

Effect of Breast-feeding and Formula- feeding on Antibody Response of Hepatitis B VaccinationMohsenzadeh A¹, Ahmadipour SH¹, Firouzi M¹, Babaei Homa², Anbari KH³

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Abstract: Background and aim: About one-third of the world's population has some serologic evidence of suffering infection by hepatitis B virus (HBV). One of the effective measures for reducing the mortality because of hepatitis B virus is vaccination at birth time, 2 months and 4 months old. The criterion for effectiveness of hepatitis B vaccine is enough production of antibody. The aim of this study is to investigate the immunity response to hepatitis B vaccination in breast-fed and formula-fed infants vaccinated at the age of 12-24 months. **Material and methods:** This descriptive study is conducted using accessible sampling based on entrance criteria on healthy 12-24 months old infants who received 3 dose of hepatitis B vaccine. one group was 51 formula-fed infants and the other 56 breastfed infants. Blood sample was taken from both groups to measure the antibody titer of hepatitis B using ELISA experimental method, and finally both groups were compared based on their antibody level and age, sex and the type of feeding. **Results:** Both groups were the same with regard to age average and sex. Overall 9 infants (8.4%) had antibody titer of lower than 10 mIU/ml and didn't response well to vaccine, among whom 1 was breast-fed and 8 ones were formula-fed infants. HBS-Ab average was 124.4±55.9 mIU/ml and 91.7±51.1mIU/ml in the first and second group respectively (p=0.002). The response in the first group (98.2%) had a meaningful difference with the second group (84.3%) (p=0.009). In each group, there was no significant statistical difference between girls and boys in responding the vaccine, but in both groups overall, the boys had more immune response to hepatitis B vaccine compared to girls (p=0.004). **Conclusion:** The results of this study showed that breastfed infants respond well to hepatitis B vaccine and the average antibody titer is higher in boys.

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Introduction

One of the common viral infections in the world is hepatitis B virus and about 1/3 of the world's population have the serologic evidence of being infected by hepatitis B virus (HBV) (1). In 1-5% of adults and 90% of infants infected with hepatitis B virus, there is chronic infection (2). Although right now there is no definitive treatment for hepatitis B complications like hepatic cirrhosis and carcinoma, but one of the basic steps to reduce the mortality and complications related to HBV, is timely vaccination, and according the recommendation of the World Health Organization (WHO) hepatitis B vaccination is performed routinely in 3 dose, at the birth time, 2 months and 6 months old (0.5cc intra muscular) but for infants lower than 2 kg birth weight ,it is performed in 4 doses (3,4). About 5-15% of infants do not respond well to this vaccine (5) and the effectiveness criteria for hepatitis B vaccination is producing enough antibodies (HBS-Abs). If the antibody titer in 10 mIU/ml or higher, the body has responded to the vaccine appropriately and the higher antibody titer, means the more immune response (6,7).

HBV is not transferred through breastfeeding, so in mothers with HBS-Ag⁺ there is no limitation for breastfeeding, but the hepatitis B immunoglobulin (HBIG) should be injected to the infants in addition to Hepatitis B vaccine in the first hours after birth to ensure enough immunity against hepatitis B (8). In addition to the role that breastfeeding plays in nutrition and growth of infants, it is full of different antibodies, cytokines and immune cells and results in the evolution of immune and nervous systems and protection against infections and allergies in infants (9,10,11).

By increasing the period of breastfeeding, 1.5 million mortality is prevented in the countries with high mortality rate (12,13).

Oligosaccharides play an important role in promoting the immune system quality and human milk has higher neutral and acidic oligosaccharide compared to the cow milk (14,15).

During pregnancy, IgG antibodies from mother enter the fetus body through placenta and give immunity to the infant. 6-12 months after birth the antibodies with maternal origin decrease in the infants' body, although by appropriate and enough

breastfeeding the infant can receive enough antibody from mother, (16). In 1903, Schlossman and Moro detected antibodies in mothers' milk, which play an important role in the immune system of the infants (17).

Breastfeeding has important effects on the first six months' vaccination of infants and helps the mother and infant to bear the stressful situations when prescribing injective vaccines and also promotes the effects of vaccines, elevates the immunologic and Entero-hepatic system in infants and can alter the ethyl-mercury metabolism derived from some vaccines (18). There is much evidence, which shows that breastfeeding can empower the immunologic response after vaccination (19).

With regard to the mothers' milk and the importance of hepatitis B infection and the possibility of inadequate response after hepatitis B vaccination in some infants, this study has been conducted to investigate and compare the immune response after full hepatitis B vaccination in breastfed and formula-fed infants.

Materials and methods

This study is a partial descriptive research conducted using accessible sampling based on the entrance criteria on the healthy infants of 12-24 months old referred to Pediatrics clinic of Shahid Madani educational hospital, Khorram Abad, Lorestan, Iran.

In this study, 56 and 51 infants who were breastfed and formula-fed respectively from birth were investigated. All of them were term infants with the gestational age of ≥ 37 weeks and ≥ 2500 gr birth weight.

In both of the groups no hospitalization, Immunodeficiency disorder and immunoglobulin or blood products receiving was found and their mothers were HSBS-Ag negative. All of the infants had received hepatitis B vaccine (Hepavax-Gen, made in Korea) according to the national routine for about 0.5 cc intra-muscular at the birth time, 2 months and 6 months.

The information related to the negativity of HSBS-Ag in mothers and full hepatitis B vaccination was received from infants' health care records.

In the case of the parents' written consent to conduct the test, 1cc of blood clot was taken in Sina laboratory in aseptic conditions and HSB-Ab level was calculated through ELISA method (Biomerieux, USA). If the HSB-Ab titer was 10mlU/ml or more, it was taken as the appropriate response to the vaccine.

The test was conducted free and no cost was incurred for parents. The information was analyzed using SPSS software and statistical tests of Chi-squared test and t-test and Pearson's correlation

coefficient were analyzed statistically. In this study, the significance level of lower than 0.05 is considered.

Results

In the population under study, 80 ones (74.8%) were under 18 months and 27 subjects (25.2%) were between 18-24 months. The average age in breastfed and formula-fed groups was 15.3 ± 4.1 and 16.2 ± 4.2 months, respectively.

In breastfed group, there was 31 girls (55.4%) and 25 boys (44.6%), while in formula-fed group there was 25 girls (49%) and 26 boys (51%) and according to the statistical t-test and Chi-squared test, there is no statistical difference between two groups with regard to age ($p=0.35$) and sex ($p=0.41$).

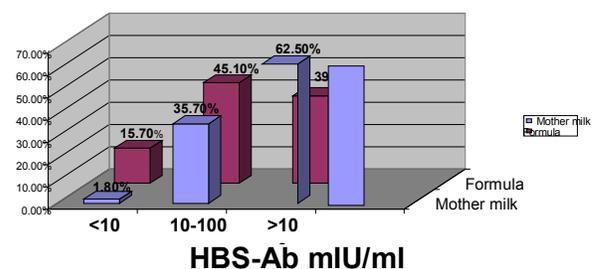
Hepatitis B antibody titer in both groups is divided into 3 levels of 10, 10-100 and above 100 mlU/ml.

The study of frequency distribution in different levels of antibody according to the feeding type and sex in all of the infants under study showed antibody response was lower than 10 mlU/ml in 10% of girls and 7% of boys.

In 107 infants studied, 9 ones responded inadequate to hepatitis B vaccine, in a way that in 1 of the breastfed infants (1.8%) and 8 ones in formula-fed group (15.7%) the HBS-Ab titer was lower than 10 mlU/ml.

In 20 breast-fed infants (35.7%) and 23 formula-fed ones (45.1%) the hepatitis B antibody titer was between 10-100 mlU/ml.

In 35 breastfed infants (62.5%) and 20 formula-fed ones (39.2%) the HBS-Ab titer was above 100 mlU/ml. (graph 1)



Graph 1: Relative frequency distribution of Anti HBS-Ab level in the infants under study based on feeding type

Fisher exact tests the difference in antibody level of infants under study is significant based on the feeding type ($p=0.009$), but it is not significant based on their sex ($p=0.35$) (table 1).

Table 1- Frequency distribution of different levels of Anti HBS-Ab in the infants under study based on sex and feeding type

Pvalue	total	>100	10-100	<10	HBS-Ab levels mIU/ml	
					Female	Male
0.35	50(100%)	22(44%)	23(46%)	5(10%)	Female	Sex
	57(100%)	23(57.9%)	20(35.1%)	4(7%)	Male	
0.009	56(100%)	35(62.5%)	20(35.7%)	1(1.8%)	Breastfed	Feeding type
	51(100%)	20(39.2%)	23(45.1%)	8(15.7%)	formula	

In table 2, the comparison between average and standard deviation of HBS-Ab values is presented for all of the infants under study based on age, sex and feeding type.

In the infants under 18 months the average antibody was higher than 18-24 months infants, but

based on the statistical independent t-test this difference was not significant statistically (p=0.11).

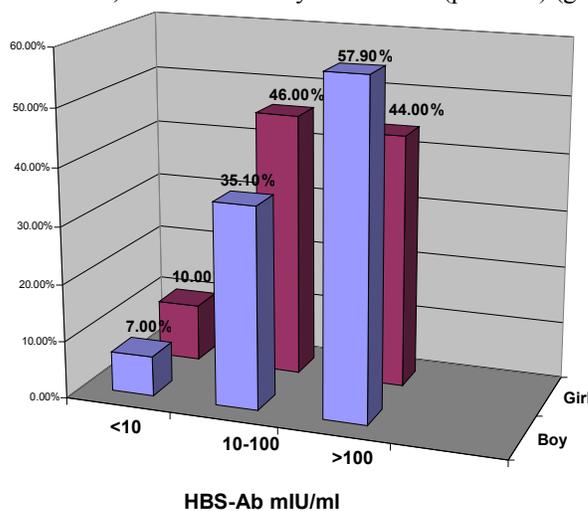
In addition, by comparing the average antibody value based on nutrition according to independent t-test the average antibody value in breastfed infants is higher than formula-fed ones (p=0.002).

Table 2- Comparison between the average and standard deviation values of anti HBS-Ab in infants based on age, sex and feeding type

P value	The type of statistical test	variable		
		Value	Category	Variable
0.11	T test	113.1±59.8	Under 18	Age (month)
		96.4±40.8	18-24	
0.044	T test	97.3±53.1	female	Sex
		119±56.9	Male	
0.002	T test	124.4±55.9	breastfed	Feeding Type
		91.7±51.1	formula	

The difference between antibody values average is significant based on sex, in such a way

that in girls the antibody average is lower than boys are (p=0.044) (graph 2).



Graph 2: The relative frequency distribution of anti HBS-Ab levels in infants under study based on sex

In table 3 and 4 the average antibody values in breastfed and formula-fed infants is studied based on age and sex; although in breastfed and formula-fed group the antibody value is higher in boys than girls, but based on independent t-test this difference is not significant (p=0.15) and (p=0.18).

The average antibody is significantly higher in breastfed infants younger than 18 months infants are (p=0.45), but in formula-fed infants the difference was not statistically significant (p=0.86).

Table 3- Comparison between average and standard deviation of anti HBS-Ab values in breastfed infants based on age and sex

P value	The type of statistical test		variable	
0.045	T test	128.9±59.9	Under 18	(month) Age
		104.1±25.1	18-24	
0.15	T test	112.6±51.6	female	sex
		134±58.2	male	

Table 4- Comparison between average and standard deviation of anti HBS-Ab values in formula-fed infants based on age and sex

Pvalue	The type of statistical test		variable	
0.86	T test	94.6±53.4	Under 18	Age (month)
		89.5±47.7	18-24	
0.18	T test	82.1±51.7	female	sex
		101±50.7	male	

In all of the infants in both groups, there is a reversed linear relationship between age and antibody titer based on Pearson correlation coefficient and the antibody value decreases by age ($P=0.024$, $r=-0.31$).

Discussion

The results of this study showed that the response to hepatitis B vaccine is higher in infants who are breastfed from birth compared to formula-fed infants.

In the study conducted by Hahn and colleagues the antibody value in infants was measured after OPV vaccine injection and in spite of the fact that the antibody value was higher in low-protein formula-fed infants than the group who received conventional formula, but it was lower compared to the solely breastfed infants (20). In addition, the immunity value was higher in breastfed infants after diphtheria, tetanus and pertussis vaccination (DTap).

Azzari and colleagues in Italy measured the subclasses of HBSAb IgG in healthy infants born from HBS-Ag positive mothers and received full hepatitis B vaccine, they found that the HBS-Ab value in IgG2 subclass was 3 times higher in breastfed group compared to formula-fed infants but HBS-Ab IgG1 in formula-fed group was higher. There was no difference in IgG3 subclass between two groups (21). In this study, the total HBS-Ab titer in healthy 12-24 months infants with HBS-Ag negative mothers was measured and antibody titer was significantly higher in the group who were solely breastfed for 6 months compared to formula-fed group.

In a study, Pichichoro found that breast-feeding is very effective in increasing the response of oral rhesus rotavirus vaccine sero-conversion (22).

In a study by John et al, no difference was found in the response to the first and third doses of

oral live poliovirus vaccine type 1,2 and 3 (OPV) between breastfed and formula-fed infants and breastfeeding has no effect on the amount of antibody production in infant after receiving OPV vaccine (23). However, Chandra reported in 1978 that poliovirus antibodies in mother's milk might interfere with live attenuated poliovirus vaccine (24).

The results of the study by Silfverdal et al. shows that infant solely breastfed for 90 days or more had better serologic response to conjugate Hib and pneumococcal serotype 6B and 14 vaccines compared to the infants breastfed less than 90 days (25). In this study, HBS-Ab production in infants solely breastfed for at least 6 months is higher than bottle-fed ones.

In a research by West et al., the immunologic response to Hib vaccine in breastfed infants for less than 6 months is significantly higher than the infants who were breastfed for 6 months or more (26).

In a study by Wold et al. antibody production against live viral vaccines (MMR) is lower in breastfed compared to bottle-fed infants and it is probably because of the present of IgA in mother's milk which has antiviral feature and the virus in the vaccine cannot replicate in the digestive system and stimulate the infant immune system (27).

Furthermore in a study in Kermanshah carried out by Hemmati et al, on premature infants born from HBS-Ag⁻ mothers, 1 month after injecting the last turn of hepatitis B vaccine (in month 7) 86.8% of premature infants responded 100% to hepatitis B vaccine (28).

In a study by Zahedpasha et al. in Babol, on the amount of response to hepatitis B vaccine in premature and mature infants of 12-24 months, it was evaluated that both groups responded 100% to the vaccine and in premature infants there was no

statistical difference according to antibody titer in boys and girls, but in term infants boys had higher titer than girls. In addition, in all of the infants the average antibody titer was significantly higher in boys than girls (29).

In this study in term infants, 91.6% responded well to the hepatitis B vaccine, but in 8.4% there was inadequate response (titer less than 10 mIU/ml) which was found more in the group who did not receive their mother's milk, in addition the average antibody value was higher in all of the boys compared to girls, but no significant difference was found between infants in breastfed and formula-fed groups according to their sex.

In the study conducted by Yang et al. 37% and 94% of premature and mature infants responded well to hepatitis B vaccine at the age of 4-7 years old (30).

In a study on term infants by Pickering et al, the antibody titer of tetanus and OPV was higher in infants who were fed by human milk compared to those who were fed by formula containing nucleotide, but antibody titer of Hib and Diphtheria was higher significantly in nucleotide-formula-fed (31).

In this study, although antibody decreases by age and antibody titer in less than 18 months infants is higher than 12-24 months infants, but this difference is not significant statistically. In comparing sex with average hepatitis B antibody, in all of the infants studied in this research, boys had more antibody production compared to girls and there is a meaningful relationship between the amount of hepatitis B antibody (HBS-Ab) and the feeding type. In the solely breastfed group, the higher antibody titer was seen compared to the formula-fed infants who did not receive their mother's milk. The reason for higher average antibody in boys compared to girls is not clear and it needs to be investigated in future researches.

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