Differential Immunogenicity of A Recombinant Hepatitis B Vaccine in Iranian Neonates: Influence of Ethnicity and Environmental Factors

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ABSTRACT

Objective: To compare immunogenicity of a recombinant hepatitis B (HB) vaccine in two groups of neonates born in two cities of Iran with different geographic and ethnic backgrounds. **Materials and Methods:** Ten micrograms of a recombinant HB vaccine was administered under field condition to Iranian healthy neonates at 0, 1.5 and 9 months intervals. The subjects consisted of two groups of 290 and 231 neonates selected from two cities located at north-west (Urmia) and south-east (Kerman) of Iran, respectively. The level of anti-HBs antibody was quantitated in serum 2-4 weeks after administration of the last vaccine dose, by sandwich ELISA. **Results:** A higher seroprotection rate (anti-HBs> 10 IU/L) (98.3% vs. 96.1%) and significantly increased serum anti- HBs antibody titer (11869 vs. 6104 IU/L) ($P<0.001$) were induced in vaccinated neonates from Urmia city, compared to those born in Kerman. **Conclusion:** These findings suggest contribution of ethnic and/or environmental factors in the antibody response to recombinant HB vaccine in human.

Key words: Anti-HBs antibody, Hepatitis B, Immunogenicity, Neonate, Seroprotection, Vaccination

INTRODUCTION

Hepatitis B virus infection and its sequelae which include cirrhosis and hepatocellular carcinoma has remained a major public health problem worldwide. One third of the world population shows a past history of infection and more than 350 million individuals have been estimated to be persistently infected (1). In areas of high endemicity, especially in some parts of Africa and south-east Asia, 7-20% of individuals are chronically

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infected and more than 70% of the adults show evidence of prior infection (2). In these populations the infection is predominantly transmitted vertically during perinatal period from carrier mothers to their neonates (1). In areas of intermediate endemicity, such as Iran (3), however, disease transmission is mixed and disease occurs at all ages, but again the predominant period of transmission seems to be at younger ages (4). Effective control of HBV transmission in areas of high and intermediate endemicity, therefore, would not be possible without vaccination of this vulnerable group of the population (5). Mass vaccination of neonates and children within the framework of Expanded Program on Immunization (EPI) has been recommended by WHO (6). This program has been incorporated in the national immunization scheme in Iran since 1992 (7). Recent results reported in many countries clearly indicated that in areas of high endemicity such as some parts of Asia, highly effective vaccination program has shifted this pattern toward intermediate or low endemicity (8). Similar epidemiological studies have demonstrated the long term positive influence of universal vaccination on the effective control of fulminant hepatitis, cirrhosis and hepatocellular carcinoma (9,10). However, the results obtained from a large number of studies indicate that 1-10% of healthy neonates and adults fail to respond to vaccine (7,11-13). A number of variables such as type and dose of vaccine, together with genetic background and environmental factors may contribute to the quality of immune response to a given antigen. Implementation of different vaccination schedules, however, does not allow distinctive assessment of the variables. In this study a common vaccination program with exactly the same vaccine type and dose was employed to investigate immunogenicity of a recombinant HB vaccine in two groups of neonates born in two provinces of Iran with different environmental and demographic backgrounds.

MATERIALS AND METHODS

Subjects and vaccination Scheme. A total of 521 healthy neonates attending the health centers of Kerman and Urmia (two cities that located south- east and north- west of Iran, respectively) were included in this study. Gestational age, birthweight and sex of the neonates were registered and only physically healthy neonates with a minimum weight of 2500 g were enrolled into study. Collectively, 231 neonates in Kerman (113 males and 118 females) and 290 neonates in Urmia (145 males and 145 females) were included in the study. Triple 10 microgram doses of a recombinant hepatitis B vaccine (Heberbiovac, Heberbiotec Co, Cuba) were administered into the quadriceps muscle at 0, 1.5 and 9 months intervals. Two milliliters of peripheral blood were taken from all vaccinees 2-4 weeks after completion of the vaccination course and the sera were collected and stored at – 20^{o}C until use.

Detection of HBV markers. HBs antigen, anti-HBs and anti-HBc antibodies were detected by enzyme linked immunosorbent assay (ELISA) using commercial kits (Behring, Germany). Anti-HBs antibody was quantitated using appropriate dilution of a positive sample with a known concentration of anti-HBs expressed as IU/L, provided by the manufacturer.

Statistical analysis. Differences in variables were analyzed using the Mann-Whitney

U-test, Chi-square and Fisher exact tests when appropriate, and P values of less than 0.05 were considered significant.

RESULTS

After completion of the vaccination course 96.1% and 98.3% of vaccinees in Kerman and Urmia cities developed protective titer of anti-HBs antibody (>10 IU/L), with a geometric mean titer (GMT) of 6104 and 11869 IU/L, respectively. No significant differences were observed in seroprotection rate between neonates of two cities, but the GMT was found to be significantly higher in Urmian neonates as compared to vaccinees of Kerman city (P<0.001). Distribution of anti-HBs titer in both groups of neonates is illustrated in Figure 1.

Vaccinated neonates, could be arbitrary classified into non-responders (anti-HBs<10 IU/L), low (anti-HBs: 10-100 IU/L), intermediate (anti-HBs: 100-1000 IU/L) and high (anti-HBs>1000 IU/L) responders, based on the serum titer of anti- HBs antibody (table 1). The percentage of non-responders, low responders and intermediate responders, collectively, was found to be higher in Kermanian neonates than Urmian vaccinees (P<0.01). Consequently, the proportion of high responder neonates was lower in the Kermanian group compared to Urmian vaccine recipients (P<0.01).

Seroprotection rate and GMT were similarly expressed in male and female vaccinees of both cities. Although the GMT was higher in females compared to males, this difference was not statistically significant (table 2). The GMT of both male and female neonates from Urmia (11433 and 12309 IU/L) was significantly (P<0.001) higher than

Table 1. Classification of vaccinees from Kerman and Urmia cities based on serum concentration of anti-HBs antibody

Group(anti-HBs IU/L)	Kerman			Urmia		
	No	%	GMT	No	%	GMT
NR (<10)	9	3.9	6.3	5	1.7	7.6
LR (10-100)	23	10	44	9	3.1	48
IR (100-1000)	42	18.2	365	29	10	336
HR (>1000)	157	68	8859	247	85.2	13654
Total	231	100	6104	290	100	11869

NR: non-responders, LR: low responders, IR: intermediate responders, HR: high responders, GMT: geometric mean titer

those from Kerman (5772 and 6400 IU/L).

To evaluate the influence of prior HB virus infection on unresponsiveness to vaccination, HBsAg and anti-HBc antibody were detected in sera collected from all non-responder neonates after completion of vaccination. Among the Kermanian non-responders, 3 neonates were $HBsAg^{+}$ and anti-HBc antibody was detected in 6 samples in this group. In Urmian non-responders, all neonates were found to be HBsAg and anti-HBc negative.

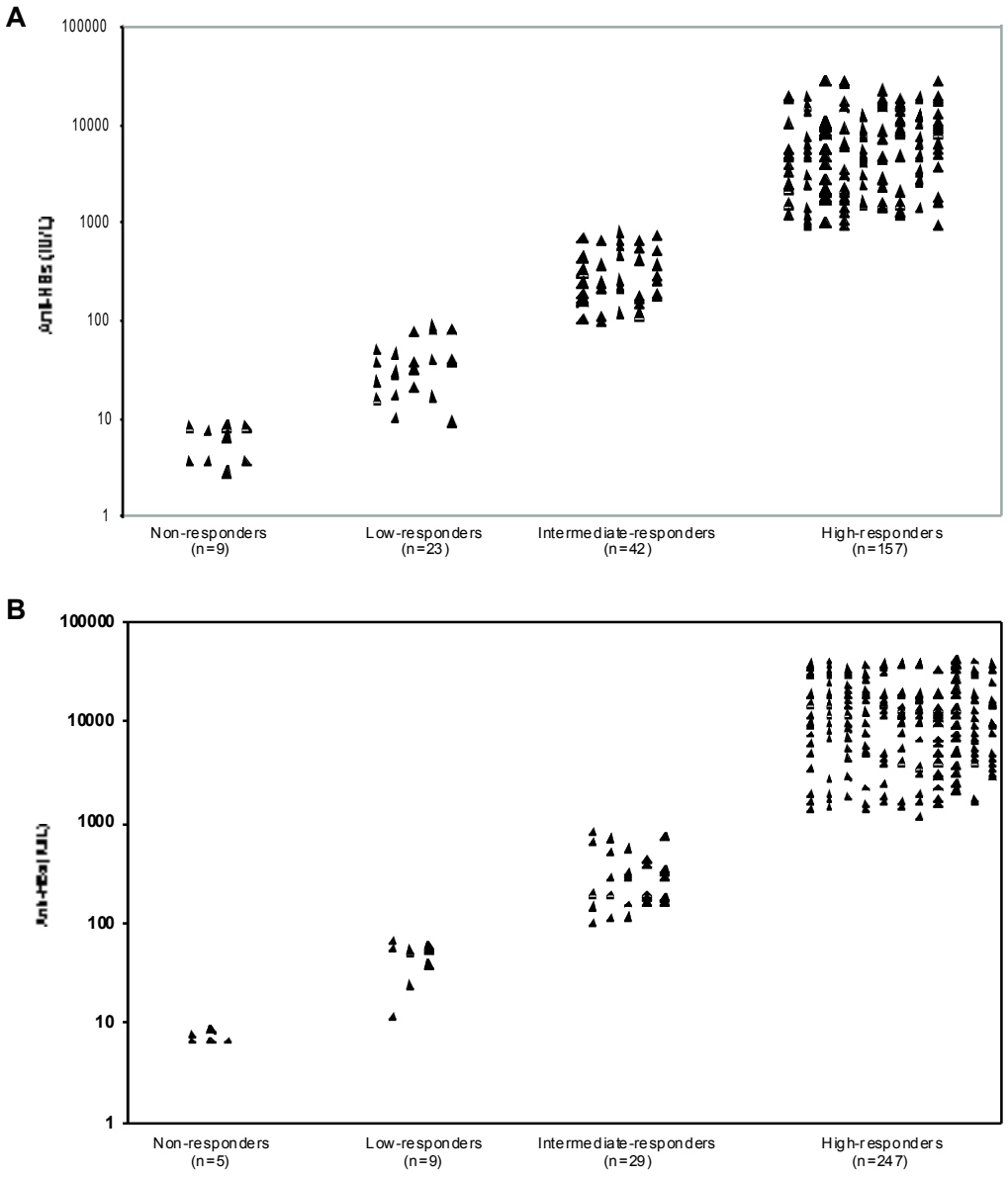

Figure 1: Distribution of serum anti-HBs antibody levels in vaccinated neonates from Kerman (A) and Urmia (B)

DISCUSSION

The results obtained from a large number of studies have indicated that vaccination of healthy neonates and adults with recombinant HBs Ag induces a protective antibody response in 90-99% of vaccines (7,11-13). In the present study, which is the first report

on differential immunogenicity of a recombinant HB vaccine in Iranian neonates, a strong protective antibody response was observed in the majority of healthy vaccinated neonates from both Kerman and Urmia cities. However, a small proportion of vaccinees fail to respond, accounting for 1.7% and 3.9% of Urmian and Kermanian neonates, respectively. Lack of response to HBs Ag has been attributed to a variety of mechanisms, including defects in HBs Ag-specific T-cell and/or B-cell repertoires (14-16), expression of certain HLA antigens and haplotype (16-18), expression of certain TCR-Vβ genes

Table 2. Comparison of seroprotection rate and GMT of anti-HBs antibody between vaccinated neonates from Urmia and Kerman cities

City	Sex	No	Seroprotection	GMT (IU/L)± SD
Kerman	Male	113	107 (94.7%)	5772 ± 7354
	Female	118	115 (97.5%)	6400 ± 7758
	Total	231	222 (96.1%)	6104 ± 7553
Urmia	Male	145	143 (98.6%)	11433 ± 11079*
	Female	145	142 (97.9%)	12309 ± 11781*
	Total	290	285 (98.3%)	11869 ± 11422*

* GMT was significantly higher in Urmian vaccinees as compared to those born in Kerman (p<0.001).

(15), inadequate production of TH1 and/or TH2 cytokines (19,20), destruction of HBs Ag-specific B-cells by antigen specific cytotoyic T-cells (21) and immunological tolerance (22).

Unresponsiveness to HB vaccination in neonates has been shown to be influenced by prior HBV infection during prenatal period (23). A similar finding has also been reported in adults (24). Immunological tolerance due to encounter of B or T lymphocyte with HBe or HBs Ag, through clonal anergy or deletion, has already been demonstrated in transgenic mice and also in human neonates born to HBV carrier mothers (22,23). To explore the role of prior HBV infection in unresponsiveness to the vaccine in our vaccinees, the frequency of HBV markers was studied in all non-responders. In our previous study, no significant correlation was found between HBV infection and lack of response to vaccination (7). In the present study, however, a high proportion of non-responders from the Kerman vaccinees was found to be either HBs Ag and /or anti-HBc antibody positive. Although pre-immunization blood samples could not be taken from neonates, due to practical and ethical limitations, HBV infection seems to have occurred prior to vaccination, perhaps during the prenatal period. This is strongly supported by the fact that the HBV markers were also detected in serum from mothers of corresponding neonates (data not presented). Our finding of higher HBV markers frequency in non-responders from the Kerman city, suggests that the lower seroprotection rate observed in this city may partly be due to prior HBV infection. This, however, could not explain the differences observed in GMT of anti-HBs antibody in the responder neonates from both cities.

Our results indicate that GMT was significantly higher in Urmian neonates as compared to Kermanian vaccine recipients. Parameters influencing the antibody response to HB vaccine might be overally classified into host and environmental factors. Increasing age and weight have been shown to correlate independently with a decreasing response to the vaccine (25,26).

Gender does not seem to play an important role in the immune response to HB vaccination. Initial studies found that females often responded to the vaccine with higher antibody levels than did males (26). In the present study, however, no significant differences were observed between male and female vaccinees with regard to seroprotection rate and GMT in both cities, though the GMT was slightly higher in females compared to males in both groups of vaccinees. Immunosuppressed groups, such as hemodialysis (27) and diabetic (28) patients and those on chemotherapy (29) respond poorly to vaccination. Smoking and alcohol consumption (25) also appears to have adverse effect on antibody response to HBsAg. None of these parameters, however, seems to contribute to lack or low antibody response in our subjects.

Genetic background plays a pivotal role in determining the strength of the immune response to HB vaccine. Both HLA and non-HLA genes are involved in regulation of the immune response to infant vaccination (30,31). Several studies have demonstrated an association between non- and poor responsiveness to HB vaccine with certain HLA specificities (16-18).

Characteristics of the immunization program that influence immunogenicity include type of vaccine (32), dosage (12), number and timing of inoculations (33). Storage of vaccine (25), site and route of inoculations (34) and use of adjuvants could also influence antibody responses (35). In this study, however, identical immunization protocols were employed for vaccination of neonates in both cities. Moreover, the most important host characteristics such as age, weight and sex were matched between the two groups. Therefore, the difference in immunogenicity may reflect environmental and/ or ethnic differences between the two groups of vaccinees. This highlights importance of such studies to improve immunogenicity of the vaccine in different geographic and ethnic areas. Comparative determination of the expressed HLA antigens and the habitual dietary supplements between the two study groups may provide insights into the mechanisms involved in differential antibody response to HB vaccination.

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