ORIGINAL ARTICLE

Hepatitis B Vaccination and Factors Related with Unresponsiveness in Healthy Children

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ABSTRACT

Background: *The aim of this study was to assess the effectiveness of hepatitis B vaccination and factors associated with vaccine unresponsiveness in healthy children.*

Methods: *A total of 141 healthy children aged between two and five years were included in the study. All of the cases had received 20 µg of recombinant DNA vaccine for hepatitis B (0, 1 and 6 months). Demographic features and factors such as duration of breastfeeding, exposure to HBsAg-positive family members, administration of concomitant vaccines and exposure to smoke were determined. Hepatitis B vaccination serological markers were evaluated. Post-vaccination serologic evaluation was performed one month after the last dose of primary vaccination, one month after the booster dose. Human leukocyte antigens typing was performed in non-responders.*

Results: *Only 87.9% of the children achieved seroprotection antibodies to hepatitis B surface antigen (anti-HBsAG titers ≥ 10 mIU/ml) one month after primary vaccination. No difference was observed between vaccine responsiveness and age, gender, birthweight, maturity, duration of breastfeeding, exposure to HBsAg-positive family members, and mid-upper arm circumference (*p *> 0.05). HLA types, DRB 111 (64.7%), B5 (52.9%), DRB 104 (52.9%) and DRB 11001 (47%) were detected at increased frequency in non-responders. The antibody titers were significantly higher in children who breastfed for the first six months and longer and who were vaccinated concomitantly with other common vaccines.*

Conclusion: *The seroprotection antibodies to hepatitis B surface antigen correlated with breast feeding and hepatitis B vaccination concomitant with other common vaccines. HLA types DRB 111, B5, DRB 104 and DRB 11001 had increased frequency in non-responders.*

Keywords: Children, hepatitis B vaccination, responsiveness

INTRODUCTION

Hepatitis B vaccines are safe and highly effective in preventing hepatitis B infection and its consequences such as cirrhosis or hepatocellular carcinoma. Children who become infected with hepatitis B virus are more likely than adults to develop chronic infections. Ninety per cent of infants who are infected from their mothers at birth, and 30% to 50% of those infected before age five years, become chronic HBV carriers. The primary vaccine series induces protective antibody levels in more than 95% of infants, children and young adults (1). However, approximately 4% to 10% of healthy individuals do not develop an adequate immune response against the hepatitis B vaccine after the primary vaccination series (2, 3).

A multifactorial mechanism plays a role in hepatitis B vaccine unresponsiveness, with a cumulative effect of different environmental and genetic factors such as age, weight, gender, smoking and haplotype of human leukocyte antigens (HLA). In this study, we investigated the factors that influence hepatitis B vaccine responsiveness and unresponsiveness in healthy children.

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SUBJECTS AND METHODS

A total of 141 healthy children aged between two to five years who were followed-up at the Well Child Clinic of Sisli Etfal Training and Research Hospital (Istanbul, Turkey) were included in the study. All of the cases received three doses of hepatitis B vaccine (20 µg, in 0.5 ml, Genhevac B, Sanofi Pasteur Diagnostic, France) at months 0, 1 and 6 intramuscularly. Infants born to HBsAg-positive mothers received hepatitis B immunoglobulin (HBIG) within 12 hours of birth in addition to hepatitis B vaccine. Demographic features and the factors such as duration of breastfeeding, exposure to HBsAg-positive family members except mother, administration of concomitant vaccines, and exposure to smoke were determined.

Children were followed by testing anti-HBs and anti-HBc titers one month after the last dose of vaccinations, one month after the booster dose given to cases who did not respond to primary vaccination. Infants born to HBsAg-positive mothers were tested for HBsAg, anti-HBs and anti-HBc titers after the completion of the vaccination series at age 9 to 18 months. Seroconversion was considered if anti-HBs levels were above ≥ 10 mIU/ml. Based on the anti-HBs titers, subjects were identified as non-responders (< 10 mIU/ml) and hypo-responders (10–100 mIU/ml).

HBsAg, antibody to hepatitis B antigen (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc IgG) were estimated in all children. HBsAg was measured by ELISA (Hepanostika, Biomeriuex, Boxtel, The Netherlands). HBeAg, anti-HBe, anti-HBs and anti-HBc were tested by IgG vitros Eci system (Ortho-Clinical Diagnostic, Johnson and Johnson Company, Dutra, Brazil) and HBV DNA by the Hibrid Capture (Digeme, Gaithersburg, MD) method. HLA typing was performed by the microlymphocytotoxicity method.

Mid-upper arm circumference was obtained by the same physician from the left arm, at the mid-point between the acromion and olecranon, with the elbow flexed at an angle of 90º, using a non-extendable measuring tape, with a width of 1.0 cm and subdivisions of 0.1 cm. A total of three consecutive measurements were taken for each child, and the mean value was considered for analysis.

Informed consents were obtained from all of the parents before procedures and the study was approved by the Hospital Institutional Review Board.

Statistical analysis

Statistical analyses were performed using SPSS (version 11, 2000; Chicago, IL, USA) software. Comparison of groups was performed by Chi-square test, Fisher's exact test, Kruskal–Wallis test, Mann–Whitney *U* test, Pearson correlation, and linear regression. A value of $p < 0.05$ was considered statistically significant.

RESULTS

One hundred forty-one healthy children (M/F:1.12) aged between two and five years (35.54 ± 8.93) months were included in the study. All of the children received hepatitis B vaccine and 87.9% of them achieved seroprotection (anti-HBs titers ≥ 10 mIU/ml) one month after primary vaccination. Seventeen patients were unresponsive to the primary hepatitis B vaccination. DRB 111 (64.7%), B5 (52.9%), DRB 104 (52.9%) and DRB 11001 (47%) haplotypes were detected at increased frequency in non-responder children.

No difference was observed between vaccine responsiveness and age, gender, birthweight, maturity, duration of breastfeeding, exposure to HBsAg-positive family members, and mid-upper arm circumference ($p > 0.05$) (Table 1). The antibody titers were significantly higher in children who breastfed for the first six months and longer than the children who breastfed for a shorter length ($p = 0.005$). The rate of unresponsiveness to vaccination was higher in children who were exposed to secondhand smoke ($p = 0.049$) (Table 1).

Although no correlation was observed between unresponsiveness and administration of concomitant vaccines (Table 2), the antibody titers were found to be higher in children who were vaccinated concomitantly with other common vaccines (87.897 ± 57.686 *vs* 217.837 ± 40.252, $p = 0.001$). No significant correlation was seen between antibody titers and age, gender, birthweight, maturity, exposure to HBsAg-positive family members, and HBsAg-positivity of mothers (Table 3).

HBsAg positivity rate was found to be 2.8% among mothers. All of their infants received HBIG in addition to hepatitis B vaccine soon after birth. All of them achieved seroprotection after vaccination.

Those 17 children received a booster dose and none of them achieved seroprotective antibody titers. The overall seroprotection rate was 87.9% in children after the whole hepatitis B vaccination series.

DISCUSSION

The age at the time of HBV acquisition is the major determinant of chronicity. Up to 90% of infected neonates develop chronic infection, whereas only 3% to 5% of adults develop this. Immunization of all newborn infants and adolescents for HBV is the most effective means of

Table 1: The comparison of responders and non-responders among children

	Total patients (n = 141)	Responders (n = 124)	Nonresponders (n = 17)	*p*
Age (month)	35.54 ± 8.93	34.6 ± 9.1	35.38 ± 7.1	0.67
Gender (male/female)	1.13 (75/66)	1.17 (67/57)	0.8 (8/9%)	0.61
Birth weight	3273 ± 534.9	3254 ± 231.8	3284 ± 192.6	0.21
Maturity				
Preterm	8 (5.6%)	6 (4.8%)	2 (11.7%)	0.24
Term	133 (94.3%)	118 (95.1%)	15 (88.2%)	
Duration of breast feeding (months)	14.07 ± 8.23	14.12 ± 7.15	14.32 ± 9.85	
HBsAg carrier state in mothers				
Yes	4 (2.8%)	4 (3.2%)	0	1.00
No	137 (97.1%)	120 (96.7%)	17 (100%)	
HBsAg positivity in first degree relatives				
HBsAg (+)	10 (7.6%)	8 (6.4%)	2 (25%)	0.34
HBsAg (–)	131 (92.9%)	116 (93.5%)	15 (88.2%)	
Exposure to smoke				
Yes	43 (30.4%)	34 (27.4%)	7 (41.1%)	0.049
No	98 (69.5%)	90 (72.5%)	10 (58.8%)	
Concomitant vaccine administration	98 (69.5%)	89 (71.8%)	6 (35.2%)	0.004
Mid-upper arm circumference	90.71 ± 3.31	90.74 ± 3.34	89.98 ± 2.87	0.53

$p < 0.05$ is statistically significant.

preventing HBV infection and its consequences. The standard three-dose regimen of hepatitis B vaccines, with the second and third doses being given one and six months after the initial dose, elicits protective serum titers of anti-HBs (greater than 10 IU/L) in 95% to 99% of healthy infants, children, and young adults (4, 5). Approximately 4% to 10% of healthy individuals do not develop an adequate immune response against the hepatitis B vaccine after the primary vaccination series (2, 3).

The unresponsiveness to hepatitis B vaccines has been attributed to several environmental and genetic factors. Besides the well-known demographic factors such as increasing age, male gender, smoking and obesity, inadequate Th1- and Th2-like cytokine production, defect in HBsAg-specific T and/or B-cell repertoires, defect in expression of certain HLA and haplotypes, destruction of HBsAg-specific B-cells by antigen-specific cytotoxic T cells and immunologic tolerance have been identified (6–11). Several studies demonstrated that the HLA class II alleles HLA-DR3, -DR7, -DQ2

Table 2: Concomitant vaccine administration among responders and non-responders

Concomitant vaccine	Total patients (n = 141)	Responders (n = 124)	Nonresponders (n = 17)	*p*
None	43 (30.4%)	35 (28.2)	11 (64.7%)	0.05
DTP+OPV (1 dose)	23 (17.45%)	22 (17.7%)	2 (11.7%)	1.00
DTP+OPV (2 doses)	40 (30.3%)	39 (31.4%)	2 (11.7%)	0.43
Measles	2 (1.41%)	1(0.8%)	–	1.00
Hib	6 (4.55)	5 (4%)	2 (11.7%)	0.31
BCG	2 (1.41%)	1(0.8%)	–	1.00
DTP+OPV (1 dose)+ Measles	3 (2.27%)	3 (2.4%)	–	1.00
DTP+OPV (2 doses) +Measles	12 (9%)	12 (9.6%)	–	1.00
DTP+OPV (1 dose)+ Hib	3 (2.12%)	1(0.8%)	–	1.00
DTP+OPV (2 doses)+Hib	2 (1.41%)	1(0.8%)	–	1.00
DTP+OPV (1 dose)+ BCG	3 (2.27%)	3 (2.4%)	–	1.00
DTP+OPV (1 dose)+ Hib+Measles	2 (1.41%)	1(0.8%)	–	1.00

BCG = Bacille Calmette-Guérin; DTP = diphteria, tetanus toxoids, and pertussis vaccine; OPV = oral poliovirus; Hib= haemophilus influenzae type B vaccine. $p < 0.05$ is statistically significant.

Table 3: Concomitant vaccine administration and antibody titers

	Anti-HBs antibody titers (geometric means, IU/ml)	*p*
Gender		
Male	142.060 ± 47.400	0.48
Female	189.382 ± 47.718	
Maturity		
Preterm	94.762 ± 164.130	0.58
Term	171.516 ± 34.378	
Concomitant vaccine administration		
Yes	217.837 ± 40.252	0.001
No	87.897 ± 57.686	
HBsAg carrier state in mothers		
Yes	107.696 ± 231.903	0.76
No	167.694 ± 34.077	
Exposure to smoke		
Yes	128.1446 ± 64.5324	0.45
No	184.1766 ± 39.5338	
Exposure to HBsAg positive family members		
Yes	819 271 ± 967 575	0.46
No	175.2717 ± 35.3420	

$p < 0.05$ is statistically significant.

and -DP11 are associated with low or unresponsiveness to HBsAg vaccination (12). We observed increased frequencies of DRB 111 (64.7%), B5 (52.9%), DRB 104 (52.9%) and DRB 11001 (47%) haplotypes in non-responder children.

There was no gender difference between hepatitis B vaccine responders and non-responders in our study as similarly reported in a study conducted by Karaoglu *et al* (13). It has been reported that birthweight and prematurity was not associated with the development of a protective immune response (14–16). We found no correlation between response rate, anti-HBs titers, birthweight and prematurity.

Rey-Cuille *et al* (17) found that nutritional status was significantly correlated with the response to hepatitis B vaccination ($p < 0.001$), with 85% of children with normal nutrition status being protected versus 60% in moderate-to-severe malnutrition. el-Gamal *et al* (18) reported that seroconvertion rate was lower in children with protein calorie malnutrition than healthy children (87% *vs* 100%, respectively). In contrast with these reports, Karimi *et al* (19) and Karaoglu *et al* (13) reported that severity of malnutrition does not affect vaccine-induced antibody level and seroprotection rate. No correlation was found between response rate and mid-upper arm circumference.

Breastfeeding also improves vaccine responses. Several studies showed that milk may actively stimulate the immune system of the offspring via transfer of anti-idiotypic antibodies and lymphocytes (20–22). We observed that the antibody titers were significantly higher in children who breastfed for the first six months and longer than the children who breastfed for a shorter period.

The negative effect of smoking on immunological response to hepatitis B vaccine has been reported (23). We found that the rate of unresponsiveness to vaccination was higher in children who were exposed to secondhand smoke.

It has been reported that hepatitis B vaccine given simultaneously with other common vaccines elicits a strong anti-HBs response and does not interfere with the immune response to the other antigens (24, 25). In our study, although no correlation was observed between unresponsiveness and administration of concomitant vaccines, the antibody titers were found to be higher in children who were vaccinated concomitantly with other common vaccines.

In conclusion, the seroprotective anti-HBs antibody titers are correlated with breastfeeding and vaccination concomitantly with other common vaccines. Exposure to smoke seems to negatively influence the response to hepatitis B vaccine. Early identification of potential non-responders is important for optimally reducing the risk of acquiring chronic infection during childhood. It is possible to obtain seroconversion by using vaccination strategies to improve the responsiveness to HBV vaccine in non-responders. Thus, it is advisable to check the anti-HbsAg concentration in non-responder children and administer boosters to those with lack of seroprotection against HBV.

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