

Immunogenicity of Recombinant Hepatitis B Vaccine in Thalassaemic Children

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Hepatitis B virus (HBV) infection is a world-wide major health problem(1). Patients with thalassaemia major who receive blood transfusions regularly to maintain optimal hemoglobin (Hb) level frequently suffer from the hepatitis infection. Screening of blood for HBsAg reduces the risk of transmission, but cannot eliminate it entirely, because of window period and low titer HBV infections, with

HBV variants(2). Therefore, it is necessary to vaccinate all thalassaemia major patients for hepatitis B infection as soon as they are diagnosed.

The plasma derived vaccine for hepatitis B became commercially available in 1982 but its availability was limited. The new generation of hepatitis B vaccine (recombinant rDNA yeast derived) can be produced in greater quantities at a comparable cost. Recombinant HBV vaccine given in infancy is highly immunogenic. Most infants develop antibody titre well above 10 mIU/ml, the level associated with seroprotection(3,4).

Recombinant rDNA Hepatitis B vaccine derived from *Pichia pastoris* yeast has been recently introduced in India. *Pichia pastoris* yeast express heterologous genes, which overcome some of the disadvantages of the *S. cerevisiae* system. Since immune response is imperfect in children with thalassaemia, the present study was designed to assess the immunogenicity of three doses of recombinant Hepatitis B vaccine in high-risk hypertrans-fused children with thalassaemia.

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Subjects and Methods

The present prospective study was conducted in children of either sex below the age of 12 years at LN hospital, New Delhi. Written informed consent from parents or guardians of the children was obtained prior to inclusion in the study. All children below 12 years of age attending clinics and high-risk thalassaemic children were screened for HBsAg and HBeAg.

Serum tests for hepatitis B surface antigen, antibody to hepatitis B surface antigen (anti-HBs) and antibody to hepatitis B core antigen were measured by semiquantitative method using ELISA kit (MELOTEK, Spain) on entry into the study. Children with immunodeficiency disorder or history of allergy to any drug or receiving immunosuppressive therapy were not considered for this study. Children with any severe hepatic, renal, cardiac or respiratory diseases were also not eligible.

Enrolled children received three doses of recombinant Hepatitis B vaccine (Enivac HB®. Batch No. 42915. Manufacture Date 18/08/1994, samples registered as Heberhiovac HB) at interval of 0, 1 and 6 months. Children below 10 years were given 10 µg while older children received the adult dose (20 µg). All doses were given intra-muscularly in the anterolateral thigh or deltoid muscle. Blood specimens were drawn before administering hepatitis B vaccine and then at 1 month. The third sample was collected between 3 to 4 months and fourth sample was drawn at 6 months. The blood was immediately separated and the serum was frozen at -20° to -70°C. Seroprotection was defined when a titre of anti-HBs was above 10 mIU/ml. The lower limit of detection of the assay was 0.1 mIU/ml.

After each dose, study subjects were observed for 30 minutes and reactions during next 3 days were ascertained by parental reporting. Parents were instructed to record the child's temperature, use of antipyretics, irritability, drowsiness, crying for longer than 3 hours, decreased appetite, vomiting, vaccine site redness, swelling or tenderness or any other problem.

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Results

A total of 58 (25 females and 33 male) children were enrolled in the study. Of these, 30 children were suffering from thalassaemia major and had a history of receiving repeated blood transfusions. Out of 30 thalassaemia major patients, 7 appeared reactive on ELISA and one healthy subject were positive for HBsAg and therefore, was excluded from the study. Fifty subjects (27 healthy, 23 thalassaemic) thus received the first dose of recombinant Hepatitis B vaccine.

Of these, eight healthy subjects and 2 children with thalassemia were lost during follow up and, therefore were not considered for analysis. One healthy subject developed non hepatitis B jaundice due to which his second dose was delayed and was therefore, not included for analysis. A total of 39 children (21 thalassemic, 18 healthy subjects) completed the study protocol.

Three healthy subjects and 10 thalassemic children above 10 years of age received 20µg of hepatitis B vaccine. Nineteen (10 healthy, 9 thalassemic) children were between 5 to 10 years of age. Two subjects each in healthy and thalassemia group belonged to 2-5 years group. Three healthy subjects were below 2 years of age.

All children including the high risk subjects seroconverted before 6 months, *i.e.*, before the administration of third dose of recombinant Hepatitis B vaccine. The cumulative seroconversion rate with recombinant Hepatitis B vaccine in healthy and thalassemic children was 44% vs 48% at 1 month, 72% vs 71 % at 3 months and 100% in both groups at 6 months (*Table 1*). No serious adverse effects were reported with the administration of this recombinant Hepatitis B vaccine. Mild pain at the vaccine site occurred in 8 subjects. No other serious symptoms were reported.

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Discussion

Thalassemic patients receive monthly packed red cells as a standard transfusion regimen to maintain hemoglobin concentration above 10g/dl. Being the most transfused population with such blood products, thalassemic patients have been heavily contaminated in the past with transmitted viruses. The prevalence of HBV markers differs among thalassemic patients from various countries, reflecting variations of prevalence of infection among donors. In a European study, between 1.2 and 6% of thalassemic patients were positive for the hepatitis B surface antigen (HBsAg) varying with the country(5). In the present study, we observed a high prevalence (23.33%) of viral markers in thalassemic patients while only 3.7% of healthy children were positive for HBsAg.

The lack of small animal models of HBV infection has precluded the precise definition of antibody concentration required to prevent HBV infection. However, controlled clinical trials in adults have shown that HBsAg-positive infections occur in persons with a poor response to vaccine (HBs<10mIU/ml)(6,7). In addition, achievement of a post third dose anti-HBs concentration ≥10 mIU/ml correlates with long term protection in studies in both adults and infants at high risk of HBV infection, (homosexuals and people living in endemic area) despite the fall in antibody in many individuals to low or undetectable values(8,9). Nevertheless, the persistence of detectable antibody is directly related to the maximal antibody response(10,11).

Immune response in thalassemia patients is usually imperfect. However, in the present study antibody production in healthy children and thalassemics at 1, 3 and 6 months was

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TABLE I—Cumulative Seroconversion Rates for 1, 2 and 3 Samples After Initiation of Vaccination.

	Healthy subjects			Thalassemics		
			Sample No.			Sample No.
	1	2	3	1	2	3
No. of sera tested	18	10	5	21	11	6
No. of newly converted sera	8	5	5	10	5	6
Cumulative % of seroconversion	44	72	100	48	71	100

comparable to other reports in thalassemics(12) The recombinant rDNA Hepatitis

B vaccine used in present study was efficacious and well tolerated vaccine in neonates, healthy children and high-risk thalassemic children. There were no serious side effects noted in the children vaccinated in the present study. Neither age nor thalassaemia inhibited the immune response which was excellent, despite the fact that the usual dose was administered. Although 100% seroconversion rate was elicited by 180 days in all the children, Anti HBsAg surveillance should be carried out to confirm immunity after 6-12 months and need for a booster dose of vaccine in-patients with a low titre.

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REFERENCES

1. West DJ, Calandra GB, Ellis RW. Vaccination of infants and children against hepatitis B. *Pediatr Clin North Am* 1990; 37: 585-601.
2. Rasenack JWF, Schlayer H, Hettler F. Hepatitis B virus infection without immunological markers after open-heart surgery. *Lancet* 1995; 345: 355-357.
3. West DJ. Clinical experience with recombinant hepatitis B vaccine. *Am J Infect Control* 1989; 17: 172-180.
4. Greenberg DP. Pediatric experience with recombinant hepatitis B vaccines and relevant safety and immunogenicity studies. *Pediatr Infect Dis J* 1993; 12: 438-445.
5. De Montalembert M, Costagliola D, Lefrere JJ. Prevalence of markers for human immunodeficiency virus type 1, cytomegalovirus, and hepatitis B and C in multiply transfused thalassaemia patients. *Transfusion* 1992; 32: 509-512.
6. Szmunes W, Stevens CE, Harley EJ, Zang EA, Oleszko WR, Williams DC, *et al.* Hepatitis B vaccine: Demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Eng J Med* 1980; 303: 833-841.
7. Francis DP, Hadler SC, Thompson SE, Maynard JE, Ostrow DG, Altman N, *et al.* Prevention of hepatitis B with vaccine: Report from the Centers for Disease Control multi-center efficacy trial among homosexual men. *Ann Intern Med.* 1982; 97: 362-366.
8. Lo KJ, Lee SD, Tsai YT, Wu TC, Chen CY, Chen GH. *et al.* Long-term immunogenicity and efficacy of hepatitis B vaccine in infants born to HBeAg-positive HBsAg-carrier mothers. *Hepatology* 1988; 8: 1647-1650.
9. Coursaget P, Yvonnet B, Chotard J, Sarr M, Vincelot P, N'doye R, *et al.* Seven year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). *Lancet* 1986; 2: 1143-1155.
10. Goh KT, Tan KL, Oon CJ, Chan SH. Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: A four-year follow-up study. *Bull WHO* 1992; 70: 233-239.
11. Jilg W, Schmidt M, Deinhardt F. Vaccination against hepatitis B: Comparison of three different vaccination schedules. *J Infect Dis* 1989; 160: 766-769.
12. Matsaniotis N, Kattamis C, Laskari S, Liapaki K, Valassi-Adam H, Dionissopoulou E. Immune response to hepatitis B vaccine. *Lancet* 1981; i: 210.

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