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HEPATITIS B VACCINE ALONE OR IN COMBINATION WITH ANTI-HBS IMMUNOGLOBULIN IN THE PERINATAL PROPHYLAXIS OF BABIES BORN TO HBSAG CARRIER MOTHERS

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Summary. - The efficacy of hepatitis B virus (HBV) vaccine alone (group I) or in combination with hepatitis B immunoglobulin (HBIG) (group II) for prevention of perinatal transmission of the virus was assessed in 21 and 24 neonates, respectively. 58 infants who could not be vaccinated constituted the control group. It was observed that in the unvaccinated group ~ 70 % of the infants became infected. In both the vaccinated groups, the seroconversion and seroprotection rates (anti-HBs ≥10 IU/1) were almost similar at 6 months of follow up, but, at 12 months, infants given HBIG and vaccine showed better seroprotection rate (85 %) than those given vaccine alone (58.8 %). Immune response to the vaccine was also better in both the groups if the mothers were anti-HBe positive. Despite immunization, 14.2 % and 25 % infants in group I and II, respectively, became chronic carriers if their mothers were HBeAg positive.

Key words: hepatitis B virus vaccine; immunoprophylaxis; neonates

Introduction

HBV belongs to the family of Hepadnaviridae and affects more than 200 million people around the world. The virus may cause complications like acute fulminant hepatitis, cirrhosis, glomerulonephritis and hepatocellular carcinoma (Beasley, 1982; Di Bisceglie, 1988). Infection is usually acquired by parenteral route due to blood transfusion, contaminated syringes or by sexual intercourse. In addition, perinatal transmission from infected carrier mothers to their offspring is very common. However, this transmission varies with the serological status of the mother, 70-100 % babies becoming infected if mother

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is both HBsAg and HBeAg positive, while the risk is much lower (12–20 %) if the mother is HBsAg and anti-HBe positive (Stevens *et al.*, 1975; Alagille, 1985; CDC, 1985). With the availability of specific HBV vaccine for about a decade now, millions of doses have been administered without any inconvenient effects. Recently, a genetically engineered HBV vaccine has also been prepared and has undergone extensive application in clinical trials; it appears to be safe, antigenic and free from major side effect (Andre, 1989).

Hepatitis B prevalence in India has been reported about 2–10 %. Since there is a wide prevalence of antibody to HBV, the hepatitis B prevalence may be in fact much higher. It has been estimated that around 138 000 infants acquire the infection during their perinatal period each year (Nayak *et al.*, 1987). Hence, to control the transmission of the infection, immunoprophylaxis is urgently needed. The World Health Organization has already recommended the inclusion of HBV vaccine in the expanded programme of immunization (WHO, 1988).

We have already assessed the efficacy of HBV vaccine alone or in combination with hepatitis B immunoglobulin (HBIG) in the prevention of perinatal transmission for 6 months after birth (Sehgal *et al.*, unpublished results). The present study is a report of follow up including antibody titers for 12 months.

Materials and Methods

Subjects. The study was conducted in a total of 4137 pregnant women admitted in the labour wards of Nehru Hospital, Postgraduate Institute of Medical Education and Research, Chandigarh from January 1987 to December 1989.

A detailed history was taken regarding the past and present pregnancy with special reference to any episode of jaundice or liver disease in each case. Also, thorough clinical examination was performed. Collection of blood samples from all the pregnant mothers was done after obtaining an informed written consent at the time of delivery. Simultaneously, blood sample's were collected from the newborn. The sera were separated and stored in aliquots at −70 °C for further use.

Screening of maternal blood samples for HBsAg. Reverse passive haemagglutination assay (RPHA, Cellognost, Hoechst Diagnostics India Ltd.) of maternal samples was carried out daily according to manufacturer's instructions. Samples from babies of the HBsAg positive mothers were collected before vaccination and the sera were stored in aliquots at −70 °C.

Vaccination of neonates born to HBsAg positive mothers. Group I consisted of neonates who were given 10 μg of plasma derived HBV vaccine (Green Cross Corporation, South Korea) within 24 hr of birth intramuscularly into the thigh muscle. Second and third dose of vaccine was given at 4 and 8 weeks, respectively.

Group II consisted of neonates who were given HBV vaccine along with HBIG. The dose and route of vaccination was the same as in the group I. In addition, 0.5 ml of HBIG was given in the contralateral thigh muscle (Hepabig, Green Cross Corporation), within 24 hr of birth. Subsequent doses of vaccine were given as in the group I subjects.

Group III consisted of neonates who were not vaccinated. Initially, the vaccine was not available until April 1988. Though the mothers were explained the purpose of the vaccination, some did not give consent but agreed to come for follow up. Hence, these babies were included in the group III and served as controls.

Follow up of mothers and neonates. All the three groups were called for follow up at 3, 6 and 12 months from birth. Blood samples were collected from all the mothers and babies at the follow up.

The sera were separated and stored at −70 °C till use. All the HBsAg positive (by RPHA) and subsequent samples of the mothers and babies were checked for HBsAg, HBeAg, anti-HBeAg and anti-HBsAg antibodies with the use of ELISA test. HBsAg was checked by ELISA using the kit obtained from Biotest Diagnostics, FRG. HBeAg and anti-HBeAg antibodies were detected with the help of ELISA kit obtained from Hoechst Diagnostics India Ltd. The tests were performed according to the manufacturer's instructions.

Anti-HBsAg antibodies were detected with the help of ELISA kit obtained from Hoechst Diagnostics India Ltd. The standard curve was drawn from the dilution of the WHO anti-HBsAg antibody standard, and the results expressed as IU/1 of anti-HBsAg.

Results

Out of 4137 pregnant women screened, 109 (2.6 %) were positive for HBsAg. 24 and 27 neonates were enrolled in the group I and II, respectively, and 58 mothers who did not agree with the vaccination of their babies either because of the distance or due to non-availability of the vaccine, were considered as the group III.

Table 1 shows the results obtained in the children vaccinated with HBV vaccine alone (group I) or with HBV vaccine in combination with HBIG (group II). The seroconversion rates with the two schedules of the vaccine are almost similar, except that the seroconversion is marginally better in the group II. A similar trend was seen with the seroprotection rates (percentage of children with anti-HBsAg titers $>$ 10 IU/l) till 9 months of follow up, but, at 12 months after vaccination, the seroprotection rate in the group I was significantly ($P < 0.05$) lower (58.8 %) than in the group II (85 %). When the mean antibody titers at different time intervals were calculated in these children, the titers were similar in both groups till 9 months post vaccination. Subsequently, there was a significant decrease in the antibody titers in the group I subjects (15.02 ± 12.79 IU/l) as compared to the group II (21.97 ± 10.89 IU/l).

Table 2 and 3 show the maternal HBV markers and seroprotection rates in the babies in the group I and II subjects. It was observed that 42.8 % and 50 % of babies in the group I and II, respectively, showed seroprotection when mothers were HBeAg positive, whereas all the babies in the group I and 66.6 % in the group II were seroprotected when mothers were HBsAg positive only. When mothers were anti-HBeAg positive, then the immune response was better, i.e. 66.9 % and 90 % in the group I and II, respectively, were immune at 12 months follow up.

It was also observed that despite immunization the chronic carrier rate in babies born to HBeAg positive mothers was 14.2 % and 25 % in the group I and II, respectively, whereas in the case of babies born to HBsAg positive mothers, the carrier rate was 0 % and 16.6 % in the two groups, respectively. However, in the mothers with anti-HBeAg, the chronic carrier rate in babies decreased to 10 % in the group II while no carrier state was detected in the group I.

On the other hand in the group III (unvaccinated controls) by 12 months, 66.6 % of babies born to HBsAg and HBeAg positive mothers became positive

Table 1. Antibody levels, seroprotection rates and seroconversion rates after two schedules of vaccination

Time of follw-up (months)	Seroconversion rate*		Seroprotection rate*		Antibody titers	
	Vaccine	HBIG plus vaccine	Vaccine	HBIG plus vaccine	Vaccine	HBIG plus vaccine
3	17/21 (76.2 %)	20/24 (83.3 %)	14/21 (66.6 %)	17/24 (70.9 %)	22.27 ± 13.18	20.25 ± 14.2
6	18/21 (85.7 %)	22/24 (91.6 %)	17/21 (80.9 %)	19/24 (79.2 %)	29.18 ± 11.37	15.12 ± 14.2
9	18/21 (85.7 %)	22/24 (91.6 %)	17/21 (80.9 %)	20/24 (83.3 %)	21.3 ± 12.9	28.9 ± 13.8
12	14/17 (82.3 %)	18/20 (90.0 %)	10/17 (58.8 %)	17/20 (85.0 %)	15.02 ± 12.8	21.0 ± 10.9

* Results are expressed in ratios of No. of babies with anti-HBsAg antibodies to No. of babies followed up.

for HBsAg, whereas only 13 % babies became positive when the mothers were only HBsAg positive. Only 9 % babies acquired the infection when the mothers were HBsAg and anti-HBeAg positive.

Discussion

Various studies have reported that 70 % babies born to HBeAg positive mothers acquire the virus during their prenatal period, and a majority of these babies become chronic carriers (Alagille *et al.*, 1985; CDC, 1985; Smego and

Table 2. Correlation of maternal viral markers and seroprojective rates in the group I at 12 months follow up

No. of neonates	Maternal viral markers		
	HBeAg (+)	HBeAg (-) HBsAg (+)	Anti-HBeAg (+)
Total	7	6	8
Lost to follow up	0	2	2
Immune at 12 months (anti-HBsAg $\geq$ 10 IU/l)	2	4	4
HBsAg carriers	1	0	0

Table 3. Correlation of maternal viral markers and seroprojective rates in the group II at 12 months follow up

No. of neonates	Maternal viral markers		
	HBeAg (+)	HBeAg (-)	Anti-HBeAg (+)
Total	7	7	10
Lost to follow up	3	1	0
Immune at 12 months (anti-HBsAg $\geq$ 10 IU/l)	3	4	9
HBsAg carriers	1	1	1

Table 4. Viral marker follow up studies in unvaccinated babies and their mothers at 12 months

Antigen status of mother at parturition	No. of babies available for follow up*	No. of HBsAg positive during subsequent follow up					
		Mothers (months)			Babies (months)		
		3	6	12	3	6	12
HBsAg (+)**	23	20 (86.9)	18 (78)	17 (73.9)	4 (17.4)	3 (13)	3 (13)
HBsAg (+) and HBeAg (+)	15	14 (93)	14 (93)	13 (86.6)	8 (53.3)	10 (66.6)	11 (73.3)
HBsAg (+) and anti-HBeAg (+)	11	7 (63.6)	6 (54.5)	6 (54.5)	1 (9)	1 (9)	1 (9)
Total	49						

Figures in paranthesis represent percentages.

* Six babies were positive for HBsAg at birth. They are excluded form the above table.

** Two mothers and babies did not come for follow up. One baby expired at 2 months of age due to gastroenteritis.

Halsey, 1987; Ghaffar *et al.,* 1989). In the present study it was also observed that more than 70 % of the babies born to carrier mothers became positive for HBsAg if not vaccinated at birth.

The efficacy of HBV vaccine was assessed in two randomly allocated groups. It was observed that antibody titers and seroprotection rates after the two schedules of the vaccine were almost similar, except that in the group I patients, seroprotection was significantly lower as compared to the group II children at 12 months follow up.

The risk of babies becoming chronic carriers of HBV was maximum even after vaccination if the mothers were HBeAg positive (Tables 2 and 3). Immune response was better in the neonates if the mothers were only HBsAg positive or HBsAg and anti-HBeAg positive. Altogether, 14–15 % of the vaccinated infants became chronic HBsAg carriers. This is similar to the results obtained by Wheeley *et al.* (1990) by use of four doses of the vaccine. These authors reported that 71 % of the vaccinated neonates born to HBeAg positive mothers became immune and about 26 % become chronic carriers. In unvaccinated neonates, 70 % of them became chronic carriers, which is similar to our results and also results obtained by other workers (Ip *et al.,* 1989).

The overall antibody response in the neonates in the present study appears to be lower than previously reported by other workers (Polakoff and Vandervelde, 1988; Ip *et al.,* 1989). However, there are studies where the antibody response after 2 or even 3 doses of the vaccine is low; 4.7 and 10.9 IU/l, respectively (Andre, 1989). The seroconversion rate after 2 doses of the vaccine (10 μg) was only 70 % in children of 1–2 years age group in the same study. Even adults may not produce high levels of antibodies after 3 doses (Jilg *et al.,* 1990). These persons may be so called non-responders or poor responders (Hollinger, 1989). These groups of patients may require single or multiple doses of boosters. In addition, the other mechanisms which have been postulated as leading to a failure of the immune response include (a) intrauterine infection with HBV (Beasley and Hwang, 1983; Li *et al.,* 1986), in which case the vaccine is ineffective, and (b) a subgroup of "superinfective" mothers, in whom the infectivity is linked to maternal HBV DNA level (Ip *et al.,* 1989; Wheeley *et al.,* 1990). In the latter group, the dose of the virus may be high so that the vaccine may not be effective. In the present study, the seroprotection rates were much higher (85 %) in neonates given a combination of HBIG and vaccine as compared to those given vaccine alone. In the case of neonates whose mothers are HBeAg positive, HBIG has to be given along with the vaccine (Ip *et al.,* 1989; Wheeley *et al.,* 1990). Hence, it is suggested that a combination of HBIG and vaccine should be used. In addition, a booster dose of the vaccine should be given at 6/12 months so that the babies are protected for a further 2–3 years.

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