Assessment of Anti-HBs Antigen in 6- to 9-Year-Old Children Routinely Vaccinated via Vaccination Program in Iran

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Key Words

Hepatitis • Vaccination • Anti-HBV antigen • Children • Iran

Abstract

Objective: To determine persisting antibody levels to hepatitis B virus (HBV) antigen in healthy children, aged 6–9 years, vaccinated at birth. **Methods:** Blood samples were collected from 374 vaccinated children (178 girls and 196 boys) and 57 unvaccinated children, attending Shiraz Primary School, Shiraz, Iran from September 2002 to April 2003. An HBV surface antibody (anti-HBs) was determined using enzyme-linked immunosorbent assay. **Results:** The anti-HBs titer was detected in 17% of the 8-year-old children, 7.7% of the 7-year-old children and 46.6% of the 6-year-old children. The decrease was greatest in the 9-year-old children; more than half (54.3%) had a titer of less than 10 IU/ml, indicating a decrease in antibody levels with increasing age/time. Conversely, more than 35% of the 6-year-old children had a titer greater than 150 IU/ml compared with 24, 12 and 7% of children at the age of 7, 8 and 9, respectively, whose antibody titer was less than 150 IU/ml. **Conclusion:** Antibody titer declined with time. In comparison with other countries, the antibody titer in Iranian children was much lower.

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Introduction

More than 300 million people worldwide are persistently infected with hepatitis B virus (HBV) and a significant proportion of these people develop severe pathologic consequences such as chronic hepatitis, cirrhosis and hepatocellular carcinoma [1]. In Iran, hepatitis B carrier rate was reported to be 1.7% in 1996 [2]. Among Iranian patients with cirrhosis, 70–84% had evidence of exposure to HBV and 51–56% are carriers. Patients with hepatocellular carcinoma show a 72% rate of exposure [3].

Immunization with hepatitis B vaccine is considered an effective means of prevention of infection with HBV and reducing occurrence of chronic sequelae related to the illness [4]. However, the disease still remains a global problem. Many factors contribute to the failure to control hepatitis B infection and morbidity, including the limiting nature of vaccination programs implemented initially. Universal childhood HBV immunization may have a significant impact in decreasing the number of people with chronic HBV infection and this may limit its spread. The most common method employed for the prevention of HBV infection in the Middle East is by active immunization [5]. In Iran, since 1984 HBV vaccination of children at birth and high-risk groups was implemented by the Expanded Program of Immunization (EPI) [6].

Table 1. Distribution of antibody against HBs antigen (mIU/ml) by age and gender

Age	Girls				Boys				Both			
	n (%)	mean (median)	SD	SE	n (%)	mean (median)	SD	SE	n (%)	mean (median)	SD	SE
6 years	14 (7.1)	127 (118)	105.5	8.2	37 (20.8)	92.5 (55.4)	82.7	13.6	51 (13.6)	101.9 (70)	89.8	12.6
7 years	51 (26)	97.2 (84.2)	85.5	11.9	55 (30.9)	73.2 (43)	75.3	10.1	106 (28.4)	84.8 (63.8)	80.9	7.8
8 years	67 (34.2)	52.4 (12.5)	68.6	8.4	58 (32.6)	52.6 (19.4)	68.8	9.0	125 (33.4)	52.5 (15.4)	68.5	6.1
9 years	64 (32.7)	38.4 (6.47)	60.5	7.5	28 (15.7)	18.4 (5.7)	26.9	5.1	92 (24.6)	32.3 (6.5)	53.3	5.5
Total	196 (100)	64.8 (22.8)	78.9	5.6	178 (100)	61.9 (25.9)	72.9	5.5	374 (100)	63.4 (23.7)	76	3.9

p < 0.001.

HBV vaccine is shown to be immunogenic and effective in preventing infection [7]. For ensuring that sustained immunity is conferred, antibody titer could be monitored. However, in Shiraz, it is not a general policy to monitor the long-term persistence of antibodies induced by vaccination.

The age at which HBV is acquired is an important factor in the development of chronic HBV infection. Follow-up studies of children vaccinated at birth have shown that a continued high level of protection against chronic infection persists for at least 5 years [8–10]. However, the exact duration of protection which the vaccine confers is unclear. The Center for Disease Control and Prevention continues to study children immunized at birth to determine whether or not boosters are required later in life. Also, in the interest of containing this infection, many studies have been conducted in different countries in order to assess the effect and duration of the recombinant vaccine [14–16]. Similarly, it is necessary to know the level of antibody response sustained in Iranian children included in the International Vaccine Program to receive recombinant vaccine and monitor antibody levels.

In this study, the duration of persisting anti-HBs antigen was determined among 6- to 9-year-old schoolchildren vaccinated at birth and who received two booster doses of HBV vaccine via a routine vaccination program.

Subjects and Methods

Blood samples were obtained from 374 (178 girls and 196 boys) healthy primary schoolchildren aged 6–9 years, living in Shiraz, southern Iran, from September 2002 to April 2003. Using the cluster random sampling method, questionnaires were given to the parents of the children, to provide the medical history of their

children. Written informed consent was obtained from all of the parents. As recorded on their vaccination cards, the children were vaccinated with HBV: each with a total of three doses: one at birth and two booster doses at 2 and 6 months. Unvaccinated children (57 children, 25 boys and 32 girls) were selected as controls.

The sera from the blood samples were preserved at –20°C. Enzyme-linked immunosorbent assay kits (Equipar, Italy) were used for detecting HBs antibody in the sera. One of the two purified HBs antigens was bound to the microplate and the second one was conjugated with the peroxidase (horseradish peroxidase). The antigen and antibody formed a sandwich complex with the antibodies, when present in the sample. The enzymatic activity was detected by incubation with the specific chromogen/substrate 3,3',5,5'-tetramethylbenzidine (TMB). The color intensity of TMB was proportional to the amount of antibody in the specimens. The TMB levels were quantified using the reference standard (WHO) [11]. Microplates were read at 450 nm and a subtract fitting system was used and the concentrations of the HBsAb in the samples were determined on the standard curve.

Data were analyzed using chi-square, one-way ANOVA and cross-tabulation for assessing any difference in antibody titer levels between and within groups, using SPSS 12.5, probability value of p < 0.05.

Results

HBsAb concentrations higher than 10 IU/ml were considered to be sufficiently high to afford immunity against HBV infection. The specificity and sensitivity of the assay were >99 and 98%, respectively.

Measurement of antibody titer against HBs antigen showed a decrease in mean antibody titer at 7, 8, and 9 years of age (table 1).

About one third of all vaccinated children at birth (35.2%, n = 69 girls, and 33.7%, n = 59 boys) had antibody titers less than 10 IU/ml. A quarter of them (25% of girls, n = 49, and 27.5% of boys, n = 49, respectively) had titers

Fig. 1. Distribution of antibody titer (IU/ml) against antigen in various age groups.

of 10–50 IU/ml. High titer levels, 50–100 IU/ml, were noted in considerably fewer individuals: 12.45% girls and 14.9% boys ($n = 24$ and $n = 26$, respectively); 100–150 IU/ml in 8.16% girls and 5.61% boys, and 150–200 IU/ml in 9.8% girls ($n = 19$) and 11.79% boys ($n = 20$). A small number of children (2.67%, $n = 10$) had a titer greater than 200 IU/ml. Antibody titer was not detected in the unvaccinated group.

A substantial decrease in antibody titer was noted at 9 years, so that 54.3% ($n = 50$) had titers less than 10 IU/ml. Only a small percentage (19.6%, $n = 18$) had a titer above 50 IU/ml.

Children who were 8 years of age (39.7% of the boys, $n = 23$, and 43.3% of the girls, $n = 29$) had a titer of less than 10 IU/ml, but 23% of this group had 10–50 IU/ml. Compared to the titers of 50–99 IU/ml of 9-year-old children, there were a greater number of children with antibody titers of 50–99 IU/ml (15.2%, $n = 19$).

Of the 106 individuals who were 7 years old, 17% ($n = 18$) had titers less than 10 IU/ml. More than 30% of these children ($n = 32$) had titers between 10 and 49 IU/ml, whereas 17.9% ($n = 19$), 10.4% ($n = 11$) and 14.3% ($n = 15$) had titers in the range of 50–99, 100–140, and 150–199 IU/ml, respectively. A small percentage (10.4%, $n = 11$) had titers >200 IU/ml. In the 6-year-old group, out of 51 children 9 had less than 10 IU/ml (a total of 17.7%). A greater number of children (25%, $n = 13$) had titers between 10 and 40 IU/ml and the rest (60%, $n = 30$) had titers greater than 50 IU/ml (fig. 1).

The difference in titer levels between genders was not statistically significant but the differences between and within age groups were statistically significant ($p < 0.001$), showing that circulating levels of antibody titers decreased with time.

Discussion

Several studies are being conducted to assess the duration of immune response in individuals initially vaccinated during infancy with a recombinant vaccine [15–17]. This is to determine if immunity lasts to confer protection into adulthood.

Our results showed that the duration of vaccine efficacy in Iranian children was lower compared to that reported in other countries. About 50% of 9-year-old children had an antibody titer less than 10 IU/ml.

Overall, the antibody titer was considerably low, but the trend in reduction of antibody titer is similar to the study on neonates in New Zealand who were vaccinated at birth; the vaccine proved to be highly immunogenic. In that study of 704 children who were given HBV vaccine at birth, it was shown that 96.7% of 1- to 4-year-old children had detectable anti-HBs, compared to 91% of 5- to 9-year-old children and 81% of 10- to 14-year-old children [12]. In another study, 66% of children aged 5–9 years had antibody titers above 50 IU/ml [13].

Since only a few (17%) 6-year-old children had very low antibody titers (<10 IU/ml), it can be concluded that vaccination performed at birth had a good response and immunogenicity persists in most individuals. The reason as to why 17% of children did not have a good response needs further investigation.

It is interesting to note and investigate why immunity declines very much more rapidly among children in this study in Shiraz, as compared to other countries. Also, the question arises, since immunity diminishes significantly by 9–10 years following vaccination, whether there should be a policy of giving a booster dose to children older than 10 years.

Conclusion

Among Iranian children vaccinated at birth, immunogenicity, i.e. antibody titer to HBV, diminished at a higher rate than reported elsewhere.

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