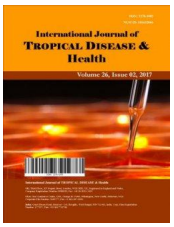

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Prevalence and Associated Risk Factors of Hepatitis B Viral Infection among Pregnant Women Accessing Antenatal Care at Mbarara Regional Referral Hospital, South West, Uganda

Masajjage Derick[1], Kyeyune Lyavala Davis[1], Mubangizi Morris[1], Ogwang Samuel[1], Were Rebecca[1] and Okongo Benson[1*]

[1]Department of Medical Laboratory Sciences, Faculty of Medicine, Mbarara University of Science and Technology, P.O.Box 1410, Mbarara, Uganda.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MD and OS designed the study, wrote the protocol and managed the analyses of the study. Authors KLD, WR and MM managed the literature searches and performed the statistical analysis. Author OB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the prevalence and associated risk factors of Hepatitis B surface antigen (HBsAg) among pregnant women.
Study Design: A cross-sectional study.
Place and Duration of Study: Antenatal clinic of Mbarara Regional Referral Hospital, between April and July 2016.
Methodology: We included 160 pregnant women (age range 18-45 years). 4ml of blood samples were taken in EDTA tubes; plasma was separated and tested for Hepatitis B surface antigen (HBsAg) using the visual immunochromatographic HBsAg Card (Cypress Diagnostics). Positive

**Corresponding author: Email: bokongo@must.ac.ug;*

samples with HBsAg were further retested using (Axysm immunochemical Technology) for hepatitis B envelope antigen (HBeAg).

The Results: Out of the 160 participants, 4 (2.5%) tested positive for HBsAg and the prevalence by gravidity was highest in primigravida, 5.88% *(n=51, p-value =0.28*). The gestational age groups of 1-13 and 28-42 weeks had the highest prevalence 3.1% (*p-value 0.05*). The highest prevalence of HBsAg by age was seen in age groups 18-26 to 4.7% (n=84). All the HBsAg positive became HBeAg negative on ELISA technique. There was no association between history of surgery and HBV infection *(Odds Ratio 2.03, p value= 0.54*) similarly with the history of blood transfusion (*Odds Ratio 0.66*).

Conclusion: Despite the reported high prevalence of HBV infection among pregnant women in other areas within the country, HBV prevalence was low in this study. Routine testing for HBV infection at all ante-natal care clinics countrywide is recommended.

Keywords: HBsAg; HBeAg; pregnant women; prevalence; risk factors; Uganda.

1. INTRODUCTION

Hepatitis B viral infection continues to be a significant public health burden worldwide; approximately half a billion people in the world still harbor hepatitis B viral infection [1]. The burden of hepatitis infection differs world over with an estimated 50% of the world's population living in the countries where HBV infection is endemic, in most parts of Asia, the Pacific Islands, Africa and the Arab region [2]. Recent studies indicate that 686,000 deaths occur due to HBV infection and about 300,000 die due to liver cancer secondary to HBV every year worldwide [3]. Perinatal and early childhood transmission are the significant ways of hepatitis B infection in endemic regions [4] Vaccination coverage of 90% to all newborn within 24 hours from birth can avert 84% of world Hepatitis virus-associated mortality [5]. The prevalence is decreasing in many endemic countries due to improved socioeconomic status, Hepatitis B vaccination exercise and perhaps effective antiviral treatments [6]. Nevertheless, immigration is presently altering the prevalent in most of the low endemic developed continents, owing to higher HBsAg prevalence rates in migrants and refugees from developing countries compared with the indigenous population [7,8]. The most significant percentage of these individuals acquired the infection during or around the time of birth and early childhood [9]. Pregnant mothers who test positive for both hepatitis HBsAg and HBeAg have 70-90% risk of transmitting the infection to their newborn babies and "10-40% when only HBsAg positive" [10]. Therefore, pregnant women must be routinely screened for HBsAg and infants whose mothers test positive for HBsAg be administered Hepatitis B vaccine [11]. The acquisition of hepatitis B infection early in life escalates the danger of advancement to chronic liver disease, the development of cirrhosis, and hepatocellular carcinoma. Nowadays, newborn vaccination is the most accepted way for elimination of hepatitis B viral infection [12].

Uganda has long been considered to be among the highly endemic countries of sub-Saharan Africa, with more than 8% of the population expected to harbour chronic infection with transmission occurring during childhood [13]. In adulthood, more than 1.4 million adults are chronically infected and some communities are disproportionately affected [13]. HBV vaccine was introduced in Uganda in 2002 as part of the Expanded Programme for Immunization (EPI) and is given at 6, 10 and 14 weeks of age [14]. There was no government policy on the vaccination of adults against HBV in Uganda until 2012 when reported outbreaks in several parts of Northern and Northeastern districts of Uganda occurred. Following these outbreaks, the Ministry of Health rolled out Hepatitis B mass vaccination in 25 districts of the 39 highly burdened districts identified by the Government in 2013 and prioritized them in the first phase of the Hepatitis B vaccination drive as it moves to combat the disease affecting over 1 million people. The Ministry of Health hopes that the funding from development partners will be provided to ensure that the entire country is covered. Other districts have not yet started the vaccination exercise and in those districts, private health facilities offer vaccination at a cost which is unaffordable to the majority who live on less than a dollar per day. Majority of these pregnant women who are harbouring chronic hepatitis B (CHB) may not be aware of the infection and hence fail to seek appropriate medical attention, and this may progress to chronic liver disease, cirrhosis, and hepatocellular carcinoma. These pregnant

women with CHB constitute a severe health risk not only to their unborn child, but also to the society at large. Although studies have been carried out on HBV infection in other parts of the country like in Gulu Northern Uganda, where the prevalence was reported to be 11.8% [15]. Information is still very scarce on the prevalence of HBV among pregnant women in southwestern Uganda, and Mbarara Regional Referral Hospital in particular. The primary aim of this study was to determine the prevalence of Hepatitis B and its risk factors among pregnant women at Mbarara Regional Referral Hospital, Southwestern Uganda.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Mbarara Regional Referral Hospital located 250km southwest of Kampala capital city, it is a government-owned referral hospital and a teaching hospital for The Medical School of Mbarara University of Science and Technology. The hospital was founded in 1940 and it has a bed capacity of 600. It is the referral hospital for the Western region and serves neighbouring countries like DR Congo, Rwanda, and Tanzania as well.

2.2 Study Design

A cross-sectional descriptive study was carried out between April 2016 and July 2016 to determine the prevalence and Risk factors of Hepatitis B viral infection among pregnant women attending antenatal care in Mbarara Regional Referral Hospital, in southwestern Uganda. All pregnant women who consented to participate after explaining the aim and objectives of the study were included in this study. A simple random sampling method was used to recruit the participant while those who declined were excluded.

2.3 Sample Size Estimation

We calculated a sample size of 160 based on an estimated 11.8% HBV prevalence [15] to have 80% power with a 95% confidence estimate precision of ~5%.

2.4 Subjects

One hundred sixty (160) blood samples were collected from consented pregnant women who were attending antenatal care in Mbarara Regional Referral Hospital. Structured questionnaires were used to obtain risk factors associated with Hepatitis viral infection (History of blood transfusion, surgery and whether vaccinated) and demographic data from the study participants like (age, gestational week, occupation).

2.5 Sample Collection

4 ml of venous blood was collected in EDTA vacutainers from each participant. The blood samples were centrifuged and 1ml of the plasma obtained was transferred into cryovials and stored at –80°C for further confirmation of active infection by testing for HBeAg.

2.6 Laboratory Analysis

With the use of a micropipette, 50µl of plasma was obtained and delivered into the sample pad of HBsAg test card (Cypress Diagnostic, Langdorpsesteenweg, 3201 Langdorp, Belgium) and was incubated at room temperature for 15 minutes. Those that were positive for HBsAg were retested for HBeAg using AxSYM HBe 2.0 reagent and AxSYM immunochemical automated analyser from Abbott Diagnostics Germany. It is based on Microparticle Enzyme Immunoassay (MEIA) technology and utilises the principle of direct binding of the HBeAg in the sample to the anti-HBe coated on the microparticles followed by the detection of the bound HBeAg by the anti-HBe. The alkaline phosphatase-labelled conjugate catalyses the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-methylumbelliferone. The MEIA optical assembly measures this fluorescent product. Those who were positive were retested using ELISA technique for HBeAg and referred to the gastroenterology clinic for proper management and those who tested negative were advised to undergo the Hepatitis B vaccination.

2.7 Data Analysis

Data generated were subjected to comparative statistical analyses using the statistical program SPSS version 17 for the variables. Percentages of positive HBsAg cases were calculated. Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated to determine any association between Hepatitis B and Risk factors, (history of surgery and Blood transfusion). *P value ≤ 0.05* was taken as significant.

3. RESULTS

Of the 160 pregnant women recruited into the study, 128 (80%) were between age groups of 18 - 28, 29 -39 were 28 (17.5%) and those of age groups >40 years were 4 (2.5%). The distribution according to their occupation are peasants 82 (51%), Businesswomen 40 (25%) and 0thers 38 (24%) (Table 1).

The overall "prevalence" of HBsAg positivity was 2.5% (95% CI 1.8 – 3.5). The 4 (2.5%) who were positive for HBsAg were retested using an AxSYM immunochemical automated analyser for confirmation of active infection; all were negative for HBeAg (Table 2). Hepatitis B viral infection was more prevalent in primigravida mothers with 5.8% (n=51), followed by multigravida mothers who had a prevalence of 0.9% (n=109) (Table 1). The gestational age groups of 28-42 weeks and 1-13 weeks had the same prevalence of HBsAg positivity of 3.1%, while the gestational age group of 14-27 weeks had a prevalence of 1.6% (Table 1). The highest HBsAg positivity according to age was seen in women aged 18-24 years (4.7%) and those >25 years of age were not affected (Table 1).

3.1 Risk Factors for HBV Infection

Risk factors for Hepatitis B viral infection that were assessed using questionnaires included the history of blood transfusion and history of surgery. The results showed no association between history of blood transfusion and positivity for Hepatitis B virus, Odds Ratio 0.6641 (95% CI; 0.1528 to 15.1966, *p*-value 0.7196). There was no significant association between history of surgery and Hepatitis B infection with Odds Ratio 2.0303 (95% CI; 0.2020 to 20.4069, *p=value* .5475) (Table 1). However, the study was not powered enough to determine the association between history of surgery and HBV infection as indicated above with (*p-value* .5475). Only 1.3% (n=2) were vaccinated against hepatitis B viral infection (Table 1). There was meagre hepatitis B vaccination coverage with 1.3% of the participants vaccinated while 98.7% were not vaccinated.

Table 1. Showing demographic characteristics of the study participants and HBsAg results

(n=160)	HBsAg		P value
Age group (Years)	**Negative**	**Positive**	
18 –24	80	4	0.29
25 – 31	55	0	
32 – 38	18	0	
39 -45	3	0	
Occupation			
Peasant	82	4	0.30
Business	40	0	
Others	38	0	
Gestational age (weeks)			
1 - 13	32	1	0.86
14 – 27	60	1	
28 – 42	64	2	
History of surgery			
Previous surgery	22	1	0.54
No previous surgery	134	3	
History of blood transfusion	159	1	0.72
HBV vaccination			
HBV vaccinated	01	1	<0.005
Not vaccinated	155	3	
Parity			
Nulliparous	48	3	0.09
Multiparous	108	1	
Gravidity			
Primigravida	48	3	0.09
Multigravida	108	1	

Table 2. Showing demographic characteristics of the study participants and HBeAg results

(n=4)	HBeAg		P-value
Age group (Years)	**Negative**	**Positive**	
18 –24	84	0	0.29
25 – 31	55	0	
32 – 38	18	0	
39 -45	3	0	
Occupation			
Peasant	84	0	0.30
Business	40	0	
Others	38	0	
Gestational age (weeks)			
1 - 13	33	0	0.86
14 – 27	61	0	
28 – 42	66	0	
History of surgery			
Previous surgery	23	0	0.54
No previous surgery	137	0	
History of blood transfusion	160	0	0.72
HBV vaccination			
HBV vaccinated	02	0	<0.005
Not vaccinated	158	0	
Parity			
Nulliparous	51	0	0.09
Multiparous	109	0	
Gravidity			
Primigravida	51	0	0.09
Multigravida	109	0	

4. DISCUSSION

The study revealed a low prevalence of HBV infection (2.5%) among pregnant women attending the antenatal clinic at the Mbarara Regional Referral Hospital. This prevalence closely correlates with studies in Mwanza, Tanzania and Mbagathi District Hospital in Nairobi, Kenya [16] with 3.8% prevalence, but disagrees with a similar study done in 2 hospitals in northern Uganda with a prevalence of 11.8% [15]. The high prevalence of HBV in northern Uganda may be associated with the prolonged civil conflict, though no causal relationship between HBV infection and civil conflict can be drawn [15]. It also disagrees with the study on the prevalence of Hepatitis B viral infection among pregnant women in Makurdi, Nigeria, which had shown a prevalence of 11% [17].

The high prevalence of HBV infection (5%) in the age group of 18-24 years in comparison with other age groups correlate with similar findings from a study in Rakai district, which had the highest level of HBsAg positivity in the age group of 20-29 years [18]. This study also agrees with the study in Mbagathi District Hospital in Nairobi, [19] with the highest prevalence in age groups 20-24 but it disagrees with the study in Tanzania with the highest prevalence occurring in the age group 16-20 years [16]. It is clear that sexual activity is common among individuals in the reproductive ages, and many of the behaviours that they engage put them at risk for contracting sexually transmitted infections.

This study revealed a high prevalence of HBsAg among the peasants (unemployed), and this correlates with the finding in Nigeria, which indicated that unemployed pregnant women formed the bulk of positive cases [20]. This may be explained by the low economic status, introducing women to multiple sexual partners and unprotected sex thus making them vulnerable to sexually transmitted diseases [15].

Results from this study showed a gradual decrease in the prevalence of HBV infection by gestational age, the gestational age of 1-13 and 28-42 weeks had a prevalence of 3.1 %, while 14-17 weeks had 1.7%. These findings are in agreement with a study in Nigeria, which found a high prevalence of 20% in the gestational age of 1-13 weeks and 17.4% in gestation age of 28-42

weeks while the gestational age of 14-27 weeks had the least prevalence of 14.9% [21]. Pregnant women are considered to be a unique population group due to their specific susceptibility to some infectious diseases because of the unique "immunological" condition caused by pregnancy. This could be explained from the fact that pregnancy progresses with a decrease in immunity of the expectant mother [22] a reason that might put the mothers in higher gestational ages at higher risk of acquiring HBV.

There was no significant association between HBV and history of surgery (Odds Ratio 2.03, 95% CI, 0.2020-20.4069, *p= value* .55). This disagrees with a similar study in Ethiopia that showed a relation of the history of surgery and HBV infection with Odds Ratio 11.1 [23]. Similar studies conducted from Bamako, Mali [24] and Gabon [25] identified that history of blood transfusion and history of surgery had a significant association with HBV infection.

Blood transfusion and surgery are generally indicated as risk factors for HBV infection, but this study showed no association with a history of blood transfusion (Odds Ratio 0.664, 95% CI 0.1528-15.1966, *p-value.*72), this disagreed with [18] a study in northwest Ethiopia that found pregnant women with a previous history of blood transfusion had been 3.7 times more likely to be positive for HBsAg [23]. This difference of blood transfusion services not to be associated with HBV infection in Uganda is a clear manifestation of a successful campaign of blood being screened for transfusion-transmissible infections (TTI's) before transfusion.

The finding of the present study revealed that nulliparous pregnant women had the highest HBsAg positivity (5.8%) compared to multiparous (0.9%). This finding does not correlate with the finding in China, where multiparous women with a prior history of abortion were at a higher risk of maternal HBV, compared to nulliparous women [26]. This study results showed a similar finding with a study in Mbagathi District Hospital in Nairobi, where 21% prevalence was found among young women between 11-15 years [19]. The most likely route of HBV horizontal transmission is sexual intercourse. It appears that the high prevalence of HBV infection in nulliparous women is due to early age at first sexual encounter among young girls in low-income countries [19].

Hepatitis B viral infection was more prevalent in primigravida women (5.9%), followed by multigravida women who had a prevalence of (0.9%). These findings disagree with a similar study in Osogbo city in Nigeria, which showed the highest prevalence among multigravida mothers (20.7%) while primigravida mothers had the least prevalence of 10% [21]. The high prevalence seen in primigravida in this study could be due to early age at first sexual encounter among young girls in low-income countries [19].

5. CONCLUSION

This study showed a low prevalence of HBsAg positivity in southwestern Uganda among pregnant women compared to other regions of the country. The highest prevalence was seen among primigravida, age 18-24 years, with the gestational age of 1-13 and 14-27 weeks. The Ministry of Health Uganda and its partners should put in place a policy that will ensure routine testing for HBV infection at all ante-natal care clinics countrywide, this will enable early detection of HBV among pregnant women and allow for proper planning for availability of Hepatitis B vaccine and Hepatitis immunoglobulins (HBIg) in delivery rooms for babies born to Hepatitis B positive mothers.

6. LIMITATION

Small sample size and inability to determine whether the Hepatitis B e antigen (HBeAg)-negative samples could be HBeAg-negative CHB which have a naturally occurring mutant form of HBV that does not produce HBeAg antigen.

CONSENT AND ETHICAL CLEARANCE

Ethical clearance was sought and granted from the Faculty Research Committee and Research Ethical Committee of Mbarara University of Science and Technology. Permission to carry out this study was also sought from the Director Mbarara Regional Referral Hospital. Written informed consent was obtained from each participant included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (1964).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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