Seifudin Usman, Berhanu Seyoum, Bezatu Mengiste, Azeb Tarekegne, Fiker Bekele, Adane Mihret, Abraham Aseffa, Rawleigh Howe. *Ethiop Med J, 2019, Supp. 2*

ORIGINAL ARTICLE

SERUM LEVEL OF ANTI-HEPATITIS B SURFACE ANTIGEN AND HBV ASSOCIATED FACTORS AMONG VACCINATED AND UNVACCINATED CHILDREN IN HARAR, EASTERN ETHIOPIA: A COMMUNITY-BASED STUDY

Seifudin Usman, BSc[1], Berhanu Seyoum, PhD[1], Bezatu Mengiste, BSc[1], Azeb Tarekegne, BSc[2], Fiker Bekele, MSC[2], Adane Mihret, DVM, PhD[2], Abraham Aseffa, MD, PhD[2], Rawleigh Howe, MD, PhD[2]

ABSTRACT

Background: *Hepatitis B virus infection is a major global health problem which is known to be the main cause of liver failure, cirrhosis, and hepatocellular carcinoma. Production of anti-HBs which is stimulated by HBV vaccine, provides protection against HBV infection. However, not all vaccinated children develop protective or durable levels of antibody against HBsAg. Therefore, testing for anti-HBs levels after HBV vaccination is important.*

Objective*: The main objective of this study was to assess serum level of antibody against hepatitis B surface antigen among vaccinated and unvaccinated children in Harar, Eastern Ethiopia.*

Method*: A community-based comparative cross-sectional study design was used. 540 children within the ages of 5 -8 years (284 vaccinated and 256 unvaccinated) were enrolled in the study using simple random sampling in selected kebeles. Three to five milliliters of blood was collected from each study participant. Serum was separated and anti-HBsAg level was determined using ELISA. A pre-tested, structured questionnaire was used to collect socio-demographic and HBV associated factors exposure information of the study participants and their parents. Data entry and statistical analysis were done using SPSS statistical software version 21. Logistic regressions with 95% CI were used to identify independent predictors of anti-HBs. A p-value of less than 0.05 was considered statistically significant.*

Results: *The overall seroprotection rate detected in this study was 95.4% among vaccinated children, whereas it was only 6.2% among unvaccinated children. 3.1% of unvaccinated children were positive for HBsAg, indicating chronic disease, whereas 1.1% of vaccinated children were HBsAg+. Anti-HBs levels declined from 414 U/ml at 5 years after vaccination to 105 U/ml after 8 years.*

Conclusion and Recommendation: *Protective levels of anti-HBs were detected in 95.4% of vaccinated children suggesting that there is no need for a further booster dose for these children.*

INTRODUCTION

Hepatitis B virus causes liver infection resulting in a lifelong (chronic) infection that can lead to liver scarring (cirrhosis) and liver cancer (1). Universal immunization against HBV which induces specific antibodies is the best method of preventing HBV infection. Duration of protection against HBV after Hepatitis B vaccination depends on the presence of hepatitis B surface antigen antibody (anti-HBsAb) levels in serum (2).

Immunization with this vaccine starting at birth has dramatically reduced the subsequent development of chronic hepatitis B in young children who have perinatal or early childhood exposure to HBV (3).

Following the completion of the vaccination series, the concentration of anti-HBs can decline over the years and reach levels less than 10mlU/ml. The success of childhood vaccination against hepatitis B relies on the persistence of immunity into adolescence and adulthood (4).

Ethiopia has also integrated the HBV vaccine combined in the routine Expanded Program on Immunization (EPI) since 2008 (FMOH, 2010). However, there is a paucity of information on the effectiveness of the HBV vaccination program among vaccinated children.

[1]College of health and Medical sciences, Haramaya University.
[2]Armauer Hansen Research Institute.
*Corresponding author e-mail: ahbabseifudin@gmail.com

MATERIALS AND METHODS

A community-based comparative cross-sectional study was carried out between May and October 2016 in Harar, Eastern Ethiopia. From 19 city kebele sample collection sites, 12 were selected by a lottery method. Children in the age range of 5-8 years were enrolled.

Ethical approval was obtained from the AHRI/ ALERT Research Ethics Review Committee and the Institutional Health Research Ethics Review Committee of the College of Health and Medical Science (CHMS), Haramaya University. Parents or guardians of the study participants provided written informed consent prior to enrollment in the study. Data on sociodemographic information and HBV associated factors were collected through face-to-face interviews using a structured questionnaire. Information on participants' HBV vaccination status was obtained from vaccination cards and parents' interviews. For quality control, data and specimen collectors were given three days training on the data and specimen collection tools, the objectives of the research and ethical considerations. Standard operating procedures were followed for safe sample collection and appropriate laboratory procedures.

Laboratory analysis: Three to five milliliters of blood was aseptically collected from 5-8 years old children by vein puncture in EDTA tubes. Sera were separated and stored at -20°C in the laboratory at CHMS, Haramaya University. Serum anti-HBc, HBsAg, and anti-HBs levels were detected using commercially available enzyme immunoassays (Monolisa^{TM} BIO-RAD) according to the manufacturer's instructions. Anti-HBs level ≥ 10mIU/ml was considered protective against HBV infection. All experiments were carried out at the Dire Dawa Blood Bank laboratory.

***Statistical analysis*:** Serological findings were categorized in 2 groups according to anti-HBs levels: 1) non-responders or seronegative, children with <10mIU/ml, and 2) responders, children with serum antibody level ≥10mIU/ml (1). Data were entered, checked for errors and analyzed using SPSS version 20. Results were summarized as mean, frequencies and percentages. Significance was tested with chi-square and odds ratio, and 95% CI were calculated. Independent variables with a p-value ≤0.25 in bivariate analysis were analyzed using multiple logistic regressions in order to control confounding variables. All statistical analyses were performed with SPSS software, and $p < 0.05$ was considered significant.

RESULTS

Sociodemographic characteristics of the study participants: A total of 540 study participants aged 5-8 years were enrolled in the study. The overall respondent rate was 98%. Among these, female children 307(56.9%) were slightly more represented. Mean age of the participants was 6.42 years (SD + 1.14) (Table 1). Mid Upper Arm Circumstance (MUAC) measurements of most children (504) (93.3%) were normal.

Sociodemographic information of parents: Most mothers of participants (259,48%) were merchants. Most mothers (492, 91.1%) gave birth to their child at health institutions. Almost all of the mothers (538, 99.6%) breastfed their children for more than 12 months, and 198 (36.7%) had an elementary level of education (Table 2).

Serum level of anti-HBs: Out of all study participants, 284 (52.6%), were vaccinated and 256 (47.4%) were unvaccinated. Among vaccinated children, 273 (96.1%) received the complete series of doses of HBV vaccine (Table 3).

Quantification of anti-HBs titers revealed that 5%, 35% and 60% of the vaccinated children in this study were non-responders, low responders, and good responders, respectively. On the other hand, 94%, 3% and 2.5% of unvaccinated children were non-responders, low responders and good responders, respectively. It is important to note that though 94.5% of vaccinated children had a protective anti-HBs response (anti-HBs >10mIU/mL), only 6% of unvaccinated children had a protective anti-HBs response (anti-HBs >10mIU/mL). This difference in protective anti-HBs response between vaccinated and unvaccinated children was highly significant ($p<0.000$) (Figure 1).

The mean level of anti-HBs in vaccinated children was 413.7 mIU/ml five years after vaccination. It declined to 355.2, 271 and 105.4 mIU/ml after 6, 7, and 8 years, respectively.

The overall rate of protection among vaccinated children was 95.4% but only 6.2% among unvaccinated children. Table 2 shows the distribution of vaccinated children according to their protection status by gender and age. No significant difference was detected between male and female children ($p=0.62$).

Table 1: Sociodemographic characteristics of vaccinated and unvaccinated children in Harar, Eastern Ethiopia, 2016.

	Vaccinated children		Unvaccinated children	
Variables	**Frequency (N=284)**	**N, %**	**Frequency (N=256)**	**N, %**
Age (in years)*				
5	116	40.8	41	16
6	71	25.0	55	22.7
7	65	22.9	59	23
8	32	11.3	98	38.3
Sex				
Male	131	46.1	102	39.8
Female	153	53.9	154	60.2
Ethnicity**				
Amhara	78	27.5	101	39.5
Oromo	115	40.5	61	23.8
Adare	57	20.1	80	31.3
Other	9	3.2	6	2.3
Nutritional status				
Normal	276	97.2	228	89.1
Moderate	8	2.8	28	10.9

*1 missing age value from unvaccinated children ** 1 missing ethnicity values from vaccinated and 8 from unvaccinated

Table 2: Sociodemographic information of parents of participants.

	Vaccinated children		Unvaccinated children	
Variables	**Frequency**	**%**	**Frequency**	**%**
Occupation				
Government employed	64	22.5	16	6.3
Merchant	124	44	134	52.3
Farmer	39	13.7	88	34.4
Other	56	19.7	18	7.0
Educational status				
Illiterate	44	15.5	86	33.6
Elementary	67	23.6	131	51.2
High school	99	34.6	17	6.6
College and above	74	26.1	22	8.6
Place of birth				
Home	10	3.5	38	14.8
Health institution	274	96.5	218	85.2
Duration of breast feeding (in months)				
>12	281	98.9	241	94.1
<=12	3	1.1	15	5.9
Economic status (birr/ month)				
>1000	120	42.3	12	4.7
500-1000	104	36.6	9	3.5
100-500	37	13.0	77	30.1
<100	23	8.1	158	61.7

Table 3. Vaccination status information of participants.

Variables	Frequency	Percentages
Vaccination status		
Vaccinated	284	52.4%
Unvaccinated	256	47.6%
Dose of vaccine		
Zero dose	256	47.6%
One dose	3	0.6%
Two dose	8	1.5%
Three (full) dose	273	50.6%
Year after vaccination		
5-6	176	32.6%
7-8	108	20%

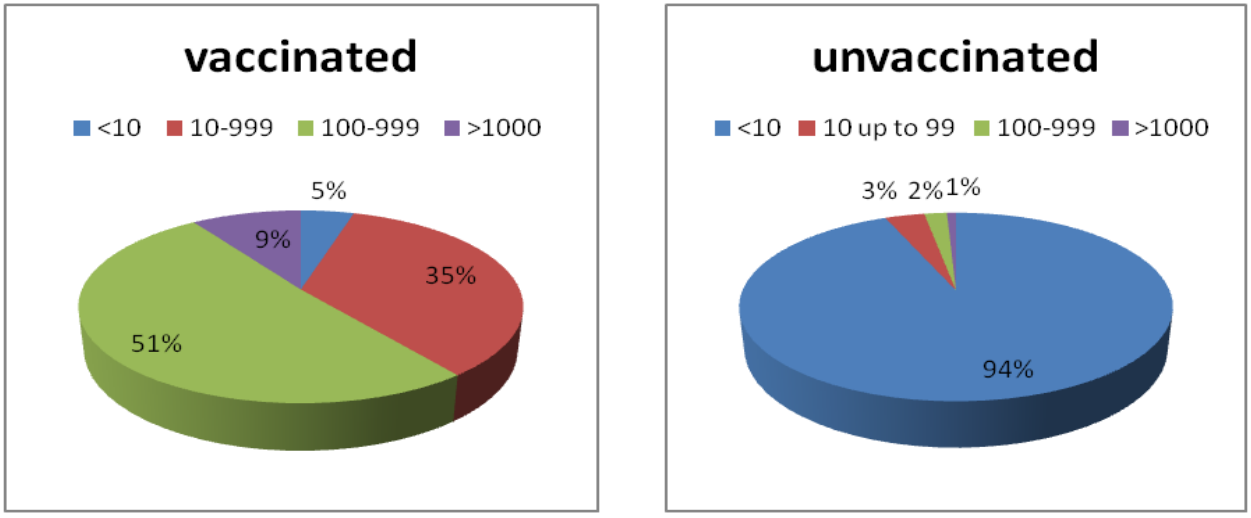

Figure 1. Serum level of anti-HBs among vaccinated and unvaccinated children in Harar, Eastern Ethiopia, 2016.

However, the mean levels of anti-HBs decreased significantly with increasing age (p=0.0001). As the age of vaccinated children increased, there was a significant decrease in both protection rate and GMT (p=0.0001). Nonetheless, these differences were not observed with unvaccinated children (P=3.24) (Table 4).

HBV infection: 23 (4.3%) out of the total of 540 children had HBV infection. Among these, 3(1.1%) were vaccinated and were positive for HBsAg, while 8 (3.1%) were unvaccinated and positive for HBsAg. Further, six (2.1%) of the vaccinated children were positive for anti-HBc, whereas 14 (5.5%) of unvaccinated children were positive for anti-HBc (Table 5).

Table 4. Prevalence of anti-HBs by gender and age among vaccinated children in Harar, Eastern Ethiopia, 2016.

Variables	Anti-HBs <10mIU/ ml, No. (%)	Anti-HBs >10mIU/ ml, No. (%)	GMT, mIU/ml, +SD	P value
Gender				
Male	5(1.7)	126(44.3)	410.8+82.5	
Female	8(2.8)	145(51.1)	207.1+ 76.5	0.62
Age, years				
5	1(0.4)	115(40.5)	413.7+ 346.7	
6	1(0.4)	70(24.6)	355.2+ 105.5	
7	4(1.4)	61(21.5))	271+ 112.3	0.000**
8	7(2.5)	25(8.8)	105.4+ 98.4	
Total	13(4.5)	271(95.4)	286.3+ 123.1	

Table 5**.** Hepatitis B virus breakthrough infection among vaccinated and unvaccinated children 5-8 years old.

Vaccination status	Age in years	Seroprotected (n)	HBV infection marker	
		Anti-HBs >10mIU/ml	Anti-HBc(+)	HBsAg (+)
Vaccinated	5	115(40.5)	4	2
	6	70(24.6)	2	1
	7	61(21.5)	0	0
	8	25(8.8)	0	0
Total		271(95.4)	6(6.2)	3(1.1)
Unvaccinated	5	5(2.0)	1	2
	6	1(0.4)	6	1
	7	4(1.6)	0	1
	8	6(2.3)	7	4
Total		16(6.3)	14(5.5)	8(3.1)

DISCUSSION

In this study, it was found that 95.4% of vaccinated children had produced a protective level of anti-HBs. However, only 6.2% of unvaccinated children had developed an anti-HBs $\geq$10mIU/mL. According to our study, the serum level of anti-HBs declined significantly overtime following vaccination to an undetectable level. Additionally, HBsAg seropositivity was 3.1% among unvaccinated children but only 1.1% among vaccinated children. As would be expected there was a significant difference between vaccinated and unvaccinated children regarding levels of anti-HBs ($p<0.001$).

A very high proportion of (95.4%) vaccinated children had a protective level of anti-HBs. This proportion was greater than that reported in a study conducted in Yazd, Iran, where only 60.2% of vaccinated children had seroprotective level of anti-HBs (5). This variation might be due to a difference in the age group of study participants. Our finding suggests a higher rate of protection against HBV than what was reported in studies from several middle and high -income countries in Asia and the Americas; in New Zeeland, 85% (6), China, 54.9% (7), USA 41% (8). This might be due to the difference in geographical location and types of vaccines.

However, it compared well with studies conducted in Turkey which reported that 96.7% of 210 HBV vaccinated children within the ages of 1-3 were protected (9).

Anti-HBs levels declined from 414 U/ml at 5 years after vaccination to 105 U/ml after 8 years. It has been widely reported that HBV vaccine induced anti-HBs titers decline to low or undetectable levels with time after vaccination. The rate of decline could vary between reports. A study conducted in Ahvaz, Iran, for example, reported that 75.4% of children still had protective anti-HBs antibody level 5 years after vaccination (2). Another study in Isfahan, Iran observed protective anti-HBsAb in 29.2% of children 6 years after vaccination (10).

The number of vaccine doses can another factor influencing serum levels of anti-HBs. In our study, nearly all vaccination children received the complete 3 doses, and 95.4% of children who received 3 doses of vaccine at infancy had persistent protective anti-HBs levels. A recent study in UK showed that 84.6% of children immunized with 3 doses of vaccine have persistence immunity (16).

From this study, it can be concluded that the vaccination of children in infancy produces adequate protection for at least 5 to 8 years post vaccination. Serum levels of anti-HBs varied significantly by age after initial vaccination, and vaccination status. In order to reduce the endemicity of HBV infection in the study area, special focus should be given to those unvaccinated children who are susceptible to HBV infection.

ACKNOWLEDGMENTS

We like to extend our deepest gratitude to the Ministry of Health for funding this study through the Clinical Research capacity Building program at the Armauer Hansen Research Institute (AHRI). We would also like to express our special thanks to AHRI and the Department of Medical Laboratory Sciences at Haramaya University College of Health and Medical Sciences for the technical support they provided.

REFERENCES

1. CDC.. Long Term Immunogenecity and Effectiveness of Hepatitis B Virus Vaccination.2012.
2. Norouzirad, R., Shakurnia, A., Assarehzadegan, M., Serajian, A. and Khabazkhoob, M. Serum Levels of Anti-Hepatitis B Surface Antibody among Vaccinated Population Aged 1 to 18 Years in Ahvaz City Southwest of Iran. TheJournal of Infectious Diseases. 2014. 14(1): e13625.
3. McMahon, B., Dentinger, C.M., Bruden, D., Zanis, C., Peters, H., Hurlburt, D., Bulkow, L., Fiore, AE., Bell, B.P. and Hennessy, T.B. Antibody Levels and Protection after Hepatitis B Vaccine: Results of a 22-Year Follow-up Study and Response to a Booster Dose. The Journal of Infectious Diseases. 2009. 200(1): 1390-6.
4. Su, T. and Chen, P. Emerging Hepatitis B Virus Infection in Vaccinated Populations: A Rising Concern? Emerging Microbes and Infections . 2012. 1: e27
5. Karimi, M., Raee, A., Baghianimoghadam, B. and Fallahzadeh, M.H. Vaccine-Induced Anti-Hbs Level in 5-6 Year-Old Malnourished Children. Hepatitis B Monthly. 2013. 13(2): e7048.
6. Mendy, M., Peterson, I., Hossin, S., Peto, T., Jobarteh, M., Jeng-Barry A., Sidibeh, M., Jatta, A., Sophie, E.M., Andrew, J.H. and Whittle, H. Observational Study of Vaccine Efficacy 24 Years after the Start of Hepatitis B Vaccination in Two Gambian Villages: No Need for a Booster Dose. PLoS ONE. 2013. 8(3): e58029.
7. Luo, Zh., Li, L. and Ruan, B. Impact of Implemantation of Vaccination Strategy on Hepatitis B Virus Infection in China over 20 Years. The Journal of Infectious Diseases. 2012. 16: e82-e88.
8. Elizabeth, W., Hwang, M. and Cheung, R. Global Epidemiology of Hepatitis B Virus (Hbv) Infection. American journal of medicine and science. 2011. 4(1): 7-13.
9. Ergunay, K., Balaban, Y., Cosgun, E., Alp, A., Simsek, H., Sener, B., Tatar, G. and Harscelik, G. Epidemiologic Trends in Hepatitis B Virus Infection at Reference Center in Turkey: 11 Year Retrospective Analysis. Annals of Hepathology. 2012. 11(5): 672-8.
10. Aghakhani, A., Banifazl, M., Izadi, N., McFarland, W., Sofian, M., Khademsadegh, A., Pournasiri, Z., Foroughi, M., Eslamifar, A. and Rida, A. Persistence of Antibody to Hepatitis B Surface Antigen among Vaccinated Children in a Low Hepatitis B Virus Endemic Area. Wolrd Journal of Pediatrics. 2011. 7(4): 358-60.
11. Keck, J., Bulkow, L., Gregory, A., Raczniak, R., Susan, E., Negus N., Carolyn, L.Z., Michael, G.B., Philip, R.S., Eyasu, H.T. and Brian, J.M. Hepatitis B Virus Antibody Levels 7 to 9 Years after Booster Vaccination in Alaska Native Persons. Journal of Clinical and Vaccine Immunology . 2014. 21(9): 1339-42
12. Jorgensen, P.,Poethko, M.C.,Hellenbrand, W.,Jilg, W.,Thierfelder, W.,Meyer, C.,Erheiden, M., Schlaud, M. and Radun, D. Low Hepatitis B Immunogenicity of a Hexavalent Vaccine Widely Used in Germany: Results of the German Health Survey for Children and Adolescents, 2003–2006. Journal of Epidemiological Infectious Diseases. 2010. 138: 1621-29.
13. Elian, F. and Shubair, M. Evaluation of the Efficacy of Hepatitis B Vaccine in Different Age Groups of Immunized Children in Gaza Strip. Islamic University Journal. 2006. 14(2): 91-103.
14. Salama, I.I., Sami, S.M., Salama, S.I., Foud, W.A., Abdelhamid, A.T. and Said, Z.N. 2014. "Persistence of Protection to Hepatitis B Vaccine and Response to Booster Dose among Children and Adolescents in Dakahleya- Egypt. Egyptian Journal of Immunology. 2014. 21(1): 13-26
15. Sami, S.M., Nabil, Z., Said, A., Salama, I.I., El-Sayed, M.H., Eletreby, L.A., Rabah, Th.M., Elmosalami, D., Abdelhamid, A.T., Salama, S.I., Abdelmohsen, A.M., Emam, H.M., Elserougy, S.M., Hassanain, A.I., Abdulhalim, N.F., Shaaban, F.A., Hemeda, S.A., Ibrahim, N.A. and Metwall, A.M. Effectiveness of Hepatitis B Virus Vaccination Program in Egypt: Multicenter National Project. World journal of Hepatology. 2015. 7(22): 2418-26
16. Park, S. H. Trends in the Seroprevalence of Hepatitis B Surface Antigen in the South Korean Population. International Journal of Infectious Diseases. 2012. 16: e669-72.