The effect of concurrent use of hepatitis B and Bacille Calmette–Guérin vaccination on anti–hepatitis B response

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ABSTRACT

Objectives: Bacille Calmette-Guérin (BCG) is given at 2-months of age; diphtheria toxoid, tetanus toxoid, whole cell pertussis, oral polio are given at 2, 3, 4 months, and hepatitis B virus (HBV) vaccine is given at 3, 4, 9 months of age. The aim was to evaluate the sero-protection rate of HBV vaccine which has been given at 2, 3, and 9 months of age and coincided with BCG vaccine at the first dose.

Methods: Hepatitis B virus vaccine was administered to 3 groups of infants at 2, 3, 9 months (n=20), 3, 4, 9 months (n=20) concurrently with BCG or other vaccines, and at 0, 1, 6 months with no other coinciding another vaccine (n=20). These 60 infants who were born between June 2001 and September 2001 have been vaccinated at the Akdeniz University School of Medicine, Antalya, Turkey. Antibodies to hepatitis B surface antigen analyzed by enzyme-linked immunosorbent assay.

Results: The simultaneous administration of BCG and HBV vaccines did not influence the immune response to HBV vaccine. We showed that all 3 schedules can induce the sero-protective levels of antibody concentrations to HBV both one month and one year after the vaccination.

Conclusion: Bacille Calmette-Guérin and HBV vaccinations can be performed at the same time in endemic countries.

Saudi Med J 2004; Vol. 25 (12): 1939-1942

Turkey is a moderately endemic country for hepatitis B virus (HBV) infection.[1,2] Although variations are observed in different geographic areas, average chronic carriage rate for HBV is reported to be 5% (range 3.1-10.4) in Turkey. There is a high well-established data indicating that HBV vaccination should be given not only to high risk groups, but to all newborns, especially in populations which do not have a screening program for HBV sero-profile during pregnancy.[3,4] Based on epidemiological data and country's resources, universal infant immunization program at 0, 3, and 9 months of age has been adopted in Turkey, since 1998. However, since our national health organization does not have a routine follow-up visit for infants during the first month of life, the program turned out to be 3, 4, and 9 months of age in most of our children. This schedule enabled the integration of HBV vaccine with routine expanded program on immunization vaccines, without causing extra visit for preventive health care. At 3 and 4 months of age, HBV vaccine is given concurrently with diphtheria toxoid, tetanus toxoid, whole cell pertussis (DTwP) vaccine and oral polio vaccine (OPV) and at 9 months with measles vaccine. Hepatitis B virus vaccine has not been recommended at 2 months of age, in some reason interaction between Bacille Calmette-Guérin (BCG)

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Received 29th May 2004. Accepted for publication in final form 28th July 2004.

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and HBV is not well known. Thus, our well-established national immunization program includes BCG, DTwP and OPV vaccines at 2 months of age. However, the addition of this new vaccine (HBV) to the old vaccination program caused some misunderstandings and some of our children have been vaccinated at the second, third, and ninth months. The aim of this study is to evaluate the sero-protection rate of HBV vaccine, which has been given at 2, 3, and 9 months of age and coincided with BCG vaccine at the first dose.

Methods. Sixty infants born between June 2001 and September 2001 were randomly assigned to the study and vaccinated with 3 different protocols, arranged in order on admission (**Table 1**). Group I included 20 infants vaccinated at 2, 3, and 9 months of age. The vaccination program of these infants included BCG, DTwP, OPV, and HBV vaccines at the second month; DTwP, OPV, and HBV vaccines at the third month; and measles and HBV vaccines at the ninth month. Group II consisted of 20 infants vaccinated at, 3, 4, and 9 months of age. Their vaccination protocol included DTwP, OPV, and HBV vaccines at the third and fourth months, and measles at the ninth month. Twenty infants in group III were vaccinated with HBV vaccine at 0, 1, and 6 months of age. This program did not coincide with any other vaccine within the national immunization program. The first dose of the HBV vaccine was given at the first 5-days of life.

The exclusion criteria for the study were to have a history of (i) amniocentesis or cordocentesis, (ii) being born prematurely or with a low birth weight, (iii) transfusion of blood, blood products, or immunoglobulin preparations, (iv) any known chronic illness or (v) moderate to severe infection at the time of vaccination, and (vi) born from a mother who has seropositivity for HBV.

The same vaccine (Euvax 10µg/dose, Goldstar, Seoul, South Korea) was introduced intramuscularly to the anterolateral thigh of infants, at the Akdeniz University School of Medicine, outpatient clinic of the Pediatrics, Antalya, Turkey. Two sera samples were collected from each infant and stored at -20^{0}C until testing. The first sample named "next to the vaccination" was drawn 4-6 weeks after the last dose of vaccination while the second sample named "one year after the vaccination" was collected 13 months after the last dose. Sera were assayed in duplicate simultaneously for antibody to hepatitis B surface antigen (anti-HBs) by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Hepanostika anti-HBs, Organon, The Netherlands) according to the manufacturer's recommendations. We accepted the concentrations of 1 -10 IU/L as "seropositivity," and over 10 IU/L as "sero-protection" levels of antibodies. The significance of differences between geometric mean titers, and sero-protection rates were determined using non-parametric tests by Statistical Package for Social Sciences software.

We got an informed consent from the mothers. Every infant was observed for 15 minutes after vaccination. The parents were asked for 3 adverse events (severe pain, swelling >20 mm, fever >38^{0}C) when they came to analysis.

Results. In all schedules, HBV vaccine was well-tolerated. No side effect was observed and notified in early postvaccination period. We found no difference in seroconversion rates among 3 groups. Antibody levels over 1 IU/L were obtained in all infants. In Group I and Group III, the

Table 1 - Immunization protocols of the study (N=60).

Study group	0-5 days	First month	Second month	Third month	Fourth month	Sixth month	Ninth month
Group I (n=20)	-	-	DTwP OPV BCG HBV	DTwP OPV HBV	DTwP OPV	-	Measles HBV
Group II* (n=20)	-	-	DTwP OPV BCG	DTwP OPV HBV	DTwP OPV HBV	-	Measles HBV
Group III (n=20)	HBV	HBV	DTwP OPV BCG	DTwP OPV	DTwP OPV	HBV	Measles

*as in the national immunization program
DTwP - diphtheria toxoid, tetanus toxoid, whole cell pertussis, OPV - oral polio vaccine, HBV - hepatitis B vaccine, BCG - bacillus Calmette-GuÈrin

Table 2 - The means of the early and late antibody concentrations of HBV vaccinated infants at 2, 3, 9 (Group I), 3, 4, 9 (Group II), 0, 1, 6 (Group III) months (IU/L).

Study groups	Group I	Group II	Group III
Next to the vaccination	773 (155-1000)	970 (660-1000)	812 (66-1000)
One year after vaccination	537 (35-1000)	518 (4-1000)	534 (13-1000)
	r= 0.68 p<0.001	r= 0.16 p<0.001	r= 0.56 p<0.001

HBV - hepatitis B vaccine,

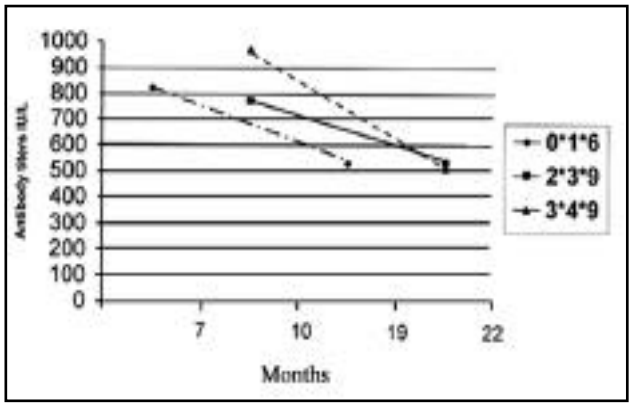

Figure 1 - The means of antibodies to hepatitis B surface antigen in three other schedules, next to the vaccination and one year after the vaccination.

sero-protection rates were invariably 100% next to vaccination, also one year after vaccination. Although in Group II, sero-protection rate was 95% next to the vaccination, it declined to 90% at one year after the vaccination. We found no difference between the mean antibody concentrations of Group I and Group III, next to the vaccination ($p>0.05$). But, the mean was higher in Group II than Group I and Group III ($p<0.0001$). We found no differences in means of antibody titers between each group, one year after vaccination ($p>0.05$). We found a significant correlation in both Group I and Group III for antibody concentrations measured with one year interval (**Table 2**). We observed a decrease in antibody titers in each group, one year after vaccination than next to the vaccination ($p<0.0001$) (**Figure 1**). One infant in Group II showed a low response to vaccine when tested next to vaccination although her antibody concentration was found to be >10 mIU/ml one year later. Except for 2 infants in Group II we found the presence of sero-protective antibodies in all infants one year after the vaccination. These infants do not have a health problem. We found the sero-protective level one month after the vaccination with an additional 20µg dose.

Discussion. The HBV vaccination is introduced at 3, 4, 9 months of age in Turkey. Contrary to this practice, routine immunization of newborns within 12 hours after birth is recommended in countries where HBV carriage status of the pregnant women is unknown and the suggested schedule is 0, 1, and 6 months.[5,6] We observed no side effect of HBV vaccine when given concurrently with BCG or other vaccines. Fever during the first 3 days of vaccination in the newborns had been reported.[7] No unexpected adverse events had been reported in neonates and infants given hepatitis B vaccine, despite the use of at least 12 million doses of vaccine in these age groups.[8] Sero-protection rates observed next to the vaccination by 2, 3, 9 months schedule were 100%, 3, 4, 9 months schedules were 95% and 0, 1, 6 months schedules were 100%. These results are comparable with the formerly reported rates.[2,9] Twelve-month antibody concentration is known to be a strong predictor of antibody persistence. Furthermore, antibody loss does not necessarily mean loss of immunity to HBV.[2,9,10]

Alikasifoglu et al[10] reported a sero-protection rate of 100% in 180 infants vaccinated with 6 different schedules of 2 other recombinant HBV vaccines. A sero-protection rate of 96% was as well-reported in infants vaccinated at the first week, sixth week, and 5.5-months.[11] Our results may be better than the last study due to the completion of HBV vaccine series before 6 months of age is not routinely recommended.[10] We could not find any record relevant to HBV vaccination of infants at 2, 3, and 9 months. Although case number is very limited, our data show that satisfactory sero-protection rates could be obtained by this schedule. Available data suggest that simultaneous administration of HBV vaccine with DTP and OPV neither increases the side effects nor decreases the sero-protection rates.[12-14] In another study in which HBV vaccine was given with *Haemophilus influenzae* type b, DTP and polio vaccines, no change in the sero-protection rates were recorded.[15] We encountered only one study for HBV and BCG vaccines given concurrently. The adverse effects and sero-protection rates of 360 infants given HBV, BCG and meningococcal vaccines alone or together in 5 different schedules were investigated by Yuan.[16] They did not observe any difference in antibody responses or side effect frequencies between the different schedules. In this study, we found that the serological responses to HBV vaccine

given simultaneously with either BCG, or other vaccines are similar. We showed that all 3 schedules can induce sero-protective levels of antibody concentrations to HBV. The permanent protective immunity to HBV is reported to be especially dependent to B lymphocytes. In general, there is a rapid decline in protective antibody in the first 12 months after the third dose and a more gradual decline over time.[2,17] The recent data for the mechanism of anamnestic immune response to HBV in long term indicates the presence of an immune memory even when antibodies decrease or disappear after the primary vaccination.[2,18]

In conclusion, BCG vaccine does not change the serological response to HBV vaccine given concurrently. Hepatitis B virus vaccination schedule of 2, 3, 9 months induce protective levels of anti-HBs both next to vaccination and one year after vaccination, similar to 3, 4, 9 months and the 0, 1, 6. months schedule. In a moderately endemic country, if all the pregnant could not be screened for HBV sero-profile or all newborns could not be reached, the HBV vaccination might be started at 2 months with BCG.

Acknowledgment. We would like to thank the families who joined in this study, the staff of the Central Biochemistry Laboratory of Akdeniz University Medical School, Medila Laboratory, Biostatistics Department of Akdeniz University Medical School who made their facilities available for us. This study was supported by a grant from the Research Foundation of Akdeniz University, Antalya, Turkey.

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