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ORIGINAL ARTICLE

IMMUNE PROTECTION OF HEPATITIS B VACCINE AMONG CHILDREN LIVING IN GONDAR, NORTHWEST ETHIOPIA

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ABSTRACT

Background: *Hepatitis B virus infection is a worldwide health problem and highly endemic in developing countries including Ethiopia. Hepatitis B vaccine is included in the routine Expanded Program on Immunization since 2007 in Ethiopia.*

Objective: *The aim of this study is to assess the seroprotection level of hepatitis B vaccination among children who have received the vaccine.*

Methods: *A cross-sectional study was conducted on children attending kindergarten and elementary school in Gondar. A pretested structured questionnaire was used to collect the sociodemographic data. Blood samples were collected and serum separated to measure anti-HBs, anti-HBc, and HBsAg levels. Data were analyzed using SPSS statistical software version 21. Binary logistic regression analysis was done. P-value less than 0.05 was considered as statistically significant.*

Results: *Out of 431 children screened, 27 were excluded from analysis because they were positive for anti-HBc (27/431, 6.3%) and/or for HBsAg (18/431 or 4.2%). Out of the rest 404 children, 130 (32.2%) had anti-HBs titers >10 mIU/ml (seroprotected), while 274 (68.8%) had anti-HBs titers <10 mIU/ml (non-protected). Among 130 sero -protected children, 99 (76.2%) were hypo-responders (antibody titer 10-100 mIU/ml) and 31 (23.8%) were good responders (antibody titer >100 mIU/ml). In multivariate analysis, children of age 6 and 8 years old were 2.4 times (AOR: 2.436, 95% CI 1.049-5.654) (P=0.038) and 3.3 times (AOR: 3.397, 95% CI1.306-8.837) (p=0.012) better responders compared to 9 years old children, respectively. Moreover, children whose mothers had no previous history of hepatitis were 2.0 times (AOR: 2.009, 95% CI 1.101-3.665) (P= 0.023) better responders compared to their counterparts.*

Conclusion: *The seroprotection level among vaccinated children in Gondar was surprisingly low. Age and children from mothers with a history of hepatitis B infection were associated with seroprotection. The preliminary findings obtained in this study call for a thorough assessment of the effectiveness of the current hepatitis B vaccination program in this study region.*

Key words: *Hepatitis B Virus, Vaccine, EPI, Ethiopia*

INTRODUCTION

Hepatitis B virus infection is a major health problem and may lead to acute, fulminant, or chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (1 -3). Based on the prevalence of HBsAg seropositivity, HBV endemicity in the world has been classified into three categories; high (>8%), intermediate (2–8%), and low (<2%). Hepatitis B is highly endemic in developing regions with large populations, such as South East Asia, sub-Saharan Africa, and the Amazon Basin, where at least 8% of the population are HBV chronic carriers (4). As a continent, Africa has the second largest number of chronic carriers after Asia and is considered a region of high endemicity (5).

The reported prevalence rate of HBsAg seropositivity in Ethiopia ranges from 3.9% to 10.8% in community based studies (6-10).

While perinatal transmission is the most common mode of transmission of HBV infection in Asia, horizontal transmission in childhood is thought to be predominant in Africa. A lower prevalence of HBeAg positivity has been observed in mothers from Sub-Saharan Africa compared with mothers in Asia, suggesting a lower potential for vertical transmission (5). A major reason for the relatively low rate of perinatal transmission in Africa might be the lower prevalence of HBeAg positivity, which is considered a marker of active viral replication.

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From the various preventive measures employed, active immunization with hepatitis B vaccine remains to be the most important hepatitis B prevention measure to reduce the global incidence of hepatitis B virus (11).

In 2007, Ethiopia introduced hepatitis B vaccine as part of the expanded program on immunization (EPI), where it is administered at 6, 10 and 14 weeks of age in the form of pentavalent combination (12). However, there is paucity of data regarding seroprotection among the recipients of hepatitis B vaccine in Ethiopia. Therefore, the objective of this study is to evaluate the seroprotection status of children who have received the vaccine. This study can enhance our understanding of the seroprotective potential of hepatitis B vaccine in Ethiopia and can be used to influence policy makers to take the right action against hepatitis B infection.

POPULATION AND METHODS

Study design and area: A cross sectional study was conducted from December 2015 to March 2016 among children from Gondar, Northwest Ethiopia. According to Gondar Statistics Office Census report in 2015, it has a population of 315,856 living in 13 urban and 11 rural kebeles. The city has 43 public first cycle elementary schools and 46 private schools, of which 25 are kindergarten (KG) and 21 first cycle elementary schools. Children between 5 to 9 years of age from selected KG, first cycle elementary school and kebeles were considered for this study.

Sample size and sampling technique: Three private schools, two governmental schools, and three kebeles were randomly selected for this study. Children were recruited at the first day of school and during house to house visits, if they were not attending school. Parents/guardians were asked to provide an immunization chart of their child. Health extension workers working in the selected kebeles identified eligible children based on the vaccination history on their immunization chart. Only children who had hepatitis B vaccine were included in the study. In the end 431 school age children (5-9 years old) were enrolled in the study.

Data collection: Sociodemographic data, including mothers' previous history of hepatitis, were obtained using a structured pretested questionnaire administered by trained health extension workers. Further, each child's weight and height were measured**.**

Sample collection and transportation: 3-5 ml of venous blood was collected using serum separator vacutainer tubes. These samples were then transported in an box to University of Gondar, School of Biomedical and Laboratory Sciences laboratory, where serum was separated and stored at -80^{o}C until further use.

Laboratory analysis: The HBsAg enzyme immunoassay and the anti-hepatitis B nucleocapsid core (anti-HBc) antigen monolisa assay (Bio-Rad, France) were used to exclude active or previous HBV infection. The anti-HBs Monolisa Plus assay (Bio-Rad, France) was used to measure the plasma levels of anti-HBs antibodies. The assays were run according to the instructions provided by the manufacturer and all samples and standards were tested in duplicate.

Seroprotective level of antibody*:* Anti-HBsAg antibody titer ≥ 10 mIU/ml; **Non-seroprotective level of antibody**: Anti-HBsAg antibody titer < 10 mIU/ml; **Good responders:** Anti-HBsAg antibody titer > 100 mIU/ml; **Hyporesponder:** Anti-HBsAg antibody titer 10-100 mIU/ml; **Non-responders:** Anti-HBsAg antibody titer < 10 mIU/ml [13-15].

Data analysis: The data were entered into SPSS (version 21) and double checked before analysis. Descriptive statistics (means, percentages or frequency) were calculated and associations were tested using logistic regression analysis. A p-value < 0.05 was considered significant.

Ethical considerations: Ethical approvals were obtained from AHRI/ALERT research ethics review committee and the ethical and review committee of the School of Biomedical and Laboratory Sciences, University of Gondar. A support letter was obtained from the North Gondar Health Bureau. The purpose, potential risk and benefit of the study were informed to the parents/guardians. Written informed consent was obtained from the parents or lawful guardians before children were recruited into the study.

RESULTS

Table1: Serum HBsAg, HBcAb, and HBsAb levels segregated by sociodemographic status and anthropometric measurement.

Variables	Frequency Number (%) (n= 431)	Anti-Hbs> 10 m IU/ml (n= 404)		HBsAg (n= 431)		Anti-HBc (n= 431)	
		Yes N (%)	No N (%)	No N(%)	Yes N(%)	No N(%)	Yes N(%)
Sex							
Female	213 (49.4)	54(41.6)	143(52.2)	203(49.2)	10(55.6)	197(48.8)	16(59.3)
Male	218 (50.6)	76(58.4)	131(47.8)	210(50.8)	8(44.4)	207(51.2)	11(40.3)
Total	431	130	274	413	18	404	27
Age							
5 years age	91(21.1)	25(19.2)	55(20.1)	81(19.6)	10(55.6)	80(19.8)	11(40.7)
6 years age	104(24.1)	34(26.2)	65(23.4)	100(24.2)	4(22.2)	98(24.3)	6(22.2)
7 years age	139(32.3)	44(33.8)	89(32.5)	135(32.7)	4(22.2)	133(32.9)	6(22.2)
8 years age	43(10.0)	18(13.8)	24(8.8)	43(10.4)	0(0.0)	42(10.4)	1(3.7)
9 years age	54(12.5)	9(6.9)	42(15.3)	54(13.1)	0(0.0)	51(12.6)	3(11.1)
BMI percentile							
Underweight	88(20.4)	21(16.2)	57(20.8)	80(19.4)	8(44.4)	78(19.3)	10(37.0)
Normal BMI	299(69.4)	97(74.6)	189(69.0)	291(70.5)	8(44.4)	286(70.8)	13(48.1)
Over weight	22(5.1)	5(3.8)	14(5.1)	20(4.8)	2(11.2)	19(4.7)	3(11.1)
Obese	22(5.1)	7(5.4)	14(5.1)	22(5.3)	0(0.0)	21(5.2)	1(3.7)
Mothers' hepatitis history							
No	376(87.2)	106(81.5)	246(89.8)	361(87.4)	15(83.3)	352 (87.1)	24(88.9)
Yes	55(12.8)	24(18.5)	28(10.2)	52(12.6)	3(16.7)	52(12.9)	3(11.1)

% = percentile, BMI= Body Mass Index, Underweight = < 5th %ile, Normal BMI = 5th - 85th %ile, Overweight = >85th %ile, Obese = >95th %ile

Sociodemographic characteristics of the study participants: A total of 431 children were included in the study out of which 49.4% (213/431) were female. The median age of the study participants was 7 years. 69.4% (299/431) of children had normal a body mass index (BMI), while 20.4% (88/431) and 10.2% (44/431) of children were underweight and overweight, respectively. Among them, 12.8% (55/431) were born to mothers who had a previous history of hepatitis (Table 1).

Seroprevalence of HBsAg and Anti-HBc: From a total of 431 children screened, 27 (6.3%) were positive for anti-HBc. These children were excluded from further analysis. 18 (4.2%) of them were also positive for HBsAg.

From 18 children positive for HBsAg, 10 (55.6%) of the children were females and 10 (55.6%) of the children were 5 years old. From 27 children positive for anti-HBc, 16 (7.5%) of the children were female and 11 (5.0%) of the children were male. Further, higher prevalence of anti-HBc was found among 5-years old children compared to >5 years old children (Table 1).

***Seroprotection level of children to HBV vaccine*:** Most of the children (68.8% or 274/404) were non-responders (anti-HBs titre < 10 mIU/mL). However, 32.2% (130/404) had seroprotective titers of anti-HBs, which is defined as an anti-HBs titer > 10 mIU/mL. From these, 31 were good responders (anti- HBs titer > 100 mIU/mL), while the remaining 99 were hypo-responders (anti-HBs titer of 10-100 mIU/mL).

Further, from the 130 children with protective antibody levels, 54 (41.6) were female and 76 (58.4) were male and 106 of the children were born from mothers who have no previous history of hepatitis (Table1). It was observed that serum anti-HBs level was increasing with age from 19.2% at 5 years up to 33.8% at 7years. Nonetheless, a significant decline was observed at 9 years 6.9% of age.

High 9 (29%) proportion of good responders (anti-HBs titer > 100 mIU/mL) were shown at 7 years old children and the lowest 3 (0.7%) proportion of good responder (anti-HBs titer > 100 mIU/mL) were shown at 9 years old children. However, the proportion of good responders (anti-HBs titer > 100 mIU/mL) were almost equivalent in males 16 (51.6%) and females 15(48.4%) (Table 2).

Table 2: Distribution of seroprotection levels among children who received previous vaccination segregated by age and sex.

Variables	Antibody concentration in mIU/mL			Total
	Antibody titer <10 No. (%)	Antibody titer 10- 100 No. (%)	Antibody titer >100 No. (%)	
Age				
5 years	55(20.1)	18(18.2)	7(22.6)	80(19.8%)
6 years	64(23.4)	27(27.3)	7(22.6)	98(24.3%)
7 years	89(32.5)	35(35.4)	9(29.0)	133(32.9%)
8 years	24(8.8)	13(12.1)	5(16.1)	42(10.4%)
9 years	42(15.3)	6(6.1)	3(9.7)	51(12.6%)
Total	274	99	31	404
Sex				
Male	131(47.8)	60(60.6)	16(51.6)	207(51.2)
Female	143(52.2)	39(39.4)	15(48.4)	197(48.8)
Total	274	99	31	404 (100.0%)

Antibody titer < 10 mIU/mL= non-responder, Antibody titer 10 – 100 mIU/mL= hyporesponder, Antibody titer > 100 mIU/mL= good responder.

Factors associated with seroprotection level: In the bivariate analysis, male gender (COR: 1.590, 95% CI 1.048– 2.414) (P=0.039), 6 years of age (COR: 2.576, 95% I 1.127 – 5.887) (P=0.035)-), 7 years of age (COR: 2.500, 95% C.I 1.123 – 5.565) (P=0.026)), 8 years of age (COR: 3.958, 95% CI (1.554 – 10.084) (P=0.004)), and children born from mothers with history of hepatitis B infection (COR:1.848, 95% (CI 1.038 – 3.292), P=0.037) were significantly associated with seroprotection.

In multivariate analysis, children who were 6 and 8 years old were, respectively, 2.4 and 3.3 times more likely to be responders, (AOR: 2.436, 95% C. I (1.049-5.654) (p=0. 038)) and (AOR: 3.397, 95% C.I (1.306-8.837) (p=0.012)) compared to 9 years old children.

Moreover, children who had mothers with a previous history of hepatitis were 2 times more likely to respond to HBV vaccine (AOR: 2.009, 95% C. I (1.101-3.665) (p= 0.023)) compared to their counterparts. In contrast, sex and BMI had no statistically significant association with seroprotection levels (Table 3).

Table 3: Factors associated with sero-protection among vaccinated children at Gondar town, 15 December 2015 to 30 December 2016.

Variables	Frequency No. (%) (n=404)	Anti-HBs> 10 mIU/ml (n= 404) Yes No. (%)	No No. (%)	COR (95%CI)	AOR(95%CI)	P-value
Sex						
Female	213 (49.4)	54(27.4)	143(72.6)	1.00	1.00	
Male	218 (50.6)	76(36.7)	131(63.3)	1.536(1.008– 2.342)*	1.504(0.979-2.311)	0.063
Age						
5 years age	91(21.1)	25(31.2)	55(68.8)	2.121(0.896 – 5.019)	2.084(0.870-4.993)	0.099
6 years age	104(24.1)	34(34.7)	65(65.3)	2.479(1.079 – 5.694)*	2.436(1.049-5.654)	0.038
7 years age	139(32.3)	44(33.1)	89(66.9)	2.307(1.031 – 5.163)*	2.249(0.994-5.087)	0.052
8 years age	43(10.0)	18(42.9)	24(57.1)	3.500(1.361 – 8.999)**	3.397(1.306-8.837)	0.012
9 years age	54(12.5)	9(17.6)	42(82.4)	1.00	1.00	
BMI percentile						
Underweight	88(20.4)	21(26.9)	57(73.1)	0.737(0.261 – 2.077)	-----	
Normal BMI	299(69.4)	97(33.9)	189(66.1)	1.026(0.401 – 2.627)	-----	
Over weight	22(5.1)	5(26.3)	14(73.7)	0.714(0.182 – 2.800)	----	
Obese	22(5.1)	7(33.3)	14(66.7)	1.00	----	
MHH						
Yes	55(12.8)	24(46.2)	28(53.8)	1.00	1.00	
No	376(87.2)	106(30.1)	246(69.9)	0.503(0.278 – 0.908)*	2.009(1.101-3.665)	0.023

*, p<0.05;**, p<0.005, A= adjusted odds ratio, MHH=Mother Hepatitis History

DISCUSSION

In this study, the overall seroprotection level for hepatitis B vaccination was found to be very low. Among all vaccinated children, 7.7% were good responders and 24.5% were hyporesponders, while 68.8% (274/404) non-responders, meaning the majority of children were not protected from hepatitis B despite taking the vaccine. Younger age and children born from mothers with history of hepatitis B infection had association with increases seroprotection level (anti-HBs> 10 mIU/mL).

In the present study, the seroprotection level of HBV vaccine among 5-9 years old children was 32.2%. Similar findings were reported in Taiwan (37%)(16), Iran (37%)(17) and Saudi Arabia (38%)(18). Even though the seroprotection level of HBV vaccine in these studies were similar to ours, the studies were conducted on children much older (15-20 years old) than our study participants.

On the other hand, the seroprotection level of HBV vaccine in our study was lower than studies from Egypt (57.7%-67%)(19-21), Senegal (58%)(22), Iran (59.6%-79%)(23-25),and Bulgaria (67.9%)(26), and much lower than Cameroon (92%) (22) and Malaysia (96.7%)(27).

The seroprotection differences might be due to different vaccination systems, especially immunization logistics and the cold chain system of each country. For example, in Ethiopia, the nation's cold chain system was investigated in 2002. This revealed that there are various problems with the function of the country's cold chain equipment and system. Some of the identified problems were old and inadequate number of cold chain equipment, lack of maintenance, lack of spare parts and the use of different brands of refrigerators and freezers. In addition, the function of the equipment used in the cold chain system was also assessed.

According to the report, 35% of equipment were not functional, 83% of the functional equipment were aged 10 years and more than 14% of the functional equipment were sub-standard (12). Socioeconomic status may also have great influence on the seroprotection level of the children. Some of the studies were done in upper-middle-income countries like Malaysia (27) and Bulgaria (26), where endemicity of HBV infection and vaccination schedule difference among countries may play its own role on the immune protection level of the vaccine. On the other hand, the seroprotection level observed in this study is higher than what was observed in a study conducted in Egypt (8.9%) (28). A possible reason may be due to difference in age of study participants; the study participants in the Egypt were much older than the study participants in this study.

Out of the 130 children who had anti-HBs titers > 10m UL/ml (seroprotected), 76.2% and 23.8% were hypo-responders and good responders, respectively. This finding is in agreement with studies conducted elsewhere (21). In this study, as the age increased the seroprotection level for HBV vaccine steadily increased from 31.2% at 5 years to 34.7% at 6 years, 33.1% at 7 years, and 42.9% at 8 years. There was a significant decline at 9 years. Similarly studies from Bulgaria (26), Iran (23), and Egypt (9) have demonstrated that seroprotection level decreased significantly with age.

Children from mothers who have a previous history of hepatitis were 2 times more likely to be seroprotected after vaccination. This is discordant with a study conducted in Malaysia (27), where children from mothers who have a previous history of hepatitis had the same level of seroprotection as children from mothers who have no history of hepatitis. This discrepancy might be due to the recall bias in our study, since mothers were asked about their previous history of hepatitis infection.

Although several studies have shown significant association between sex and seroprotection, there was no association between sex and seroprotection in our study. The finding from this report was similar with findings from Iran (25, 29), Brazil (30) and Malaysia (27). One of the limitations of this study was that the findings of this cross-sectional study show the seroprotection level at one time point after receiving the HBV vaccination in the EPI schedule.

However, it is difficult to evaluate the immune response longevity and vaccine breakthrough infection. Therefore, further studies are required on a large scale, where influencing factors such as type of vaccine, dose, vaccine schedule, route of administration, and health status of vaccine recipients are controlled to determine the seroprotective effect of HBV vaccination at a national level.

Conclusion

The overall seroprotection level was surprisingly very low among the children included in this study. Age and children born from mothers with a history of hepatitis B infection was associated with anti-HBs > 10 mIU/mL (seroprotection level). Even though the number of children included in the study is limited to extrapolate the findings to the general population, the preliminary findings obtained from this study emphasize the need to assess the efficacy and other factors of hepatitis B vaccination being implemented in Ethiopia. As the results from this study are of great public health concern, further studies with larger sample size are recommended.

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Author's contribution: GA designed the study, participated in the collection of specimens, performed all laboratory experiments, reviewed all data and wrote the manuscript; conducted the data analyses and modified the text upon revision of manuscript. EA and AA designed the study, supervised the experiments, participated in data analysis, and participated in the writing of the manuscript. MG assisted in data collection and reviewed the initial and final drafts of the manuscript. AM, RH, AA and AA interpreted the results and reviewed the initial and final drafts of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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