

# Anti-HBs Response and its Protective Effect in Children and Adults Receiving Hepatitis B Recombinant Vaccine in Tehran

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## Abstract

**Background:** Following WHO recommendation, HBV vaccination has been integrated into EPI program in Iran since 1996.

**Objective:** To evaluate the immunogenicity and protective effect of HB vaccine (recombinant Heberbiovac ,Cuba ) in vaccinated children and adults .

**Methods:** A total of 542 cases (340 children and 202 adults) were vaccinated using a three-doses schedule of zero, one and six month. Blood samples were collected before the last injection, one month, one and two years after vaccination. Sera were tested for anti-HBs as indicator of immune response.

**Results:** Seroconversion rates for anti-HBs after second dose was 97.9% in children and 87% in adults. After the third injection this response increased to 100% and 93.7% in children and adults respectively. The anti-HBs titer decreased after two years in both groups.

**Conclusion:** General vaccination with Heberbiovac vaccine in Iran has been successful in provoking immune response and protection against HBV infection. It seems that the persistence of the produced immunity is higher in children than adults.

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**Keywords** • Hepatitis B • antibody response • vaccination • immunization programs

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## Introduction

The need for control and prevention of hepatitis B infection as an important public health problem has been recognized worldwide. There are more than 350 million carriers as the reservoir of HBV agent throughout the world<sup>1</sup>. As many as 30% of these individuals are expected to die of the chronic liver disease, cirrhosis or primary hepatocellular carcinoma.<sup>2</sup>

There is established evidence that protection against HBV infection has been successfully achieved through vaccination and immunization programs have significantly proved to reduce spread of the disease<sup>3</sup>. According to the WHO recommendation in 1991<sup>4</sup>, HBV vaccination should have been included in all countries national

**Table 1:** Seroconversion rates and geometric mean titers of anti-HBs antibody in children and adults in different times after vaccination.

Groups		Follow up after vaccination		
		1 month	1 year	2 years
Children	GMT*	5262.45	2433.82	2068.3
	Seroconversion (%)	297/297(100)	124/132(93.9)	40/41(97.6)
Adults	GMT*	761.84	349.86	325.84
	Seroconversion (%)	148/158(93.7)	112/123(91.1)	9/11(81.8)

\*GMT = geometric mean of anti-HBs titer IU/L

immunization program to reduce the regional infection rate of HBV with a particular emphasis on vaccination of infants from 1997.<sup>5</sup> According to the Disease Control Department (DCD) of Iran, mass vaccination against HBV has been included in Expanded Program of Immunization (EPI) since 1992. Immune response to vaccination has been shown to be related to factors like the age at which the vaccine is administered, the site of injection and the number of injected doses.<sup>6</sup> Moreover, it has been shown that the antibody titer decreases after a few years following vaccination. Evaluation of these parameters for a successful vaccination program therefore, is of particular importance.<sup>6</sup>

At present, a few different recombinant vaccines against HBV are being used throughout the world. Recently a new recombinant HB vaccine, (Heberbiovac, S.A.Havana, Cuba), has been introduced for general vaccination, especially for newborns, in Iran. It has already been shown that, 95.2% of healthy adult individuals after vaccination with this vaccine developed protective antibody response.<sup>7</sup> However, in our country with a carrier rate of 2-7%,<sup>8</sup> the immune response and persistence of protective antibody to HBV vaccine has not yet been evaluated.

The purpose of this study was to evaluate the immunogenic and protective effect of HB vaccine in children and adults in Tehran.

### Materials and Methods

This study was performed from 1996 to 1999 in Tehran. Two groups were recruited: The first group consisted of 355 randomly selected adults with a mean age of 27.8, SD± 9.8 (ten years and over) who attended Pasteur Institute of Iran for vaccination against HBV and volunteered for this study.

The second group was 343 children with a mean age of 4.7, SD± 1 (under ten years) with no background of vaccination attending nurseries under authorization of Tehran Welfare Organization.

At the beginning of this study, adult cases and parents of the children were informed about the aim of the study and, with their consent, a 2-5 ml blood sample was taken from each case.

Totally 698 blood samples of individuals were collected before vaccination. Sera were stored at -

20 °C till used. Sera sample were tested for hepatitis B surface antigen (HBs-Ag) using Biotest kits (Biotest ELISA HBsAg), antibody to hepatitis B core antigen (anti-HBc) using Behring Kits (Enzygnost Anti-HBc micro) and antibody to hepatitis B surface Antigen (anti-HBs) using Behring kits (Enzygnost Anti-HBs micro) according to manufacturer instructions.

After initial tests for HBV markers a total of 542 cases (340 children and 202 adults) who had none of the HBV markers were chosen. All cases were vaccinated by three doses (zero, one and six month) of recombinant HB vaccine (Heberbiovac, S.A. Havana, Cuba) intramuscularly in the deltoid muscle, using 10 µg for children and 20 µg for adults. Blood samples (2-5 ml) were collected before injection of last dose and one month, 1 year and 2 years after completion of the vaccination cycle and tested for presence of anti-HBs. Seroconversion was defined as >10 IU/L, for anti-HBs antibodies. Anti-HBs levels were measured by quantitative ELISA method using Behring kits (Enzygnost Anti-HBs Micro) to evaluate the immunogenicity of the vaccine. In order to quantitate anti-HBs titer, ten times dilutions of positive sera were prepared and tested for anti-HBs. The anti-HBs concentration of the diluted sera were obtained by linear interpolation.

The geometric mean titers (GMT) were calculated from values observed in responders and seroconversion rates were compared using Fisher's exact and  $\chi^2$  test.

To assess the vaccine induced protection against infection, sera of individuals were also tested for the presence of anti-HBc antibody, after completion of vaccination cycle, one and two years after vaccination.

### Results

Our study indicated that seroconversion rates for anti-HBs in children and adults were 100% and 93.7% respectively after receiving three doses of vaccine. Geometric mean of anti-HBs titers (GMT) in children was, with 5262.5, significantly ( $p < 0.001$ ) higher than adults with GMT of 761.48 (Table 1). Persistence of antibody in children and adults was evaluated by determination of GMT for one and two

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**Table 2:** Distribution of seroconversion rates of anti-HBs in different times and doses with 95% confidence interval in children and adults.

Time after vaccination	Children		Adults	
	Seroconversion %	95%CI	Seroconversion %	95% CI
2 <sup>nd</sup> doses	97.9	96.3–99.4	87.0	79.5–94.5
3 <sup>rd</sup> doses	100	100	93.7	89.9–97.5
One year	93.9	91.8–95.9	91.1	86.1–96.1
Two years	97.6	92.9–100	81.8	59.0–100

years following the last injection dose (Table1). In both groups GMT was significantly higher after third dose when compared with the first year (2433 for children, 349 for adults,  $p < 0.05$ ) and after two years (2068 for children, 325 for adults). None of the vaccinees were positive for anti-HBc at one month, one and 2 years after vaccination, indicating the protective effect of vaccine in these groups.

Table 2 shows the seroconversion rate and the 95% confidence interval (CI) in two groups under study. The seroconversion rate was 97.9% (95% CI: 96-99) in children after the second dose and increased to 100 % following the third dose of injection but decreased to 93.9% (95% CI: 92-96) after one year and reached 97.6% (95% CI: 93-100) after two years. The seroconversion rate was 87% (95% CI 79- 94) in adults after second dose, increased to 93.7% (95% CI: 90-97) following the third dose but decreased to 91.1% (95 % CI: 86-96) after one year, and reached 81.8 % (95% CI: 59-100) after two years, indicating that the vaccine is protective in both groups of children and adults, but the response is higher and lasts longer in children.

### Discussion

According to WHO recommendation, HBV infection may be prevented by general vaccination with available vaccines. Therefore from an international point of view, evaluation of seroconversion and persistence of antibody produced by vaccination especially in children and newborns is of paramount importance for final aims of the prevention and protection throughout the world. There are miscellaneous investigations on different HBV vaccines with emphasis on aspects like efficacy, immunogenicity, side effects and persistence of the immune response after vaccination in different countries<sup>4</sup>. In Iran, a recombinant HB vaccine produced in yeast (Heberbiovac, S.A. Havana, Cuba) has been used for general vaccination since 1992. However, there is no study on evaluation of immune response to vaccination in children and newborns in our country. Seroconversion for anti-HBs antibodies, was defined  $>10$  IU/L as a generally accepted minimum

level required to indicate protective immunity against HBV.<sup>9</sup> Seroconversion rate has already been determined to be 95% in French Polynesian newborns vaccinated by GenHevac B<sup>10</sup>, and 97% in Italian children receiving Engerix B<sup>11</sup>, using three-dose schedule of 0,1,6 months during 1988-1991 and 1991-1994, respectively. However, our results showed that seroconversion rates for vaccinees under study were 100% in children and 93.7% in adults, which are identical to the results of another study performed by Shokrgozar and his colleagues in 1999<sup>7</sup> using the same vaccine in Iran, indicating 95% response to vaccination in adults. Also, results of other studies performed by S.Thoelen et al<sup>2</sup> in Belgium during 1996, showed a seroconversion rate of 100% in adults vaccinated by recombinant HB vaccine Engerix B with the novel adjuvant system.

According to our study, about 6% of adults were non-responder, which is in accordance with the results of Shokrgozar et al<sup>7</sup> when measuring HLA associated antibody response to recombinant HB vaccine (Heber Biovac, Havana, Cuba) in their study about 5% of individuals were non-responder, which decreased to 2% after receiving two booster injections with one month interval. Therefore, considering the percentage of the responsiveness in children, it seems that booster injection in HBV vaccination may not be cost effective and it may only be recommended for non-responder individuals.<sup>12</sup>

Our results showed that seroconversion rate and geometric means of anti-HBs titer i.e. the protective effect of vaccine was higher in vaccinated children than adults at the beginning and in follow up periods. Although, protective efficacy may not be solely achieved by the level of antibody alone since a person may be protected from a given disease in the absence of specific antibodies, because of involvement of other mechanisms such as cell mediated immunity, immunological memory and antibody titer. However, incidence of the disease in vaccinated individuals by epidemiological studies may provide more information about duration of immunity.<sup>11</sup>

It has been shown that in three doses vaccination schedules (0, 1, 6 month) usually the first two

injections suffice to initiate the antibody response. Moreover the third injection stimulates the secondary response, in which the appearance of antibody is earlier and titer is higher than primary response and immunogenic memory remains for at least 15 years following the completion of the vaccination program.<sup>13</sup> So, there will be no necessity for any booster dose in healthy individuals within this period.<sup>13</sup>

Our study indicates that seroconversion rate after second dose was 97.9% in children and 87% in adults. However, after third injection this response increased to 100% and 93.7% in children and adults respectively. It seems that booster injection dose is neither required nor cost-effective for children. So, booster dose may be recommended for those who are classified as high risk groups, e.g. in health care workers and others at occupational risk, intravenous drug users, close contacts of HBsAg carriers, after measuring antibody level.<sup>13</sup>

It is shown that persistence and stability of anti-HBs has close relation to the degree of immune response. Antibody rate will decrease promptly in the first few years following vaccination<sup>14</sup> and persistence of the antibody titer after long times directly depends on the produced titer of antibody at the time of vaccination.<sup>15</sup> Davilla and his colleagues also showed that antibody response was 97.6% for newborns early after vaccination with recombinant HB vaccine, Engrix B, but, immunogenicity decreased to 76.9% after 10 years period of the study.<sup>6</sup> Our results indicates, that in agreement with Davilla et al in 1996 and Moulia et al in 1994, persistence of anti-HBs in vaccinees decreased to 97.6% for children and 81.8% for adults who were positive for anti-HBs after 2 years, this decline is more prominent in adults and even some of them became antibody negative (Table 1). It is obvious that protective levels of anti-HBs in children are more prevalent in comparison to adults. However, since the number of our vaccinees after two years follow up are limited, a precise conclusion could not be ascertained.

Considering the results of our study it is apparent that age could also be an important factor for vaccination and immune response decreases with increasing age of the individuals, since, cellular and humoral immunity declines during aging.<sup>16</sup> In addition, it seems that in adults immuno reactivity decreases as a result of other conditions that occur as a consequence of secondary age-dependent alterations, such as malnutrition, insufficient blood supply, metabolic changes, drug etc.<sup>16</sup>

In conclusion general vaccination with Heber Biovac HB vaccine in Iran has been successful in provoking immune response and protection against HBV infection and it seems that the persistence of the produced immunity, i.e. anti-HBs directly de-

pends on the level of the primary antibody titer after the last injection, which is higher in children than adults: it could be concluded that continuation of HBV vaccination in EPI program is the best method of achieving control and eradication of HBV infection.

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