PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	Ndububa, Dennis; Kuti, Oluwafemi; Awowole, Ibraheem; Adekanle, Olusegun; Ijarotimi, Oluwasegun; Makinde, Olufemiwa; Adeyemi, Adebanjo B; Anyabolu, Chineme; Ijadunola, Macellina

VERSION 1 – REVIEW

REVIEWER	Shevanthi Nayagam Imperial College
REVIEW RETURNED	20-May-2022

GENERAL COMMENTS	The authors present a study on HBV mother-to-child transmission in Nigeria, which is a high burden setting. This is large prospective study where children born to HBsAg positive women were followed up at 9 months of age. Such studies are important and add to the limited evidence base on HBV MTCT in Africa. It highlights some of the real-life challenges of implementing all the WHO recommended PMTCT interventions in the region. However, I have some comments about the manuscript, which I hope the authors will find helpful. Methods I think overall the manuscript would benefit from more information about the methodology including the following additions: How was the sample size calculated? Can the authors add some details on sampling methodology – were all consecutive pregnant women recruited, was it convenience sampling etc? I note that the study duration was over a 6-year period. Was this in order to recruit a specific sample size (related to point above and needs to be detailed), due to study interruptions (eg Covid) or another reason(s)? The limitations of the long recruitment period need to be discussed. Was the study performed alongside routine antenatal services and who did the screening – was it performed by existing antenatal care staff or a dedicated member of the research team? Describe any inclusion/exclusion criteria.

	Was HIV status checked and recorded? This would be useful information to add. The authors mention that the HBV panel was 'subsidized significantly' by the research team. Can you specify whether the patients had to pay anything out of pocket? It is not clear whether HBV DNA was also subsidized – can the authors clarify? Can the authors provide more details about the 'pre-existing' children who were tested, for example, how many 'pre-existing' children did the mothers have in total and how many accepted testing? What were their ages? Was information collected on their vaccination status? How was the information on history of perinatal transmission in previous pregnancies gathered? How do the authors differentiate between perinatal and horizontal transmission in these 'pre-existing' children? Can the authors clarify who was responsible for administering the vaccination and HBIG? Was it the national EPI team, midwives or the research team? Who alerted the vaccinator to an out of facility birth and how? How was adherence to Tenofovir assessed? How long after birth was it continued? Results How many pregnant women were eligible for the study and how many accepted to be recruited to study? We are only given the number who were tested, not the number eligible over the 6 year period. The authors state that 54% of pregnant women were screened before week 28 of pregnancy. For those who were screened after 28 weeks, were they still offered Tenofovir if they met your eligibility criteria? Related to the point above in the methods, in the results it is noted that 46% did not have full HBV assay due to financial constraints. Can the authors please clarify as the suggestion in the methods was that the cost was subsidized. In the flow chart it would be useful to discuss how many women were eligible for screening and number of women eligible for antiviral treatment as per HBV DNA criteria. The manuscript would benefit from some results tables with descriptive statistics on maternal and child characteristics. I may have missed this but can the authors add how many women had an HBV viral load tested and if any had a high viral load? In the abstract the authors state that the intervention includes TDF if HBV VL over 200,000. However, it is a notable limitation if there was a deviation from a pre-defined protocol. Discussion

	In the first paragraph the authors state that in the study all targets were met. However, the authors don't specify how many of the pregnant women were screened, and not all women in the study were assessed for Tenofovir eligibility. Therefore it is not possible to know whether the '90% coverage of antenatal screening' and '90% coverage with antivirals for those eligible' targets were met. Advise rephrasing to more accurately represent the study results. In the discussion the authors mention that there is no difference in transmission between HBV3 alone and HBV3 plus HBIG. I appreciate the challenges of HBIG administration in many settings and these are important to discuss. However, this study was not designed and powered to answer the comparative effectiveness, and the authors provide no information in the difference in HBeAg status/HBV DNA levels between the two groups, therefore this is a misleading conclusion. Also there is likely to be bias in those who received the HBIG and those who didn't since administration was based on ability to pay.

REVIEWER	Y.H Zhou Nanjing University Medical School, Departments of Laboratory Medicine and Infectious Diseases
REVIEW RETURNED	31-May-2022

GENERAL COMMENTS	The authors reported the efficacy of recommended immunoprophylaxis, hepatitis B immunoglobulin and/or hepatitis B vaccine, in the prevention of mother-to-child transmission of hepatitis B in 395 infants born to 395 pregnant women with positive HBsAg. None of the 364 infants followed up at the age of 9-10 months was HBsAg positive. Overall, this study added more evidence that the recommended immunoprophylaxis against mother-to-child transmission of hepatitis B is highly effective, which is an important study from the African region. However, the manuscript requires extensive revisions. The Introduction and Discussion are verbose and contain too many contents that are not closely associated with the study topic. Moreover, some statements are inadequate. For an example, page 5 of 17, "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". It is not adequate. Indeed, a proportion of HBV infection was not detected until the occurrence of hepatic decompensation. However, a big proportion of HBV infection can be detected in health-related actions, such as essential medical examinations such as entering schools, marriage, blood donation, and in other medical activities. In addition, HCC is used only once, and the abbreviation is not required. Statistical analysis methods are absent in the manuscript, and they should be added in the Methods section. The Results section: Were there any co-infections in these HBsAg-positive women? On page 8 of 17, the authors stated that 395 (3.64%) pregnant women were HBsAg positive. Of these 395 women, 249 were tested for other hepatitis B serological markers (anti-HBs, anti-HBc, HBeAg, and anti-HBe), and 146 were not tested for these markers.

	Based on 13 women with positive HBeAg, the authors stated that 3.3% of the 395 HBsAg-positive women were HBeAg positive. This is not correct. The positive rate of HBeAg should be calculated based on the number of tested women, and it should be 5.2% (13/249). Similarly, the positive rate of anti-HBe was not correctly calculated. There were 6 stillbirths and 5 early neonatal deaths in this study. It is essential to present the details of these 11 severe adverse events. How many occurred in women who received TDF? Were these adverse events associated with the use of TDF in the pregnant women? Other comments: The English editing is required. For an example, page 8 of 17, “Of the 395 women that tested positive to the HBsAg”---“Of the 395 women who were positive HBsAg”, or “Of the 395 women with positive HBsAg”. Please use all abbreviations based on the general rules, such as HBV, and many others. “HBV” should not be used to represent “Hepatitis B Vaccine”. HBV after the first appearance of ‘hepatitis B virus’ is used in the manuscript, please use HBV to replace hepatitis B virus throughout the m The “hepatitis B viral panel assay” is actually hepatitis B serological markers. Please use widely accepted terms to replace “self-made” or laboratory jargons. “hepatitis B viral panel assay” --- “hepatitis B serological markers”, “hepatitis B viral DNA” --- “hepatitis B virus DNA”. “iu/ml”---“IU/ml”

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
How was the sample size calculated?

Can the authors add some details on sampling methodology – were all consecutive pregnant women recruited, was it convenience sampling etc?
Consecutive pregnant women were recruited. This has been added to the methodolody section.

I note that the study duration was over a 6-year period.
The study was deliberated conducted over the study duration in order to get a sizeable study population. Consequently, the sample size represents the largest in any study on hepatitis b in pregnancy from Nigeria. We do not think this has affected the outcome of the study in any way. Throughout the duration of the study, it was financed by members of the research team. . It was however conceived by the research team that the hospital management and indeed the government of Nigeria might be encouraged to take over ownership of the prevention of mother to child transmission (PMTCT) programme if the study results showed significant benefits.

Was the study performed alongside routine antenatal services and who did the screening – was it performed by existing antenatal care staff or a dedicated member of the research team?
The study was conducted by the pre-existing antennal care staff and dedicated research staff. This is now reflected in the manuscript.

Describe any inclusion/exclusion criteria.
The inclusion and exclusion criteria are now included.

Was HIV status checked and recorded? This would be useful information to add.
HIV status was checked. The co-infection rate is now included in the manuscript.

The authors mention that the HBV panel was 'subsidized significantly' by the research team. Can you specify whether the patients had to pay anything out of pocket? It is not clear whether HBV DNA was also subsidized – can the authors clarify?
95% subsidy was provided for the patient on the hepatitis b serological markers assay by the research team, while the patients paid the balance. The HBV DNA assay was not subsidised. This is now included in the manuscript.

Can the authors provide more details about the 'pre-existing' children who were tested, for example, how many 'pre-existing' children did the mothers have in total and how many accepted testing? What were their ages? Was information collected on their vaccination status?
The number of pre-existing children, and the proportion that accepted to be tested, including their age range is now included in the manuscript.

How was the information on history of perinatal transmission in previous pregnancies gathered? How do the authors differentiate between perinatal and horizontal transmission in these 'pre-existing' children?
The authors acknowledge that it is difficult to differentiate between vertical and perinatal transmission for children that were delivered before the study started. This was stated as a limitation in the manuscript.

Can the authors clarify who was responsible for administering the vaccination and HBIG? Was it the national EPI team, midwives or the research team? Who alerted the vaccinator to an out of facility birth and how?
The National Programme on Immunization Unit was responsible for the vaccination. This is now included in the manuscript.

How was adherence to Tenofovir assessed? How long after birth was it continued?
Adherence was assessed by the Gastroenterologists, who are also members of the research team during clinic visits. The medication was discontinued at delivery. This is now included in the manuscript.

Results

How many pregnant women were eligible for the study and how many accepted to be recruited to study? We are only given the number who were tested, not the number eligible over the 6 year period.
All the eligible women accepted to be tested, as HBsAg screening is a routine part of the antenatal services. This is now included in the results section.

The authors state that 54% of pregnant women were screened before week 28 of pregnancy. For those who were screened after 28 weeks, were they still offered Tenofovir if they met your eligibility criteria?

All eligible women, including those diagnosed after 28weeks were offered TDF treatment. This is now included in the manuscript.

Related to the point above in the methods, in the results it is noted that 46% did not have full HBV assay due to financial constraints. Can the authors please clarify as the suggestion in the methods was that the cost was subsidized.
This is the true reflection of the events.

In the flow chart it would be useful to discuss how many women were eligible for screening and number of women eligible for antiviral treatment as per HBV DNA criteria.
This is now reflected on the flow chart.

The manuscript would benefit from some results tables with descriptive statistics on maternal and child characteristics.
The data on maternal and newborn characteristics have been presented as texts. Re-presenting them as tables may result in duplicate data presentation. A table has however been added to depict the identifiable causes among the 11 women that had perinatal deaths.

I may have missed this but can the authors add how many women had an HBV viral load tested and if any had a high viral load? In the abstract the authors state that the intervention includes TDF if HBV VL over 200,000. However, it is a notable limitation if there was a deviation from a pre-defined protocol.
The number of women that had HBV DNA testing done is now included in the manuscript. None of them attained the pre-defined trigger for TDF treatment.

Discussion

In the first paragraph the authors state that in the study all targets were met. However, the authors don't specify how many of the pregnant women were screened, and not all women in the study were assessed for Tenofovir eligibility. Therefore it is not possible to know whether the '90% coverage of antenatal screening' and '90% coverage with antivirals for those eligible' targets were met. Advise rephrasing to more accurately represent the study results.
All eligible pregnant women were screened, so this target was met. The inability to perform HBV DNA assay in all the women is acknowledged and has been stated as a limitation in the manuscript.

In the discussion the authors mention that there is no difference in transmission between HBV3 alone and HBV3 plus HBIG. I appreciate the challenges of HBIG administration in many settings and these are important to discuss. However, this study was not designed and powered to answer the comparative effectiveness, and the authors provide no information in the difference in HBeAg status/HBV DNA levels between the two groups, therefore this is a misleading conclusion. Also there is likely to be bias in those who received the HBIG and those who didn't since administration was based on ability to pay.
The authors acknowledge that an RCT may be needed to make a definite conclusion on the role of HBIG, and this was stated as a limitation in the manuscript. The authors also acknowledge that until conclusive evidence is available, it will be unethical to deliberately withhold HBIG from any patient that could afford it. The percentage of HBeAg positive women that had HBIG administered to their babies is now included in the manuscript. The result of the study, which showed zero transmission rate between newborns that had HBIG and those that did not, do not suggest a bias in favour of any group or otherwise.

Reviewer: 2
Dr. Y.H Zhou, Nanjing University Medical School
Comments to the Author:

However, the manuscript requires extensive revisions. Some statements are inadequate. For an example, page 5 of 17, “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”. It is not adequate. Indeed, a proportion of HBV infection was not detected until the occurrence of hepatic decompensation. However, a big proportion of HBV infection can be detected in health-related actions, such as essential medical examinations such as entering schools, marriage, blood donation, and in other medical activities. In addition, HCC is used only once, and the abbreviation is not required.
Some contents have been deleted from the introduction and discussion sections.
The highlighted statement has been revised as advised, and the abbreviation has been deleted.

Statistical analysis methods are absent in the manuscript, and they should be added in the Methods section.
This has been added to the methods section.

The Results section:

Were there any co-infections in these HBsAg-positive women?
The co-infection rate is now included in the manuscript

On page 8 of 17, the authors stated that 395 (3.64%) pregnant women were HBsAg positive. Of these 395 women, 249 were tested for other hepatitis B serological markers (anti-HBs, anti-HBc, HBeAg, and anti-HBe), and 146 were not tested for these markers. Based on 13 women with positive HBeAg, the authors stated that 3.3% of the 395 HBsAg-positive women were HBeAg positive. This is not correct. The positive rate of HBeAg should be calculated based on the number of tested women, and it should be 5.2% (13/249). Similarly, the positive rate of anti-HBe was not correctly calculated.
The authors acknowledge this error, which has now been corrected.

There were 6 stillbirths and 5 early neonatal deaths in this study. It is essential to present the details of these 11 severe adverse events. How many occurred in women who received TDF? Were these adverse events associated with the use of TDF in the pregnant women?
This information is now depicted on Table 1.

Other comments:

The English editing is required. For an example, page 8 of 17, “Of the 395 women that tested positive to the HBsAg”---“Of the 395 women who were positive HBsAg”, or “Of the 395 women with positive HBsAg”.
This has now been revised.

Please use all abbreviations based on the general rules, such as HBV, and many others. “HBV” should not be used to represent “Hepatitis B Vaccine”.
This observation has been effected.

HBV after the first appearance of ‘hepatitis B virus’ is used in the manuscript, please use HBV to replace hepatitis B virus throughout the m
This is now effected.

The “hepatitis B viral panel assay” is actually hepatitis B serological markers. Please use widely accepted terms to replace “self-made” or laboratory jargons. “hepatitis B viral panel assay” --- “hepatitis B serological markers”, “hepatitis B viral DNA” --- “hepatitis B virus DNA”.
“iu/ml”---“IU/ml”
These observations have been effected.

VERSION 2 – REVIEW

REVIEWER	Y.H Zhou Nanjing University Medical School, Departments of Laboratory Medicine and Infectious Diseases
REVIEW RETURNED	28-Aug-2022

GENERAL COMMENTS	The revisions have improved the quality of the manuscript. However, the Introduction and Discussion still contain too many contents that are not closely associated with the topic. The first paragraph of the Introduction can be replaced by one sentence with some references, “Chronic hepatitis B infection is a serious public health problem globally, especially in the African and the Western Pacific regions”. The 2nd paragraph can also be shortened to be a sentence, “Mother-to-child transmission (MTCT) of HBV is a major cause of HBV infection” Table 1 and figure 1 require reorganization. The English editing is required.

VERSION 2 – AUTHOR RESPONSE

2. The revisions have improved the quality of the manuscript. However, the Introduction and Discussion still contain too many contents that are not closely associated with the topic. The first paragraph of the Introduction can be replaced by one sentence with some references, “Chronic hepatitis B infection is a serious public health problem globally, especially in the African and the Western Pacific regions”. The 2nd paragraph can also be shortened to be a sentence, “Mother-to-child transmission (MTCT) of HBV is a major cause of HBV infection.

The authors wish to respectfully state that summarizing the paragraphs into single sentences as suggested by the reviewer will result in loss of the necessary information and data that are required to present the background of the study to the readers. Nevertheless, the first 2 paragraphs have been significantly shortened and collapsed into a single paragraph in compliance with the reviewer’s comment. The introduction and the discussion segments have been shortened significantly as recommended.

3. The English editing is required.

This has been done.

4. Table 1 and figure 1 require reorganization.

This has been done.