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RESULTS OF VACCINATED INFANTS BORN TO HBsAg – POSITIVE MOTHERS WITH DIFFERENT HEPATITIS B VACCINES AND DOSES*

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SUMMARY: Kuru Ü, Turan Ö, Kuru N, Sağlam Z, Alver A. (Department of Pediatrics, Bakırköy Maternity and Children's Social Security Hospital, İstanbul, Turkey). Results of vaccinated infants born to HBsAg-positive mothers with different hepatitis B vaccines and doses. Turk J Pediatr 1995; 37: 93-102.

Seventy-eight infants born to HBsAg-positive women were randomly assigned to receive either the plasma-derived vaccine or 0.5 ml (10 µg HBsAg) yeast-derived recombinant hepatitis B vaccine within 24 hours of birth, simultaneously with hepatitis B hyperimmunoglobulin. In 67 infants who received the plasma-derived vaccine, one of the doses of 0.5 ml (2.5 µg HBsAg) was used randomly. In all of the infants, the second and third doses of both vaccines were given at one and two months of age, respectively. The booster doses were given at 12 month of age in all of the infants. These vaccinated infants were followed up until 13 months of age.

There were differences in the seroconversion rates with different vaccines and doses. The recipients of the half-dose of plasma-derived vaccine showed lower seroconversion rates than the others, and the newborns in this group showed more seronegativity (13.2%) than the others ($p < 0.05$).

The lowest anti-HBs geometric mean titers (GMTs) were obtained in newborns vaccinated with Hevac B 0.5 ml. Sixty percent of the anti-HBs GMTs in this group were under 100 mIU/ml.

There were statistically significant differences between males and females in anti-HBs seronegativity rates, with males having lower anti-HBs GMTs than females. The difference was particulary significant among male newborns vaccinated with a half-dose of plasma-drived vaccine. *Key words: hepatitis B virus, hepatitis B vaccine, immunization, reduced vaccination schedule, sex.*

Transmission of the hepatitis B virus (HBV) during the perinatal period leads to the most devastating consequences of HBV infection. Women who are positive for the surface antigen of HBV (HBsAg) at the time of delivery and who have hepatitis Be antigen (HBeAg) in their sera have a 70 percent chance or greater of transmitting the infection to their newborn infants. At least 90 percent of these infected infants become chronic carriers of HBV.These HBV-carrier children have

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a life-time risk of dying from primary hepatocellular carcinoma or cirrhosis, usually during adulthood[1]. In addition, they serve as a reservoir of HBV infection for their families and communities[2,3].

Fortunately, treatment of these newborns shortly after birth with a combination of hepatitis B immune globulin (HBIg) and hepatitis B vaccine is 85-95 percent effective in preventing development of the HBV-chronic carrier state[4]. Both of the hepatitis B (HB) vaccines, plasma-derived vaccine and recombinant vaccine, are available in Turkey. When these vaccines are used by physicians in indicated cases, the dose and vaccination schedule which are advised by the manufacturer must be applied. However, in Turkey both of these HB vaccines are used in newborns of HBV-carrier-mothers at a dose of 0.5 ml, usually at zero, one and six months of age. However, the plasma-derived vaccine, Hevac B, is recommended by the manufacturer to be used at a dose of 1 ml (5 µg) and in a schedule of zero, one, two and 12 months of age for newborns of HBsAg-positive carriers.

Our study was conducted to determine the long-term effect of immunization against HBV infection with a one-half-dose of the plasma-derived vaccine, as well as to examine any differences in response to the hepatitis B vaccines between males and females.

Material and Methods

Hepatitis B Immunoglobulin and Passive Immunization:

Immuneglobulin containing 200 IU/ml of the antibody to HBsAg (Anti-HBs) (Hepuman Berna, Switzerland) was used within 24 hours of birth. HBIg was applied intramuscularly to the anterolateral thigh at a dose of 0.5 ml.

Vaccination and Active Immunization Schedule:

Active immunization was accomplished with a total of four injections of two different vaccines. Plasma-derived (Hevac B, Pasteur Institute) and recombinant vaccine (Engerix B, Smith Kline Biologicals) were used in the study. The plasma-derived vaccine was composed of 5 µg/ml of HBsAg, and the recombinant vaccine consisted of 20 µg/ml of HBsAg. Two doses of plasma-derived vaccine were used randomly: 0.5 ml (2.5 µg HBsAg) and 1 ml (5 µg HBsAg). The dose of recombinant vaccine which we used was 0.5 ml (10 µg HBsAg). These vaccines and doses were used in newborns randomly. The manufacturer of yeast-derived recombinant vaccine has marketed this vaccine only recently in Turkey; thus the number of cases vaccinated with this vaccine was lower than that of plasma-derived vaccine. The first doses were given within 24 hours after birth, as was HBIg. The second dose was administered at one month of age, and the third at two months of age. The booster doses were given at twelve months of age.

Mothers and Infants:

A total of 5366 pregnant Turkish women living in İstanbul and attending antenatal clinics of Bakırköy Maternity and Children's Social Security Hospital between February 1, 1991, and December 1, 1991, were screened for HBsAg by Elisa. The prevalence of HBV infection among the pregnant women was 4.2 percent.

Two hundred and twenty-five pregnant women with HBsAg-positive tests were informed of their status and of the risk of infection for their infants. Seventy-eight healthy full-term infants (33 males and 45 females) born to HBsAg-positive mothers were included in the study. All of these infants were vaccinated with one of the vaccines and doses, and HBIg was administered in all cases with 24 hours after birth. All of these infants were followed up for 13 months. Seven of the 78 mothers were both HBsAg and HBeAg-positive, and 71 of 78 were positive for HBsAg and the antibody to hepatitis Be antigen (anti-HBe).

Of the 78, twenty-five infants were vaccinated with Hevac B 0.5 ml, 42 infants with Hevac B 1 ml, and 11 infants with Engerix B 0.5 ml.

Laboratory Tests:

All of the infants were followed up until 13 months of age and were revisited at one, two, three, six, nine, 12 and 13 months of age. At the time of their follow-up visits, their blood samples were obtained by venipuncture. Serum was separated from clot within one hour of collection and stored at -30 °C until assayed and studied within 15 days. All of the HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe) and the antibody to hepatitis B core antigen [anti-HBc]) were evaluated. Anti-HBs values were expressed in geometrical mean titer (GMT) as milli-international units per millilitre (mIU/ml) Anti-HBs values were found by diluting the serum samples between 1/2 and 1/4096 according to the case. World Health Organization (WHO) standard anti-HBs serum which contained 10 mIU/ml anti-HBs was used as a reference. The absorbance value of the final positive dilution was multiplied by a dilution factor, and anti-HBs was calculated as mIU/ml.

It was accepted that there was seroconversion after the hepatitis B vaccination when the anti-HBs titer was greater than or equal to 10 mIU/ml.

Statistics:

Data were analyzed with statistical software (Extatix and StatView^{Tm}) by MacIntosh LC computer. Student's t test, Chi-Square, Kruskal Wallis and Mann Whitney U tests were used according to the situation. A p value of < 0.05 was considered to be significant.

Results

The seroconversion rates with different vaccines and different doses are presented in Table I. There was a statistical difference in seronegativeness and vaccination, with Hevac B 0.5 ml having the lowest seroconversion rate overall ($p < 0.05$).

Table I: Seroconversion Rates Which are Obtained with Different Hepatitis B Vaccinations

Vaccine	Dose ml	Total Test Number	Anti-HBs < 10 mIU/ml n	%	Anti-HBs ≥ 10 mIU/ml n	%
Hevac B	0.5	135	20	14.8	115	85.2
Hevac B	1	269	15	5.6	254	94.4
Engerix B	0.5	70	5	7.1	65	92.9

($p < 0.05$, chi-square)

Protective levels of anti-HBs with different vaccines and different doses at different time intervals are shown in Table II. One month after the booster dose, seroconversion rates were 97-100 percent in all groups. However, the seroconversion rates in the group vaccinated with Hevac B 0.5 ml were lower than the other groups in the first three months, and the difference in the first month was statistically significant ($p < 0.05$).

Table II: Seroconversions at Different Time Intervals with Different HB Vaccinations

Vaccine	Dose ml (µg)	n	Anti-HBs > 10 mIU/ml (%) Months 1	2	3	6	9	12	13
Hevac B	0.5 (2.5)	25	17/24 (70.8)	19/22 (86.4)	19/22 (86.4)	22/23 (95.7)	18/21 (85.7)	20/23 (87)	25/25 (100)
Hevac B	1 (5)	42	36/38 (94.7)	38/39 (97.4)	36/39 (92.3)	35/36 (97.2)	33/34 (97.1)	25/31 (85.4)	41/42 (97.6)
Engerix B	0.5 (10)	11	10/11 (91.9)	11/11 (100)	10/10 (100)	5/6 (83.3)	8/10 (80)	10/11 (91.9)	11/11 (100)

Table III shows the distributions of GMTs of anti-HBs at different time intervals. The lowest GMTs were obtained in the group of infants who were vaccinated with Hevac B 0.5 ml, but it was not statistically significant ($p > 0.05$). When we compared the anti-HBs GMTs obtained by different vaccines and doses according to months, there were statisticaly significant differences at one and six months ($p \leq 0.05$). As shown in Table IV, 60 percent of anti-HBs titers were less than 100 mIU/ml with Hevac B 0.5 ml usage. However, there was no statistical difference between the groups.

Table III: Anti-HBs GMTs at Different Time Intervals with Different Hepatitis B Vaccine and Dose

Vaccine	Dose ml	GMTs (mIU/ml) Months 1	2	3	6	9	12	13
Hevac B	0.5	19.9	30.4	23.4	51.2	113	119	1882
Hevac B	1	68	49.2	51	216	290	131	2997
Engerix B	0.5	50	35.7	68.2	43.5	108.5	122	7698

Table IV: Anti-HBs GMTs According to the Vaccines and Doses

Vaccine	Dose ml (µg)	n*	GMTs (mIU/ml) (%)				
			< 10	10-99	100-999	1000-10000	> 10000
Hevac B	0.5 (2.5)	160	20/160 (12.5)	76/160 (47.5)	40/160 (25)	19/160 (11.9)	5/1600 (3.1)
	1 (5)	269	15/269 (5.5)	108/269 (40)	88/269 (32.7)	44/269 (16.3)	15/269 (5.5)
Engerix B	0.5	70	5/70	31/70	18/70	11/70	5/70
	10		(7.1)	(44.4)	(25.7)	(15.7)	(7.1)

* n: shows total test numbers ($p > 0.05$), Chi-square).

Table V shows seroconversion rates according to sex. Of 215 tests done during a period of 13 months, 25 seronegative cases were found (13.2%) in the male newborn group. But among female newborns, the seronegative rate was 4.94 percent (14/283) and there was a statistically significant difference between sexes ($p < 0.01$). We concluded that male newborns showed more seronegativeness than females.

Table V: Seroconversion Rates According to Sex

Sex	n	Anti-HBs > 10 mIU/ml (%) Months						
		1	2	3	6	9	12	13
Male	33	26/32 (81.3)	30/31 (96.8)	31/33 (94)	25/27 (92.6)	21/27 (77.8)	24/32 (75)	33/33 (100)
Female	45	41/41 (100)	36/40 (90)	36/38 (94.7)	34/38 (89.5)	37/38 (97.4)	41/43 (95.3)	44/45 (97.8)

As for the GMTs according to sex, male newborns showed lower anti-HBs titers than female newborns; these results were statistically significant until 12 months ($p \leq 0.05$), but after the booster doses the difference was not significant ($p > 0.05$). Female newborns showed higher anti-HBs GMTs than males (Table VI).

Table VI: Anti-HBs GMTs According to Sex at Different Time Intervals

Sex	n	Anti-HBs GMTs mIU/ml (n) Months						
		1	2	3	6	9	12	13
Male	33	31.6 (32)	40.1 (31)	32.5 (33)	66.7 (27)	57.3 (27)	46.6 (32)	2261 (33)
Female	45	54.2 (41)	44.4 (40)	51.9 (38)	163.6 (38)	421 (38)	264 (43)	3504 (45)

Table VII shows anti-HBs GMTs at different times according to sex, vaccine and dose. There was a statistical difference between males who were vaccinated with Hevac B 0.5 ml and female newborns who were vaccinated with 1 ml Hevac B before the booster dose ($p < 0.05$). After the booster doses there was no significant difference.

Table VII: Anti-HBs GMTs Which were Obtained with Different Vaccines and Doses and Changes According to Sex

				GMTs (mIU/ml)						
				Months						
Sex	Vaccine	Dose (ml)	n	1	2	3	6	9	12	13
Male	Hevac B	0.5	10	10	36.6	23.6	55.1	36.6	52.1	1160
		1	17	62.3	49.7	32.7	117	133	42.5	2806
	Engerix B	0.5	6	42.7	27.7	54.4	13.5	14.2	49.6	3720
Female	Hevac B	0.5	15	32.7	23.2	23.2	48.8	264.2	223	2610
		1	25	72.5	61	81.8	364	469	270	3018
	Engerix B	0.5	5	62.4	48.5	96.8	104	822	350	18410

The same significant difference occurred when comparing male newborns vaccinated with Hevac B 0.5 ml and female newborns vaccinated with Engerix B 0.5 ml ($p < 0.05$). However, there was no significant difference between female newborns vaccinated with Hevac B 0.5 ml and male newborns vaccinated with Hevac B 1 ml ($p > 0.05$). The male newborns vaccinated with Hevac B 0.5 ml showed lower anti-HBs GMTs than male newborns vaccinated with Hevac B 1 ml, but there was no statistically significant difference between the two groups ($p > 0.05$). The female newborns vaccinated with Hevac B 0.5 ml had lower anti-HBs GMTs than those vaccinated with Hevac B 1 ml, and there was a significant difference between the two groups before the booster doses ($p < 0.05$).

Discussion

HBV-related liver diseases continue to be important health-care problems in developing countries. In areas of hyperendemicity, HBV is transmitted from asymptomatic carrier-mothers to their babies, especially when mothers are seropositive for hepatitis Be antigen. Almost all of such babies become persistent HBsAg carriers and constitute a reservoir of HBV for further spreading the virus in their families and the community. Also, chronic carriers of HBsAg are at high risk of developing chronic liver diseases such as cirrhosis and primary hepatocellular carcinoma later in life.

Vaccination with the hepatitis B vaccine and HBIg at birth is a safe and effective way of preventing HBV infection in endemic areas[5-7]. In order to eradicate HBV,

measures must be taken not only to interrupt mother-to-baby transmission in the perinatal period, but also to prevent horizontal transmission in later life[8, 9]. To date, approximately 50,000,000 doses of plasma-derived hepatitis B vaccine have been administered around the world. Although the vaccine is prepared from material obtained from the blood of HBsAg carriers, there have been no cases of acquired immune deficiency syndrome (AIDS) in plasma-derived hepatitis B vaccine recipients[9]. It has also been shown that recombinant-hepatitis B vaccines are effective and safe vaccines to prevent HBV infections[10].

The major factor affecting the immunogenicity will be the structure and source of antigen. HBsAg is a glycosylated protein composed of pre-S and s gene encoded regions. Vaccines differ in mode of purification and inactivation. In the process of isolation and purification from plasma, the structure of native HBsAg could be affected. Therefore recommendations from manifacturers as to the optimal doses and vaccination schedule of different vaccines which will produce 90 to 95 percent seroconversion, as well as suitable geometric mean titers at the end of immunization, can differ. Between two required criteria of HB vaccine for effective immunization, the dose which produces a 90-95 percent seroconversion rate is reasonable and universally accepted. The criteria for high titers of anti-HBs is more controversial. Some authors believe that once immunized above the so-called protective level of 10 mU/ml, subjects retain immunologic memory and sufficient immunity to be resistant to carrier-state infections even in the absence of detectable anti-HBs titers[11, 12]. But there are some conflicting data about this conclusion.

In our study, 78 infants born to HBsAg-positive mothers underwent active-passive immunization starting in the newborn period. In all groups, 97-100 percent of vaccinated infants showed protective antibody titers at 13 months of age. These high rates of seroconversion, which were obtained by hepatitis B vaccines, were also found in other studies[13-15]. Although the number of yeast-derived vaccines was lower than plasma-derived vaccines in our study, the vaccines elicited similiar immune responses. We also saw no side effects in the infants after the vaccination.

Although hepatitis B vaccine produces reliable immunity to infection by HBV, the high cost of the vaccine diminishes common use of it. Recombinant hepatitis B vaccines decreased this high cost, but did not solve the problem. The doses and the numbers of vaccinations were reduced to diminish the costs and were shown effective in several studies[16-18]. The intradermal route (id) was thought to be an alternative way to immunize against hepatitis B infection. Several studies have indicated that the less expensive id route will induce or boost immunity[19-20]. Some investigators have found that immunity following id vaccination was delayed and resulted in lower serum concentrations of anti-HBs than those observed following intramuscular vaccination[21]. Lee et al[22]. have demonstrated that infants

vaccinated with low-dose hepatitis B vaccine were more likely produce the HBV carrier state than those vaccinated with the full dose of vaccine. They concluded that low doses of hepatitis B vaccine and the id route were not recommended in newborns.

In our study, the low GMTs of anti-HBs and the high percentages of seronegativity were obtained after administration of the half-dose of plasma-derived vaccine. This can be explained by low presentation of HBsAg to the newborn and/or deficiency in their immune systems in this period. The low anti-HBs titers and high rates of seronegativity after low-dose hepatitis B vaccination were obtained in the first three months of age. We know that these infants are more dependent on their HBsAg-positive mothers in these first months than in other months of their lives. Despite hepatitis B vaccination, infants may be at risk of hepatitis B infection because of low immune response. We have planned to follow up the infants in our study for five years. Most of the infants are three years old now. HBsAg positivity was determined in one infant at two years of age. He was vaccinated with the half-dose plasma-derived vaccine and showed seropositivity at the 13-month follow-up. However, he was a low responder. Therefore, although some authors concluded that high anti-HBs titers were not important, and nor were high seroconversion rates at the end of hepatitis B vaccination as in our low-dose vaccinated infant pattern, HBV can in fact be obtained later by vaccinated infants who show seroconversion but have low anti-HBs titers. In our study, the vaccination schedule was chosen to be at birth and at one, two and 12 months of age. If a half-dose of plasma-derived vaccine were administered in three doses of hepatitis B vaccine, which is the schedule usually used in our country (at birth and at one and six months of age), then lower seroconversion rates and anti-HBs titers would be obtained. This means that more infants would be at risk of HBV infection by horizontal transmission.

It has been demonstrated by some investigators that female hepatitis B vaccine recipients had better seroconversion rates and higher anti-HBs titers than male recipients[23-25]. We too have found higher seroconversion rates and anti-HBs titers in female than male infants. There was a statistically significant lower response in male infants who were vaccinated with the half-dose plasma-derived vaccine.

Both plasma-derived and recombinant hepatitis B vaccine are available in Turkey. Both are effective and safe vaccines. There are some differences in the doses and vaccination schedules of different hepatitis B vaccines. We conclude that all newborns of HBsAg-positive mothers must be vaccinated at the dose and schedule which is advised by the manufacturer of that vaccine.

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