal transmission in SSA.

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COMPETING INTERESTS

We declare that we have no conflict of interest.

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AUTHOR CONTRIBUTIONS

YS drafted the manuscript, and all the authors reviewed and approved it. HW initiated and MM maintained the cohort. YS, ML, RN, and MTh were responsible for the design of the liver assessment 2012-2013; YS and AJ for fieldwork; ML, GN, and RN for clinical work; HFN and AJB for laboratory assays; RDG for histopathological analysis; YS and CB for statistical analysis. RW, SM, IB, MTa, and UDA supported the conduct of the study.

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FIGURE LEGENDS

Figure 1. Flow diagram of study participants

Figure 2. Proportion of chronic HBV carriers who cleared HBeAg and HBsAg as a function of age*

* The number at risk is smaller at 5 and 15 years than at 25 years in the figure for HBsAg because the median age of recruitment was 10.8 years.

Figure 3. Changes with age in serum HBV DNA (A) and ALT levels (B) by maternal HBsAg status (- and + denote negative and positive maternal HBsAg, respectively) amongst chronic HBV carriers*

* Two outliers (ALT: 166 and 351 IU/L) in positive maternal HBsAg group are not presented in the figure 3-B.

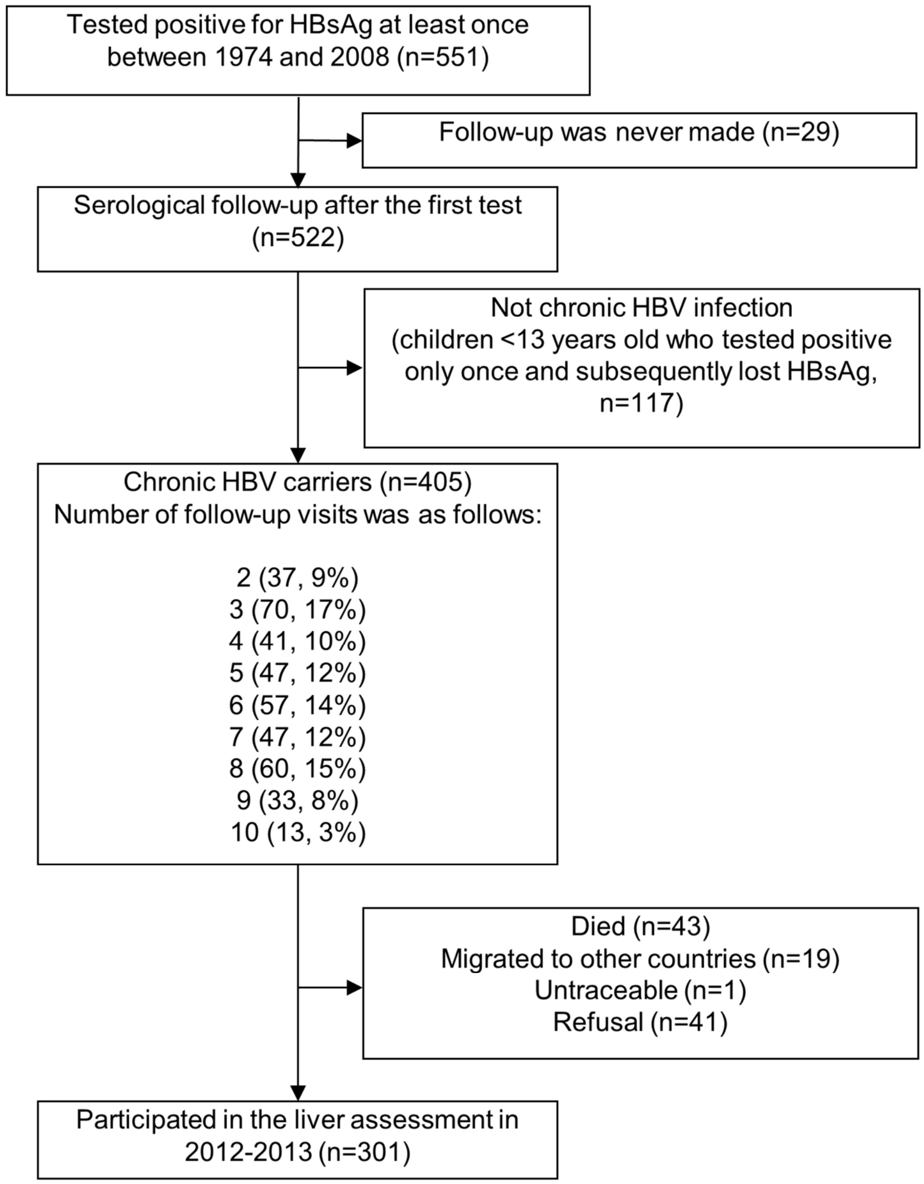

Figure 1. Flow diagram of study participants
159x203mm (300 x 300 DPI)

Figure 2. Proportion of chronic HBV carriers who cleared HBeAg and HBsAg as a function of age*

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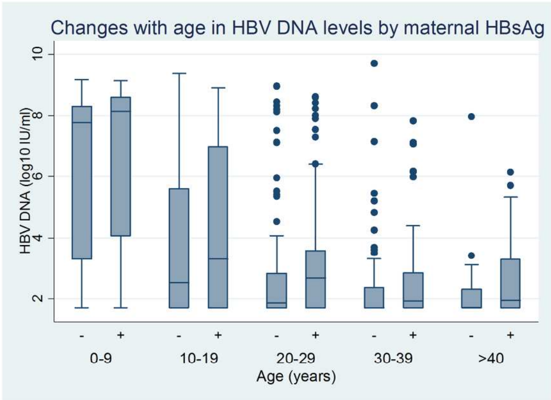

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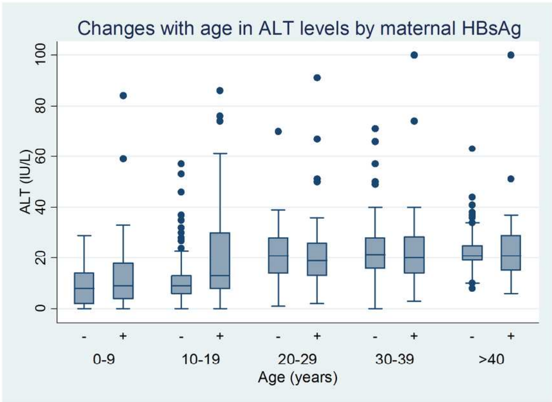

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190x142mm (300 x 300 DPI)

Supplementary Table 1. Participation, numbers who previously tested HBsAg-positive and number of newly identified HBsAg-positive in sero-surveys between 1974 and 2013.

Year	Target population	Total tested	Previously tested HBsAg-positive		Newly identified in the current survey	Laboratory tests performed			
			Total	Participated in the current survey (% follow-up)		HBsAg	HBeAg	HBV DNA	ALT
1974	All villagers	1317	-	-	136	RIA	-	-	-
1980	Children <15 years & mothers	802	136	65 (48%)	104	RPHA[1]	RIA	-	-
1984	Children <20 years	936	240	99 (41%)	143	RPHA[1]	RIA	q-PCR	Cobas Mira
1985	Children <20 years	937	383	242 (63%)	4	RPHA[1]	RIA	-	-
1989	Children <20 years & mothers	1358	387	271 (70%)	49	RPHA[1]	RIA	q-PCR	-
1992	HBsAg carriers	366	436	270 (62%)	1	RPHA[1]	RIA	-	Cobas Mira
1993	Children <20 years & mothers	1478	437	175 (40%)	30	RPHA[1]	RIA	q-PCR	-
1998	HBsAg carriers & vaccinees	1476	467	171 (37%)	12	RPHA[1]	RIA	-	-
2003	All villagers	1640	479	294 (61%)	67	IC	EIA	q-PCR	-
2008	HBsAg carriers & vaccinees	2078	546	323 (59%)	5	IC	EIA	q-PCR	Vitros DT60-II
2012-13	Carriers	332	551	332 (60%)	0	CMIA	EIA	q-PCR	Vitros 350

Abbreviations: CMIA, chemiluminescent microparticle immunoassay; EIA, enzyme immunoassay; IC, immunochromatography; q-PCR, quantitative real-time polymerase chain reaction; RIA, radioimmunoassay; RPHA, reverse passive hemagglutination assay

[1] Positive results using RPHA were confirmed by neutralization with rabbit anti-HBs.

Supplementary Table 2. Phases of the natural history of chronic HBV infection (adapted from the EASL/AASLD guidelines)

Phase		HBsAg	HBeAg	HBV DNA (IU/ml)	ALT (U/L)
Immune tolerant phase		Positive	Positive	≥20,000	<40
Chronic hepatitis B disease	HBeAg-positive chronic hepatitis B	Positive	Positive	Any	≥40
	HBeAg-negative chronic hepatitis B	Positive	Negative	≥2,000	≥40
Inactive HBV carrier state		Positive	Negative	<2,000	<40
Occult HBV infection		Negative	Negative	Detectable	Any
Resolved hepatitis B		Negative	Negative	Undetectable	<40
Unclassified	HBeAg-positive	Positive	Positive	<20,000	<40
	HBeAg-negative	Positive	Negative	≥2,000	<40
				<2,000	≥40

Supplementary Table 3. Predictors of HBeAg sero-clearance (n=173)

Variables		Person-years	No. of subjects cleared HBeAg	Rate (% per annum)	Crude RR		Adjusted RR[3]	
					RR (95% CI)	p-value[1]	RR (95% CI)	p-value[1]
Sex	Male	1231	86	7.0	1.0 (ref)	0.3	1.0 (ref)	0.3
	Female	682	56	8.2	1.2 (0.9 – 1.6)		1.2 (0.9-1.6)	
Current age group (years)[2]	0-9	663	34	5.1	1.0 (ref)	0.02	1.0 (ref)	0.5
	10-19	761	66	8.7	1.7 (1.1 – 2.5)		1.4 (0.9-2.2)	
	≥20	488	42	8.6	1.7 (1.1 – 2.6)		1.2 (0.7-1.9)	
Birthplace	Keneba	869	67	7.7	1.0	0.6	1.0 (ref)	0.8
	Manduar	1043	75	7.2	0.9 (0.7 – 1.3)		1.0 (0.7-1.4)	
Maternal HBsAg	Negative	1027	86	8.4	1.0 (ref)	0.1	1.0 (ref)	0.2
	Positive	673	43	6.4	0.8 (0.5 – 1.1)		0.8 (0.5 – 1.2)	
HBV DNA (IU/ml) at baseline[2]	<2,000	333	31	9.3	1.0 (ref)	0.009	1.0 (ref)	0.02
	2,000-10^8	601	51	8.5	0.9 (0.7-1.2)		1.0 (0.7-1.4)	
	≥10^8	930	56	6.0	0.6 (0.5-0.9)		0.7 (0.4-0.9)	
ALT (IU/L) at baseline	<40	1736	129	7.4	1.0 (ref)	0.9	1.0 (ref)	0.7
	≥40	150	11	7.3	1.0 (0.6-1.5)		0.9 (0.6-1.5)	

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Test for linear trend.

[3] Rate ratio adjusted for sex, current age, calendar year and birthplace.

Supplementary Table 4. Predictors of HBsAg sero-clearance (n=405)

Variables		Person-years	No. of subjects cleared HBsAg	Rate (% per annum)	Crude RR		Adjusted RR[3]	
					RR (95% CI)	p-value[1]	RR (95% CI)	p-value[1]
Sex	Male	4076	32	0.79	1.0 (ref)	0.05	1.0 (ref)	0.8
	Female	4426	53	1.20	1.5 (1.0 – 2.3)		1.1 (0.7-1.7)	
Current age group (years)[2]	0-9	957	1	0.10	1.0 (ref)	<0.001	1.0 (ref)	<0.001
	10-19	2189	10	0.46	4.4 (0.6 – 34.1)		5.5 (0.7-42.5)	
	20-29	2382	24	1.01	9.6 (1.3 – 71.0)		16.2 (2.2-120.4)	
	30-39	1528	16	1.05	10.0 (1.3 – 76.0)		16.6 (2.2-125.9)	
	40-49	820	19	2.32	22.2 (3.0 – 165.7)		35.7 (4.8-264.2)	
	50-70	627	15	2.39	22.9 (3.0 – 174.6)		42.5 (5.6-321.1)	
Birthplace	Keneba	4344	49	1.13	1.0 (ref)	0.3	1.0 (ref)	0.3
	Manduar	4159	36	0.87	0.8 (0.5 – 1.2)		0.8 (0.5-1.2)	
Maternal HBsAg	Negative	3913	27	0.69	1.0 (ref)	0.1	1.0 (ref)	0.1
	Positive	2006	7	0.35	0.5 (0.2 – 1.2)		0.5 (0.2 – 1.2)	
HBeAg at baseline	Negative	4353	51	1.17	1.0 (ref)	<0.001	1.0 (ref)	0.3
	Positive	4000	16	0.40	0.3 (0.2-0.6)		0.7 (0.3-1.3)	
HBV DNA (IU/ml) at baseline[2]	<2,000	4490	68	1.52	1.0 (ref)	<0.001	1.0 (ref)	0.03
	$2,000-10^{8}$	2111	9	0.43	0.3 (0.1-0.5)		0.5 (0.2-1.0)	
	$\geq 10^{8}$	1776	5	0.28	0.2 (0.1-0.4)		0.4 (0.2-1.2)	
ALT (IU/L) at baseline	<40	8066	79	0.98	1.0 (ref)	0.9	1.0 (ref)	1.0
	≥40	339	3	0.88	0.9 (0.3-2.9)		1.0 (0.3-3.6)	

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Test for linear trend.

[3] Rate ratio adjusted for sex, current age, calendar year and birthplace.

Supplementary Table 5. Characteristics of individuals who died of ESLD (includes HCC and non-malignant ESLD)

Cause of death	Age at enrolment	Age at diagnosis	Sex	Birth place	Maternal HBsAg	HBeAg at baseline	HBV DNA at baseline (IU/ml)	ALT at baseline (IU/L)	HBsAg loss during follow-up
HCC	43	45	M	Keneba	N/A	Negative	N/A	N/A	No
HCC[1]	29	67	M	Manduar	N/A	Negative	2,800	43	No
HCC	23	57	M	Manduar	N/A	Negative	N/A	13	No
HCC	20	50	M	Manduar	Positive	Negative	1,345,000	10	No
HCC	21	42	M	Manduar	Positive	Positive	300,000	15	No
HCC	21	38	M	Manduar	N/A	Negative	N/A	N/A	Yes
Non-malignant ESLD	21	57	M	Keneba	N/A	Negative	N/A	6	No
Non-malignant ESLD	7	19	F	Keneba	Positive	Positive	N/A	8	No

[1] This patient had genotype A.

Supplementary Table 6. Predictors of geometric mean HBV DNA and mean ALT levels (n=405)

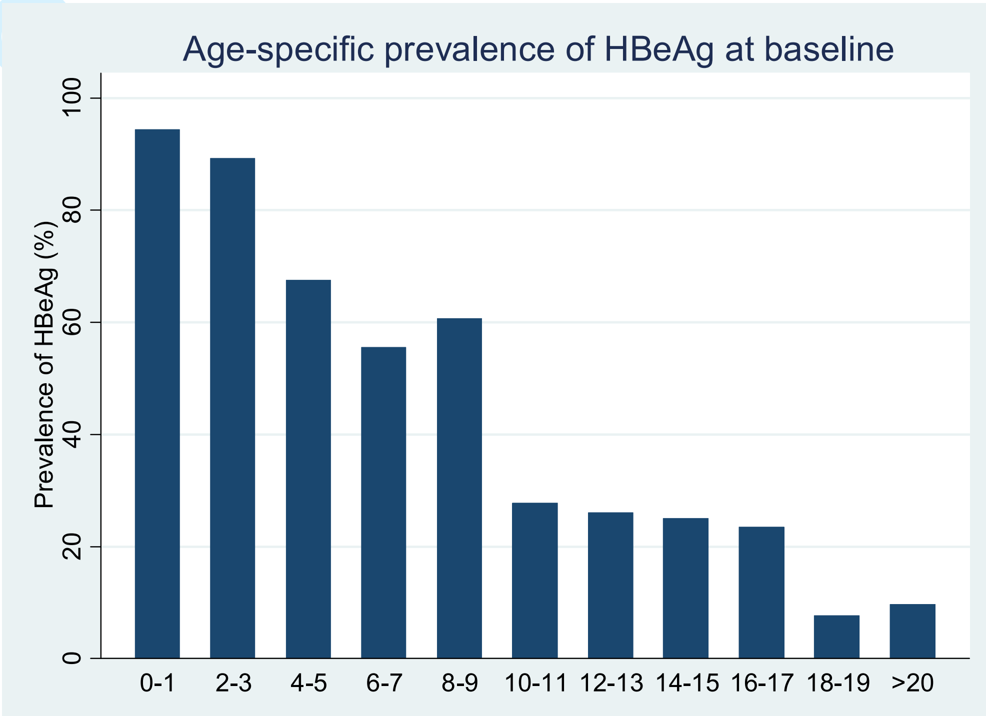
Variables		HBV DNA levels			ALT levels		
		Geometric mean HBV DNA (IU/ml)	Adjusted ratio of geometric mean HBV DNA (95% CI)[1,3]	p-value[1]	Mean ALT (IU/L)	Adjusted mean difference (95% CI)[1,3]	p-value[1]
Sex	Male	10,093	1.0 (ref)	0.04	21.0	0.0 (ref)	0.01
	Female	916	0.5 (0.3 – 0.7)		18.7	-3.5 (-6.3 – 0.8)	
Current age group (years)[2]	0-9	6,505,734	1.0 (ref)	<0.001	10.9	0.0 (ref)	<0.001
	10-19	36,785	$4x10^{-3}$ ($2x10^{-3}$ – $9x10^{-3}$)		15.3	5.8 (1.4 – 10.2)	
	20-29	947	$1x10^{-4}$ ($4x10^{-5}$ – $2x10^{-4}$)		25.3	14.8 (10.3 – 19.4)	
	30-39	318	$3x10^{-5}$ ($1x10^{-5}$ – $7x10^{-5}$)		23.4	17.1 (12.8 – 21.4)	
	40-49	170	$7x10^{-6}$ ($2x10^{-6}$ – $2x10^{-5}$)		21.8	20.3 (14.5 – 26.0)	
	50-70	145	$2x10^{-6}$ ($4x10^{-7}$ – $1x10^{-5}$)		20.5	28.2 (21.3 – 35.0)	
Birthplace	Keneba	1,723	1.0 (ref)	0.6	20.6	0.0 (ref)	0.4
	Manduar	5,704	1.2 (0.6 – 2.2)		18.9	-1.2 (-3.9 – 1.4)	
Maternal HBsAg	Negative	3,607	1.0 (ref)	<0.001	17.6	0.0 (ref)	0.005
	Positive	21,499	4.7 (2.0 – 11.1)		22.5	4.0 (1.2 – 6.8)	

[1] Mean difference, p-value and 95% CI estimated using a linear mixed models to account for repeated measurements within participants.

[2] Test for linear trend.

[3] Mean difference adjusted for sex, current age, age at study entry and birthplace.

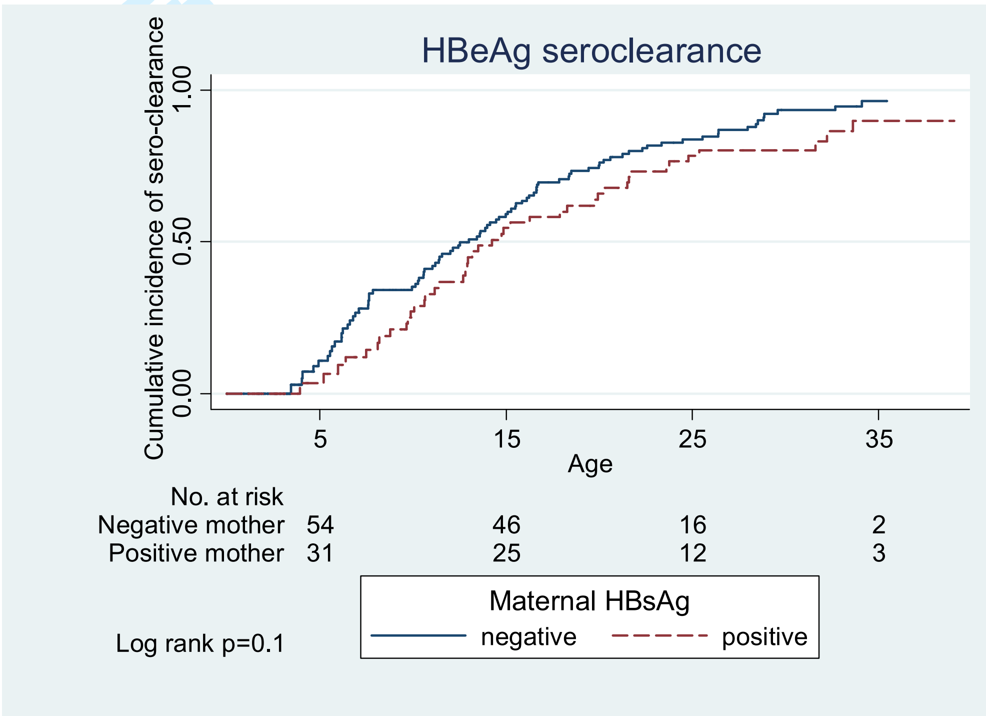
Supplementary Figure 1. Age-specific prevalence of HBeAg in chronic HBV carriers at baseline (n=405)



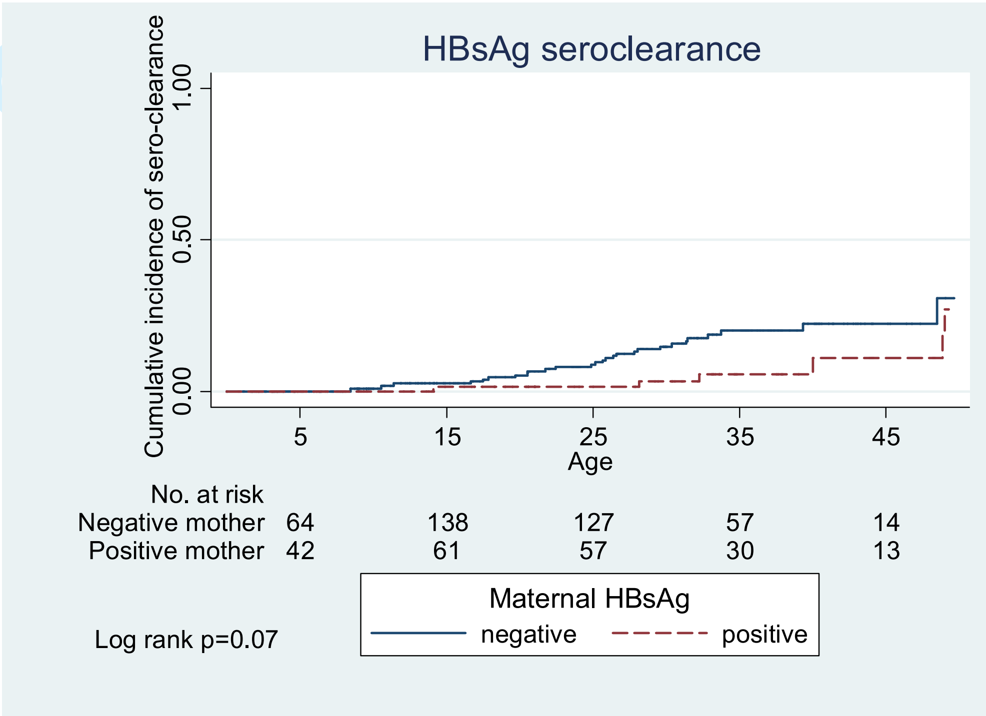
Supplementary Figure 2. Proportion of chronic HBV carriers who cleared HBeAg (A) and HBsAg (B) as a function of age and according to maternal HBsAg positivity*

* The number at risk is smaller at 5 years than at 15 years in supplementary figure 2-B because the median age of recruitment was 10.8 years.

Supplementary Figure 2-A



Supplementary Figure 2-B



Supplementary Figure 3. Changes in phase of natural history between baseline (n=405) and 2012-2013 liver assessment (n=301)