

Serum Level of Anti-hepatitis B Surface Antigen among Newborns and Fully Vaccinated Infants and Children Aged 6 to 11 Years

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Abstract: Objectives: To assess the antibody level against hepatitis B surface antigen (anti-HBs) among newborns and their mothers and among recently vaccinated infants at age of 9 and 12 months. It also aimed at assessing the long-term immunity to hepatitis B among vaccinated children aged 6–11 years according to Egyptian expanded immunizing protocol. Methodology: Total no of 323 vaccinated healthy infants and children plus 45 newborns and their respective mothers were included in this study. Anti-HBs titer was assessed among 45 newborns and their mothers, recently vaccinated infants at age of 9 and 12 months (30, 51) respectively and 245 children aged 6–11 years. Quantitative ELISA technique and mean anti-HBs level \pm Standard Error (SE) were detected. Results: No detectable antibody level was found among newborns or their respective mothers. The mean level of anti-HBs among 30 vaccinated infants aged 9 months were 426.8 ± 71.2 IU/L and 367.4 ± 52.6 IU/L among 51 vaccinated infants aged 12 months. In a group of children (n=242) aged 6-11 years, the total mean anti-HBs level was 54.8 ± 19.5 IU/L. Among children aged 6-11 years, only 95 (39.3%) of them had protective level of anti-HBs (≥ 10 IU/L). The mean level of anti-HBs decreased significantly with increasing age ($P = 0.026$). A significant negative correlation was found between current age and anti-HBs levels ($P < 0.0005$). No significant difference was detected between males and females or among different level of socioeconomic status ($P > 0.05$). Conclusion: More than half of the studied children had non-protective level of anti-HBs and this puts them at risk of infection. The failure to achieve satisfactory seroprotection level by the national immunization programme reflects the need to re-evaluate the current hepatitis B vaccination strategy in Egypt.

Key words: Hepatitis B virus, anti-HBs level, primary vaccination, Egypt.

INTRODUCTION

Hepatitis B infection is major public health problems in Egypt. Egypt is considered as a region of intermediate prevalence for HBV infection with reported figure of 4.5% (WHO, 2007). The prevalence of chronic HBV infection varies geographically, from high ($> 8\%$), intermediate (2-7%) to low ($< 2\%$) prevalence (Hou *et al.*, 2005). Infection with HBV in infancy or early childhood may lead to a high rate of persistent infection (25–90%), while the rates are lower if infection occurs during adulthood (5–10%). In most endemic areas, infection occurs mainly during early childhood and mother-to-infant transmission accounts for approximately 50% of the chronic infection cases (Chang, 2007).

Neonatal HBV vaccination is the most effective measure for prevention of HBV infection in countries with intermediate to high levels of HBV endemicity (Puvacic *et al.* 2004). A compulsory vaccination programme against hepatitis B infection among infants was started in Egypt in 1992 using a yeast recombinant DNA vaccine (10 μ g) and with a schedule of 2, 4 and 6 months of age (Mansour *et al.*, 1993). HBV is acquired by vertical transmission from an HBsAg positive mother or via horizontal transmission in childhood. (Sung, 1997). The Advisory Committee on Immunization Practices (ACIP) that updates the strategy to eliminate

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transmission in the United States recommended that all infants born to women without documentation of HBsAg test results should receive the first dose of single-antigen hepatitis B vaccine (without HBIG) <12 hours of birth (Mast *et al.*, 2005).

Seroprotection is assured when hepatitis B surface antibody (anti-HBs) level is ≥ 10 IU/L (Floreani *et al.*, 2004 and Yu *et al.*, 2004) but more needs to be learned about the duration of protection and indication for booster doses (Lu *et al.*, 2004). According to the Viral Hepatitis Prevention Board, the 2 schedules most widely used for the hepatitis B vaccine are 0, 1, 6 months and 0, 1, 2, 12 months, both of which have been shown to be equally effective and can control perinatal infection. Increasing the time between the 1st and 2nd doses and 2nd and 3rd doses appears to increase antibody levels (Middleman *et al.*, 2001).

Current guidelines for vaccination is 3-doses of hepatitis B vaccine to be initiated on the first day or at the latest by two months of age. At least 96% of vaccinated infants developed an anti-HBs level of at least 10mIU/ml. Efficacy trials are associated with protection against acute HB disease or chronic HBV infection in the US (Centers for Disease Control, 2008). Long term protection of 10-12 years appears to occur in those infants who are at high risk or whose mothers were positive for hepatitis B surface antigen (HBsAg) (Huang *et al.*, 1999). However, the duration of protection in low risk infants whose mothers are negative for HBsAg and who receive hepatitis B vaccine from birth is unknown. In these populations, the risk of HBV infection increases during adolescent and early adulthood (CDC, 2007).

The aim of this study was to assess the presence of anti-HBs in new born and their mothers, and to assess anti-HBs titer level among recently fully vaccinated infants at age of 9 and 12 months. As well as to evaluate the fading titer in children aged 6–11 years who had received a full vaccination course according to Egyptian Expanded Immunizing Protocol (EEIP) during infancy.

MATERIALS AND METHODS

This cross-sectional study was conducted on 45 newborns & their respective mothers, 81 infants and 242 children. Infants were randomly recruited from Pediatric Hospitals Inpatient Department and Outpatient Clinics, Ain Shams University in a period from Jan 2004 to Dec 2004. They were divided into three groups: newborns (n= 45), infants aged 9 months (n= 30) and infants aged 12 months (n= 51).

The 242 children age 6-11 years were randomly selected from children attending the Pediatric Clinic of Health Insurance seeking medical advice for illnesses such as anemia, headache, visual problems and school accidents. All children were received three doses of hepatitis B vaccine as recorded on the back of the child's birth certificate. A questionnaire was designed and administered to the parents or caretakers of the children to collect demographic data (age, sex and socioeconomic status) and history of hepatitis B vaccination in infancy. Socioeconomic status was determined according to Fahmy and Sherbiny (1983). Parents were informed about the aim of the study and their consent for their children to be included in the study was taken. The study exclude infants and children with history of hepatitis or having chronic diseases and all infants included in the study were subjected to careful history taking and thorough clinical examination.

Blood was drawn aseptically by vein puncture and serum was separated by centrifugation and stored at -70°C . Samples were thawed for the quantitative determination of antibody to HBV by competitive enzyme-linked immunosorbent assay and according to the manufacture instructions (DiaPro, Milan, Italy). Antibody level was determined quantitatively by means of a standard curve calibrated against the World Health Organization reference preparation. Antibodies to hepatitis B surface antigen (anti-HBs) ≥ 10 mIU/ml, was considered, according to international standards, to be protective against this infection (Mast *et al.*, 2004).

Data were analyzed using SPSS, version 13. Descriptive analysis was done using mean \pm SE (Standard Error). To test for difference in anti-HBs protective levels chi square test was used. As the data are not normally distributed, the Mann–Whitney test was used to compare differences between 2 means and kruskal wallis test to compare between more than two means. Correlation test was used to test relation between age and level of anti-HBs.

RESULTS AND DISCUSSION

No detectable antibody against HBsAg was found in sera of newborns or their respective mothers. The mean level of anti-HBs among 30 vaccinated infants aged 9 months were 426.8 ± 71.2 IU/L and 367.4 ± 52.6 IU/l among 51 vaccinated infants aged 12 months. Among 242 children aged 6-11 years, the total mean of anti-HBs level was 54.8 ± 19.5 IU/ml. Only 95 (39.3%) of the children had protective anti-HBs (≥ 10 IU/L). The mean level of anti-HBs decreased significantly with increasing age ($P = 0.026$) (Table 1). Only 9.9% of

them had titres > 100 IU/L. The percentage of children with a titre < 10 IU/L increased with age. There was a significant negative correlation between current age and anti-HBs levels ($r = -0.31$, $P = 0.041$). Figure 1 shows mean level of anti-HBs at different age groups.

Table (2) shows the distribution of the children according to gender and protection status. No significant difference was detected between males and females. Although the seroprotection level (10 IU/L) of anti-HBs increased gradually with increase in the socioeconomic status, it was not statistically significant ($P = 0.364$) (Table 3).

Table1: Distribution of children according to the level hepatitis B surface antibodies (anti-HBs) and age.

Variable	anti-HBs level (IU/l)<10 IU/L		anti-HBs level (IU/l) ≥ 10 IU/L		Total	Anti-HBs level(IU/l) (SE)	
	NO	%	NO	%		Mean	SE
Age							
9m	1	3.3%	29	96.7%	30	426.8	(71.2)
1y	8	15.7%	43	84.3%	51	376.4	(52.6)
6y	21	52.5%	19	47.5%	40	79.2	(24.4)
7y	21	52.5%	19	47.5%	40	70.8	(23.1)
8y	20	51.3%	19	48.7%	39	61.5	(19.2)
9y	25	61.0%	16	39.0%	41	52.9	(21.1)
10y	35	81.4%	8	18.6%	43	19.4	(11.1)
11y	25	64.1%	14	35.9%	39	44.8	(18.1)
Total	156	48.3%	167	51.7%	323	139.8	(14.7)

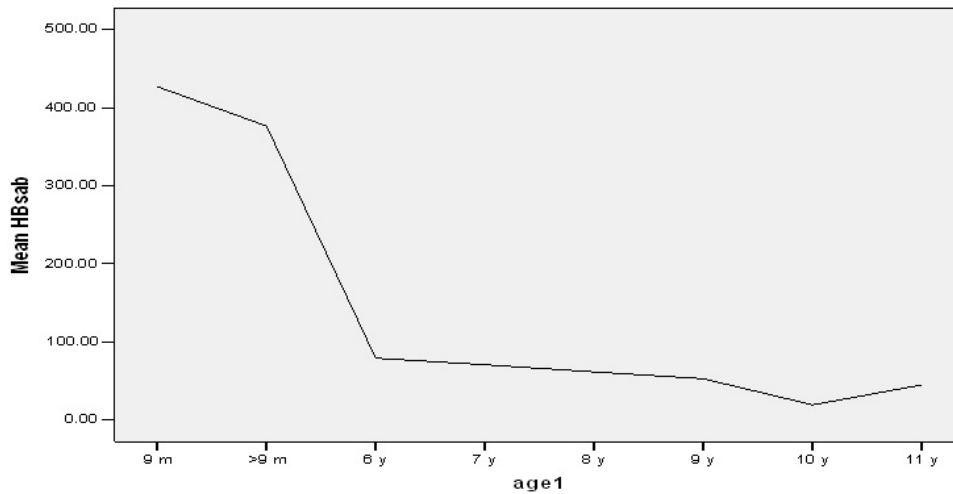


Fig. 1: Mean level of anti-HBs in relation to the age of children.

Table 2: Distribution of children according to the level hepatitis B surface antibodies and Sex

Sex	Anti-HBs level (IU/L)<10 IU/L		Anti-HBs level (IU/L) ≥ 10 IU/L		Total no	Anti-HBs level(IU/L) (SE)	
	No	%	No	%		Mean	SE
Males	43.4	72	56.6	4	166	154.1	(21.7)
Females	53.5	84	46.5	73	157	124.7	(19.6)
Total	48.3	156	51.7	167	323	139.8	(14.7)

P value of Chi Square > 0.05

Table 3: Distribution of children according to the mean level anti-HBs and socioeconomic status

Socio- economic status	anti-HBs level (IU/L)<10 IU/L		anti-HBs level (IU/L)≥10 IU/L		Total no	Anti-HBs level(IU/L) (SE)	
	No	%	No	%		Mean	SE
Low	68	50.7	66	49.3	134	143.3	(23.2)
Middle	51	44.3	64	55.7	115	152.1	(25.8)
High	17	39.5	26	60.5	43	168.3	(43.5)

P value of Chi Square > 0.05

Discussion:

HBV is a very contagious human pathogen. Two billion people (about one-third of the global population) have been infected with hepatitis B virus and 600,000 die each year from HBV-related liver disease (Shepard

et al., 2006). Approximately 400 million people worldwide are chronically infected with HBV (Crovari, 2003). Despite effective vaccination strategies, roughly 50 million new infections continue to occur annually around the world (Nidhi & Reau, 2008). Hepatitis B is preventable with a safe and effective vaccine. Inclusion of hepatitis B vaccine into national infant immunization programs could prevent >80% of HBV-related deaths (Goldstein *et al.*, 2005). As recommended by world Health Organization (WHO), all infants should receive the hepatitis B vaccine. Neonatal HBV vaccination is the best effective measure for prevention of HBV infection in countries with intermediate to high level of HBV endemicity. The duration of protection induced by plasma-derived and recombinant vaccines against HBV was investigated by several authors. Even if there is a decline of antibody titers over time, there is evidence that immunological memory persists for at least 9–15 years after immunization (Floreani *et al.*, 2004 and Saffar *et al.*, 2004).

In the present study, all the tested newborns and their respective mothers were negative for antibodies against hepatitis B surface antigen. WHO recommends administering the first HBV vaccine dose <24 hours after birth to prevent perinatal HBV transmission (WHO, 2004). WHO/UNICEF (2007) reported that complete implementation of routine newborn HB vaccination globally would reduce the substantial morbidity and mortality caused by perinatally acquired HBV infection. Disease modeling suggests that implementing HBV vaccine birth dose in regions with relatively low prevalence of chronic HBV infection, such as the Americas or Europe, might reduce HBV mortality by an additional 10%–20% compared with following a HBV vaccination schedule without a birth dose. For this reason, a substantial number of countries in areas with intermediate or low hepatitis B endemicity have implemented newborn HBV vaccination (Goldstein, 2005). In highly endemic areas where 8 to 15% of people are chronically infected with hepatitis B virus, the risk for the neonate to be perinatally infected by the chronically infected mother, then to become chronically infected themselves is very high. In those countries, the World Health Organization recommends hepatitis B vaccination systematically at birth, independent of hepatitis B virus maternal status. This vaccination program has begun to induce a rapid decrease in the number of acute hepatitis B virus infections and has also had a secondary effect of a decrease in related sequels (Ranger-Rogez *et al.*, 2004).

Hepatitis B surface antigen (HBsAg), used in current vaccines, contains an (a) determinant which is believed to be the major immunogen inducing polyclonal antibody against HBsAg (anti-HBs). Egypt includes HBV vaccination in its Expanded Program of Immunization (EPI) in 1992 with coverage rate of about 95%, where 3 doses of recombinant vaccine were given at 2, 4, 6 months. The schedule adopted by the Egyptian Ministry of Health was three doses of a yeast-recombinant HB vaccine administered to all infants (Engerix B, Smith Kline Beecham) at 2, 4 and 6 months of age to coincide with other compulsory vaccines [diphtheria, tetanus, pertussis and oral polio (DTP-OPV)]. Each pediatric dose (0.5 ml = 10 µg) had been administered intramuscularly in the anterolateral region of the middle third of the right thigh.

In this study, the overall seroprotection at 9 months, one year and at 6–11 years after immunization were (96.7%, 84.3% and 39.3%) respectively. Similarly, an Egyptian study for evaluation of immunogenicity and efficacy of vaccination among 180 infants and children whose time lapse since last vaccination varied between 1 month and 5 years showed that, although a high seroprotection rate (93.3%) was elicited 1 month after vaccination, there were low initial anti-HBs concentrations and both declined rapidly over time (El-Sawy and Mohamed, 1999). Lu *et al.* (2004) found that 62.6% of the 15-year-olds had non-protective HBsAb levels after primary neonatal immunization with plasma-derived hepatitis B vaccines. In the United States Petersen *et al.* (2004) reported that anti-HBs disappeared by 5 years of age in most of the studied children who had been vaccinated with hepatitis B vaccine from birth. On the other hand, some studies performed on children aged 6–12 years, detected that greater proportions of children had protective anti-HBs levels, ranging from 81.6% to 95% as reported by Floreani *et al.* (2004) and Yu *et al.* (2004) and 71.4% by Al-Faleh *et al.* (1999).

In the current study, the seroprotection level (≥ 10 IU/L) was detected in 47.5 % at 6–7 years and in 39% at 9 year after vaccination. Similarly low proportions of children with seroprotective antibody levels (41% at 5 years and 39% at 9 years) were reported by Williams *et al.* (2003). On the other hands, higher rates was reported by Reda *et al.* (2003) in Egypt (67%) 5 years after vaccination. In the older age group, in Taiwan, the percentage of children with seroprotective levels of anti-HBs gradually decreased from 71.1% at age 7 years to 37.4% at age 12 years (Lin *et al.*, 2003). Serologic studies have shown that the titer of antibodies against hepatitis B surface antigen drops within the first few years after vaccination and that one-third to one-half of children vaccinated as infants will have titers below 10 IU/L by 10–15 years of age (Dentinger *et al.*, 2005). Williams *et al.* (2003) found that persistence of protective levels for a longer period occurred when the vaccine doses were administered soon after birth. Increasing the time between the 1st and 2nd doses and 2nd and 3rd doses appears to increase antibody levels (Middleman *et al.*, 2001). This study showed that, there was no significant difference in the frequency of HBV seroprotection between males and females. This is in

agreement with (Middleman *et al.*, 2001), while others, have found that male sex is a predictor of non-response (Hossein *et al.*, 2008).

The low level of anti-HBs reported in the current study and the diversity of results in the different studies can be attributed to several factors; the type of the vaccine, whether it is a plasma-derived or yeast-derived vaccine, age at initial vaccination, the dose of vaccine and the schedule of immunization. Da Villa *et al.* (1996) found that the DNA recombinant vaccine gave a higher titre (97.6%) than the plasma-derived vaccine (80.4%), while Floreani *et al.* (2004) recorded a slightly higher titre with plasma-derived vaccine than with yeast-derived vaccine (87.8% and 81.6% respectively). A study of children at 12 years of age who had received a plasma derived vaccine in infancy and were at low risk for hepatitis B exposure found that none had HBsAb < 10 mIU/mL (Petersen, 2004). Another study in Iran reported that over half of the children (52%) had titres ≤ 10 mIU/mL with no difference between the sexes, while 81 (29.7%) had no anti-HBsAg (0 mIU/mL) (Kazemi *et al.*, 2008). Some studies suggested that starting the initial vaccination series later in infancy may result in better persistence of anti-HBsAg as the prevalence of anti-HBsAg titres ≥ 10 mIU/mL ranged from 79% to 85% at 10–12 years of age (Lemon & Thomas, 1997 and Yuen, 2003). Zuckerman *et al.* (1997) suggested that increasing the dosage of the vaccine leads to significantly higher levels of anti-HBs. El-Sawy and Mohamed (1999) reported that the short interval (2 months) between the second and third doses of vaccine is less desirable for the long term immunity. They recommended booster inoculations for all previously vaccinated children and a new vaccination schedule at 1, 2 and 9 months. Mackie *et al.* (2009) suggested that universal vaccination of infants in the first year of life and at 11 years of age has a greater efficiency on the improvement on the endemic status of the infection in the general population.

Result of this study suggest that universal vaccination of infants in the first year of life and at 11 years of age has a greater efficiency on the improvement on the endemic status of the infection in the general population which was similar to the study by Christopher *et al.* (2009) who review the evidence for long-term effectiveness of vaccination programs for infants and adolescents.

In conclusion, more than half of the studied children aged 6-11 years had non-protective level of anti-HBs and this puts them at risk of infection. The failure to achieve satisfactory seroprotection level by the national immunization programme reflects the need to re-evaluate the current hepatitis B vaccination strategy in Egypt. Further studies are needed to explain whether this low seroprotective level is due to waning immunity with time or due to an initial low response. A booster dose is suggested for maintaining a high seroprotective level.

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