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Outcome of the Protocol for Prevention of Mother to Child Transmission of Hepatitis B Infection and Nine Months Follow-up of Hepatitis B-exposed Infants at Ile-Ife, Nigeria.

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Outcome of the Protocol for Prevention of Mother to Child Transmission of Hepatitis B Infection and Nine Months Follow-up of Hepatitis B-exposed Infants at Ile-Ife, Nigeria.

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Abstract

Objectives: Eliminating Mother-to-Child-Transmission (MTCT) of Hepatitis B Virus (HBV) is central to the WHO's target of reducing hepatitis B infection in children to <0.1% by 2030. While Nigeria alone accounts for 8.3% of the global burden, interventional studies on prevention of MTCT of HBV are hardly available. This study aims to assess the impact of prevention of MTCT interventions on vertical transmission of HBV among pregnant women in Nigeria.

Design: A prospective cohort study

Setting: A University Teaching Hospitals Complex in Nigeria between 2015-2021.

Participants: 10,866 pregnant women and their pre-existing children.

Interventions: The pregnant women were screened for HBsAg using chromatographic immunoassay (Micropoint®, USA). HBsAg-positive women had the HBV panel assay done and their pre-existing children were screened. Women with HBV DNA ≥200,000IU/ml and those positive for HBeAg had 300mg daily of Tenofovir Disoproxil Fumarate (TDF) in the 3rd trimester. The newborns had HBV vaccines and HB Immunoglobulin (HBIG) administered, followed by testing for HBsAg at 9months postnatally.

Primary outcome measures: Prevalence of chronic hepatitis B infection in pregnancy, and the incidence of MTCT of HBV.

Results: Overall, 395 women had chronic HBV infection, giving a prevalence of 3.64%. Their mean age was 31.51± 5.71years, with a median parity of 1.2. Thirteen women (3.3%) were positive for HBeAg, seven (3.1%) of the 225 pre-existing hepatitis B-exposed children were HBsAg positive, and 17women had prenatal TDF. Overall, 376 women completed the study, with mean birthweight of 3.21±1.86kg and perinatal mortality rate of 29.2/1,000births. HBV vaccine-HBIG combination was administered to 260 newborns, while the others had HBV vaccine alone. All the children tested negative to the HBsAg at 9months.

Conclusion: Eliminating MTCT of hepatitis B infection through validated protocols in LMICs with the highest burden of chronic HBV infections is feasible. A national scale-up of such protocols is recommended.

Article Summary

Strengths and limitations of this study

- This study involves the largest cohort of hepatitis B pregnant women to be prospectively recruited and actively managed in Nigeria.
- The multidisciplinary nature of the study of the managing team made implementation of the hepatitis B EMTCT interventions feasible.
- This research is the only study to actively intervene, prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months in Nigeria.
- HBV DNA assay could not be routinely done in the HBsAg-positive women due to financial constraints.

Keywords: Chronic hepatitis B, Hepatitis B virus, Hepatitis B vaccine, Hepatitis B immunoglobulin, Mother-to-child transmission, Nigeria

Word count: 3,193

Introduction

The World Health Organization (WHO) estimates that 1.4 million deaths are recorded annually due to viral hepatitis, and hepatitis-associated mortality now ranks as the 7th leading cause of death globally, ahead of deaths due to HIV/AIDS, Malaria and Tuberculosis. This burden is mainly associated with Hepatitis B virus (48%), Hepatitis C virus (48%) and the remaining from acute hepatitis A and E infection[1]. Overall, about 2 billion people are estimated to have evidence of past or present hepatitis B infection, and at least 296 million people, corresponding to about 3.8% of the global population are estimated to be chronically infected with the hepatitis B virus in 2019[1]. The burden of hepatitis B infection is however unevenly distributed, with developing countries, especially the African and the Western Pacific regions suffering disproportionately; the 2 regions account for 67% of the global burden of hepatitis B infection[1-3], with at least 5% of the population estimated to be living with the hepatitis B infection. Worse still, the current figures are possibly

underestimates, as probably less than 5% of people that are chronically infected in these regions have undergone testing to diagnose their status[3].

Transmission of hepatitis B virus may be horizontal (through contact with body fluids including blood, semen, and sharp objects) or vertical, through mother to child transmission. Vertical transmission has served as a means of maintaining the reservoir of infection in low-and middle-income countries, due to its propensity to progress into chronicity. While acute hepatitis B infections in adults are likely to be cleared within 6months in 95% of cases, chronicity is recorded in 20-60% of children under the age of 5years, and at least 90% of perinatally transmitted cases. As these newborns often remain asymptomatic, diagnosis is unlikely until they reach the stage of hepatic decompensation, liver cirrhosis or hepatocellular carcinoma (HCC) in adulthood.

The Center for Disease Control (CDC) estimated the prevalence of chronic hepatitis B infection in Nigeria to be at least 8%[4]. This was further supported by the Federal Ministry of Health's estimate of 11.1%, amounting to 8.3% of the global chronic hepatitis B infection, thereby making Nigeria the country with the highest burden globally[5]. Despite the need to strive to attain the set targets for Elimination of Mother to Child Transmission (EMTCT) of hepatitis B infection, and the proven benefits of the afore-mentioned interventions, they are not always provided as standard components of the Reproductive, Maternal, Newborn and Child Health (RMNCH) programmes in Nigeria and other developing countries. A case in-point is the birth dose of hepatitis B vaccine coverage, which is reported to be only 6% in the African region in 2020[6]. Although the HBV vaccine is administered at no cost to newborns in Nigeria, only 52.4 % of Nigerian children reportedly had the birth dose of hepatitis B vaccine, while just 4% had the dose administered within the first 24hours of life as recommended, and overall, only 50.3% of Nigerian children completed the 3 additional doses of hepatitis B vaccine[7].

In response to the bothersome statistics of hepatitis B infection, the Global Health Sector Strategy (GHSS) on viral hepatitis was launched at the World Health Assembly in 2016, with the aim of eliminating viral hepatitis B and C infections[8]. For this goal of eliminating viral hepatitis as a public health threat to be achieved, WHO has set the target of reducing HBsAg prevalence in children to 0.1% by 2030[8]. To evaluate the impact of the EMTCT measures and identify bottlenecks to the successful attainment of the GHSS targets in Low- and Middle-Income Countries (LMICs) such as Nigeria, appropriately designed prospective studies are imperative.

Although some Nigerian researchers have evaluated the outcomes of pregnancies among women with hepatitis infection, many of these studies were either retrospectively conducted or simply prevalence studies that did not control for or report the quality of prevention of mother to child transmission of hepatitis B care that the study subjects received[9, 10]; none had therefore reported on the outcome of implementing a well designed and implemented protocol. While Onakewhor et al reported the administration of hepatitis B vaccine and HBIG immunoprophylaxis for PMTCT among a Nigerian cohort, the study had a sample size of only 45patients[11]. This study was therefore designed to meticulously implement the EMTCT of hepatitis B measures, and to follow the hepatitis B-exposed babies till the age of 9months, for the purpose of identifying perinatal vertical and early childhood horizontal transmissions.

Methods

Study setting: The study was undertaken at the Perinatal Unit of the Department of Obstetrics, Gynaecology and Perinatology, and the Gastroenterology Unit of the Department of Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria between November 2015 and March 2021.

Design: The study is a prospective cohort study.

Study participants: All pregnant women that booked for antenatal care at the institution were prospectively recruited for the study after appropriate counseling.

Patient and public involvement: It was not possible to involve patients in the design and conduct of the study, but they will be involved in the dissemination of the study findings, for ease of widespread dissemination.

Ethics Approval: Research approval for the study was obtained from the Obafemi Awolowo University Teaching Hospitals Complex Ethics and Research Board (IRB/IEC/0004553: ERC/20/02/010).

Intervention: After obtaining informed consent from each study participant, relevant data regarding the socio-demographic characteristics of the women were captured using a purpose-designed proforma. Provider-initiated counselling and testing of the participants' serum for HBsAg was thereafter undertaken, using chromatographic immunoassay (Micropoint®, USA)

rapid diagnostic kit. Women who tested positive subsequently had the complete hepatitis B viral panel assay done, comprising the Hepatitis B surface Antibody (HBsAb), Hepatitis B core antibody (HBcAb: Total and IgG). Hepatitis B e-antigen (HBeAg) and the Hepatitis B e-antibody (HBeAb), using Micropoint® kit, for the purpose of categorization and prognostication. To ensure compliance, the cost of the complete hepatitis B viral panel assay was subsidized significantly by the research team. Serum hepatitis B viral DNA assay was also routinely requested for all the HBsAg positive patients. Chronic Hepatitis B (CHB) infection was defined as the presence of HBsAg for more than 6 months in the participants, or the presence of the IgG fraction of the Hepatitis B core antibody, with absent HBsAb and IgM fraction. All the pregnant women diagnosed with CHB were encouraged to bring their pre-existing children for testing and those identified as HBsAg positive were referred for necessary treatment at the Paediatric and Child Health Department of the hospital. Three categories of women were considered for antenatal treatment with Tenofovir Disoproxil Fumarate (TDF), namely, those with serum HBV DNA $\geq$ 200,000iu/ml, women positive for HBeAg without serum HBeAb, and women with history of perinatal transmission in their previous pregnancies. Pregnant women in any of these categories were placed on TDF 300mg orally once daily starting from the 28th week of gestation till delivery. The mode of delivery was determined strictly by obstetric indications. Serial phone calls were placed to all the study participants as their pregnancies advanced, to ensure compliance with antenatal care and postpartum EMTCT of hepatitis B advisories. Those who delivered out-of-facility were therefore identified and necessary EMTCT of hepatitis B measures were implemented without delay. All the babies that were delivered within the study period, irrespective of the place of birth or the day of the week, had the birth dose of the hepatitis B vaccine administered within 24hours of delivery. When affordable to the patient, 200iu of the Hepatitis B Immunoglobulin (HBIG) was also administered to the newborns of the hepatitis B positive parturients within 24hours of delivery, while the mothers were referred to the Gastroenterology Unit for continuity of care. All the babies were followed up on phone to ensure strict compliance with the national vaccination schedule, including the hepatitis B vaccination. The infants were tested at the age of 9months, to assess their hepatitis B infection status. The primary outcome measure was the proportion of infants that tested positive to the HBsAg at the age of 9months. Secondary outcomes were the proportion of hepatitis-B exposed babies that had the birth dose of hepatitis B vaccine within 24hours, the proportion of babies that completed the 3doses of the hepatitis B vaccine at

the appropriate time, spontaneous miscarriage rate, preterm delivery rate, mean birthweight and the perinatal mortality rate of the hepatitis B-exposed infants.

Results

A total of 10,866 pregnant women were screened during the study period, of which 395 tested positive to the HBsAg, giving a prevalence of 3.64%. The mean age of the women was 31.51± 5.71years, with a median parity of 1.2. More than half (53.9%) were screened before the 28th week of pregnancy, and 249 women (63%) had the complete hepatitis B viral ~~antibody~~ assay done. The remaining expectant mothers (146:37%) could not do the complete hepatitis B viral serology due to financial constraints. All the HBsAg positive had chronic hepatitis B infection. In addition, 13 (3.3%) of the 395 positive women expressed HBeAg in their serum, while 162 women (41%) had developed the anti-HBe antibody. Three patients had both the HBeAg and the HBeAb contemporaneously. Of the 225 pre-existing hepatitis-B exposed children before the index pregnancy that were tested, seven (3.1%) were HBsAg positive. Seventeen women had prenatal TDF administered from 28weeks gestation till delivery.

Of the 395 women that tested positive to the HBsAg, there was one spontaneous miscarriage, while 18 patients were lost to follow-up. Further analysis was therefore based on 376 women that completed the study with known perinatal outcomes. With respect to mode of delivery, 219 women (58.2%) had spontaneous vaginal delivery, with a Caesarean section rate of 41.8%. The mean birth weight of the babies was 3.21 ± 1.86kg. Forty women (10.2%) had preterm births, defined as spontaneous or provider-initiated delivery for obstetric reasons, before 37completed weeks, and there were 11 perinatal mortalities (comprising six stillbirths and five early neonatal deaths), with a stillbirth rate of 15.9/1,000 births and a perinatal mortality rate of 29.2/1,000 births. All the babies were breastfed (Figure 1: Flow chart of study protocol).

Hepatitis B Vaccine-Birth Dose (HBV-BD) was administered to all the 365 newborns; 327 babies (89.6%) had the HBV-BD administered within 24hours of delivery, while the remaining 38 babies (10.4%) had delayed vaccination within 2-4 days postpartum due to logistic reasons. There was a diarrhoea-related infant mortality among the cohort, leaving 364 babies (99.7%) that completed the HBV-3 at the appropriate time. In addition, 260 (71.3%) babies had both HBV vaccine and the Hepatitis B immunoglobulin combination; the remaining 105 babies (28.7%) did not have the HBIG administered due to financial constraints. The entire hepatitis-B exposed cohort of 364

children tested HBsAg negative at the exit hepatitis B screening that was conducted between 9-10 months postnatally.

Discussion

In 2020, the WHO Interim guidance for country validation of viral hepatitis elimination was launched, with absolute targets that include HBsAg prevalence ≤0.1% in those aged 5 years or less, 95% reduction in mother to child transmission rates and an absolute hepatitis B related annual mortality rate of <4 per 100,000 persons. To achieve this, each country is expected to target at least 90% coverage of maternal antenatal HBsAg testing, ≥90% coverage with antivirals for those eligible, 90% timely HBV vaccine birth dose (HBV-BD), and ≥90% of 3 doses (HepB3) vaccine coverage[1]. This vaccination is expected to confer immunity on at least 95% of the vaccinated infants. Co-administration of hepatitis B immunoglobulin (HBIG) may also be used to further reduce the risk of MTCT of HBV. This study met all the set targets, with absolute prevention of vertical and early childhood transmission of hepatitis B virus up to the age of 9months among the cohort. This conforms to the findings of some studies that had earlier reported 0% transmission rate following initiation of immunoprophylaxis within 24hours of birth[11,12]. Meanwhile, seven (3.1%) of the 225 already existing children that were delivered to the same cohort of women before the commencement of this protocol tested positive to the hepatitis B virus during screening. This further underpins the immense role of the hepatitis B preventative measures at eliminating vertical transmission.

Expression of HBeAg significantly heightens the risk of vertical transmission of HBV. In a systematic review of 15 articles from 11 African countries including Nigeria by Keane et al, the pooled risks of vertical transmission of hepatitis B virus among 34 HBeAg positive mothers was 38.3% (95% CI: 7.0–74.4%) without prophylaxis, and 4.8% (95% CI: 0.1–13.3%) among HBeAg negative mothers, without prophylaxis. These rates are similar to the 40% and 5% transmission rates for HBeAg-positive and -negative mothers respectively, by WHO (3) This risk was however eliminated among the 13 HBeAg positive women in the index study due to the combination of prenatal TDF from the 28th week of gestation and postpartum prophylaxis in the exposed children. This approach has been proven to be more effective that vaccination at birth alone, among HBeAg positive mothers, at preventing vertical transmission[3,13]. Prenatal TDF is already supplied to HIV positive patients at no cost. Such privilege is, however, not yet available to HBV positive

women[11,14]. It is advisable that the country leverages on the supply chain that is already available for HIV PMTCT programmes, for the prevention of HBV as well.

Almost 30% of the children in this study did not have the HBIG administered due to financial constraints. This is not unexpected, as such medications are expensive and only available on an "out-of-pocket" payment basis, and according to the World Bank and the National Bureau of Statistics (NBS) of Nigeria, about 40% of Nigeria's population, amounting to about 83 million people, live below the country's poverty line of $381.75 per annum[15]. There was, however, no difference in the outcome of the children in the Hepatitis B vaccine-HBIG combination and the hepatitis B vaccine alone groups. This observation aligns with an earlier report that the efficacy of appropriately administered HepB3 is comparable to that of HBV vaccine-HBIG combination[16], and the WHO recommendation of HepB3 as the primary preventative measure for hepatitis B-exposed children, with expected immunity rate of 95%. This finding is of significant relevance, especially in low- and Middle-income countries such as Nigeria, where a dose (200iu) of HBIG as at the time of the study costs about 70,000 Naira, equivalent to 166 US Dollars. Meanwhile, the HBV vaccine is made available free to all newborns as a national policy through governmental efforts in Nigeria and many of the developing countries. The use of HBV vaccine is recorded to have prevented 210million new chronic infections so far, with over a million deaths averted within the next 8years. It is therefore essential that health care facilities and systems in LMICs, including Nigeria prioritize achievement of the set targets for HBV-BD and HepB3, to maximize the benefits from the already existing governmental policies. Without doing this, however, about 63 million new chronic infections and 17million deaths could be recorded within the same period[17].

With respect to obstetric outcomes, the prematurity rate is comparable with the report for the general obstetric population. The Caesarean section rate was comparable to the 47% reported from an earlier study, and the mean birthweight was also comparable to the 3210 ± 490g reported by Awowole et al from the same center 3 years earlier[18]. Hepatitis B infection in pregnancy was not associated with increased stillbirth or perinatal mortality rate among the cohort in this study, as the findings were comparable to the stillbirth rate of 15/1,000 births recorded by Kuti et al among unselected booked patients at the same facility[19]. The perinatal mortality rate of 29.2/1,000 births from this study, though high, is comparable to the perinatal mortality rate of 38/1,000 births reported by the Nigerian Demographic and Health Survey for the southwest geopolitical zone, where the OAUTHC is situated[7]. Chronic asymptomatic hepatitis B infection in pregnancy was

therefore not associated with increased Caesarean section, low birth weight, stillbirth, and perinatal mortality rates in this study.

The mother to child transmission of hepatitis B infection rate of 0% at 9 months in this study is comparable to the findings from some earlier studies that reported similar transmission rates, following initiation of the HBV-BD within 24hours of delivery[11,12,14]. Meanwhile, seven (3.1%) of the 225 children delivered to the same cohort of women before the commencement of this protocol tested positive to the hepatitis B virus during screening. This further underpins the immense role of the hepatitis B preventative measures at eliminating vertical transmission.

One of the strengths of this study is the sample size, which involves the largest cohort of hepatitis B pregnant women to be prospectively recruited and actively managed in Nigeria, translating to generation of reliable and credible data. The multidisciplinary nature of the study, with involvement of Specialists from Obstetrics, Gastroenterology, Paediatrics and Child Health, Public Health and the Childhood Immunization Sub-unit and Community Health Extension Workers made in-facility and community monitoring, surveillance, and painstaking implementation of the hepatitis B EMTCT interventions feasible in the study cohort. This research is also the only study in Nigeria, to prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months, following implementation of HBV EMTCT measures. In addition, this is the first study from Nigeria that endeavored to screen all the pre-existing children of hepatitis B pregnant women, with appropriate follow-up at the Paediatric and Child Health Unit of the Teaching Hospital, thereby using the antenatal period as a window of opportunity to provide a family-centered, holistic care to the affected women.

This study also has some identifiable limitations. Since 2020, WHO has updated its recommendation to include the administration of TDF to pregnant women with HBV DNA ≥200 000 IU/mL to prevent mother-to-child transmission (PMTCT) of HBV[1,20]. One of the limitations of this study is however the inability to undertake the quantitative HBV DNA in all the women due to financial constraints This, however, did not significantly affect the outcome of the study. Secondly, the additional benefit of HBIG among HBV-positive women in low-resource settings could have been better assessed using a randomized controlled trial. Withholding such treatment from the control group, especially for patients that could afford it would however have been unethical. It is also not clear whether the pre-existing children that tested positive to the

hepatitis B virus acquired the infections at birth or in early childhood. Discerning this may however not be significant, as the endpoint of chronic childhood hepatitis B infection are similar. Lastly, screening for anti-HBs titre has been recommended for HBsAg-negative infants as anti-HBs levels ≥ 100 mIU/ml are considered protective with no need for further medical management[21]. Screening for anti-HBs was not done in this study for the 364 babies that were HBsAg-negative at 9 months of age due to financial constraints.

In conclusion, this study demonstrates that achieving the set goals for eliminating mother to child transmission of hepatitis B infection is feasible, even in the absence of high-end investigations such as HBV DNA assay, and expensive interventions such as the HBIG, especially in LMICs with the highest burden of chronic HBV infections and limited resources. Chronic hepatitis B infection did not demonstrate adverse effect on the outcome of the pregnancies. A national scale-up of these interventions in Nigeria through committed vaccination policies, sustained governmental funding and commitment, as well as universal health coverage are required to bridge the gap for the patients that live below poverty line in Nigeria and would therefore not be able to afford even the most basic of these interventions.

Author Contributions: DN and OK conceived the study. IA, OA, OI participated in literature search and proposal writing. All the authors participated in study design, supervision of sample collection, patient management, critical review of the manuscript for intellectual content, and approval of the final draft and agree to take responsibility for the integrity of the research.

Data sharing statement: The raw data for this study is available

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Competing interests: All the authors hereby declare no competing interests.

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Figure Legend

Figure 1: Figure 1: Flow chart of study protocol

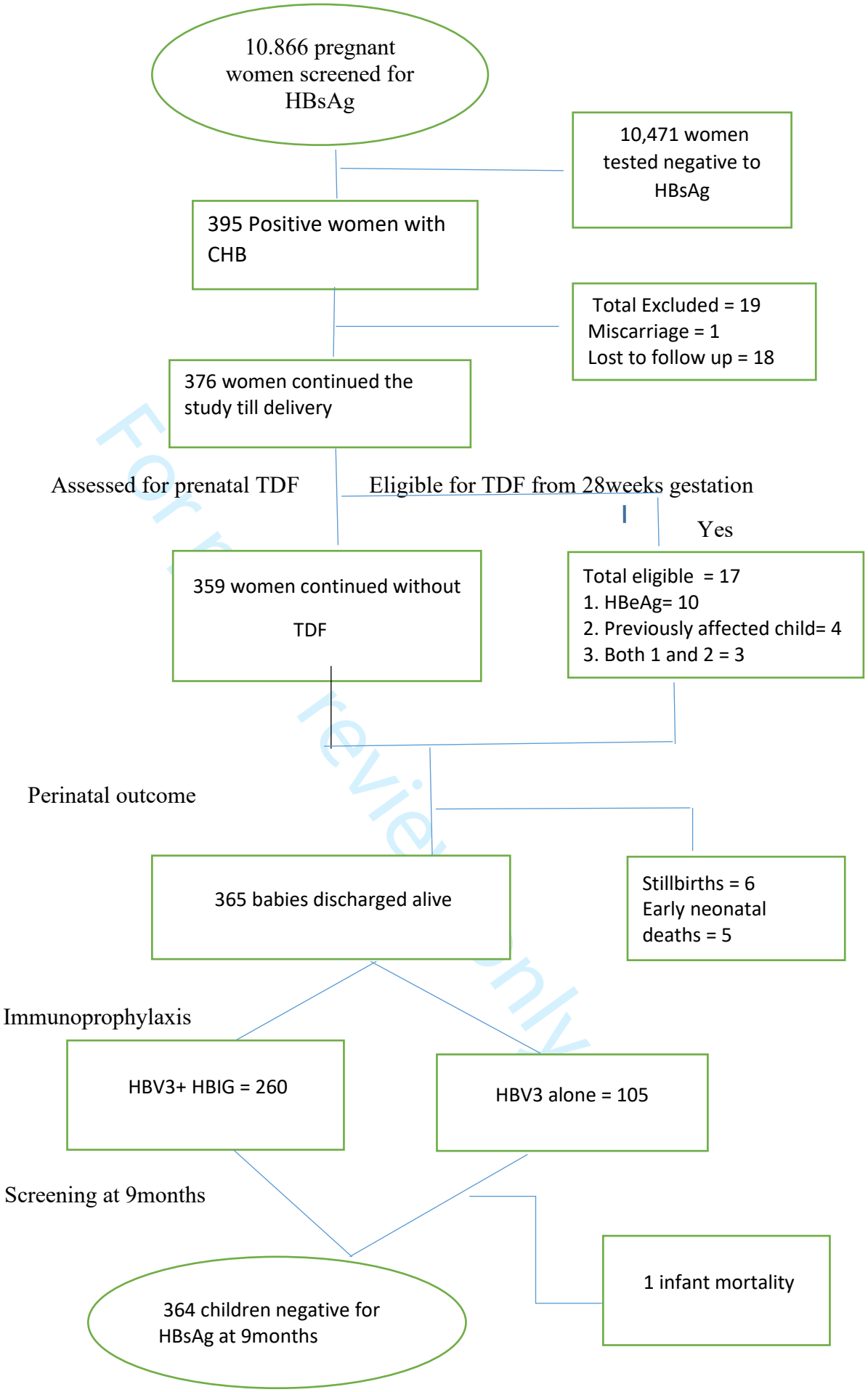

Figure 1: Flow chart of study protocol

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	Yes
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalisability	21	Discuss the generalisability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Prospective Cohort Study of Prevention of Mother to Child Transmission of Hepatitis B Infection and Nine Months Follow-up of Hepatitis B-exposed Infants at Ile-Ife, Nigeria.

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Abstract

Objectives: Eliminating Mother-to-Child-Transmission (MTCT) of Hepatitis B Virus (HBV) is central to WHO's target of reducing hepatitis B infection in children to <0.1% by 2030. While Nigeria accounts for 8.3% of the global burden, interventional studies on prevention of MTCT of HBV are hardly available. This study ~~aims~~ aimed to assess the impact of prevention of MTCT interventions on vertical transmission of HBV among pregnant women in Nigeria.

Design: A prospective cohort study

Setting: A University Teaching Hospitals Complex in Nigeria between 2015-2021.

Participants: 10,866 pregnant women and their pre-existing children.

Interventions: Eligible pregnant women were screened for HBsAg using chromatographic immunoassay (Micropoint®, USA). HbsAg-positive women had HBV serological assay done and their pre-existing children were screened. Women with HBV DNA ≥200,000IU/ml and those positive for HBeAg had 300mg daily of Tenofovir Disoproxil Fumarate (TDF) in the 3rd trimester. The newborns had hepatitis B vaccines and HB Immunoglobulin(HBIG) administered, followed by testing for HBsAg at 9months post-natally.

Primary outcome measures: Prevalence of chronic hepatitis B infection in pregnancy, and the incidence of MTCT of HBV.

Results: Overall, 395 women had chronic HBV infection, giving a prevalence of 3.64%. Their mean age was 31.51± 5.71years, with a median parity of 1.2. Thirteen women (5.2%) were positive for HBeAg, seven (3.1%) of the 225 pre-existing hepatitis B-exposed children were HbsAg positive, and 17women had prenatal TDF. Overall, 376 women completed the study, with mean birthweight of 3.21±1.86kg and perinatal mortality rate of 29.2/1,000births. Hepatitis Bvaccine-HBIG combination was administered to 260 newborns, while the others had hepatitis B vaccine alone. All the children tested negative to the HbsAg at 9months.

Conclusion: Eliminating MTCT of hepatitis B virus infection through validated protocols in Low- and Middle-Income Countries (LMICs) with the highest burden of chronic HBV infections is feasible. National scale-up of such protocols is recommended.

Article Summary

Strengths and limitations of this study

- This study involves the largest cohort of hepatitis B pregnant women to be prospectively recruited and actively managed in Nigeria.
- The multidisciplinary nature of the study of the managing team made implementation of the hepatitis B EMTCT interventions feasible.
- This research is the only study to actively intervene, prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months in Nigeria.
- HBV DNA assay could not be routinely done in the HBsAg-positive women due to financial constraints.

Keywords: Chronic hepatitis B, Hepatitis B virus, Hepatitis B vaccine, Hepatitis B immunoglobulin, Mother-to-child transmission, Nigeria

Word count: 3,586

Introduction

The World Health Organization (WHO) estimates that 1.4 million deaths are recorded annually due to viral hepatitis, and hepatitis-associated mortality now ranks as the 7th leading cause of death globally, ahead of deaths due to HIV/AIDS, Malaria and Tuberculosis. This burden is mainly associated with Hepatitis B virus (48%), Hepatitis C virus (48%) and the remaining from acute hepatitis A and E infection[1]. Overall, about 2 billion people are estimated to have evidence of past or present hepatitis B infection, and at least 296 million people, corresponding to about 3.8% of the global population ~~are~~ were estimated to be chronically infected with the Hepatitis B Virus (HBV) in 2019[1]. The burden of hepatitis B infection is however unevenly distributed, with developing countries, especially the African and the Western Pacific regions suffering disproportionately; the 2 regions account for 67% of the global burden of hepatitis B infection[1-3], with at least 5% of the population estimated to be living with the hepatitis B infection.

Transmission of HBV may be horizontal (through contact with body fluids including blood, semen, and sharp objects) or vertical, through mother to child transmission. Vertical transmission has served as a means of maintaining the reservoir of infection in low-and middle-income countries (LMIC), due to its propensity to progress into chronicity. While acute hepatitis B infections in adults are likely to be cleared within 6 months in 95% of cases, chronicity is recorded in 20-60% of children under the age of 5years, and at least 90% of perinatally transmitted cases. As these newborns often remain asymptomatic, some diagnoses may only be made incidentally during routine screening for blood donation, admissions into schools and blood donation. Others may however not be diagnosed until they reach the stage of hepatic decompensation, liver cirrhosis or hepatocellular carcinoma in adulthood.

The Center for Disease Control (CDC) estimated the prevalence of chronic hepatitis B infection in Nigeria to be at least 8%[4]. This was further supported by the Federal Ministry of Health's estimate of 11.1%, amounting to 8.3% of the global chronic hepatitis B infection, thereby making Nigeria the country with the highest burden globally[5]. Despite the need to strive to attain the set targets for Elimination of Mother to Child Transmission (EMTCT) of hepatitis B infection, and the proven benefits of the afore-mentioned interventions, they are not always provided as standard components of the Reproductive, Maternal, Newborn and Child Health (RMNCH) programmes in Nigeria and other developing countries. A case in-point is the birth dose of hepatitis B vaccine coverage, which ~~is~~ was reported to be only 6% in the African region in 2020[6]. Although the HBV vaccine is administered at no cost to newborns in Nigeria, only 52.4 % of Nigerian children reportedly had the birth dose of hepatitis B vaccine, while just 4% had the dose administered within the first 24hours of life as recommended, and overall, only 50.3% of Nigerian children completed the 3 additional doses of hepatitis B vaccine[7].

In response to the bothersome statistics of hepatitis B infection, the Global Health Sector Strategy (GHSS) on viral hepatitis was launched at the World Health Assembly in 2016, with the aim of eliminating viral hepatitis B and C infections[8]. For this goal of eliminating viral hepatitis as a public health threat to be achieved, WHO has set the target of reducing HBsAg prevalence in children to 0.1% by 2030[8]. To evaluate the impact of the EMTCT measures and identify bottlenecks to the successful attainment of the GHSS targets in LMICs such as Nigeria, appropriately designed prospective studies are imperative. Although some Nigerian researchers

have evaluated the outcomes of pregnancies among women with hepatitis B virus infection, many of these studies were either retrospectively conducted or simply prevalence studies that did not control for or report the quality of prevention of mother to child transmission of hepatitis B care that the study subjects received[9, 10]; none had therefore reported on the outcome of implementing a well designed and implemented protocol. While Onakewhor et al reported the administration of hepatitis B vaccine and HBIG immunoprophylaxis for PMTCT among a Nigerian cohort, the study had a sample size of only 45patients[11]. This study was therefore designed to meticulously implement the EMTCT of hepatitis B measures, and to follow the hepatitis B-exposed babies till the age of 9 months, for the purpose of identifying perinatal vertical and early childhood horizontal transmissions.

Methods

Study setting: The study was undertaken at the Perinatal Unit of the Department of Obstetrics, Gynaecology and Perinatology, and the Gastroenterology Unit of the Department of Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria between November 2015, and March 2021.

Design: The study is a prospective cohort study.

Study participants: All consecutive pregnant women that booked for antenatal care at the institution were prospectively recruited for the study after appropriate counseling. As the study was descriptive without any hypothesis testing, a specific sample size wasn't calculated.

Inclusion criteria: All pregnant women that received antenatal care at the Obafemi Awolowo University Teaching Hospitals Complex, Ife, Osun State, Nigeria during the study period.

Exclusion criteria: Pregnant women that refused to consent to HBV screening.

Statistical analysis: the data was analysed with IBM SPSS 20.0. Categorical variables were presented in frequencies and percentages while continues variables were presented as means and standard deviations.

Patient and public involvement: It was not possible to involve patients in the design and conduct of the study, but they will be involved in the dissemination of the study findings, for ease of widespread dissemination.

Ethics Approval: Research approval for the study was obtained from the Obafemi Awolowo University Teaching Hospitals Complex Ethics and Research Board (IRB/IEC/0004553: ERC/20/02/010).

Intervention: After obtaining informed consent from each study participant, relevant data regarding the socio-demographic characteristics of the women were captured using a purpose-designed proforma. Provider-initiated counselling and testing of the participants' serum for HBsAg was undertaken for all consecutive pregnant women by the Nurses at the antenatal clinic, using chromatographic immunoassay (Micropoint®, USA) rapid diagnostic kit. In addition to HBsAg testing, all the women also had retroviral screening done after due counselling. HBsAg positive women subsequently had the complete hepatitis B serological markers assay done, comprising the Hepatitis B surface Antibody (HBsAb), Hepatitis B core antibody (HBcAb: Total and IgG). Hepatitis B e-antigen (HBeAg) and the Hepatitis B e-antibody (HBeAb), using Micropoint® kit, for the purpose of categorization and prognostication. To ensure compliance, 95% subsidy on the cost of the hepatitis B serological marker was provided by the research team. HBV DNA assay was also routinely requested for all the HBsAg positive patients, but this was not subsidised. Chronic Hepatitis B (CHB) infection was defined as the presence of HBsAg for more than 6 months in the participants, or the presence of the IgG fraction of the Hepatitis B core antibody, with absent HBsAb and IgM fraction. All the pregnant women diagnosed with CHB were encouraged to bring their pre-existing children for testing and those identified as HBsAg positive were referred for necessary treatment at the Paediatric and Child Health Department of the hospital. Three categories of women were considered for antenatal treatment with Tenofovir Disoproxil Fumarate (TDF), namely, those with serum HBV DNA $\geq$ 200,000IU/ml, women positive for HBeAg without serum HBeAb, and women with history of perinatal transmission in their previous pregnancies. Pregnant women in any of these categories were placed on TDF 300mg orally once daily starting from the 28th week of gestation till delivery, or as soon as possible, if the diagnosis was made after the 28th week of gestation. Adherence to medication was assessed during outpatient follow-up by the Gastroenterologists, who were also members of the research team. The mode of delivery was determined strictly by obstetric indications. Serial phone calls were placed to all the study participants by dedicated research staff and community health workers as their pregnancies advanced, to ensure compliance with antenatal care and postpartum EMTCT of hepatitis B advisories. Those who delivered out-of-facility were therefore identified and necessary

EMTCT of hepatitis B measures were implemented without delay. All the babies that were delivered within the study period, irrespective of the place of birth or the day of the week, had the birth dose of the hepatitis B vaccine administered within 24 hours of delivery by the National Programme on Immunization (NPI) department. When affordable to the patient, 200iu of the Hepatitis B Immunoglobulin (HBIG) was also administered to the newborns of the hepatitis B positive parturients within 24 hours of delivery, while the mothers were referred to the Gastroenterology Unit for continuity of care. All the babies were followed up on phone to ensure strict compliance with the national vaccination schedule, including the hepatitis B vaccination. The infants were tested at the age of 9 months, to assess their hepatitis B infection status. The primary outcome measure was the proportion of infants that tested positive to the HBsAg at the age of 9 months. Secondary outcomes were the proportion of hepatitis-B exposed babies that had the birth dose of hepatitis B vaccine within 24hours, the proportion of babies that completed the 3doses of the hepatitis B vaccine at the appropriate time, spontaneous miscarriage rate, preterm delivery rate, mean birthweight and the perinatal mortality rate of the hepatitis B-exposed infants.

Results

A total of 10,866 eligible pregnant women were screened during the study period, of which 395 tested positive to the HBsAg, giving a prevalence of 3.64%. The mean age of the women was 31.51± 5.71years, with a median parity of 1.2. More than half (53.9%) were screened before the 28th week of pregnancy, and 249 women (63%) had the complete hepatitis B viral assay done. The remaining expectant mothers (146:37%) could not do the complete hepatitis B serological markers due to financial constraints. All the HBsAg positive women had chronic hepatitis B infection. In addition, 13 (5.2%) of the 249 HBsAg positive women expressed HBeAg in their serum, while 162 women (65.1%) had developed the anti-HBe antibody. Three patients had both the HBeAg and the HBeAb contemporaneously. Only 21 patients had HBV DNA assay done, with values that ranged between 20IU/ml and 7,456IU/ml. Consequently, none of them attained the viral load trigger for prenatal TDF. Of the 486 pre-existing hepatitis-B exposed children before the index pregnancy, 225 (46.3%), with ages ranging between 2years and 22years were tested and seven (3.1%) were HBsAg positive. The other children were not tested because their parents did not give consent. Seventeen women had prenatal TDF administered from 28weeks gestation till delivery. Eight (2.0%) of the 395 women with chronic HBV infection had HBV-HIV co-infection.

Of the 395 women with positive HBsAg, there was one spontaneous miscarriage, while 18 patients were lost to follow-up (see figure 1). Further analysis was therefore based on 376 women that completed the study with known perinatal outcomes. With respect to mode of delivery, 219 women (58.2%) had spontaneous vaginal delivery, with a Caesarean section rate of 41.8%. The mean birth weight of the babies was 3.21 ± 1.86kg. Forty women (10.2%) had preterm births, defined as spontaneous or provider-initiated delivery for obstetric reasons, before 37completed weeks, and there were 11 perinatal mortalities (comprising six stillbirths and five early neonatal deaths), with a stillbirth rate of 15.9/1,000 births and a perinatal mortality rate of 29.2/1,000 births. The details of the perinatal mortalities are depicted on table 1. One of the 17 women that had prenatal TDF had a stillbirth from complications of obstructed labour outside the facility; there was no other complication recorded among the remaining 16 women. All the babies were breastfed (Figure 1).

The birth dose of hepatitis B vaccine was administered to all the 365 newborns; 327 babies (89.6%) had the vaccine administered within 24hours of delivery, while the remaining 38 babies (10.4%) had delayed vaccination within 2-4 days postpartum due to logistic reasons. There was a diarrhea-related infant mortality among the cohort, leaving 364 babies (99.7%) that completed the vaccination at the appropriate time. In addition, 260 (71.3%) babies had both hepatitis B vaccine and the Hepatitis B immunoglobulin combination; the remaining 105 babies (28.7%) did not have the HBIG administered due to financial constraints. Only eight (61.5%) of the 13 women with HBeAg had the hepatitis B vaccine-HBIG combination administered to their babies. The entire hepatitis-B exposed cohort of 364 children tested HbsAg negative at the exit-hepatitis B screening that was conducted at 9 ~~between 9-10~~ months postnatally.

Discussion

In 2020, the WHO Interim guidance for country validation of viral hepatitis elimination was launched, with absolute targets that include HBsAg prevalence ≤0.1% in those aged 5 years or less, 95% reduction in mother to child transmission rates and an absolute hepatitis B related annual mortality rate of <4 per 100,000 persons. To achieve this, each country is expected to target at least 90% coverage of maternal antenatal HBsAg testing, ≥90% coverage with antivirals for those eligible, 90% timely administration of the birth dose of hepatitis B vaccine, and ≥90% of 3 doses (HepB3) vaccine coverage[1]. This vaccination is expected to confer immunity on at least 95% of the vaccinated infants. Co-administration of hepatitis B immunoglobulin (HBIG) may also be used

to further reduce the risk of MTCT of HBV. This study met all the set targets, with absolute prevention of vertical and early childhood transmission of hepatitis B virus up to the age of 9 months among the cohort. This conforms to the findings of some studies that had earlier reported 0% transmission rate following initiation of immunoprophylaxis within 24 hours of birth[11,12]. Meanwhile, seven (3.1%) of the 225 already existing children that were delivered to the same cohort of women before the commencement of this protocol were HBsAg positive . This further underpins the immense role of the hepatitis B preventative measures at eliminating vertical transmission.

Expression of HBeAg significantly heightens the risk of vertical transmission of HBV. In a systematic review of 15 articles from 11 African countries including Nigeria by Keane et al, the pooled risks of vertical transmission of hepatitis B virus among 34 HBeAg positive mothers was 38.3% (95% CI: 7.0–74.4%) without prophylaxis, and 4.8% (95% CI: 0.1–13.3%) among HBeAg negative mothers, without prophylaxis. These rates are similar to the 40% and 5% transmission rates for HBeAg-positive and -negative mothers respectively, by WHO (3) This risk was however eliminated among the 13 HBeAg positive women in the index study due to the combination of prenatal TDF from the 28th week of gestation and postpartum prophylaxis in the exposed children. This approach has been proven to be more effective among HBeAg positive mothers than vaccination at birth alone, at preventing vertical transmission[3,13]. Prenatal TDF is already supplied to HIV positive patients at no cost. Such privilege is, however, not yet available to HBV positive women[11,14]. It is advisable that the country leverages on the supply chain that is already available for HIV PMTCT programmes, for the prevention of HBV as well.

Almost 30% of the children in this study did not have the HBIG administered due to financial constraints. This is not unexpected, as such medications are expensive and only available on an "out-of-pocket" payment basis, and according to the World Bank and the National Bureau of Statistics (NBS) of Nigeria, about 40% of Nigeria's population, amounting to about 83 million people, live below the country's poverty line of $381.75 per annum[15]. There was, however, no difference in the outcome of the children in the hepatitis B vaccine-HBIG combination and the hepatitis B vaccine alone groups. This observation aligns with an earlier report that the efficacy of appropriately administered hepatitis B vaccine is comparable to that of hepatitis B vaccine-HBIG combination[16], and the WHO recommendation of HepB3 as the primary preventative measure for hepatitis B-exposed children, with expected immunity rate of 95%. This finding is of significant

relevance, especially in low- and middle-income countries such as Nigeria, where a dose (200iu) of HBIG as at the time of the study costs about 70,000 Nigerian Naira, equivalent to 166 US Dollars. Meanwhile, the hepatitis B vaccine is available at no cost to all newborns as a national policy through governmental efforts in Nigeria. The use of hepatitis B vaccine is recorded to have prevented 210million new chronic infections so far, and over a million deaths will be averted within the next 8 years. It is therefore essential that health care facilities and systems in LMICs, including Nigeria prioritize achievement of the set targets for hepatitis B vaccination, to maximize the benefits from the already existing governmental policies. Without doing this, however, about 63 million new chronic infections and 17million deaths could be recorded within the same period[17]. With respect to obstetric outcomes, the prematurity rate is comparable with the report for the general obstetric population. The Caesarean section rate was comparable to the 47% reported from an earlier study, and the mean birthweight was also comparable to the 3210 ± 490g reported by Awowole et al from the same center 3 years earlier[18]. Hepatitis B infection in pregnancy was not associated with increased stillbirth or perinatal mortality rate among the cohort in this study, as the findings were comparable to the stillbirth rate of 15/1,000 births recorded by Kuti et al among unselected booked patients at the same facility[19]. The perinatal mortality rate of 29.2/1,000 births from this study, though high, is comparable to the perinatal mortality rate of 38/1,000 births reported by the Nigerian Demographic and Health Survey for the southwest geopolitical zone, where the OAUTHC is situated[7]. Chronic asymptomatic hepatitis B infection in pregnancy was therefore not associated with increased Caesarean section, low birth weight, stillbirth, and perinatal mortality rates in this study.

The mother-to-child-transmission of hepatitis B infection rate of 0% at 9 months in this study is comparable to the findings from some earlier studies that reported similar transmission rates, following initiation of the birth dose of the hepatitis B vaccine within 24hours of delivery[11,12,14]. Meanwhile, seven (3.1%) of the 225 children delivered to the same cohort of women before the commencement of this protocol were HBsAg positive. This further underpins the immense role of the hepatitis B preventative measures at eliminating vertical transmission.

One of the strengths of this study is the sample size, which involves the largest cohort of hepatitis B virus positive pregnant women to be prospectively recruited and actively managed in Nigeria, translating to generation of reliable and credible data. The multidisciplinary nature of the study,

with involvement of Specialists from Obstetrics, Gastroenterology, Paediatrics and Child Health, Public Health, the Childhood Immunization Sub-unit and the Community Health Extension Workers made in-facility and community monitoring, surveillance, and painstaking implementation of the hepatitis B EMTCT interventions feasible in the study cohort. This research is also the only study in Nigeria, to prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months, following implementation of HBV EMTCT measures. In addition, this is the first study from Nigeria that endeavored to screen all the pre-existing children of consenting hepatitis B pregnant women, with appropriate follow-up at the Paediatric and Child Health Unit of the Teaching Hospital, thereby utilising the antenatal period as a window of opportunity to provide a family-centered, holistic care to the affected women.

This study also has some identifiable limitations. Since 2020, WHO has updated its recommendation to include the administration of TDF to pregnant women with HBV DNA ≥200 000 IU/mL to prevent mother-to-child transmission (PMTCT) of HBV[1,20]. One of the limitations of this study is however the inability to undertake the quantitative HBV DNA in all the women due to financial constraints This, however, did not significantly affect the outcome of the study. Secondly, the additional benefit of HBIG among HBV-positive women in low-resource settings could have been better assessed using a randomized controlled trial, as this study was not designed to specifically detect the comparative effectiveness of Hepatitis B vaccine alone versus hepatitis B vaccine-HBIG combination therapy. Withholding HBIG from the control group, especially for patients that could afford it would however have been unethical. Nevertheless, further trials to appraise this observation will be needed in the future. It is also not clear whether the pre-existing children that tested positive to the hepatitis B virus acquired the infections vertically at birth or horizontally in early childhood. Discerning this may however not be significant, as the endpoint of chronic childhood hepatitis B infection are similar. Furthermore, the vaccination status of the pre-existing children could not be evaluated with certainty. Lastly, screening for anti-HBs titre has been recommended for HBsAg-negative infants as anti-HBs levels ≥ 100 mIU/ml are considered protective with no need for further medical management[21]. Screening for anti-HBs was not done in this study for the 364 babies that were HBsAg-negative at 9 months of age due to financial constraints.

In conclusion, this study demonstrates that achieving the set goals for eliminating mother-to-child transmission of hepatitis B infection is feasible, even in the absence of high-end investigations such as HBV DNA assay, and expensive interventions such as the HBIG, especially in LMICs with the highest burden of chronic HBV infections and limited resources. Chronic hepatitis B infection did not demonstrate adverse effect on the outcome of the pregnancies. A national scale-up of these interventions in Nigeria through committed vaccination policies, sustained governmental funding and commitment, as well as universal health coverage are required to bridge the gap for the patients that live below poverty line in Nigeria and would therefore not be able to afford even the most basic of these interventions.

Author Contributions: DN and OK conceived the study. DN, OK, IA, OA, OI, OM, AA, CA and MI all participated in participated in literature search, proposal writing, study design, supervision of sample collection, patient management, critical review of the manuscript for intellectual content, and approval of the final draft and agree to take responsibility for the integrity of the research.

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Competing interests: All the authors hereby declare no competing interests.

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Figure Legend

Figure 1: Algorithm for HBsAg screening of pregnant women and hepatitis B birth dose vaccination of exposed babies.

Table 1: Causes of perinatal deaths among women with hepatitis B infection in pregnancy at OAUTHC.

Cause	Perinatal mortality N = 11		Total
	Stillbirths	Neonatal Deaths	Frequency (%)
Antepartum haemorrhage	2	1	3 (27.3)
Eclampsia	1	1	2 (18.2)
Obstructed labour*	1	1	2 (18.2)
Out-of-facility delivery	0	2	2 (18.2)
Fetal Growth Restriction	1	0	1 (9.1)
Unexplained	1	0	1 (9.1)
Total	6	5	11(100)

*Patients presented late to the facility after initial management at unorthodox centers.

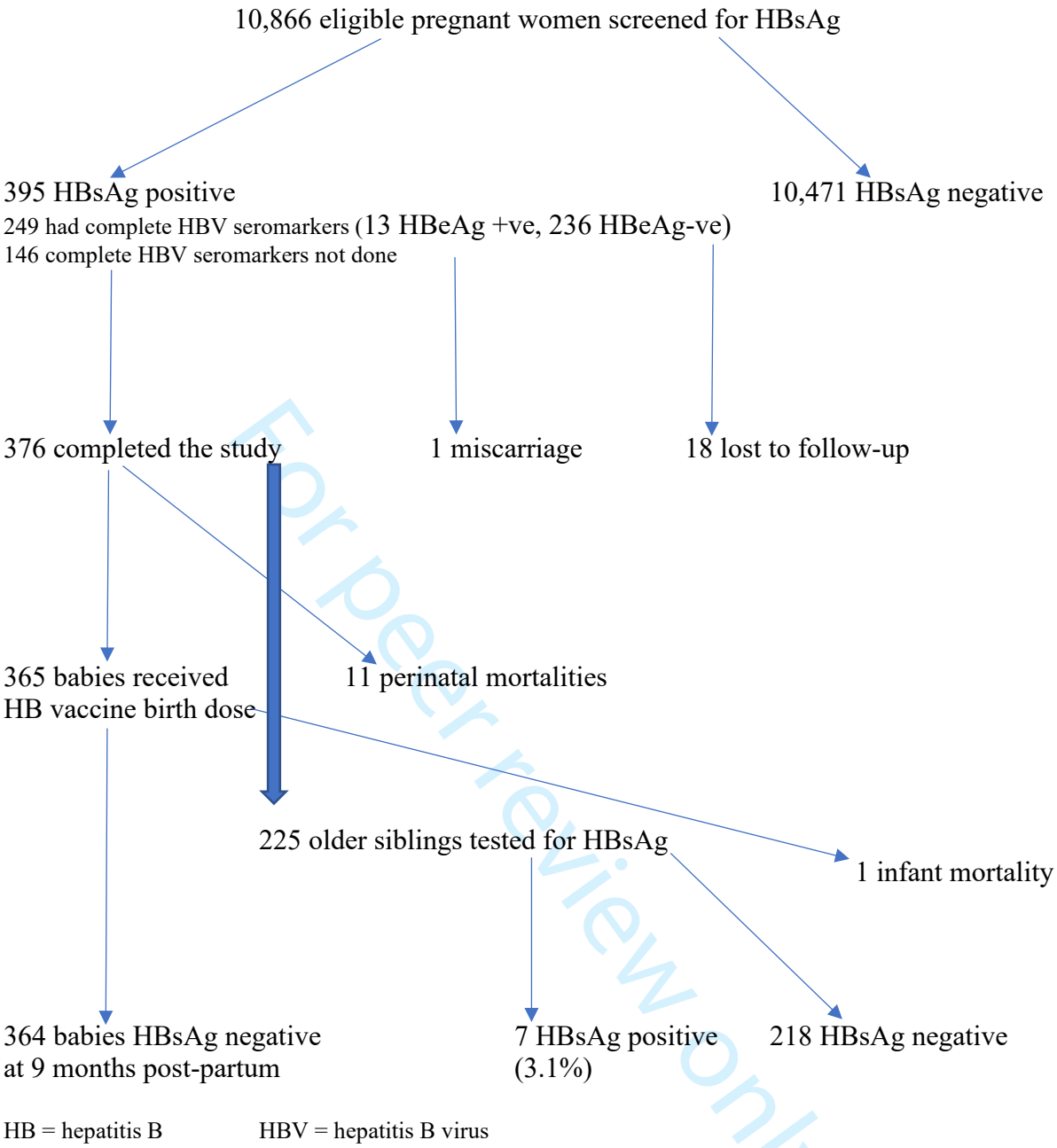

Figure 1: Algorithm for HBsAg screening of pregnant women and hepatitis B birth dose vaccination of exposed babies

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	Yes
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalisability	21	Discuss the generalisability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Prospective Cohort Study of Prevention of Mother to Child Transmission of Hepatitis B Infection and Nine Months Follow-up of Hepatitis B-exposed Infants at Ile-Ife, Nigeria.

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Prospective Cohort Study of Prevention of Mother to Child Transmission of Hepatitis B Infection and Nine Months Follow-up of Hepatitis B-exposed Infants at Ile-Ife, Nigeria.

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Abstract

Objectives: Eliminating Mother-to-Child-Transmission (MTCT) of Hepatitis B Virus (HBV) is central to WHO's target of reducing hepatitis B infection in children to <0.1% by 2030. While Nigeria accounts for 8.3% of the global burden, interventional studies on prevention of MTCT of HBV are hardly available. This study aimed to assess the impact of prevention of MTCT interventions on vertical transmission of HBV among pregnant women in Nigeria.

Design: A prospective cohort study

Setting: A University Teaching Hospitals Complex in Nigeria between 2015-2021.

Participants: 10,866 pregnant women and their pre-existing children.

Interventions: Eligible pregnant women were screened for HBsAg using chromatographic immunoassay (Micropoint®, USA). HbsAg-positive women had HBV serological assay done and their pre-existing children were screened. Women with HBV DNA ≥200,000IU/ml and those positive for HBeAg had 300mg daily of Tenofovir Disoproxil Fumarate (TDF) in the 3rd trimester. The newborns had hepatitis B vaccines and HB Immunoglobulin(HBIG) administered, followed by testing for HBsAg at 9months post-natally.

Primary outcome measures: Prevalence of chronic hepatitis B infection in pregnancy, and the incidence of MTCT of HBV.

Results: Overall, 395 women had chronic HBV infection, giving a prevalence of 3.64%. Their mean age was 31.51± 5.71years, with a median parity of 1.2. Thirteen women (5.2%) were positive for HBeAg, seven (3.1%) of the 225 pre-existing hepatitis B-exposed children were HbsAg positive, and 17women had prenatal TDF. Overall, 376 women completed the study, with mean birthweight of 3.21±1.86kg and perinatal mortality rate of 29.2/1,000births. Hepatitis Bvaccine-HBIG combination was administered to 260 newborns, while the others had hepatitis B vaccine alone. All the children tested negative to the HbsAg at 9months.

Conclusion: Eliminating MTCT of hepatitis B virus infection through validated protocols in Low- and Middle-Income Countries (LMICs) with the highest burden of chronic HBV infections is feasible. National scale-up of such protocols is recommended.

Article Summary

Strengths and limitations of this study

- This study involves the largest cohort of hepatitis B pregnant women to be prospectively recruited and actively managed in Nigeria.
- The multidisciplinary nature of the study of the managing team made implementation of the hepatitis B EMTCT interventions feasible.
- This research is the only study to actively intervene, prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months in Nigeria.
- HBV DNA assay could not be routinely done in the HBsAg-positive women due to financial constraints.

Keywords: Chronic hepatitis B, Hepatitis B virus, Hepatitis B vaccine, Hepatitis B immunoglobulin, Mother-to-child transmission, Nigeria

Word count: 2,974

Introduction

The World Health Organization (WHO) estimates that 1.4 million deaths are recorded annually due to viral hepatitis, and hepatitis-associated mortality now ranks as the 7th leading cause of death globally, ahead of deaths due to HIV/AIDS, Malaria and Tuberculosis. This burden is mainly associated with Hepatitis B virus (48%) and Hepatitis C virus (48%)[1]. Overall, about 2 billion people are estimated to have evidence of past or present hepatitis B infection, with Africa and the Western Pacific regions alone accounting for 67% of the global burden. Mother-to-child-transmission of Hepatitis B Virus (HBV) is a major route of transmitting the infection in low-and middle-income countries (LMIC), due to its propensity to progress into chronicity[1-3].

The Center for Disease Control (CDC) estimated the prevalence of chronic hepatitis B infection in Nigeria to be at least 8%[4]. This was confirmed by the recent report from the Federal Ministry of Health, making Nigeria the country with the highest burden globally[5]. Despite the high burden, most hospitals in Nigeria and other developing countries have no protocol in place to address the

problem. In 2018, only 52.4% of Nigerian children reportedly received the birth dose of hepatitis B vaccine while only 50.3% completed the three additional doses of the vaccine[6]. To achieve the Global Health Sector Strategy (GHSS) on viral hepatitis, the WHO has set the target of reducing HBsAg prevalence in children to 0.1% by 2030[7]. Appropriately designed prospective studies to generate relevant data are imperative to achieve this goal.

Although some Nigerian researchers have evaluated the outcomes of pregnancies among women with hepatitis B virus infection, many of these studies were either retrospectively conducted or simply prevalence studies that did not control for or report the quality of prevention of mother to child transmission of hepatitis B care that the study subjects received[8,9]; none had therefore reported on the outcome of implementing a well designed and implemented protocol. While Onakewhor et al reported the administration of hepatitis B vaccine and HBIG immunoprophylaxis for PMTCT among a Nigerian cohort, the study had a sample size of only 45patients[10]. This study was therefore designed to meticulously implement the EMTCT of hepatitis B measures, and to follow the hepatitis B-exposed babies till the age of 9 months, for the purpose of identifying perinatal vertical and early childhood horizontal transmissions.

Methods

Study setting: The study was undertaken at the Perinatal Unit of the Department of Obstetrics, Gynaecology and Perinatology, and the Gastroenterology Unit of the Department of Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria between November 2015, and March 2021.

Design: The study is a prospective cohort study.

Study participants: All consecutive pregnant women that booked for antenatal care at the institution were prospectively recruited for the study after appropriate counseling. As the study was descriptive without any hypothesis testing, a specific sample size wasn't calculated.

Inclusion criteria: All pregnant women that received antenatal care at the Obafemi Awolowo University Teaching Hospitals Complex, Ife, Osun State, Nigeria during the study period.

Exclusion criteria: Pregnant women that refused to consent to HBV screening.

Statistical analysis: the data was analysed with IBM SPSS 20.0. Categorical variables were presented in frequencies and percentages while continues variables were presented as means and standard deviations.

Patient and public involvement: It was not possible to involve patients in the design and conduct of the study, but they will be involved in the dissemination of the study findings, for ease of widespread dissemination.

Ethics Approval: Research approval for the study was obtained from the Obafemi Awolowo University Teaching Hospitals Complex Ethics and Research Board (IRB/IEC/0004553: ERC/20/02/010).

Intervention: After obtaining informed consent from each study participant, relevant data regarding the socio-demographic characteristics of the women were captured using a purpose-designed proforma. Provider-initiated counselling and testing of the participants' serum for HBsAg was undertaken for all consecutive pregnant women by the Nurses at the antenatal clinic, using chromatographic immunoassay (Micropoint®, USA) rapid diagnostic kit. In addition to HBsAg testing, all the women also had retroviral screening done after due counselling. HBsAg positive women subsequently had the complete hepatitis B serological markers assay done, comprising the Hepatitis B surface Antibody (HBsAb), Hepatitis B core antibody (HBcAb: Total and IgG). Hepatitis B e-antigen (HBeAg) and the Hepatitis B e-antibody (HBeAb), using Micropoint® kit, for the purpose of categorization and prognostication. To ensure compliance, 95% subsidy on the cost of the hepatitis B serological marker was provided by the research team. HBV DNA assay was also routinely requested for all the HBsAg positive patients, but this was not subsidised. Chronic Hepatitis B (CHB) infection was defined as the presence of HBsAg for more than 6 months in the participants, or the presence of the IgG fraction of the Hepatitis B core antibody, with absent HBsAb and IgM fraction. All the pregnant women diagnosed with CHB were encouraged to bring their pre-existing children for testing and those identified as HBsAg positive were referred for necessary treatment at the Paediatric and Child Health Department of the hospital. Three categories of women were considered for antenatal treatment with Tenofovir Disoproxil Fumarate (TDF), namely, those with serum HBV DNA $\geq$ 200,000IU/ml, women positive for HBeAg without serum HBeAb, and women with history of perinatal transmission in their previous pregnancies. Pregnant women in any of these categories were placed on TDF 300mg

orally once daily starting from the 28th week of gestation till delivery, or as soon as possible, if the diagnosis was made after the 28th week of gestation. Adherence to medication was assessed during outpatient follow-up by the Gastroenterologists, who were also members of the research team. The mode of delivery was determined strictly by obstetric indications. Serial phone calls were placed to all the study participants by dedicated research staff and community health workers as their pregnancies advanced, to ensure compliance with antenatal care and postpartum EMTCT of hepatitis B advisories. Those who delivered out-of-facility were therefore identified and necessary EMTCT of hepatitis B measures were implemented without delay. All the babies that were delivered within the study period, irrespective of the place of birth or the day of the week, had the birth dose of the hepatitis B vaccine administered within 24 hours of delivery by the National Programme on Immunization (NPI) department. When affordable to the patient, 200iu of the Hepatitis B Immunoglobulin (HBIG) was also administered to the newborns of the hepatitis B positive parturients within 24 hours of delivery, while the mothers were referred to the Gastroenterology Unit for continuity of care. All the babies were followed up on phone to ensure strict compliance with the national vaccination schedule, including the hepatitis B vaccination. The infants were tested at the age of 9 months, to assess their hepatitis B infection status. The primary outcome measure was the proportion of infants that tested positive to the HBsAg at the age of 9 months. Secondary outcomes were the proportion of hepatitis-B exposed babies that had the birth dose of hepatitis B vaccine within 24hours, the proportion of babies that completed the 3doses of the hepatitis B vaccine at the appropriate time, spontaneous miscarriage rate, preterm delivery rate, mean birthweight and the perinatal mortality rate of the hepatitis B-exposed infants.

Results

A total of 10,866 eligible pregnant women were screened during the study period, of which 395 tested positive to the HBsAg, giving a prevalence of 3.64%. The mean age of the women was 31.51± 5.71years, with a median parity of 1.2. More than half (53.9%) were screened before the 28th week of pregnancy, and 249 women (63%) had the complete hepatitis B viral assay done. The remaining expectant mothers (146:37%) could not do the complete hepatitis B serological markers due to financial constraints. All the HBsAg positive women had chronic hepatitis B infection. In addition, 13 (5.2%) of the 249 HBsAg positive women expressed HBeAg in their serum, while 162 women (65.1%) had developed the anti-HBe antibody. Three patients had both the HBeAg

and the HBeAb contemporaneously. Only 21 patients had HBV DNA assay done, with values that ranged between 20IU/ml and 7,456IU/ml. Consequently, none of them attained the viral load trigger for prenatal TDF. Of the 486 pre-existing hepatitis-B exposed children before the index pregnancy, 225 (46.3%), with ages ranging between 2years and 22years were tested and seven (3.1%) were HBsAg positive. The other children were not tested because their parents did not give consent. Seventeen women had prenatal TDF administered from 28weeks gestation till delivery. Eight (2.0%) of the 395 women with chronic HBV infection had HBV-HIV co-infection.

Of the 395 women with positive HBsAg, there was one spontaneous miscarriage, while 18 patients were lost to follow-up (see figure 1). Further analysis was therefore based on 376 women that completed the study with known perinatal outcomes. With respect to mode of delivery, 219 women (58.2%) had spontaneous vaginal delivery, with a Caesarean section rate of 41.8%. The mean birth weight of the babies was 3.21 ± 1.86kg. Forty women (10.2%) had preterm births, defined as spontaneous or provider-initiated delivery for obstetric reasons, before 37completed weeks, and there were 11 perinatal mortalities (comprising six stillbirths and five early neonatal deaths), with a stillbirth rate of 15.9/1,000 births and a perinatal mortality rate of 29.2/1,000 births. The details of the perinatal mortalities are depicted on table 1. One of the 17 women that had prenatal TDF had a stillbirth from complications of obstructed labour outside the facility; there was no other complication recorded among the remaining 16 women. All the babies were breastfed (Figure 1).

The birth dose of hepatitis B vaccine was administered to all the 365 newborns; 327 babies (89.6%) had the vaccine administered within 24hours of delivery, while the remaining 38 babies (10.4%) had delayed vaccination within 2-4 days postpartum due to logistic reasons. There was a diarrhea-related infant mortality among the cohort, leaving 364 babies (99.7%) that completed the vaccination at the appropriate time. In addition, 260 (71.3%) babies had both hepatitis B vaccine and the Hepatitis B immunoglobulin combination; the remaining 105 babies (28.7%) did not have the HBIG administered due to financial constraints. Only eight (61.5%) of the 13 women with HBeAg had the hepatitis B vaccine-HBIG combination administered to their babies. The entire hepatitis-B exposed cohort of 364 children tested HbsAg negative at the exit-hepatitis B screening that was conducted at 9months postnatally.

Discussion

This study met all the set targets of the WHO Interim guidance for the elimination of hepatitis B[1], with absolute prevention of vertical and early childhood transmission of hepatitis B virus up to the age of 9 months among the cohort. This conforms to the findings of some studies that had earlier reported 0% transmission rate following initiation of immunoprophylaxis within 24 hours of birth[9,10]. Meanwhile, seven (3.1%) of the 225 already existing children that were delivered to the same cohort of women before the commencement of this protocol were HBsAg positive.

Expression of HBeAg significantly heightens the risk of vertical transmission of HBV. In a systematic review of 15 articles from 11 African countries including Nigeria by Keane et al[11], the pooled risks of vertical transmission of hepatitis B virus among 34 HBeAg positive mothers was 38.3% (95% CI: 7.0–74.4%) without prophylaxis, and 4.8% (95% CI: 0.1–13.3%) among HBeAg negative mothers, without prophylaxis. These rates are similar to the 40% and 5% transmission rates for HBeAg-positive and -negative mothers respectively, by WHO[3]. This risk was however eliminated among the 13 HBeAg positive women in the index study due to the combination of prenatal TDF from the 28th week of gestation and postpartum prophylaxis in the exposed children. This approach has been proven to be more effective among HBeAg positive mothers than vaccination at birth alone, at preventing vertical transmission[3,12]. Prenatal TDF is already supplied to HIV positive patients at no cost. Such privilege is, however, not yet available to HBV positive women[10,13]. It is advisable that the country leverages on the supply chain that is already available for HIV PMTCT programmes, for the prevention of HBV as well.

Almost 30% of the children in this study did not have the HBIG administered due to financial constraints. This is not unexpected, as such medications are expensive and only available on an "out-of-pocket" payment basis. There was, however, no difference in the outcome of the children in the hepatitis B vaccine-HBIG combination and the hepatitis B vaccine alone groups. This observation aligns with an earlier report that the efficacy of appropriately administered hepatitis B vaccine is comparable to that of hepatitis B vaccine-HBIG combination[14], and the WHO recommendation of HepB3 as the primary preventative measure for hepatitis B-exposed children, with expected immunity rate of 95%. This finding is of significant relevance, especially in low- and middle-income countries such as Nigeria, where a dose (200iu) of HBIG as at the time of the study costs about 70,000 Nigerian Naira, equivalent to 166 US Dollars. Meanwhile, the hepatitis B vaccine is available at no cost to all newborns as a national policy through governmental efforts in Nigeria.

With respect to obstetric outcomes, the prematurity rate is comparable with the report for the general obstetric population. The Caesarean section rate was comparable to the 47% reported from an earlier study, and the mean birthweight was also comparable to the 3210 ± 490g reported by Awowole et al from the same center 3 years earlier[15]. Hepatitis B infection in pregnancy was not associated with increased stillbirth or perinatal mortality rate among the cohort in this study, as the findings were comparable to the stillbirth rate of 15/1,000 births recorded by Kuti et al among unselected booked patients at the same facility[16]. The perinatal mortality rate of 29.2/1,000 births from this study, though high, is comparable to the perinatal mortality rate of 38/1,000 births reported by the Nigerian Demographic and Health Survey for the southwest geopolitical zone, where the OAUTHC is situated[6]. Chronic asymptomatic hepatitis B infection in pregnancy was therefore not associated with increased Caesarean section, low birth weight, stillbirth, and perinatal mortality rates in this study.

The mother-to-child-transmission of hepatitis B infection rate of 0% at 9 months in this study is comparable to the findings from some earlier studies that reported similar transmission rates, following initiation of the birth dose of the hepatitis B vaccine within 24hours of delivery[10,11,13]. Meanwhile, seven (3.1%) of the 225 children delivered to the same cohort of women before the commencement of this protocol were HBsAg positive. This further underpins the immense role of the hepatitis B preventative measures at eliminating vertical transmission.

One of the strengths of this study is the sample size, which involves the largest cohort of hepatitis B virus positive pregnant women to be prospectively recruited and actively managed in Nigeria, translating to generation of reliable and credible data. The multidisciplinary nature of the study, with involvement of Specialists from Obstetrics, Gastroenterology, Paediatrics and Child Health, Public Health, the Childhood Immunization Sub-unit and the Community Health Extension Workers made in-facility and community monitoring, surveillance, and painstaking implementation of the hepatitis B EMTCT interventions feasible in the study cohort. This research is also the only study in Nigeria, to prospectively follow up and report the outcomes of hepatitis B exposed children till the age of 9months, following implementation of HBV EMTCT measures. In addition, this is the first study from Nigeria that endeavored to screen all the pre-existing children of consenting hepatitis B pregnant women, with appropriate follow-up at the Paediatric and Child

Health Unit of the Teaching Hospital, thereby utilising the antenatal period as a window of opportunity to provide a family-centered, holistic care to the affected women.

This study also has some identifiable limitations. Since 2020, WHO has updated its recommendation to include the administration of TDF to pregnant women with HBV DNA ≥200 000 IU/mL to prevent mother-to-child transmission (PMTCT) of HBV[1,17]. One of the limitations of this study is however the inability to undertake the quantitative HBV DNA in all the women due to financial constraints This, however, did not significantly affect the outcome of the study. Secondly, the additional benefit of HBIG among HBV-positive women in low-resource settings could have been better assessed using a randomized controlled trial, as this study was not designed to specifically detect the comparative effectiveness of Hepatitis B vaccine alone versus hepatitis B vaccine-HBIG combination therapy. Withholding HBIG from the control group, especially for patients that could afford it would however have been unethical. Nevertheless, further trials to appraise this observation will be needed in the future. It is also not clear whether the pre-existing children that tested positive to the hepatitis B virus acquired the infections vertically at birth or horizontally in early childhood. Discerning this may however not be significant, as the endpoint of chronic childhood hepatitis B infection are similar. Furthermore, the vaccination status of the pre-existing children could not be evaluated with certainty. Lastly, screening for anti-HBs titre has been recommended for HBsAg-negative infants as anti-HBs levels ≥ 100 mIU/ml are considered protective with no need for further medical management[18]. Screening for anti-HBs was not done in this study for the 364 babies that were HBsAg-negative at 9 months of age due to financial constraints.

In conclusion, this study demonstrates that achieving the set goals for eliminating mother-to-child transmission of hepatitis B infection is feasible, even in the absence of high-end investigations such as HBV DNA assay, and expensive interventions such as the HBIG, especially in LMICs with the highest burden of chronic HBV infections and limited resources. Chronic hepatitis B infection did not demonstrate adverse effect on the outcome of the pregnancies. A national scale-up of these interventions in Nigeria through committed vaccination policies, sustained governmental funding and commitment, as well as universal health coverage are required to bridge the gap for the patients that live below poverty line in Nigeria and would therefore not be able to afford even the most basic of these interventions.

Author Contributions: DN and OK conceived the study. DN, OK, IA, OA, OI, OM, AA, CA and MI all participated in participated in literature search, proposal writing, study design, supervision of sample collection, patient management, critical review of the manuscript for intellectual content, and approval of the final draft and agree to take responsibility for the integrity of the research.

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Figure Legend

Figure 1: Algorithm for HBsAg screening of pregnant women and hepatitis B birth dose vaccination of exposed babies.

Table 1: Causes of perinatal deaths among women with hepatitis B infection in pregnancy at OAUTHC.

Cause	Perinatal mortality N = 11		Total
	Stillbirths	Neonatal Deaths	Frequency (%)
Antepartum haemorrhage	2	1	3 (27.3)
Eclampsia	1	1	2 (18.2)
Obstructed labour*	1	1	2 (18.2)
Out-of-facility delivery	0	2	2 (18.2)
Fetal Growth Restriction	1	0	1 (9.1)
Unexplained	1	0	1 (9.1)
Total	6	5	11(100)

*Patients presented late to the facility after initial management at unorthodox centers.

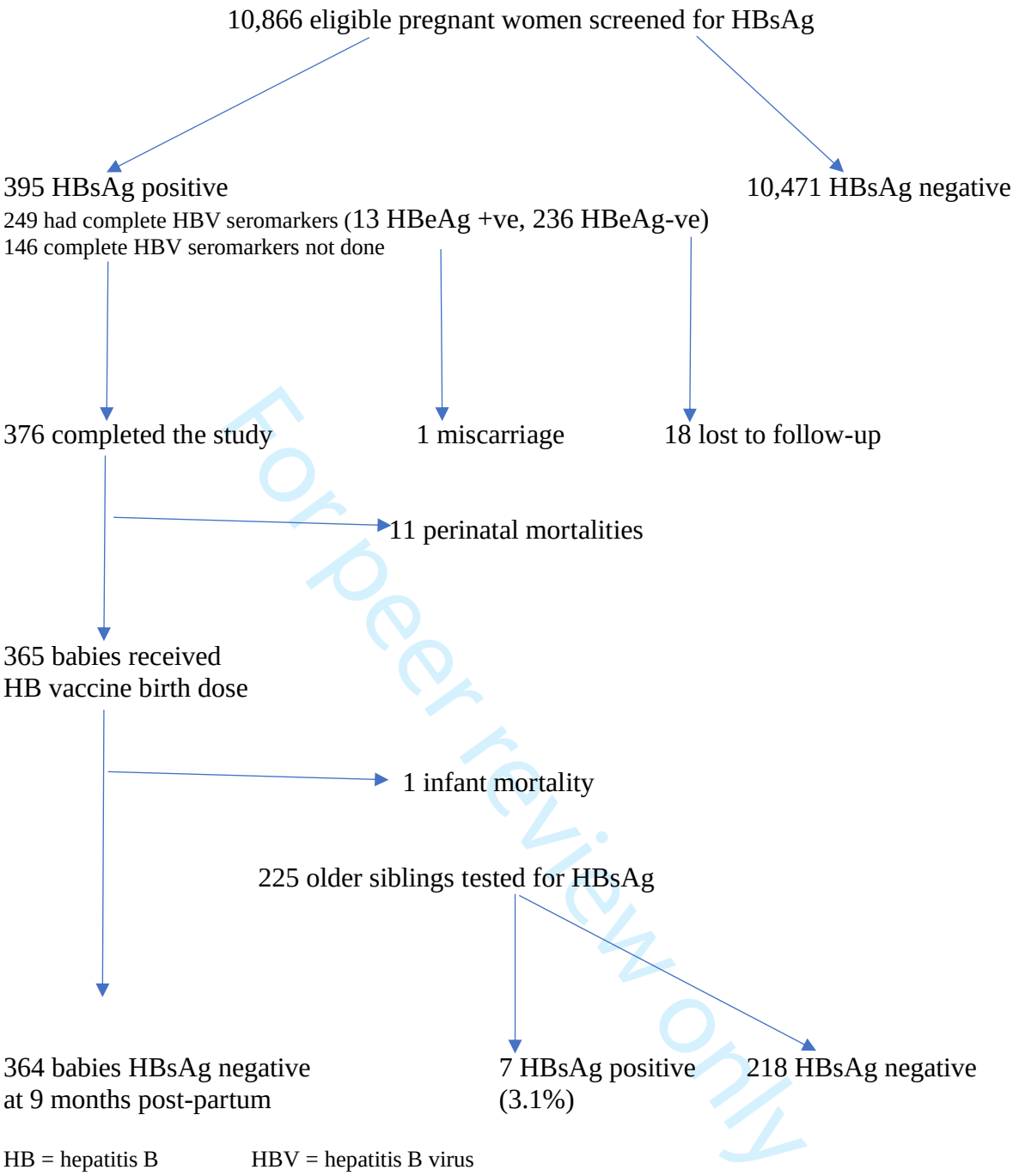

Figure 1: Algorithm for HBsAg screening of pregnant women and hepatitis B birth dose vaccination of exposed babies

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	Yes
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7
Generalisability	21	Discuss the generalisability (external validity) of the study results	8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.