Rebie Kedir, Belayneh Dimah Taye, Tesfaye Kassa, Lule Teshager, Abraham Aseffa, Rawleigh Howe, Adane Mihiret, Addisu Gezie. *Ethiop Med J, 2019, Supp. 2*

SEROPREVALENCE OF HEPATITIS B VIRUS INFECTION AND SEROPROTECTION OF HEPATITIS B VACCINE AMONG CHILDREN IN JIMMA TOWN, SOUTH-WEST ETHIOPIA

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ABSTRACT

Background*: Hepatitis B virus is the leading cause of viral hepatitis and about 240 million people worldwide are chronic carriers. The virus is reported to be widely prevalent in Ethiopia and routine vaccination of children has been initiated in the country recently. We assessed the seroprevalence of HBV infection and seroprotection of HBV vaccine among children in Jimma.*

Methods*: A community-based cross-sectional study was conducted among 900 children who were 5-9 years of age between June and December 2016. A simple random sampling technique was employed to recruit study participants by proportional allocation into different Kebeles of Jimma. Data were collected using pretested questionnaire.3-5ml of blood sample was collected from each child and it was tested for HBsAg, anti-HBc, and anti-HBs using ELISA (Bio-rad, Monolisa, Lacquote, France). Data were analyzed using chi-square and logistic regression analysis.*

Result*: HBsAg and anti-HBc prevalence among all participants was 3.5% and 3.8%, respectively. The prevalence of HBsAg among vaccinated and non-vaccinated children was 2.1% and 7.0% whereas anti-HBc positivity was 1.1% and 6.2%, respectively. It was also found that 58.4% of vaccinated children maintained a protective level of HB surface antibodies which is defined as ≥ 10 mIU/ml anti-HBs. While 1.8%(4/222) vaccinated children with protective anti-HBs levels were positive for hepatitis B core antibody, none of the vaccinated children with non-protective anti-HBs levels were positive for hepatitis B core antibody. Multi-variable logistic regression revealed that lack of vaccination (AOR =2.788, P < 0.029), children who were born at home (AOR= 3.211, P < 0.009), and children who had a history of hospital admission (AOR= 7.122, P <0.001) were more likely to be HBV surface antigen positive.*

Conclusion*: The seroprevalence of hepatitis B infection is high among children who have not received HBV vaccination. Hepatitis B vaccine has contributed to the reduction of the infection in this endemic area, though further efforts are required to improve timely vaccination and its coverage. The prevalence of protective anti-HBs is low among fully vaccinated children, hence, it is better to include the monovalent birth dose of the vaccine and conduct further studies to evaluate underlining causes for the waning of serum anti-HBs level.*

Keywords*: Hepatitis B virus, Vaccine efficacy, Sero-prevalence, Children, Vaccination*

INTRODUCTION

Hepatitis B virus (HBV) is the leading cause of viral hepatitis and about 2 billion people worldwide have been infected (1,2). Despite the availability of an effective vaccine, HBV infection remains a major health problem worldwide with estimates of nearly 240 million chronic surface antigen (HBsAg) carriers (3). Approximately 45% of the world's populations live in regions of high endemicity, defined as areas where at least 8% of the population are positive for HBsAg, such as Southeast Asia and Sub-Saharan Africa (SSA), where Ethiopia is located (4).

Africa has the second largest number of chronic carriers after Asia and is considered to be a region of high endemicity. The estimated HBsAg seroprevalence were reported to be ranging from 6 to 20% (5). About 50 million people are carriers of the virus, while 25% of these are at risk for dying from the illness. In some African regions, 90% of children have been infected and 20% have become chronic carriers (6, 7).

In Ethiopia, similar to other African countries, there is a lack of nationally representative data on hepatitis B infections. Hence, it is difficult to present or predict the prevalence and related mortality rates accurately associated to this virus.

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The regional estimates have shown wide geographic and socioeconomic variation in hepatitis B prevalence, ranging from 5.7% to 10.9% (8-10). The virus is prevalent in liver disease cases and one study reported that at least one of the hepatitis B markers was found in 78% of patients with hepatocellular carcinoma, 86% of chronic hepatitis cases, and 88% of cirrhotic patients in Ethiopia (11). Further, HBV accounts for 12% of hospital admissions and 31% of deaths in Ethiopian hospitals (12).

In high prevalence areas, HBV infection is very common in infants, particularly due to transmission from carrier mothers at birth. High carrier rates among mothers and high prevalence of perinatal infection appear to be the main mechanisms for maintaining high prevalence rate in some developing countries (13). Following acute HBV infection, the risk of developing chronic infection varies inversely with age: 90% for perinatal infection, 25–35% for infection at age 1–5 years and less than 10% for adults (14).

Hepatitis B vaccine is the mainstay of hepatitis B prevention and it has proved to be safe and highly effective in reducing the incidence of carrier rate and HBV-related mortality, in addition to providing protection against infection and disease progression (15,16). World Health Organization recommends that all infants receive HBV vaccine as soon as possible after birth, preferably within 24 hrs. and followed by 2 or 3 further monovalent or multivalent vaccines given as part of the standard infant vaccination schedule (5).

On the other hand, after primary hepatitis B immunization, anti-HBs concentrations decline rapidly within the first year and more slowly thereafter. Children who respond to a primary 3-dose vaccination series, 15–50% have low or undetectable concentrations of anti-HBs after 5–15 years of vaccination (17). Currently many years after starting the HBV vaccination program, 10% of the vaccinees still remain susceptible to HBV, especially those immunized born to infected mothers (18).

In Ethiopia, the HBV vaccine is administered as part of the pentavalent vaccine since 2007 (19). According to the Ethiopian Expanded Program on Immunization (EPI) schedules, HBV vaccine is given at 6, 10, and 14 weeks of age after delivery (20). There is insufficient evidence on the prevalence of HBV infection among children in general and to our knowledge, no data is available on the seroprotection levels of the HBV vaccination program in this part of the country since initiation of the vaccine.

The aim of this study is to assess the current prevalence of HBV infection and HBV vaccine seroprotection among children in Jimma, Southwest Ethiopia.

PARTICIPANTS AND METHODS

Study setting and design: A community-based cross-sectional study was carried out from June to December 2016 in Jimma, southwest Ethiopia. Jimma is a city located in Jimma zone of Oromia regional state in Southwestern Ethiopia. It is located at 356 km from Addis Ababa, the capital city of Ethiopia (21).

According to the data from the city health department, during the study period, the total population of Jimma city was estimated to be 194,139. Out of these, 98,907 were male while 95,233 were female. Currently, the health services of the city are supported by 53 Health Extension Workers. Single population proportion formula was used to calculate sample size by estimating the national prevalence of HBV as 7% based on a study conducted in Addis Ababa earlier (22).

All children between the ages of 5-9 years and living in Jimma were considered as the study population. Ethical approval was obtained from the AHRI/ ALERT Ethics Review Committee and Jimma University Institutional Review Board of Health Science College. Written informed consent was obtained from parents or guardians of all children who participated in this study after a clear discussion or explanation was made about the purpose of the study.

To ensure the quality of data, pre-testing of the questionnaire, standardizing of procedures and providing training for data collectors and periodic supervision was conducted. Every questionnaire was cross-checked daily for completeness and consistency. ELISA kits were checked for appropriate storage conditions and expiry dates. Internal positive and negative controls were included in each assay. Standard operation procedure (SOP) was prepared and manufacturer instructions were strictly followed. Tests were done in duplicates for samples that showed a particular pattern on the microplate and suspect samples were re-tested. For quantitative anti -HBs analysis, the team completely relied on the companys' anti-HBs standard sets.

Data collection: Face-to-face interviews were performed during house visits. Socio-demographic, cultural (behavioral) and clinical data were collected from parents or guardians.

Children were enrolled after HBV vaccination history was verified either by checking their immunization certificate (yellow card) at home or by checking their records from the nearby facility following verbal confirmation from their parents or guardians. In the absence of the vaccination card and the name of the child from the original EPI registration logbook, the child's vaccination status was recorded as unknown.

Laboratory analysis: 3-5ml of blood sample was collected from each study participant aseptically. All collected samples from different Kebeles (small administrative unit) of Jimma were transported to Jimma University Microbiology laboratory at the end of the day using a cold box with ice packs (at 40^{o}C). Serum was prepared from each blood sample, and then stored at -80^{o}C (Thermo Fisher Scientific, USA) until serologic analysis was done.

Serum level HBsAg (Bio-Rad kit, Monolisa™, HBsAg ULTRA, La Coquette, France,), Anti-HBc (using a Monolisa^{TM} Anti-HBc PLUS, Bio-Rad, La Coquette, France), and Anti-HBs (using Monolisa^{TM} Anti-HBs PLUS, Bio-Rad, La Coquette, France) were measured using commercially available Enzyme Linked Immuno Sorbent Assay (ELISA) kit. A quadratic standard curve was generated in Microplate Manager Software (Bio_Rad Microplate Manager TM version 4) using immunoglobulin anti-HBs standard sets (Bio-Rad, Monolisa™, La Coquette, France). The lower limit of detection for the anti-HBs assay was defined as ≥ 2 mIU/ml and serum titers of anti-HBs that were 10.0 mIU/ml or greater were considered as protective for vaccinated children. The tests were carried out and interpreted in accordance with the manufacturer's instructions.

Data management and statistical analysis: Completed questionnaires were brought and the data were entered into EpiData version 3.1 Data was checked for consistency and accuracy, then it was exported to SPSS version 20 software package for analysis. Chi-square test, logistic regression test, and odds ratios were used to evaluate statistically significant associations between dependent and independent variables. Variables in bivariate analysis with a p-value of < 0.25 were taken as candidates for multivariate analysis. Those independent variables which showed significant associations were reported by using p-value, odds ratios, and with 95% CI. A p-value less than 0.05 was considered as statistically significant.

RESULTS

Socio-demographic characteristics: A total of 900 5-9 years old children who were fully vaccinated, partially vaccinated, unvaccinated, or whose vaccination status was unknown were included in this study. The mean and median ages of the children were 6.6 ± 1.2 years and 7 years, respectively. From the participants, 42.4% (380) were vaccinated with three doses of HBV vaccine, 25.3% (227) were non-vaccinated, 28 were partially vaccinated, and 261 had unknown HBV vaccination status (Table 1). Further, 52.7% (472) were female while 52.5% (470) were born at home assisted by traditional birth attendants.

Distribution of HBsAg and Anti-HBc by socio-demographic variables: The overall prevalence of HBsAg among all study participants was 3.5% (31/896) with a 95% CI of 2.3% - 4.7%, and the prevalence of HBc antibody was 3.8% (34/896) with 95% CI of 2.6% - 5.1%. The prevalence of HBsAg was higher among children who were born at home (4.9%) than those born at health institutions (1.9%) (P < 0.014). Children who were born from illiterate mothers 6.4% (23/359) were more likely to be positive for HBsAg than children born from literate mothers (P < 0.001). The prevalence of hepatitis B core antibody was higher in urban children when compared to rural children (4.1% vs 1.8%). Fewer children had detectable anti-HBc among 7-9 year old children (5.2 % to 3.1 %) (**Table 2**).

Distribution of HBsAg and Anti-HBc by clinical and cultural variables: HBsAg positivity was significantly higher in non-vaccinated children, 7% (16/227) than fully vaccinated children, 2.1% (8/380) (p< 0.008). HBsAg positivity was significantly higher in those vaccinated children who had anti-HBs titer <10 mIU/ml than those who had anti-HBs titer > 10mIU/ml (p < 0.05). The prevalence of HBsAg was significantly higher among those children who had a history of hospital admission (p < 0.001) and a family history of hepatitis infection (p < 0.042). On the other hand, the prevalence of HBc antibody was significantly higher in partially vaccinated children, 10.7% (3/28), when compared to fully vaccinated children, 1.1% (4/380) (P <0.001). The antibodies to HBc persist for longer times and help to measure total HBV infection. The total HBV infection was higher in those children who had a history of hospital admission (P < 0.002) and a family history of hepatitis infection (P < 0.001) (Table 3).

Table 1. Sociodemographic characteristics of children and mothers in mother-children pair in Jimma, from June-December, 2016.

SDV#	Category	Frequency (n=900)	Valid Percent
Residence	Urban	786	87.7
	Rural	110	12.3
Marital status	Married	822	91.7
	Widowed	33	3.7
	Divorced	41	4.6
Educational level	Illiterate	359	40
	Read and write	6	0.7
	Primary (grade 1-6)	235	26.2
	Junior (7& 8)	136	15.2
	Secondary (9-10)	102	11.4
	Preparatory (11-12)	24	2.7
	Diploma & above	34	3.8
	Employed (GO/NGO)	32	3.6
Occupation	House wife	832	92.4
	Daily laborer	12	1.3
	Self-employee	17	2
	Others	7	0.7
Child's place of birth	Home	470	52.5
	Health institution	426	47.5
Child sex	Male	424	47.3
	Female	472	52.7
	5	247	27.6
Age of child	6	148	16.5
	7	239	26.7
	8	241	26.9
	9	21	2.3
Total		896	100

#: sociodemographic variable

Immunization status and mean anti-HBs distribution: Levels of anti-HBs were quantified. The highest anti-HBs titre among vaccinated children was found to be 719.45 mIU/ml. The mean, geometric mean and median anti-HBs titers among all vaccinated children were 50 mIU/ml, 14.2 mIU/ml, and 11.5 mIU/ml, respectively. The mean serum anti-HBs titer decreased from 80.62 mIU/ml at age of 5 years to 19.2 mIU/ml at the age of 9 years. Further, the anti-HBs titer was higher in female children as shown in Fig 1. Fifty of 380 (13.2%) children had anti-HBs levels above 100mIU/ml as indicated in Fig 2.

***Prevalence of protective anti-HBs concentration*:** The immune response against the HBV vaccine was assessed by quantifying anti-HBs antibody levels among all vaccinated children. A total of 222 (58.4%) of the 380 vaccinated children had a protective response to the vaccine with anti-HBs antibody levels ≥10 mIU/ml, while 158 (41.6%) of the 380 had non-protective anti-HBs antibodies level (<10 IU/ml). Of those children with protective antibody levels, 112/222 (50.5%) were females and 110/222 (49.5%) were males. About two-thirds (65.6%) of vaccinated children with protective anti-HBs level were aged between 7 and 9 years. Among all vaccinated children, 5% (19/380) did not exhibit any response. There were no significant differences in the prevalence rates of protective anti-HBs antibodies based on gender or age as summarized in Table 4.

Table 2. The distribution of children with HBsAg and Anti-HBc positivity by socio-demographic variables using c^2 tests.

Variables		HBsAg			P	Anti-HBc			P
		Negative	Positive	Total	value	Negative	Positive	Total	
Residence	Urban	758	28(3.6%)	786	0.65	754	32(4.1%)	786	0.247
	Rural	107	3 (2.7%)	110		108	2 (1.8%)	110	
Child sex	Male	409	15(3.5%)	424	0.9	410	14(3.3%)	424	0.464
	Female	456	16(3.4%)	472		452	20(4.2%)	472	
Place of birth	Home	447	23(4.9%)	470	0.014	452	18(3.8%)	470	
	Health Inst.	418	8 (1.9%)	426		410	16(3.8%)	426	0.954
Age group	5-7 Years	586	23(3.8%)	609	0.450	590	19(3.1%)	609	
	7.1-9 Years	279	8(2.8%)	287		272	15(5.2%)	287	0.124
Mom educational level	Illiterate	336	23(6.4%)	359	< 0.001	342	17(4.7%)	359	0.228
	Primary above	529	8(1.5%)	537		520	17(3.2%)	537	
Total		865	31(3.5%)	896		862	34(3.8%)	896	

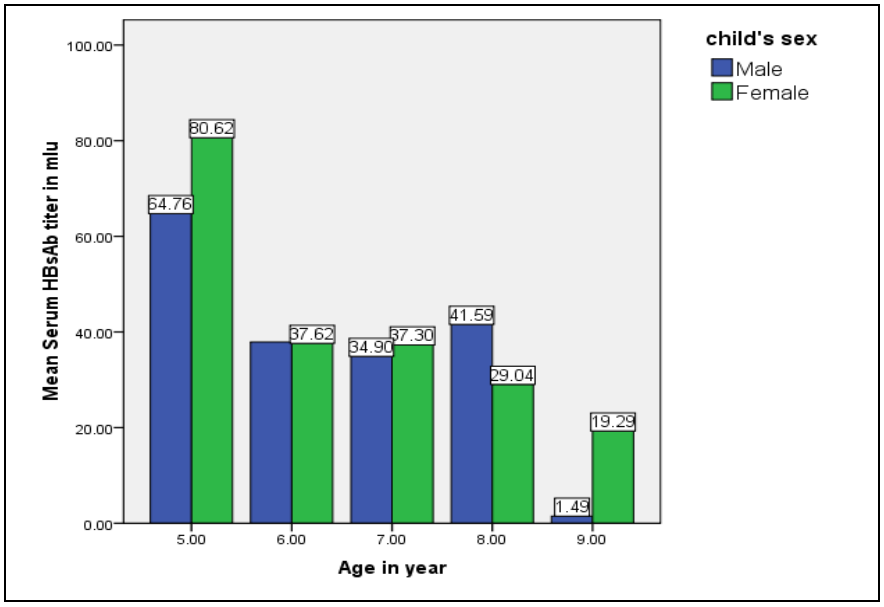

Figure 1. Distribution of anti-HBs titer among vaccinated children by age groups and sex.

Table 3. The distribution of children with HBsAg and Anti-HBc positivity by clinical and cultural variables using c^2 tests.

Clinical or cultural Variables	Category	HBsAg Negative	HBsAg Positive	HBsAg Total	P value	Anti-HBc Negative	Anti-HBc Positive	Anti-HBc Total	P value
	Vaccinated	372	8 (2.1%)	380		376	4 (1.1%)	380	
Vaccination Status	Non-vaccinated	211	16(7.0%)	227	0.008	213	14(6.2%)	227	0.001
	Partially Vaccinated	27	1(3.6%)	28		25	3(10.7%)	28	
	Unknown	255	6 (2.3%)	261		248	13(5.0%)	261	
Anti-HBs Response	<10mIU/ml	152	6(3.8%)	158	0.05	158	0(0%)	158	0.09
	>10mIU/ml	220	2(0.9%)	222		220	2(1.8%)	222	
Hospital admission	No	646	6(0.9%)	652	<	635	17(2.6%)	652	0.002
	Yes	219	25(10.2%)	244	0.001	227	17(7%)	244	
Family history of hepatitis infection	No	861	30(3.4%)	891	0.042	859	32(3.6%)	891	<
	Yes	4	1(20%)	5		3	2(40%)	5	0.001
Circumcision	No	527	20(3.7%)	547	0.908	526	21(3.8%)	547	
	Yes	337	11(3.2%)	348		335	13(3.7%)	348	0.977
Nose or ear piercing	No	617	20(3.1%)	637	0.411	611	26(4.1%)	637	
	Yes	248	11(4.2%)	259		251	8(3.1%)	259	0.481
Blood transfusion	No	864	31(3.5%)	895	0.85	861	34(3.8%)	895	
	Yes	1	.0%0	1		1	0(0%)	1	0.84
Total		865	31(3.5%)	896		862	34(3.8%)	896	

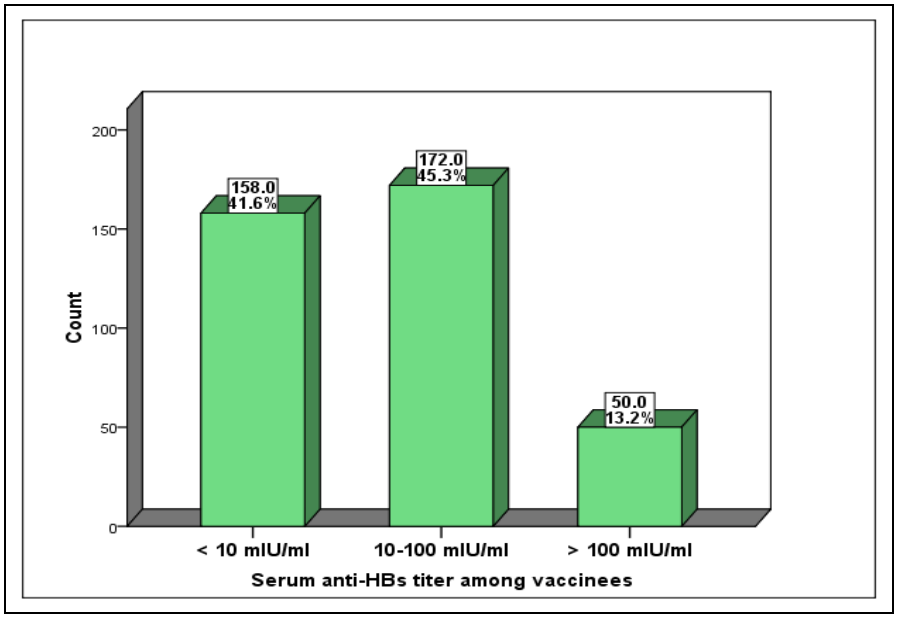

Figure 2. Serum levels of anti-HBs among vaccines.

Table 4: The prevalence of protective serum anti-HBs titers among vaccinated children according to socio-demographic/clinical/cultural variables from June-December, 2016.

Variables	Category	Anti-HBs titer		c2	P
		< 10mIU/ml	> 10mIU/ml		
Place of birth	Home	80 (46.2%)	93 (53.8%)	2.844	0.092
	Health inst.	78 (37.7%)	129 (62.3%)		
Age	5 years	55 (37.9%)	90 (62.1%)	3.395	0.494
	6 years	32 (48.5%)	34 (51.5%)		
	7years	41(44.1%)	52(55.9%)		
	8 years	27(38%)	44(62%)		
	9 years	3 (60%)	2(40%)		
Resident area	Urban	140(41.7%)	196 (58.3%)	0.009	0.924
	Rural	18(40.9%)	26 (59.1%)		
child's sex	Male	85(43.6%)	110(56.4%)	0.667	0.414
	Female	73(39.5%)	112(60.5%)		
Mother educational level	Illiterate	51(36.7%)	88(63.3%)	2.156	0.142
	Primary +	107(44.4%)	134(55.6%)		
Family history of hepatitis infection	No	156(41.4%)	221(58.6%)	0.784	0.376
	Yes	2(66.7%)	1(33.3%)		
Ear/Nose piercing	No	109(40.1%)	163(59.9%)	0.893	0.345
	Yes	49(45.4%)	59(54.6%)		
Hospital admission	No	120(42.0%)	166(58.0%)	0.068	0.794
	Yes	38(40.4%)	56(59.6%)		
Total		158 (41.6%)	222 (58.4%)		

Table 5: Efficacy of HBV vaccine against chronic infection and total HBV infection among all fully vaccinated in infancy after 5-9 years at Jimma from June - December, 2016.

HBV vaccine	**HBsAg+ %**	**Odds ratio**	**95% CI of OR**	**P value**	**Anti-HBc +%**	**Odds ratio**	**95% CI OR**	**P value**
Non-vaccinated	16/227 (7.0%)	1	-	0.004	14/227 (6.2%)	1	-	
Vaccinated	8/380 (2.1%)	0.284	0.119-0.674		4/380 (1.1%)	0.162	0.053 - 0.498	0.001

Seroprotection of HBV vaccination: There is a significant difference in HBsAg carrier status between vaccinated children (2.1%) and non-vaccinated (7%) (P-value=0.008). Additionally, HBV seroprotection (seroefficacy) is explained by using crude odds ratio for HBsAg positivity by comparing those fully vaccinated versus non-vaccinated which is 0.284, suggesting 71.6% vaccine efficacy against chronic HBsAg carriers, For anti-HBc positivity crude odds ratio was 0.162, implying 83.8% vaccine efficacy against total HBV infection as shown in Table 5**.**

Factors associated with HBsAg positivity: After adjusting for potential confounding effects, multiple logistic regression analysis was performed to identify significantly associated factors. In multiple logistic regression, hospital admission (AOR = 15.342; P <0.001), children who were born at home (AOR = 3.211, P < 0.009), and lack of HBV vaccine immunization (AOR = 2.788, P< 0.029) remained independent predictors of HBsAg seropositivity as shown in Table 6.

Table 6.Multiple logistic analysis of HBsAg prevalence for candidate variables.

Variables	Category	AOR	95% CI for AOR		P
			Lower	Upper	
Place birth	Health inst	1			
	Home	3.211	1.342	7.679	0.009
Vaccination status	Vaccinated	1			
	Non-vaccinated	2.788	1.112	6.990	0.029
Hospital admission	No	1			
	Yes	15.342	6.044	38.944	< 0.001
Family history of hepatitis infection	No	1			
	Yes	2.353	0.208	26.672	0.490

DISCUSSION

HBV infection is one of the important global public health problems, particularly in developing countries. This study is the first of its kind in Ethiopia in general and in Jimma in particular as it is the first study that used community-based cross-sectional study design to assess the seroprevalence of HBV infection and to determine the seroprotection in children after the HBV vaccine began to be administered in Ethiopia in 2007. The overall prevalence of HBsAg among 5-9 years old children was 3.5%. This finding is consistent with previous studies conducted in Karachi, Pakistan 3.3% (23) and Ivory Coast 4.2% (24). However, this figure is higher than those reported from Northwest China (25) and Central Lao (26). On the other hand, it is lower than a study reported from Northern Uganda (27). This could be explained by the difference in the age range of children, cultural practices, economic and educational status of the family, and care/place during delivery, and so on between study populations. It can also be explained by the difference in HBV vaccine commencement period, the inclusion of birth dose of the vaccine, and the difference in herd immunity of the community.

This study revealed that the presence of anti-HBc is 3.8% in the study participants, which is comparable with a previous study reported from Indonesia 3.2% once HBV infection has been acquired. Hence, the presence of this antibody may indicate past infection (that can stay chronic) or occult infection in the liver, which requires molecular methods to detect HBV DNA. High anti-HBc positivity was reported from other countries such as Colombia (6.2%), Gambia (10.2%), Ivory Coast (24%), Northern Uganda (48%), and Northwest China (14.1%) [25, 27, 29-31]. In contrast, the finding from this study was higher than what was reported from Central China (2.6%) (32). This could be due to variation in socio-demographic characteristics or cultural practices between the study populations. It may be also explained by the difference in the period for the introduction of the HBV vaccine, vaccine regimen, and the inclusion of birth dose of the vaccine.

In the present study, it was found that the frequency of HBsAg and anti-HBc positivity among the whole group of vaccinated children were 2.1% and 1.1%, respectively. The result of HBsAg positivity of this study was found to be consistent with previous studies reported from Egypt (2.0%) (33), Nicobar in India (2.4%) (34), and Sana'a in Yemen (1.8%) (35).

This figure is lower than the previous findings reported from two villages of Gambia (11.5%) (30) and Ivory Coast (17.4%) (31). On the other hand, it is higher than what was reported in studies from Nigeria (1.3%) (36) and Gambia (0.8%) (37). In addition to HBsAg prevalence, the anti-HBc positivity of vaccinated children was comparable with a previous study from Egypt (0.81%) (33) although, it may be higher than that was reported from a different location in Egypt, 0.36% (38). In contrast, our finding is lower than what was reported from a study in Guangdong Province, China (3.28%) (39). These differences may be due to the difference in the management of cold chain system, administration, and inclusion of birth dose of the vaccine. They can be also explained by the presence of vaccine escape mutants or variation in clinical or socio-demographic characteristics between study participants.

The present study found that the prevalence of HBsAg and anti-HBc among non-vaccinated children were 7.0% and 6.2%, respectively. This HBsAg prevalence of the non-vaccinated children was comparable to the previous study from Nicobar, India (9.5%) (34) and Gambia (12.4%) (37). This figure is higher than what was reported from studies in Northwest China (25) and Guangdong Province, China (39). On the other hand, the anti-HBc positivity of this study is similar to a study reported from Guangdong Province, China (5.56%) (39). However, it is lower than what was reported from a study in Nicobar, India (11.9%) (34). These differences may be due to variation in clinical or cultural practice between study participants or difference in herd immunity of the community.

According to this study, the HBsAg positivity is higher in urban areas, in 5-7years old children, in children who had lower anti-HBs titer, and in children who were born from illiterate mothers. This finding is consistent with what was reported in a previous study from Pakistan (23,40). However, our finding is inconsistent with values reported in studies from Henan, Anhui Province, China (41), Dhaka, Bangladesh (42) and Colombia (29). These differences may be explained by sociodemographic or cultural variation between study participants.

Hepatitis B vaccination of infants and children has been demonstrated to reduce the prevalence of HBsAg in many different populations that previously experienced high endemicity of HBV infection (33, 37). In this study, different HBV markers were obtained from vaccinated children. However, due to the lack of serological data either before or after vaccination among studied children, it is impossible to conclude whether these children were already infected at the time of vaccination or had been infected subsequently..

In the present study, the seroprotection (seroefficacy) against chronic HBV infection was 71.6% in the entire study population, while it was 83.8% in children that received primary vaccination 5-9 years prior to this study. Seroprotection level from this study is consistent with seroprotection level reported in a previous study from Egypt, where they had 83% seroprotection after 12 years of HBV vaccine inclusion (33). However, it is lower than what was reported in two studies from Gambia (94%) and (95.1%) (33, 37). These differences could be explained by a difference in HBV endemicity, the inclusion of birth dose of HBV vaccine, management of cold chain system, and administration schedule of the vaccine.

Moreover, the efficacy of HBV vaccine in our finding was indicated by the reduced frequency of HBsAg positivity (AOR 3.526; $p < 0.004$) and anti-HBc positivity (COR 6.178, $P < 0.001$) among vaccinated children. Hence, the hepatitis B vaccine was successful in preventing and limiting chronic carriage of HBV. The few vaccinated children who became HBsAg carriers may be due to having low anti-HBs antibody titer. In this study 6 out of the 8 HBsAg positive children had <10 mIU/ml serum anti-HBs titer. Similar findings were reported in other long-term follow-up studies from Gambia (37), China (32), and Egypt (33). Fewer children born after the introduction of the immunization program, which that included HBV vaccine, were positive for HBsAg compared to children born before the introduction of this immunization program.

In the present study, 58.4% of the vaccinated children maintained a protective level of hepatitis B surface antibodies after 5-9 years of primary infant immunization. This figure is similar to previous studies reported from Egypt (57.2%), Sana'a, Yemen (54.8%), and Alaska (50%) (35, 38, 43). It is lower than studies reported from India (72.8%), Gambia (94%), (95.1%), and Egypt (83%) (30, 33,34,37). On the other hand, this figure is higher than the findings reported from Yemen (44.2%) and East Java, Indonesia (26.5%) (28,35). The difference in these findings could be attributed to different age groups, to the different degrees of exposure to natural boosters, or to differences in nutritional status, and differences in cold chain system of vaccination.

The present study found that the protective antibody levels were higher in females, in children who were born at health institutions and in 7-9 years old children. Our finding was inconsistent with reports in studies from Yemen (35), Egypt (33) and India (34).

In contrast to our finding, higher protective titer in males was reported in a study from Yemen (35). In our study, an inverse relationship between the mean titer of anti-HBs concentration and age was observed, as antibody titer decreased with increasing age. This result is consistent with results reported in many previous studies from Egypt (33), India (34), Yemen (35), and Alaska (43).

Among the vaccinated children of our study, 41.6% had low serum anti-HBs levels (< 10 mIU/ml), indicating poor protective response even after receiving a full course of vaccine. This suggests that either these vaccinated individuals were hypo-responsive to the immunization, where their antibodies may have waned rapidly over time, or the vaccine was of poor quality. However, other studies showed that protection is still maintained among vaccinees, even in HBV-endemic country despite waning or undetectable anti-HBs levels (44, 45). Thus, the WHO does not recommend booster vaccination for individuals who have completed the three doses vaccination schedule and had primarily a response to the vaccine (5).

In this study, we also assessed the associated factors for acquiring hepatitis B virus infection. Although this study employed a cross-sectional design, it may be possible from the results that some of those independent variables are still important predictors for HBV infection after vaccine introduction. Among these factors, hospital admission and the child's birthplace were the most important independent predictors of HBV infection identified in this study. Children who were born at home were three times more likely to be infected with HBV than those who were born at health institutions. Another important predictor was lack of HBV vaccine immunization; children who have not been vaccinated for hepatitis B virus were three times more likely to be infected with HBV than those who have been vaccinated for the virus. This finding was comparable with studies reported from Henan (41), China central Lao (26), and Egypt (38). In contrast to our study, associated factors related to mothers including residence, educational level, and occupation were significantly associated with HBV infection (23,32, 40). These same studies also observed a significant association between nose/ear piercing of the child and HBV infection (23,32, 40).

In conclusion, the seroprevalence of hepatitis B infection is high among children who have not received HBV vaccine, indicating that the infant HBV vaccination can effectively prevent the transmission of the virus for a period of up to 10 years.

The prevalence of protective anti-HBs level (> 10 mIU/ml) is low among fully vaccinated children after 5-9 years of primary infant immunization, implying the inclusion of birth dose of the HBV vaccine necessity to increase its efficacy. There is a significant number of chronic carriers among non-responders of the vaccine, implying the importance of evaluating the primary response of the vaccine in children and its underlining causes. We also noted a higher prevalence among children who were born at home, suggesting likely poor sanitation and high risk of percutaneous contamination. Limitations of this study were: positive samples were not retested due to a shortage of extra ELISA kits and the risk of recall biases. Furthermore, positive samples were not checked for viral DNA markers.

ACKNOWLEDGMENTS

We would like to thank the Ethiopian Federal Ministry of Health for financial support. We are indebted to Jimma University, School of Medical Laboratory Science for facilitation of the research work. We acknowledge the support made by Jimma Zonal Health Bureau for allowing us to gather data from study participants in Jimma town. We are also thankful to all the study participants for their voluntary cooperation and involvement in this study.

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