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Research Article

Sero-prevalence of hepatitis B virus and associated factors among pregnant women attending antenatal care in Bosaso general hospital, Puntland, Somalia 2023: A cross-sectional study design

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Abstract

Background: Hepatitis is an inflammation of the liver caused by a variety of infectious and non-infectious agents, leading to a range of health problems. Hepatitis B virus (HBV) can cause both acute and chronic liver disease, and the most common virus that can be transmitted from mother to child and other modes of transmission are contact with blood or other body fluids during sexual intercourse, unsafe injections, or exposure to sharp instruments.

Objective: To determine the seroprevalence and associated factors of HBV infection among pregnant women attending the Antenatal Clinic at Bosaso General Hospital, Puntland, Somalia, in 2023.

Methods: A cross-sectional study was conducted among 302 pregnant women who underwent routine ANC at Bosaso General hospital from March 15 to May 15. Study participants were interviewed using a structured questionnaire. A systematic random sampling technique was used to identify participants. Data entry, editing, and cleanup of the collected data were performed by the principal investigator using EPI data, and data analysis was performed using SPSS software version 20 to examine the association between each independent variable and the outcome variable. Bivariate and multivariate logistic regression analyses were performed to determine associations.

Result: A total of 302 respondents participated in the study, with a response rate of 96.8%. The overall prevalence of hepatitis B infection among pregnant women was 6.6% (95% CI = 4.0–9.8). Having a history of dental procedures (AOR = 5.52, 95% CI = 1.190–25.68), a history of blood transfusion (AOR = 5.02, 95% CI = 1.088–23.19), a history surgical procedure (AOR = 4.7, 95% CI = 1.014–21.76), or having a family history of HBV infection (AOR = 10.07, 95% CI = (1.27–79.63) were significant predictors of HBV infection among pregnant women.

Conclusion: The seroprevalence of HBV was intermediate (2–8%) in the study area according to WHO classification. A history of dental procedures, blood transfusion, surgical procedures, and a family history of hepatitis B virus infection were significantly associated with HBV infection.

Keywords: Sero-prevalence; Bosaso; hepatitis B virus; pregnant women.

Background

Hepatitis is an inflammation of the liver that is caused by a variety of infectious and non-infectious agents, leading to a range of health problems, including hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), and Hepatitis E virus (HEV), some of which are fatal, while liver diseases differ in the ways of transmission and severity of illness and their preventive methods [1].

HBV is a double-stranded DNA virus of the Hepadnaviridae family that can survive for at least 7 days outside the body; if the virus enters the body of a person who is not immunized at this time, it might still cause infection. Hepatitis B virus incubation times range from 30–180 days. During the incubation period of infection, the virus can survive and cause chronic hepatitis B, especially if it is spread during infancy or childhood; it can lead to chronic infections and increases a person's risk of dying from liver cancer and cirrhosis. Moreover, hepatitis B can spread through needle sticks, tattoos, piercings, and contact with infected blood and bodily fluids like saliva, vaginal, and seminal secretions, reusing contaminated needles and syringes, while sharp objects can spread the virus among drug injectors, in the community, and in healthcare facilities. Among unvaccinated individuals who have several sexual partners, sexual transmission is more common [2].

Hepatitis B and C are leading causes of chronic liver diseases such as cirrhosis and hepatocellular carcinoma. Worldwide, 356 million people are infected with these two types of infection. Hepatitis B virus can cause both acute and chronic liver disease, and is a common virus that can be transmitted from mother to child, while other modes of transmission are contact with blood or other body fluids during sexual

intercourse, unsafe injections and/or exposure to sharp instruments [1]. Viral hepatitis is a silent epidemic, since it is largely asymptomatic. Most people are indeed ignorant of their infection One of the diseases that can be prevented by vaccination and has a safe and effective vaccine is the hepatitis B virus [3]

According to WHO estimates, 257 million people are living with chronic HBV, which can lead to cirrhosis and hepatocellular carcinoma, ultimately causing approximately 900,000 deaths annually. Southeast Asia, the eastern Mediterranean, and Sub-Saharan Africa have the highest rates of illness prevalence. The age at acquisition is closely associated with the likelihood of chronicity and is believed to be directly tied to the maturity of the thymus and the ability to distinguish between self- and non-self-antigens. Whatever the reason, rate of chronicity after HBV infection exceeds 70% in early childhood and approaches 100% during the neonatal period [4]. Hepatitis B infection during pregnancy is associated with an increase in maternal and perinatal mortality, and chronic HBV infection during pregnancy has been linked to placental abruption, premature birth, gestational hypertension, and fetal developmental restriction [5].

In 2019, the WHO estimated that 296 million of people are infected with chronic hepatitis B virus with 1.5 million new infections in each year, and also an estimated 820,000 hepatitis B virus deaths recorded within the same year due to the cirrhosis and hepatocellular carcinoma [6]. Overly for those infected chronically, 10% are diagnosed and on treatment were in America, Europe, Asia, or the Western Pacific regions; In Africa, less than 2% are diagnosed and a paltry 0.1% are treated, which is why Africa is more prevalent with hepatitis B virus when compared with other continents [7].

According to the WHO 2017 report, an estimation of 257 million people (3.5% of the population) were living with chronic HBV infection worldwide, of which the African and Western Pacific regions accounted for 68% of the infection. In 2015, viral hepatitis was responsible for 1.34 million deaths [8].

Southeast Asia, China, sub-Saharan Africa, and the Amazon Basin are among the developing nations with the highest rates of endemicity for hepatitis. Hepatitis B is widely endemic (70–95% of people in these locations) where there is serological evidence of HBV infection, either from the past or the present. Most infections occur in early childhood or infancy. There is no evidence of acute HBV-related disease in children because most infections are asymptomatic; however, the incidence of liver cancer and chronic liver disease in adults is high [9].

There are 47 WHO AFRO countries, 30 of them have prevalence >5% of HBsAg and 70% of all new hepatitis B virus infections are in these WHO AFRO regions, with 6.3 million children aged <5 years being HBsAg positive in the same region [10]

In Somalia, viral hepatitis, particularly the Hepatitis B virus, is a major public health problem; a region in the world with an intermediate prevalence of HBV infection, medical workers in Somalia are underqualified and undertrained, and there is limited access to contemporary laboratory facilities, which creates significant diagnostic challenges because of the unsettling long civil war [11]

Method and material

Study area

Bosaso is a city in Somalia's northeastern Bari province and the seat of the Bosaso district, which is located on the southern coast of the Gulf of Aden; it is the region's commercial capital and a major seaport within the autonomous Puntland state, Bosaso is situated 1,107 km from Mogadishu, the capital city of Somalia. It has an estimated population of 74,287. In Bosaso, there are seven hospitals (one government hospital, six private hospitals) and six functional health centers that provide delivery services. Bosaso General Hospital is a governmental hospital in Bosaso with a bed capacity of approximately 200 and has outpatient and inpatient wards that provide different services.

Study period: The study was employed from March 15 to May 15, 2023.

Study design: A hospital-based cross-sectional study design was employed.

Source of population: All pregnant women who visited the ANC clinic in Bosaso General Hospital during the study period.

Study population: All pregnant women who were randomly selected during the study period.

Eligibility criteria

Inclusion criteria: All pregnant women with confirmed pregnancy and who attended ANC follow-up during the study period.

Exclusion criteria: Pregnant women who were critically ill and unable to respond. Pregnant women who revisited ANC during the study period.

Sample size determination

The sample size was calculated using Epi-Info version 7.2 STAC CALC with the assumptions of 95% confidence level, 80% power, adjusted odds ratio (AOR) = 3, exposure to non-exposed ratio of 1:1, and proportion of outcomes among non-exposed for having surgical procedures in the past (7.2%) (12). Considering a non-response rate of 10%, the final sample size was 312.

Sampling technique and sampling procedure

A systematic random sampling technique was employed to identify the study subjects, by implementing a sampling interval calculation formula (k = N/n), where the k interval was calculated, the total number of pregnant women for ANC visiting the Bosaso General Hospital during the 2 months was 1,688. Dividing this by 312 (sample size) = 5, therefore every fifth mother was enrolled in the study and initially the starting point was selected by using lottery method and then the k interval was used until the length of the sample size were achieved

Data collection tool and procedure

Data were collected using a pre-tested structured questionnaire, and face-to-face interviews with eligible pregnant women were conducted to obtain socio-demographic and economic factors, hospital-related factors, and risky socio-cultural and behavioral practices, which were prepared after viewing different studies that were similar for this study and modified [12-14]. A brief explanation was given to the study participants by the data collectors regarding the aim of the study, and they were asked to voluntarily participate in the study. After the data collectors completed the interviews, they gave codes and sent them to the laboratory. Two milliliters of blood were drawn aseptically by a laboratory technologist to determine the seropositivity of hepatitis B among the study participants. Once the clients participated in the study, their information was fully recorded to avoid re-participation.

Test procedure

The HBsAg status of the participants was determined using the Wondfo one-step HBsAg test kit. The Wondfo HBsAg test kit is

a rapid immunochromatographic assay for qualitative detection of HBsAg in human whole blood serum or plasma. A one-step rapid HBsAg kit was used to test the seropositivity of HBV infection in eligible pregnant women at the ANC unit of Bosaso General Hospital during the study period. Blood samples were added to the kit according to the manufacturer's instructions and the results were interpreted after 20 minutes. Kits that indicated red or pink lines in both the test and control regions were recorded as positive; those that indicated only one line in the test region were reported as positive; one line in the control region was recorded as negative; and any kit that indicated neither the control region nor the test region was recorded as invalid. After completing these tests, known negative and positive control samples were confirmed and retested using ELISA to determine the performance of the HBsAg test kit.

Data quality assurance

The principal investigator trained the data collectors and supervisors for 2 days on the tools and methods of data collection, such as the content of the questionnaire and its context, interviewing technique, filling out questionnaires, study subject selection techniques, ethical issues, and the purpose of the study. To verify consistency or distortion in the meaning of the terms and concepts, the questionnaire was written in English, translated into Somali, and then back into English by two different language experts. The experts reviewed the questionnaire to ensure its relevance, and their feedback was considered.

The questionnaires were pre-tested a week prior to the actual data collection days in 5% of the sample size to outside the study area in order to maintain completeness and consistency as well as the precise amount of time needed to complete the questionnaire.

Supervisors, together with the principal investigator, discussed the findings of the pre-test, and the questionnaires were checked before data collection. The questionnaire was checked each day during the actual data collection period for completeness and consistency by supervisors and the principal investigator. Codes were provided for the completed questionnaire. Data double entry was performed to compare the two data clerks and to resolve any differences.

Data processing and analysis

The principal investigator checked the data manually. The data were then coded, entered, and cleaned using EPI data version 3.1 Double data entry was performed for completeness before analysis. Data were analyzed using SPSS software version 20. Simple descriptive statistics such as frequency distribution, a measure of central tendency, a measure of variability, and percentages were used to describe the demographic, socioeconomic, and other factors related to the characteristics of the respondents. This information is presented in tables and figures.

Bivariate analysis was used to determine the association between HBsAg serostatus and the independent variables. Variables with a p-value <0.25 on bivariate analysis were entered into the Multivariate Logistic Regression model, and the results were considered statistically significant when the p-value was ≤0.05. The multivariate logistic regression model (p-value less than 0.05) was considered statistically significant for hepatitis B. Multivariate logistic regression was performed to control for possible confounders and identify the true effect of selected predictor variables.

Finally, the strength of associations between outcome and predictor variables was assessed using adjusted odds ratio (AOR) with 95% confidence, and the significance of the association was declared at a p-value of less than 0.05. Model fitness was assessed using the Hosmer–Emershow test. A multicollinearity test was performed among the predictors. The results are displayed as charts, graphs, and tables.

Ethical consideration

Ethical clearance was obtained from the Review Ethics Committee of the School of Public Health, College of Health Science, Jigjiga University. A formal letter was obtained from Jigjiga University, School of Public Health, and submitted to Bosaso General Hospital. A clear description of the study title, purpose, procedure, duration, possible risks, and benefits was provided to each participant.

Additionally, the study participants were fully aware of the study's objectives and were asked to participate truthfully. An anonymous questionnaire ensured the confidentiality of the data. Participants provided informed consent after being notified of the confidentiality of their information. Each participant was given the option to leave the study whenever they wanted. For monitoring and future management, all HBsAg-positive women were linked to doctors and advised on the potential risk of transmission with invasive procedures.

Results

Socio-demographic and economic factors

A total of 312 pregnant women were enrolled in this study; 302 of them participated, giving a 96.8% response rate, the minimum and maximum ages of the respondents were 18 and 48, respectively. The average age of research participants was 29.46 with a standard deviation of 6.96. The majority of the mothers were aged between 26–35, which occupies around 46% of the total. More than half of the respondents (177; 58.6%) were married. Seventy-eight (25.8%) of the pregnant mothers were illiterate. A large number of the respondents (106; 35.1 %) were house wives by occupation, in addition to that more than half of the study participants (208; 68.9%) were multigravidas (**Table 1**).

Table 1: Socio-demographic characteristics among pregnant women attending antenatal care clinic at Bosaso General Hospital in Puntland, Somalia, in 2023 (N = 302).

Variable	*Category*	*Frequency (%)*
Age	*18–25*	*105 (34.8%)*
	26–35	*139 (46.0%)*
	36–45	*53 (17.5%)*
	>45	*5 (1.7%)*
Marital status	*Married*	*177 (58.6%)*
	Divorced	*85 (28.2%)*
	Widowed	*40 (13.2%)*
Educational status	*Do not read and write*	*67 (22.2%)*
	Read and write	*54 (17.9%)*
	Primary school	*54 (17.9%)*
	Secondary school	*84 (27.8%)*
	Above grade 12	*43 (14.2%)*
Occupation	*Trader*	*57 (18.9%)*
	Housewife	*108 (35.8%)*
	Non-employed	*62 (20.5%)*
	Formal employed	*75 (24.8%)*
Monthly income	*Less than $50*	*96 (31.8%)*
	$ 51-100	*115 (38.1%)*
	More than $100	*91 (30.1%)*
Gravidity	*Prim gravidae*	*94 (31.1%)*
	Multi gravidae	*208 (68.9%)*

Sero-positivity of HBV among pregnant women

There was a total number of 302 study participants. Of these, 20 (6.6%; 95% CI = 4.0–9.6) pregnant women were seropositive for HBV infection, while the rest (282; 93.4%) were negative **(Figure 1).**

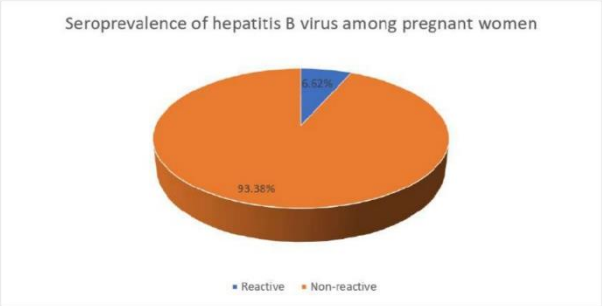

Figure 1. pie chart of sero-status of hepatitis B virus among pregnant women ANC in Bosaso general hospital, Puntland Somalia 2023.

Health service related factors and risk of socio-cultural and behavioral practices

From the total 302 study participants, 214 (70.9%) had no dental procedures in the past, and more than two-thirds of the pregnant mothers (223; 73.8%) had no history of blood transfusions although the remainder (79; 26.2%), who had a past history of blood transfusion, was still a substantial number. In addition to that the majority of the participants (208; 68.9%) had not undergone any surgical procedures. Of the 302 study participants, 250 (82.8%) had not experienced any unsafe abortion and most of the pregnant women (197; 65.2%) had not experienced FGM. A total of 186 (61.6%) study participant responded their husbands did not polygyny. Those who had no nose or ear piercing procedure totalled 261 (86.4%) and 194 (64.2%), respectively. A total of 202 (66.9%) study participants did not have a family history of HBV. Although a substantial number of the study participants (96; 31.8%) had a history of sharing sharp materials, there were still a high number (206; 68.2%) of pregnant mothers who had no history of sharing sharp materials **(Table 2).**

Predictors of HBsAg sero-positivity

Pregnant women with dental procedures in the past were five and half times (AOR = 5.52, 95% CI = 1.190–25.68) more likely to develop hepatitis B virus when compared with those who had not. Pregnant women who had received previous blood transfusions were at five times (AOR = 5.02, 95% CI = 1.088–23.19) higher risk of contracting hepatitis B virus infection when compared to those who did not. Pregnant women with surgical procedures performed in the past had a four times (AOR = 4.7, 95% CI = 1.014–21.76) higher chance to get infected with HBV infection when compared to those who had not. Pregnant women with a family history of hepatitis B virus were ten times (AOR = 10.07, 95% CI = 1.27–79.63) more at risk of developing HBV infection when compared those who did not.

Table 2: Health service related factors and risk of socio-cultural and behavioral practices among pregnant women attending an antenatal care clinic at Bosaso General Hospital in Puntland, Somalia, in 2023 (N = 302).

Variable	***Category***	***Frequency (%)***
History of dental procedure	*Yes*	*88 (29.1%)*
	No	*214 (70.9%)*
History of blood transfusion	*Yes*	*96 (31.8%)*
	No	*206 (68.2%)*
Unsafe abortion	*Yes*	*52 (17.2%)*
	No	*250 (82.8%)*
FGM	*Yes*	*52 (17.2%)*
	No	*250 (82.8%)*
Polygyny husband	*Yes*	*116 (38.4%)*
	No	*186 (61.6%)*
Nose piercing procedure	*Yes*	*41 (13.6%)*
	No	*261 (86.4%)*
Ear piercing procedure	*Yes*	*108 (35.8%)*
	No	*194 (64.2%)*
Family history of HBV	*Yes*	*100 (33.1%)*
	No	*202 (66.9%)*
Sharing sharp materials	*Yes*	*96 (31.8%)*
	No	*206 (68.2%)*

Table 3: Multiple logistic regression analysis results of factors associated with HBsAg sero-positivity of pregnant women attending ANC in Bosaso General Hospital, in 2023 (N = 302).

Variable	*Category*	*Sero-status of HBsAg*		*COR (95%)*	*AOR (95% CI)*	*P-value*
		Positive (%)	*Negative (%)*			
Polygyny husband	*Yes*	*11 (9.5%)*	*105 (90.5%)*	*0.48 (0.19–1.21)*	*0.54 (0.20–1.45)*	*0.224*
	No	*9 (4.8%)*	*177 (95.2%)*	*1*	*1*	
History of Dental procedure	*Yes*	*3 (3.4%)*	*85 (96.6%)*	*3.94 (0.89–17.39)*	*5.53 (1.19–25.68)*	*0.029***
	No	*17 (3.7%)*	*197 (96.3%)*	*1*	*1*	
History of Blood transfusion	*Yes*	*2 (2%)*	*94 (98%)*	*4.52 (1.02–19.80)*	*5.02 (1.08–23.19)*	*0.039***
	No	*18 (8.7%)*	*188 (91.3%)*	*1*	*1*	
History of Surgical procedure	*Yes*	*2 (2.1%)*	*92 (97.9%)*	*4.4 (0.99–19.18)*	*4.7 (1.014–21.76)*	*0.048***
	No	*18(8.6%)*	*190 (91.4%)*	*1*	*1*	
Unsafe abortion	*Yes*	*1 (1.9%)*	*51 (90.1%)*	*4.19 (0.55–32.05)*	*4.8 (0.588–38.83)*	*0.143*
	No	*19(7.6%)*	*231 (92.4%)*	*1*	*1*	
Family history of HBV infection	*Yes*	*1 (1%)*	*99 (99%)*	*10.3 (1.35–77.92)*	*10.1 (1.27–79.63)*	*0.029***
	No	*19 (9.4%)*	*183 (90.6%)*	*1*	*1*	
Sharing sharp materials	*Yes*	*2 (2%)*	*94 (98%)*	*4.50 (1.02–19.80)*	*4.13 (0.89–19.13)*	*0.069*
	No	*18 (8.7%)*	*188 (91.3%)*	*1*	*1*	

Significant at P < 0.05; COR, Crude odds Ratio; AOR, Adjusted Odds Ratio;95% CI, 95% Confidence Interval.

Discussion

This study aimed to assess the seroprevalence and factors associated with HBV infection among pregnant women who attended ANC at Bosaso General Hospital, and an attempt was made to determine the seroprevalence of HBV in pregnant women during the study period. The overall prevalence of pregnant mothers was 6.6% (95% CI = 4–9.6) which is intermediate endemicity According to geographical classification, the prevalence of HBV infection was low (<2%), intermediate (2–7%), and high (>8) [15].

This institution-based cross-sectional study found an overall prevalence of 6.6%, which was consistent in different studies conducted in the Sokoto metropolis, Nigeria, Addis Ababa, Hawassa, ranging from 6–6.6% [16-18].

The prevalence of this study was lower than the findings of other studies conducted in Hong Kong, Yemen, Nigeria, the northwest region of Cameroon, Uganda, Kenya, Yirgalem, Gedeo, Dire Dawa, and Jigjiga ranging from 7.2–14.1% [12, 13, 19-26]. This discrepancy might be due to geographical variation, differences in cultural and behavioral practices, risk factors for HBV infection, healthcare systems, and implementation of laboratory methods.

On the other hand, this result is higher than reported in other studies in different areas of the world, both developed and developing countries such as the USA, Brazil, northern Turkey, Saudi Arabia, Mahosot Hospital, Vientiane, Felegehiwot Referral Hospital, Mogadishu ranging from 0.1–5.4% [27-33]. This may be due to the lack of awareness among study participants, lack of hygiene practices in health facilities, and risk of socio-cultural and behavioral practices, in addition to that there is also widespread usage of vaccination belonging to these developed and developing countries.

The multivariate logistic regression analysis in this study showed that the odds of being positive for hepatitis B virus infection had a 5.5 times higher chance of contracting among pregnant women who had dental procedures than among those who did not, which is in line with the results of different studies reported in Debramarkos, Hawassa, and Shashemene [14, 34, 35]. This finding might be due to the poor hygiene of the instruments used for dental extraction and the lack of standard precaution practices during the procedure. However, using a well-cleaned and protected instrument during extraction may decrease the risk of contracting HBV infections in hospital settings.

The final multivariate analysis model revealed that pregnant women with a history of blood transfusions had five times higher odds of testing positive for HBV than those without a history of blood transfusions. This outcome is consistent with institution-based cross-sectional studies conducted in different parts of Ethiopia, such as Bahir Dar, Gedeo Zone, Felegehiwot,

Harar City, and Dire Dawa [3, 13, 20, 33, 36]. In contrast, some studies did not show any significance in that blood transfusion was an independent predictor of hepatitis B virus infection, similar to a study conducted in Wolaita sodo [37]. This finding can be explained by the lack of proper aseptic precautions and proper screening of blood after admission to the laboratory, which occurs mostly when the patient needs emergency blood transfusion and the blood type is not available in the blood bank. However, proper blood preparation and screening should decrease the risk of HBV infection during blood transfusion.

The current study indicated that pregnant mothers with a history of surgical procedures were 4.7 fold more likely to develop hepatitis B virus infection than their counterparts. Similar findings were reported in Khartoum, Bahir Dar, Harar City, and Jigjiga [3, 12, 36, 38]. Contradictory results of surgical procedures in the past were not predictors of HBV infection among pregnant women in Saudi Arabia and Kigali, Rwanda [39, 40]. This can be attributed to poor prevention practices during the surgical procedure and lack of proper sterilization of surgical instruments.

According to the results of the current study, a history of liver disease among family members is significantly associated with HBV infection among pregnant women. Moreover, pregnant mothers with a family history of hepatitis b infection were 10 times more likely to be infected than those with no family history. This result agrees with previous studies conducted in northwestern Ethiopia and Jigjiga [12, 41]. A possible explanation might be that someone who is chronically ill or a carrier has a high probability of transmitting infection to his family members, either sharing sharp materials such as nail cuts or body fluid contamination, as we are aware that the infection is highly transmittable.

Conclusion

The overall prevalence in this study was 6.6%. According to WHO classification, this study discovered that the prevalence of HBsAg seropositivity among pregnant women attending ANC in Bosaso General Hospital was at an intermediate endemicity level. A history of dental procedures, blood transfusions, surgical procedures, and a family history of hepatitis B virus infection during dental procedures were found as predictors of HBV infection in pregnant women.

This study will help raise the awareness of society and health authorities in the prevention and control of HBV infection among pregnant women in Bosaso town.

All pregnant women should be screened for HBV and treated, if necessary, to reduce their viral loads, and their children should be vaccinated at birth with the single-dose hepatitis B vaccine to break the cycle of mother-to-child transmission.

Standard aseptic techniques are used in dental procedures, blood transfusions, and surgical procedures.

Orientation regarding transmission and prevention methods should be provided to mothers with a family history of hepatitis B infection.

Sterilization of equipment while performing different hospital operations can contribute to minimizing exposure to this virus.

ELISA should be implemented as a routine screening test for HBV among pregnant mothers in hospital settings, owing to its high sensitivity and specificity.

Authors contribution

Every author has significantly contributed to the concept, method of study, data collection, analysis, and interpretation of the study, they also contributed to the manuscript's development, critically reviewed it, and decided which publication the paper should be submitted to. All authors read and agreed to the final version of the manuscript and agreed to be responsible for all contents of the manuscript.

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Conflict of interest

The authors sustain no conflict of interest.

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