0021-7557/04/80-02/113
Jornal de Pediatria

Immune response of preterm infants to hepatitis B vaccine administered within 24 hours after birth

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Abstract

Objective: To investigate the immune response of preterm infants to hepatitis B vaccination.

Methods: Three doses of recombinant hepatitis B vaccine (5 µg dose) were administered to 35 preterm and 21 full-term infants within 24 hours after birth and at one and six months of postnatal age.

Results: A protective antibody response (anti-HB > 10 mUI/mL) was observed three months after the last dose in 92.6% and 100% of preterm and full-term infants ($p > 0.05$), respectively. Newborns with gestational age below 34 weeks presented lower antibody responses in all three periods. However, gestational age was not important to determine the antibody response in the three periods analyzed. When antibody response was analyzed in terms of birth weight, it was observed that a protective response was present in 75 and 100% of newborns with birth weight $\leq$ 1,500 g and > 1,500 g, respectively. Birth weight was shown to be a relevant factor in determining a protective antibody response at six months of postnatal age. Nonresponders received a fourth vaccine dose and an adequate antibody response was obtained in 100%.

Conclusion: The antibody response of preterm infants was similar to that of term newborns. Hepatitis B vaccination can be initiated on the first day of life in preterm newborns, following the same scheme recommended for term newborns. However, in preterm infants with birth weight less than or equal to 1,500 g, whose antibody response is lower, anti-HB titers should be monitored at nine months of age, or a four-dose vaccination scheme should be provided, with doses on the first day of postnatal life and one, six and nine months later.

J Pediatr (Rio J). 2004;80(2):113-8: Newborn, vaccination, hepatitis B.

Introduction

Hepatitis B is one of the most important infectious diseases afflicting humanity that can be prevented by immunization. Safe and effective vaccines against the hepatitis B virus (HBV) have been developed that make its eradication a theoretical possibility. However, HBV remains a significant cause of morbidity and mortality in Latin American countries.[1,2]

Conditions in developing countries favor HBV infection. In these regions, where the disease is highly or intermediately endemic, perinatal transmission performs an important role in propagating the disease. It is important to highlight the fact that newborn babies (NB) who have contracted the virus exhibit elevated chances of progressing as carriers, becoming reservoirs of the virus within the community. A significant proportion of these carriers will develop conditions related to HBV. Therefore, due to the severity of this process for the individual and because it is one of the ways in which the virus maintains itself within the community, this path of transmission must be prevented.

One strategy for breaking the perinatal transmission chain is by screening all pregnant women for serum HBV. The children born to those mothers carrying the HBV surface antigen (HBAg) should be given both the vaccine and hepatitis-specific hyperimmune immunoglobulin, preferably during their first 12 hours of life.[3] This combination of active and passive immunization of neonates greatly reduces their risk of

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Manuscript received Feb 20 2003, accepted for publication Nov 19 2003.

acquiring the virus. However, research performed by Xu et al.[4] found that the administration, in isolation, of the hepatitis B vaccine during the first 12 hours of life can contribute to a substantial reduction in perinatal transmission in full-term newborns (FTN).

Due to the risk of early infection and the fact that the incidence of the disease becoming chronic increases the earlier it is contracted, the American Academy of Pediatrics,[3] and, in Brazil, the Health Ministry[5] and the Brazilian Society of Pediatrics, recommend a scheme of three doses of the hepatitis B vaccine for FTN, on the first day, at one month and at six months.

A number of different studies of the immunoresponse to vaccination in FTN reveal seroprotection percentages above 95%, when started on the first day of life. Doubts persist, however, over the vaccine immunoresponse from premature newborns (PN), when vaccination is started on the first day of life.[6-13] It is not yet clear whether the response to immunization is determined by birth weight (BW), gestational age (GA), clinical conditions, age at start of vaccination or the number of doses administered. Even the most recent research has returned conflicting data. Sood et al.[14] analyzed the seroconversion response to HBV vaccine form PN and found that just 55% dos NB com a GA of less than 33 weeks exhibited protective titers, whereas the figure was 94% for those born after more than 34 weeks' pregnancy. In contrast, a study by Bhave et al.[15] described NB with GA of less than 34 weeks exhibiting equal seroprotection rates to FTN.

In the light of this ongoing controversy, it was decided to perform a study to investigate the capacity of PN to respond to the application of three doses of recombinant vaccine, administered in accordance with standard procedure for FTN, with the production of Anti-HBAg.

Patients and methods

This is a prospective study, based on clinical trials, performed at the Nursery Annexed to the Maternity Unit of the Hospital das Clínicas of the Medical Faculty of the Universidade de São Paulo (BAM – HC - FMUSP), between January of 1999 and March of 2001. It was approved by the Commissions for Ethics and Research of the Pediatrics Department of the HC-FMUSP, on 11 March 1998, hearing 034/98.

Sixty-one newborn babies were recruited from those admitted to the BAM-HC. Inclusion criteria were ignorance of maternal HBV serology at birth, collection of cord blood at birth for HBV and HIV tracking and the signing of free and informed consent forms by the appropriate responsible adults.

Neonates were excluded if they exhibited major congenital malformations, congenital infections, severe perinatal asphyxia or respiratory insufficiency requiring mechanical ventilation or if they were born to mothers with HBAg or HIV positive.

The NB recruited were divided into two groups according to gestational age: Group I – n = 40 PN (GA < 37 weeks) and Group II – n = 21 FTN, (GA $\geq$ 37 weeks). Gestational age was estimated from the last date of menstruation as long as this coincided with 20-week ultrasound scans. When this method could not be used GA was calculated using Capurro's method for FTN[16] and the New Ballard[17] method for PN.

The babies were characterized according to birth weight, sex, type of delivery, normality of birth weight to gestational age ratio (Alexander et al. curve[18]). This data was taken from the medical records of the mothers and their babies. Cases which, after inclusion in the study, presented cord blood serology findings positive for HBAg or HIV and cases in which exsanguination transfusion was performed were excluded.

The NB received three intramuscular doses of the recombinant vaccine Recombivax® HB (Merck & Co, Inc, Rahway, NY), of 0.5 ml (5 micrograms) each, via the anterolateral surface of the thigh. Vaccines were donated by the Merck Sharp Domme laboratory (MSD). The initial dose was administered on the day of birth and the follow-up doses at one and six months. Patients were observed for signs of local (erythema, nodules, heat) or systemic (fever, cutaneous eruption, incessant crying) reactions or of unexplained clinical manifestations for five days after each dose, as recommended in published literature.[19]

Blood samples were taken at six months (before administering the third dose), and again at nine and at twelve months. The blood sample was 1.5 ml in volume. This sample was centrifuged soon after collection and the serum was stored at –20 ºC. All samples were analyzed at the same time for HBAg, anti-HBs and anti-HBc by enzyme immunoassay (Abbott Laboratories). Samples with anti-HBs positive results were analyzed quantitatively using "AUSAB Quantitation Panel" (Abbott Laboratories), to determined the titers of this antibody. Antibody levels greater the 10 mUI/ml were considered protective (Advisory Committee on Immunization Practices*).*[20]

All cases were followed-up at the Follow-up Clinic at the *Instituto da Criança*. Cases which did not present protective levels of anti-HBs at twelve months received a fourth dose at between 15 and 18 months and anti-HBs titers were measured one month later.

Data was described in terms of central tendency measurement, 95% confidence intervals for continuous variables and by frequency distribution calculations for categorical variables. Seroprotection levels in both groups were calculated for six, nine and twelve months and compared using 95% confidence intervals. Based on the number of patients followed-up in each group it was possible to discriminate differences of more than 15% (100% vs. 85%) between the seroprotection levels of the FTN and PN when significance level was set at 5% and test power at 80%.

Members of the PN group who had responded with protective antibody levels were compared with those who had not were compared for the continuous variables birth weight and gestational age using an analysis of variance. Non-parametric variables were compared with the Mann-Whitney test. Categorical variables were put into 2 x 2 tables with variables such as type of delivery, sex and normal/abnormal weight for gestational age, dichotomized between protective responses and inadequate responses and then compared with Fisher's exact test. A logistic regression model was used to evaluate the relationships between weight and GA at birth with seroprotection. The frequency of occurrences of adverse clinical events resultant from the vaccine was also studied for each group. The significance level adopted was $p < 0.05$.

Results

Sixty-one neonates started the study, 40 in group I – PN and 21 in group II – FTN. Of the 40 cases in the PN group, five cases (12.5%) were excluded having received the first dose of the vaccine. Two of these cases resulted in death during the first month, two were given exchange transfusions and anti-HBs were detected in the cord blood of one baby.

Of the 56 cases (91.8%) that were not excluded, 27 (77%) in group I and 18 (86%) in group II completed the study. There were statistically significant differences within any of the groups between babies that completed the study and those that did not. The losses were the result of difficulties inherent in a one-year follow-up period.

All the PN and FTN, received their first dose of HBV vaccine within 24 hours of birth, the second dose at 32.2 days (3.9) and 31.7 (3.3) days and the third dose at 184.1 (13.7) and 187.7 (13) days, respectively.

Seroprotection rates for each group were assayed from the three blood samples, at 6, 9 and 12 months (Table 1). When the PN were compared with the FTN according to sample date and the level of vaccination response in terms of seroprotection, no statistically significant differences were found for any period. The number of cases involved allowed for an 80% test power to discriminate differences greater than 15%.

At six months twenty of the twenty-seven PN studied (74%, 95% CI = 55-87%) presented anti-HBs titers > 10 mUI/ml as did 17 of the 18 FTN, 17 (94%, 95% CI = 74-99%). At nine and ten months, these rates were 92.6% (95% CI = 77-98%) for PN and 100.0% (95% CI = 82-100%) for the FTN. It can be observed that the 95% confidence intervals of each group have an area of coincidence and significant statistical differences are not present.

Figure 1 shows that PN with GA of less than 34 weeks responded in smaller proportions than the PN who had GA between 34 and 36.9 weeks and with the FTN, but this difference was only significant at six months.

Observe, in Figure 2, that PN with birth weight less than or equal to 1,500 g responded in smaller proportions than the other two groups and that this difference was only statistically significant at six months.

Table 2 demonstrates that, after two doses of the vaccine, birth weight, gestational age and normality for gestational age were related with seroprotective titers. At nine and twelve months the only remaining statistically significant difference relates to birth weight.

A logistic regression model was used to analyze the association between weight and gestational age at birth with a positive response to vaccination with anti-HBs titers > 10 mUI/ml. At six months, the equation proposed to predict seroprotection response showed a statistically significant level of agreement between observed and predicted and observed values when the two variables were processed with Pearson's chi-square test ($p = 0.91$). The initial analysis, in which both variables were included, revealed that birth weight was a more important factor ($p = 0.04$) than gestational age ($p = 0.53$) in terms of seroprotection.

The equation proposed to predict seroprotective response did not produce statistically significant agreement between observed and proposed values at nine or twelve months when the Pearson chi-square test was applied ($p = 0.84$). The two independent variables included in the model, birth weight ($p = 0.20$) and gestational age ($p = 0.97$), did not exhibit statistically significant associations with seroprotection.

Only one FTN suffered an adverse event that was attributable to the vaccine, characterized by hyperthermia and intense crying which passed with an antipyretic. None of the PN presented any kind of adverse event. The two cases that did not respond to three doses of the vaccine did so one month after the fourth dose.

Table 1 - Distribution of PT* and FTN† who responded to vaccination with anti-HBs titers > 10 mUI/ml during the three test periods

	Response		No response		p‡
	n	%	n	%	
6 months					
PT	20	74.0%	7	26.0%	0.08
FTN	17	94.4%	1	5.5%	
9 months					
PT	25	92.6%	2	7.4%	0.35
RNT	14	100,0%	0	0.0%	
12 months					
PT	25	92.6%	2	7.4%	0.35
FTN	18	100.0%	0	0.0%	

* PT = premature newborns.
† FTN = full-term newborns.
‡ p = Fisher's exact test.

Table 2 - Comparison between PT* who responded and did not respond with higher anti-HBs titers (> 10 mUI/ml) at 6 months of age

	Response	No response	p
n of NB vaccinated	20 (74.0%)	7 (26.0%)	
Birth weight (g)			
Mean (SD)	2,111.2 (597.8)	1,314.2 (236.7)	< 0.01†
Median	2,040.0	1,380.0	
Maximum	3,300.0	1,550.0	
Minimum	1,200.0	950.0	
95%CI	1,831.5-2,391.0	1,095.4-1,533.2	
Gestational age (weeks)			
Mean (SD)	33.7 (1.9)	31.4 (1.6)	< 0.01†
Median	34.0	31.0	
Minimum	30.0	29.0	
Maximum	36.0	33.0	
95%CI	32.8-34.6	29.9-32.9	
Sex M/F ‡	12/8	3/4	0.66 §
Adequacy BW AGA/SGA ||	16/4	2/5	0.02 §
Type of delivery C/V ¶	18/2	5/2	0.26 §

* PT = premature newborn.
† Mann-Whitney's test.
‡ M = male F = female.
§ Fisher's exact test.
|| AGA = appropriate for gestational age; SGA = small for gestational age.
¶ C = cesarean; V = vaginal.

Discussion

The results returned did not detect an statistically significant difference between the seroprotection rates of the FTN and PT, but it is interesting to point out that at least 55% of the PN and 74% of the FTN presented anti-HBs levels above those considered necessary for protection after two doses of the vaccine, reducing their risk of perinatal HBV acquisition. In contrast, it must also be pointed out that the PN presented a slower increase in the anti-HBs titers than the FTN, remaining susceptible to HBV for a longer period of time. This data should be taken into account when immunization strategies are discussed in terms of impact of perinatal transmission.

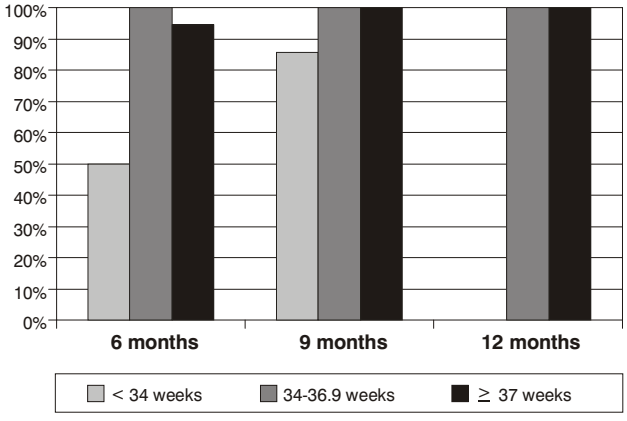

Figure 1 - Distribution of NBs who presented anti-HBs titers > 10 mUI/ml according to the gestational age (< 34 weeks, from 34 to 36.9 weeks and ≥ 37 weeks) and the test period

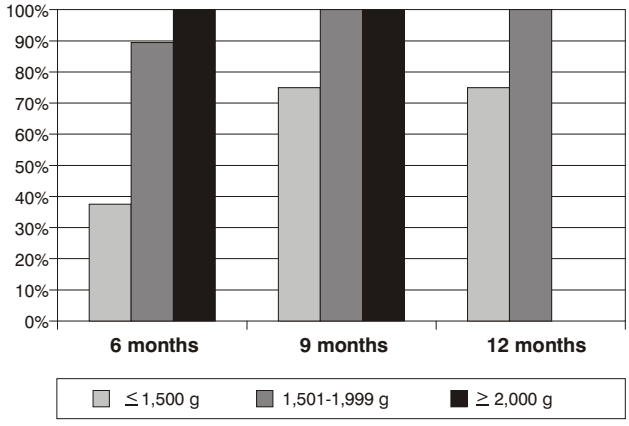

Figure 2 - Distribution of NBs who presented anti-HBs titers > 10 mUI/ml according to the birthweight (≤ 1,500 g, from 1,501 to 1,999 g and ≥ 2,000 g) and test period

Another factor to be remembered is that the dose employed may well have influenced the immunoresponse level that was different from other studies' findings. The magnitude of the immunoresponse to a vaccine is influenced by the dose employed. West[20] studied the immunoresponse post-vaccination of neonates and children to different doses (1.25; 2.5; 5 µg/doses of Recombivax®), finding that the group that received the 5.0 µg/dose presented the highest titers.

Two studies[7,21] that analyzed the immunoresponse of PN after three 10 µg doses (Engerix®), started on the day of birth, found seroprotection rates of around 90%. A different study,[10] using four doses of 10 µg each found 100%. However, Linder et al.[22] found seroprotection rates at 65% and 79% after two and three doses, respectively, among PN whose birth weights had been below 2,000 grams.

In research performed in Brazil,[23] also using a 10 µg dose, initiated during the first week of life for PN with BW < 1,800 g observed seroprotection rates were 77.4% (95% CI = 64.7 to 87.1%) after three doses. This rate, lower than that found in our study, is probably the result of differences between the populations studied since in the Motta et al. study[23] the percentage of children born weighing less than 1,500 g was 67.0% while in the current study it was 26%.

In a study performed by Lobonsky et al.[13] the percentage of NB with very low birth weights was 30% and seroprotection rates were much lower; 21% and 69.6% after two and three doses of the vaccine, respectively. Both studies used the recombinant vaccine (Recombivax®), but our study employed double the dose which may explain the higher seroprotection rates.[10,20,24]

It can be observed that PN whose GA was less than 34 weeks were less likely to respond to the three dose vaccination scheme (Figure 1). Comparing this data with the few studies that analyzed immunoresponse as a function of GA, it can be observed that Chawareewong et al.[21] found similar seroprotection rates; 78% for NB with GA < 32 weeks and 100% for those between 33 and 37 weeks, whereas Belloni et al.[6] found 100% among NB whose GA was < 32 weeks, and 40% of whom had presented GA of less than 30 weeks.

Analyzing PN whose BW was < 1,500 g, it was observed that 37.5 and 75% (Figure 2) of them responded to the second and third doses of the vaccine. Newborns whose birth weight had been > 1,500 g responded with higher rates of seroprotection. All of the PN whose BW was > 2,000 g presented seroprotection at all three test periods. Patel et al.[24] studying the immunoresponse of PN, with birth weights < 1,500 g, found seroprotection levels that were greatly reduced. Both studies used the same recombinant vaccine, but our study used double the dosage and, as was explained above, the production of antibodies is directly related to dose administered.[10,20,22] Two further studies[10,13] found low rates of seroprotection among NB whose birth weights were less than 1,000 g while among NB whose BW was from 1,000 to 1,500 g this varied from 67% to 71% and among those weighing more than 1,500 g at birth the rate was above 90%.

All of these studies, in common with this one, suggest that the NB who respond least to vaccination against HBV are those whose BW was < 1,500 g. Only Belloni et al.[6] found 100% seroprotection among NB with BW < 1,500 g and 98% among those with BW > 1,500 g.

When PN who responded with seroprotective titers were compared with those who had not, it was observed that, at six months, BW, gestational age and normality of weight for gestational age were statistically related to seroprotection (Table 2). The NB who hadn't responded had BW and GA less than had those who had. It can also be observed that babies who were born at weights adequade for their gestational ages responded better than those who were not. It was later possible, by means of multivariate analysis, to exclude GA as a variable related to response and discern that the lack of response was in fact only related to BW. These findings agree with Losonsky et al.,[13] who also analyzed large numbers of variables in terms of vaccination response.

All of the patients who had not responded to three doses did respond to the fourth. Two other studies[10,11] also observed a 100% response rate to a supplementary dose. In the case of Losonsky et al.,[13] however, this response only occurred in 50% of cases. In data reported by Motta et al.[23] it can be observed that PN who received three or four doses of the vaccine presented 90% seroprotection. In a study by Ballesteros-Trujillo et al.[25] it was observed that, after four doses of vaccine, 29 PN had responded well (89.7%) and that this response was not correlated either with BW or with GA.

In the present study, observing anti-HBs titers for twelve months, it was found that NB who had seroprotective levels after the third dose maintained these levels for their first year of life. Two studies[26,27] which assessed the behavior of anti-HBs levels for three years among both PN and FTN observed falling antibody levels in both groups, with no statistically significant difference between them.

The findings of this study suggest that the administration of three doses of the vaccine against hepatitis B to, clinically stable, premature newborns, started on the day they are born is capable of stimulating the production of anti-HBs antibodies at protective levels. This includes newborn babies whose birth weight was less than 1,500 g. However, among these last the seroprotection levels were lower as was seen in a number of recent studies.[10,13,23,24] This being so, members of this specific group should be followed-up between one and three months after the end of vaccination. Those who have not yet responded with seroprotection should be given a supplementary dose of the recombinant vaccine. Another option, primarily for use in regions where it is difficult to perform serum

assays, would be the standardization of a four-dose HBV vaccination program for these neonates which would be administered at birth and at one and six months and then again between nine and twelve months, since there was an evident increase in seroprotection levels at this point both in this research and in other studies.[10,11,23,25]

References

1. Tanaka J. Hepatitis B epidemiology in Latin America. Vaccine. 2000;18 Suppl 1:17-9.
2. Torres JR. Hepatitis B and hepatitis delta virus infection in South America. Gut. 1996;38 Suppl 2:48-55.
3. Saari TN and Committee on Infectious Diseases. American Academy of Pediatrics. Immunization of preterm and low birth weight infants. Pediatrics. 2003;112(1):193-8.
4. Xu Z-Y, Liu C-B, Francis DP, Purcell RH, Gun Z-L, Duan S-C, et al. Prevention of perinatal acquisition of hepatitis B virus carriage using vaccine: preliminary report of randomized, double-blind placebo-controlled and comparative trial. Pediatrics. 1985;76(5):713-8.
5. Manual de Normas de Vacinação – Ministério da Saúde. Fundação Nacional de Saúde. 3ª ed. Brasília: Ministério da Saúde; 2002. p. 25-26.
6. Belloni C, Chirico G, Pistorio A, Orsolini P, Tinelli C, Rondini G. Immunogenicity of hepatitis B vaccine in term and preterm infants. Acta Paediatr. 1998;87:336-8.
7. Blondhein O, Bader D, Abend M, Peniakow M, Reich D, Posteman I, et al. Immunogenicity of hepatitis B vaccine in preterm infants. Arch Dis Child Fetal Neonatal. 1998;79:F206-8.
8. Chirico G, Belloni C, Gasparoni A, Cerbo RM, Rondini G, Klersy C, et al. Hepatitis B immunization in infants of hepatitis B surface antigen-negative mothers. Pediatrics. 1993;92:717-9.
9. Del Canho R, Grosheide P, Gerards L, Heijtink R, Schalm S. Hepatitis B vaccination and preterm infants. Pediatr Infect Dis J. 1993;12(5):407-8.
10. Golebiowska M, Kardas-Sobantka D, Chlebna-Sokol D, Sabanty W. Hepatitis B vaccination in preterm infants. Eur J Pediatr. 1999;158:293-7.
11. Kim S, Chung E, Hodinka R, Demaio J, West D, Jawad A, et al. Immunogenicity of hepatitis B vaccine in preterm infants. Pediatrics. 1997;99:534-6.
12. Lau Y-L, Tam A, Ng K, Tsoi N, Lam B, Lam P, et al. Clinical and laboratory observations - response of preterm infants to hepatitis B vaccine. J Pediatr. 1992;12:962-5.
13. Losonsky GA, Wasserman SS, Stephens I, Mahoney F, Armstrong P, Gumpper K, et al. Hepatitis B vaccination of premature infants: a reassessment of current recommendations for delayed immunization. Pediatrics. 1999;103(2):1-7.
14. Sood A, Singh D, Mehta S, Midha V, Kumar R. Response to hepatitis b vaccine in preterm babies. Indian J Gastroenterol. 2002;21(2):52-4.
15. Bhave S, Bhive S, Chavan SC, Naik SS, Pusapati RV, Bavdekar A, et al. Hepatitis B vaccination in premature and low birth weight (LBW) babies. Indian Pediatr. 2002;39(7):625-31.
16. Capurro H, Konichezky S, Fonseca D, Caldeyro-Barcia R. A simplified method for diagnosis of gestational age in the newborn infant. J Pediatr. 1978;93(1):120-2.
17. Ballard JL, Khoury JC, Wedig K, Wang L, Ellers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. J Pediatr. 1991;119(3):417-23.
18. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United State national reference for fetal growth. Obstetr Gynecol. 1996;87(2):163-8.
19. Nui MT, Davis DM, Ellenberg S. Recombinant hepatitis B vaccination of neonates and infants: emerging safety data from the vaccine adverse event reporting system. Pediatr Infect Dis J. 1996;15:771-6.
20. West DJ. Clinical experience with hepatitis B vaccines. Am J Infect Control. 1989;17:172-80.
21. Charareewong S, Jirapongsa A, Lokaphadhana K. Immune response to hepatitis B vaccine in premature infants. Am J Trop Med Public Health. 1991;22:39-40.
22. Linder N, Handsher R, German B, Sirota L, Bachman M, Zinger S, et al. Controlled trial of immune response of preterm infants to recombinant hepatitis B and inactivated poliovirus vaccines administered simultaneously shortly after birth. Arch Dis Child Fetal Neonatal. 2000;83:F24-7.
23. Freitas da Motta MS, Mussi-Pinhata MM, Jorge SM, Tachibana Yoshida CF, Sandoval de Souza CB.. Immunogenicity of hepatitis B vaccine in preterm and fullterm infants vaccinated within the first week of life. Vaccine. 2002;20:1557-62.
24. Patel DM, Butler J, Feldman S, Graves GR, Rhodes PG. Immunogenicity of hepatitis B vaccine in healthy very low birth weight infants. J Pediatr. 1997;130:641-3.
25. Ballesteros-Trujillo A, Vargas-Origel A, Alvarez-Munoz T, Aldana-Valenzuela C. Response to hepatitis B vaccine in preterm infants: four dose schedule. Am J Perinatol. 2001;18(7):379-85.
26. Kesler K, Nasenbeny J, Wainwright R, McMahon B, Bulkow L. Immune responses of prematurely born infants to hepatitis B vaccination: results through three years of age. Pediatr Infect Dis J. 1998;17:116-9.
27. Khalak R, Pichichero ME, D'Angio CT. Three-year follow-up of vaccine response in extremely preterm infants. Pediatrics. 1998;101(4):597-603.

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