Original article

Seroprevalence of HBsAg and its risk factors among pregnant women in Jimma, Southwest Ethiopia

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Abstract

Background: Vertical transmission from mother to baby is an important route of transmission for hepatitis B virus infection. Neonates who contract hepatitis B will have an almost 90 % risk of developing chronic HBsAg carriage and chronic liver disease. Neonatal immunization interrupts this vertical transmission.

Objective: To determine the sero-prevalence of HBsAg among pregnant women and to identify potential risk factors associated with the infection.

Methods: A cross-sectional study was conducted from October, 2002 to March, 2003 in a total of 493 pregnant women attending Mother and Child Health clinics of Jimma university specialized hospital and its Training Health Centers. Serum was collected from each woman and tested for the presence of HBsAg using Bioline Strip, which is a qualitative, solid phase two site sandwich immunoassay (Pacific Biotech, Thailand). A pre-structured questionnaire was used to collect socio-demographic data and to find out possible risk factors.

Results: The overall prevalence of HBsAg was 3.7% ranging from 1.4% to 6.4%. It was highest in Jimma and lowest in Shebe town. The socio-demographic status of the study population shows that high proportion of HBsAg positivity was among the illiterate (61%), those whose income <500 Birr / month (88.9%) and among the Oromo ethnic subgroup (83%). Pregnant women who experience abortion had a higher prevalence of HBsAg (7.3%). The odds of having HBsAg was more than twice with those with history of abortion than with other risk factors. When pregnant women of Jimma town were separately analyzed, dental procedure, cesarean section and tattooing are associated with HBsAg positivity, unlike other towns.

Conclusion: The prevalence of HBsAg carrier state in the study area was significant enough to start routine antenatal screening for HBsAg. Except for abortion, known risk factors included in the study were not associated with Hepatitis B virus infection. The main route of HBV transmission in Jimma and its neighboring towns could be either through sexual contact or from mother to child. Further studies are required to confirm these modes of transmission. [*Ethiop.J.Health Dev.* 2005;19(1):45-50]

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Introduction

Hepatitis B virus (HBV) occurs worldwide and constitutes a serious public health problem. Globally, more than 2 billion people have been infected with HBV at some time in their lives. Of these, about 350 million people remain infected chronically and become carriers of the virus, and 1.5 million deaths occur from HBV related liver diseases, including end stage cirrhosis and hepatocellular carcinoma each year (1- 3).

Three major routes spread HBV: perinatal, horizontal, and sexual transmission (4). In developing countries, the main routes of transmission are: neonatal with HBV carrier mother infecting her infant usually during birth or soon after birth following close contact, transfer of HBV via cuts, sexual transmission, transfusion of infected blood or blood products, needle stick injury, contamination of eye, re-use of HBV contaminated needles, syringes, lancets and instruments including those used in tribal ceremonies, possibly blood sucking insects and bed bugs (2).

Adults infected with HBV usually acquire acute hepatitis B and recover, but 5-10% develop the chronic carrier state. Infected children rarely develop acute disease, but 25 to 90% become chronic carriers (5). Neonates who contract hepatitis will have an almost 90% risk of developing chronic HBsAg carriage and chronic liver disease. Infants may also spread the disease to siblings and to a community (6).

The global prevalence of chronic HBV infection varies widely, from high (>/=8%, e.g., Africa, Asia, and the western Pacific) to intermediate (2-7%) e.g. Southern and Eastern Europe) and low (<2%, e.g. Western Europe, North America and Australia (5). Most countries in Africa have high HBV endemicity, with the exceptions of Tunisia and Morocco, which have intermediate endemicity (7,8).

In an earlier study done to define the mode of transmission of Hepatitis B infection in Ethiopia, 5% of pregnant women were reported to be positive for HBsAg (9). In another study done to determine the prevalence and significance of sexually transmitted diseases among Ethiopian women attending antenatal care (ANC) in Addis Ababa hospitals, the prevalence of HBsAg among pregnant women were similar (5%) to the above study (10). A survey of Ethiopian blood donors shows that occurrence of HBsAg was 8% (11). Similar study in northwest Ethiopia reported that HBsAg was detected in 14.4 % of blood donors (12). Moreover, a communitybased sero-epidemiological survey of Addis Ababa was conducted to inform on the transmission dynamics and control of HBV infection. HBsAg prevalence was 7%. Overall HBV seroprevalence (any marker), rose steadily with age to over 70% in 40-49 year olds, indicating significant childhood and adult transmission (13).

Even though many studies on sero-epidemiology of HBV prevalence in Ethiopia have been previously done and indicated that hepatitis B is endemic in Ethiopia with regional variation (9-13), no data about the prevalence of hepatitis B among pregnant women are available in Jimma and its neighboring towns. The present study was conducted to determine the prevalence of HBsAg among pregnant women and to identify risk factors associated with hepatitis B infection in these areas.

Methods

Study design: A cross sectional study was conducted in Jimma University Specialized hospital and its four training health centers (i.e., Jimma, Asendabo, Agaro and Shebe health centers) among pregnant women attending Mother and Child Health (MCH) department from Oct. 2002 to Mar. 2003.

Study population: - A total of 493 pregnant mothers who were attending MCH clinics in the above mentioned health institutions were included in this study. Informed consent was obtained before data collection.

Steps of data collection-

I. Socio-demographic characteristics and exposure to risk factors

An MCH nurse was identified in each health institution to interview the subjects and enter the data according to the pre-structured questionnaire. The questionnaire included level of education, occupation, income, ethnicity, and history of dental and surgical procedure, tattooing, exposures to unsafe injection, history of caesarian section, abortion, liver disease, blood transfusion and ear piercing in jewelers shop.

II. Serological detection of Hepatitis B surface antigen **Sample**: - Fresh serum was obtained by centrifuging naturally clotted blood. The serum sample was kept in the refrigerator at 4°C and transported to Jimma University Medical Laboratory School.

Bioline HBsAg One Test: -The Bioline HBsAg One Test is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test band region and anti-mouse antibodies on the control band region. During testing, the

serum sample reacts with the dye conjugate (mouse antiHBsAg antibody colloidal gold conjugate) that has been coated in the test strip. The mixture then by capillary action, reacts with anti-HBsAg antibodies on the membrane and generates a red band. Presence of this red band indicates a positive result while its absence indicates a negative result. Regardless of the presence of HBsAg, as the mixture continues to migrate across the membrane to the immobilized goat anti-mouse region a red band at the control band region will always appear. The presence of this red band serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Briefly, the procedure is as follows:

1) The Bioline HBsAg test strip was removed from foil pouch.
2) The test strip in the serum samples was immersed with printed sample pointing toward the serum or plasma.
3) Then waited for the red bands to appear. The test was read after approximately 5 minutes. Results after 30 minutes were not interpreted.

Interpretation of the test:

Positive - Two distinct red bands appear, one in test region and another in the control region.

Negative - A single red band appears in the control region. No apparent red or pink band appears in the test region.

Invalid - Control band fails to appear which means improper testing procedure or deterioration of reagents probably.

Accuracy of the Bioline HBsAg strip

Bioline HBsAg was compared with a leading commercial Radio immunoassay (RIA) and an Enzyme immunoassay (EIA) test for Hepatitis B. There was 98% overall agreement between RIA and Bioline HBsAg and 97% between EIA and Bioline HBsAg. Bioline HBsAg will detect any level of HBsAg in serum higher than 5ng /ml within 10 min. However, to detect concentrations below 5ng/ml and to confirm negative results, the test should be read at the end of 15 to 20 min. All ten HBsAg subtypes (ayw1, ayw2, ayw3, ayw4, aayr, adw2, adw4, adrg +adr and adr) produce a positive result in HBsAg assay (14).

Statistical analysis: -Descriptive statistics, and Chisquare test with Yate's correction is used to determine for any association between socio-demographic characteristics, exposure to risk factors. $P< 0.05$ is considered as having significant association. Odds ratio was used to compare seropositivity of HBsAg with history exposure to risk factors.

Results

Out of 493 sera from pregnant women tested for HBsAg, 18 (3.7%) were positive, ranging from 1.4 % to 6.4% for each training health center and Jimma University's specialized hospital. It was highest in Jimma and lowest

in Shebe town. Results of area-specific serological test aresites. The seroprevalence of HBsAg in relation to age is shown in Table 1.

Table 1: **Prevalence of HBsAg of pregnant women attending Jimma University Hospital and its training health centers in 2002/03**

Site	No. tested (%)	HBsAg Positive (%)
Jimma Hospital	140 (28.4)	9 (6.4)
Jimma Health Center (H.C)	71 (14.4)	3 (4.2)
Agaro H.C	100 (20.3)	3 (3.0)
Asendabo H.C	96 (19.5)	2 (2.1)
Shebe H.C	86 (17.4)	1 (1.4)
Total	493 (100)	18 (3.7)

At least one seropositive case for HBsAg was found in all age groups except in the age range of 35 to 39. The highest age-specific prevalence was in the age group of >40; however, the total number of pregnant women tested was only four. There was no linear relationship between prevalence of HBsAg and age in all the study

shown in Table 2.

The sociodemographic status of the study population shows that a high proportion of HBsAg positivity was among the illiterate 11/18(61%), those whose income was <500 Birr / month 16/18(88.9%) and among the Oromo ethnic subgroup 15/18(83%). However, the overall prevalence of HBsAg among the illiterate (3.5%) and Oromo ethnic group (4.1%) have no significant difference ($p> 0.05$) when compared with other educational status and ethnic groups, respectively.

The prevalence among Dawro was two fold higher than among the Oromo and Keffa, and also five fold higher than among the Amhara pregnant women. HBV infection was not detected in both Gurage and Tigrean pregnant women. As far as monthly income was concerned the prevalence of HBsAg decreases as the income increases ($p<0.05$) (Table 3).

Table 2: **Prevalence of HBsAg in relation with age of pregnant women attending Jimma University hospital and its training health centers in 2001/03**

Age	Jimma hospital	Jimma* No. tested (%)	Agaro* No. tested (%)	Asendabo*	Shebe*	Total
15-9	28 (10.7)	24 (8.3)	25 (0.0)	26 (3.8)	28 (0.0)	131 (4.6)
20-24	51 (0.0)	22 (4.5)	41 (2.4)	34 (0.0)	29 (0.0)	177 (1.1)
25-29	41 (7.3)	14 (0.0)	18 (5.6)	25 (4.0)	12 (18.3)	110 (5.5)
30-34	12 (16.7)	9 (0.0)	11 (9.1)	6 (0.0)	8 (0.0)	45 (6.7)
35-39	7 (0.0)	2 (0.0)	4 (0.0)	5 (0.0)	7 (0.0)	25 (0.0)
>40	1 (100)	0 (0.0)	1 (0.0)	0 (0.0)	2 (0.0)	4 (25)
Total	**140**	**71**	**100**	**96**	**86**	**493 (3.7)**

* Health Center

Table 3: **Prevalence of HBs Ag in relation to socio demographic characteristics of pregnant women attending Jimma University hospital and its training health centers in 2002/03**

	Jimma hospital (n=140)	Jimma* (n=71)	Agaro* (n=100)	Asendabo* (n=96)	Shebe* (n=86)	Total # (%)
Education						
Illiterate	32	58	72	86	68	316 (3.5)
<6	30	2	3	15	15	52 (7.7)
7-8	19	5	12	3	3	44 (0.0)
9-12	44	5	13	3	0	65 (4.6)
12+	15	1	0	0	0	15 (0.0)
Occupation						
Government employee	27	3	4	1	4	39 (0.0)
House wife	96	64	89	94	78	418 (3.8)
Student	9	1	0	1	0	11 (18.2)
Merchant	4	0	3	0	0	7 (0.0)
House maid	1	0	0	0	2	3 (0.0)
No work	3	3	4	0	2	12 (0.0)
Income (in Birr)						
<500	81	62	90	86	64	383 (3.9)
500-1000	52	9	9	6	16	92 (3.3)
>1000	7	0	1	4	6	18 (0.0)
Ethnicity						
Oromo	82	60	73	87	62	364 (4.1)
Amhara	18	3	14	1	12	48 (2.1)
Keffa	13	0	0	0	8	21 (4.8)
Gurage	6	0	8	5	0	19 (0.0)
Dawro	8	0	0	1	0	9 (11.1)
Tigray	2	4	0	0	2	8 (0.0)
Others	11	4	5	2	2	24 (4.2)

- Health center

Pregnant women who experienced abortion had higher ($p<0.05$). Other risk factors included in the study had not prevalence of HBsAg (7.3%) and the odds of having statistically significant association ($p>0.05$) with the HBsAg was more than twice with those pregnant women HBsAg positivity (Table 4).
that had history of abortion than with other risk factors

Table 4: **Potential risk factors and prevalence of HBsAg in Jimma University's hospital and its training health centers in 2002/03**

Risk factors	No. (%)	No. HBsAg positive (%)	Odds ratio (95% CI)
Dental procedure	33 (6.7)	1 (5.6)	0.8 (0.70-0.91)
Surgical procedure	20 (4.1)	0 (0.0)	0.0
Tattooing	84 (17.0)	2 (11.2)	0.6 (0.40-0.83)
Unsafe injection	20 (4.1)	1 (5.6)	0.7 (0.65-0.75)
Caesarian section	14 (2.8)	1 (5.6)	0.5 (0.39-0.63)
Abortion	41 (8.3)	3 (16.8)	2.3 (1.0105.30)
Liver disease	6 (1.2)	0 (0.0)	0.0
Blood transfusion	5 (1.0)	0 (0.0)	0.0
Ear piercing (in jeweler's shop)	2 (0.4)	0 (0.0)	0.0
No risk factors	335 (67.9)	10 (55.6)	0.6 (0.33-0.93)
Total	**493 (100)**	**18 (100)**	

Discussion

The overall prevalence of HBsAg in pregnant women from Jimma and its neighboring towns was 3.7%. This places Ethiopia, specifically the Oromia region, as a medium endemic area (2-7 % of HBsAg positive) for HBV infection according to WHO criteria (8). But the prevalence of each town differs widely. It was highest in the relatively densely populated towns of Jimma (Jimma Hospital, 6.4% and Jimma Health Center, 4.6%) and Agaro (Agaro Health Center, 3%).

The results from this study are in agreement with the investigation done among pregnant women in Lebanon 2.9% (15), Turkey 4.3% (16) and India 4.6% (5). Lower and higher prevalence rates were also detected in similar study populations in different parts of the world. Countries with lower prevalence include United States with 0.14% – 0.97% in different races, except among Asian American where prevalence was 5.6%, (17), Brazil 1.05% (18) and Mexico 1.65% (19).

Higher prevalence of 6.5% each was reported among a similar study population in Congo and Zambia (20, 21), Hong Kong 10% (21), Papua New Guinea 11% (22), Nigeria 11.6% (23) and Taiwan 12% (24). Extremely high prevalence (25.3%) was also reported in Cameroon (25).

Since there are no previous data from our study area we are unable to compare for trend. However, there were two earlier studies conducted among pregnant women in Addis Ababa hospitals that reported 5% in both studies (9, 11), which was a little higher than our result.

The prevalence of HBsAg positivity in this study was not limited to any particular age group and was almost equally distributed among all the age subgroups of pregnant women, even though no HBsAg positive pregnant woman was detected among the age group between 35-39. Since only 7 pregnant women were in this age group, it is not representative to generalize for the status of the whole subgroup.

When age specific prevalence is considered, age group >40 years and between 30-34 years of age have a higher prevalence of 25% and 6.7% respectively. The prevalence of the latter age group agrees with a similar study done in Central America (26). Whereas in the former subgroup only 4 pregnant women participated in the study and one was positive for HBsAg as a result the prevalence may be inflated unless confirmed by another study involving a larger sample size.

The HBsAg positivity rate among those tested varies widely by ethnicity with highest in Dawro (11.1%) followed by Keffa (4.8%) Oromo (4.1%) and Amhara 2.1%. No HBsAg was detected in the Tigrean and Gurage ethnic groups. A remarkable geographical and ethnic variability of marker prevalence was shown in a previous nation wide study (9). However, our study shows only small difference and this may be due to the fact that the sample size of ours is about $1/10^{th}$ of this earlier study.

Risk factors, including blood transfusion, surgery, liver disease, dental and surgical procedures, and tattooing were not associated with HBV infection in this study as a whole. But when pregnant women of Jimma town were separately analyzed, those who history of caesarian section, dental procedure and tattooing had association which HBV infection. The odds of having HBsAg with caesarian section and dental procedure was twice and with tattooing was thrice, as compared with those who did not experience these risk factors. These differences should be confirmed in further studies.

This study revealed an association between HBsAg positivity and history of abortion. Having history of abortion increased the risk of having HBV infection more than twice as compared with those who had not suffered

such experience. As it is known, abortion is directly related to sexually active women, and one most important mode of transmission for HBV is exposure to a heterosexual partner (10). Therefore, other reasons such as instrumentation during abortion and related activities may serve as source of exposure.

A previous study identified that there was a significant association between HBsAg positivity and exposure to heterosexual partner. Because of the issue of confidentiality, information about sexual practices was not included in this study. In addition, past history of sexually transmitted diseases is significantly associated with HBsAg positivity when compared with the normal pregnant women. In general, women of child bearing age who are living in more urbanized areas may have more HBV exposure than those in the rural population, as a result of risky life style practices (26).

Previous report shows that universal prenatal screening for HBsAg is cost effective if the HBsAg carrier rate is greater than 0.06% (16). Thus, our findings affirmed the need for routine HBsAg screening among pregnant women in developing countries like Ethiopia.

Although WHO recommended that hepatitis B vaccine be incorporated into routine infant and childhood immunization program for all countries by 1997, only 130 of 216 countries introduced hepatitis B immunization into their national infant and childhood immunization programme by the beginning of 2001. The major hurdle to universal hepatitis B immunization is the cost of hepatitis B vaccine, especially for developing countries. Even though the price of hepatitis B vaccine for developing countries has dropped from about $3.00 per dose in 1990 to as low as $ 0.30 per dose in 2001, the cost of three doses of hepatitis B vaccine remains higher than the cost of other vaccines included in routine infant immunization programmes (27).

In conclusion, except abortion, known risk factors included in the study were not associated with Hepatitis B virus infection. The main route of HBV transmission in Jimma and its neighboring towns could be either through sexual contact or from mother to child. In order to determine the extent of perinatal transmission of HBV, the prevalence of HBeAg among the HBsAg carrier mothers should be determined in a further study. Due to the small sample size, although most point estimates were comparable to other published data, it was difficult to conclude the age specific prevalence, and the role of ethnic sub-groups and risk factors associated with HBV infection in this study. Hence, a further study is required. Preferably, to have a good contrast, both pregnant and non-pregnant women should be included in a future study.

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References

1. Ferriara MS. Diagnosis and treatment of hepatitis B. Rev Soc Bras Med Trop. 2000;33:389-400.
2. Cheesbrough M. District laboratory practice in tropical countries, 3rd ed. Cambridge University press, UK. 2000;pp. 250-252.
3. WHO. Global health situation and projection and estimates. Geneva: WHO, 1993
4. Edmunds WJ, Medley GF and Nokes DJ. The transmission dynamics and control of hepatitis B virus in The Gambia. Statistics in medicine. 1996;30(15):2215-33.
5. Juszozyk J. Clinical Course and consequence of hepatitis B infection. Vaccine. 2000;18 Supp 1 S23-5.
6. Sriprakash I and Anil TP. Routine prenatal screening of Indian women for HBsAg: benefits derived versus cost. Trop Doc. 1997;27:176-7.
7. Maddrey WC. Hepatits B an important Public health issue. J Med Virol 2000;61:362-6.
8. WHO / EPI Protocol for assessing prevalence of hepatitis B infection in antenatal patients. WHO / EPI/ GEN/ 90.6 1990.
9. Tesga E, Tsega M, Mengesha B, Nordenefelt E, Hansson BG and Lindberg J. Transmission of hepatitis B virus infection in Ethiopia with emphasis on the importance of vertical transmission. Int J Epidemiol. 1988;17:874-9.
10. Duncan ME, Tibaus G, Pelger A, *et al.* Prevalence and significance of sexually transmitted diseases among women attending clinics in Addis Ababa. Ethiop J Health Dev.1995; 9:31-40.
11. Kefene H, Rapicetta M, Rossi GB *et al.* Ethiopian National HBV Study. J Med Virol 1988; 24:75-84.
12. Lakew. Occurrence of HBsAg and its antibody in Ethiopian blood donors. Ethiop Med J. 1983; 21: 205-208.
13. Abebe A. Seroepidemiology of Hepatitis B Virus In Addis Ababa, Ethiopia: Transmission Patterns And Vaccine Control. Epidemiol Infect. 2003; 131:757 – 770.
14. Caldwell CW and Barret JT. Enzyme linked Immunoassay for Hepatitis B and its comparison to other methods. Clin Chem Acta. 1977;81:305.
15. Nabulsi MM, Khalil AM, Farah AE and Araj GF. Prevalence of hepatitis B surface antigen in pregnant Lebanese women. International Journal of Gynecology and Obstetrics. 1997;58:243-4.

16. Erdem M, Sahin I, Erdem A, Gursoy R, Yildiz A and Guner H. Prevalence of hepatitis B surface antigen among pregnant women in a low-risk population. International Journal of Gynecology and Obstetrics. 1994; 44: 125-8.
17. Euler GL, Worten KG, Baughman AL and Williams WW. HBsAg prevalence among pregnant women in Urban areas: Implications for testing, reporting, preventing perinatal transmission. Pediatrics 2003; 111: 1192-97.
18. Duarte G, Mussi-Pinhata MM, Martinez R, Lemos C, Figueiredo EM and Quintana SM. Frequency of HBsAg carrier status among women giving birth in a Brazilian. community Buoletin de la oficina sanitaria panamericana. 1996; 120:189-97 (Popline).
19. Vazquez-Martinez JL, Coreno-Juarez MO, MontanoEstrada LF, Attlan M and Gomez-Dantes H. Seroprevalence of hepatitis B in pregnant women in Mexico. Salud Publica Mex. 2003;45:165-70 (Popline).
20. Oshitani H, Kasoslo C, Tembo M, *et al.* hepatitis B virus infection among pregnant women in Zambia . E Afr M J. 1995;72:813-815.
21. Kong KL, Cho Y and LeeSS. The declining HBsAg carriage rate in pregnant women in Hong Kong. Epidemiol Infect. 1997;119:281-3.
22. Clegg T. Hepatitis B surface and e antigen seropositivity in mothers and cord blood at Port Moresby General Hospital: implications for a control program. Papua New Guinea Med J. 1991;34:234-7 (Popline).
23. Harry TO, Bajani MD and Moses AE. Hepatitis B virus infection among blood donors and pregnant women in Maiduguri, Nigeria. E Afr Med J. 1994; 70: 596-7.
24. In HH, Kao JH, Chang TC, Hsu HY and Chen DS. Secular trend of age-specific prevalence of hepatitis B surface and e antigenemia in pregnant women in Taiwan. J Med Vir. 2003; 69:466-70.
25. Ndumbe PM, Andela A, Nkemnkeng-Asong J, Watonsi E and Nyambi P. Prevalence of infections affecting the child among pregnant women in Yaounde, Cameroon. Med Microbiol and Immunol. 1992;181:127-30.
26. Scott-Wright A, Hakre S, Brayan JP *et al*. HBV, HIV-1, and syphilis among women attending prenatal clinics in Belize, Central America. Am J Trop Med Hyg. 1997;56:285-90.
27. Kao JH and Chen DS. Global control of hepatitis B virus infection. Lancet. 2002;2:395-403.