RESEARCH PAPER

Anti-HBs Titers Following Pentavalent Immunization (DTwP-HBV-Hib) in Term Normal Weight vs Low Birth weight Infants

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Received: December 18, 2016; ***Initial review***: March 06, 2017; ***Accepted***: January 15, 2018.

PII: S097475591600108

ABSTRACT

Objective: To compare anti HBs titers between term low birth weight (1800-2499 g) infants and normal birth weight infants, 6 weeks after last dose of primary immunization with pentavalent vaccine, and to study adverse events following immunization (AEFI) with pentavalent vaccine. **Design**: Cohort study **Setting**: Tertiary-care hospital predominantly catering to urban poor population of East Delhi. **Participants**: 265 low birth weight (1800-2499 g) and 265 normal birth weight (2500-4000 g) infants Monovalent Hepatitis B vaccine was administered within 24 hours of birth followed by three primary doses of pentavalent vaccine at 6, 10 and 14 weeks. Anti HBs titers were estimated after 6 weeks of third dose of pentavalent vaccine. Adverse events following immunization were observed after each dose of pentavalent vaccine for a month. **Main outcome measures**: Anti HBs antibody titers after 6 weeks of primary immunization; and adverse events following immunization (AEFI) with pentavalent vaccine were also observed. **Result**: Total 443 (83.5%) infants (225 low birth weight and 218 normal birth weight infants) completed the follow up. Seroprotection against hepatitis B virus was achieved in both groups after pentavalent vaccine administration. Anti HBs GMTs in low birth weight infants (194.8 mIU/mL) and normal birth weight infants (204.2 mIU/mL) were comparable (P = 0.175). No serious adverse events were observed in either group. **Conclusion**: Three primary doses of pentavalent vaccine administered along with zero dose of Hepatitis B vaccine at birth provide good seroprotection, and appears to be safe in both low birth weight and normal birth weight infants born at term.

Keywords: *Hepatitis B vaccine, Combination vaccines, Immunogenicity, Low birthweight infants, Neonate*.

INTRODUCTION

Universal immunization against hepatitis B in infancy starting at birth has resulted in marked reduction in HBV related chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. An anti-HBs concentration of ≥ 10 mIU/mL measured 1–3 months after administration of the last dose of the primary vaccination is considered a reliable marker of protection against HBV infection [1]. However, the antibody response to hepatitis B vaccine has been shown to depend on the schedule of vaccination, birth weight, gestation, chronological age, gender, genetic factors, co-morbidities and the immune status of the vaccinee [2]. Preterm infants weighing <2000g at birth may not mount an adequate response to hepatitis B vaccine. The World Health Organization (WHO) recommends that the birth dose of hepatitis B vaccine to such preterm infants should not be counted and an additional dose of hepatitis B vaccine should be given to them [3]. Term low birth weight (LBW) infants weighing 1800 to 2499g; with several of them being small for gestational age, may lie in the grey zone of the immunity where they may be vulnerable despite being born chronologically mature [4]. Immunogenicity of monovalent hepatitis B vaccine in term low birth weight babies has been found to be satisfactory [5,6]. However, there is inadequate data on the immunogenic response of hepatitis B vaccine after immunization with pentavalent vaccine (DTwP-HBV-Hib) among term LBW infants when 'zero' dose of monovalent hepatitis B vaccine is also administered at birth. This study compared anti HBs titres after 6 weeks of primary immunization with pentavalent vaccine between term infants weighing 1800-2499 g at birth and normal birth weight infants. Infants in both groups were also observed for adverse events following pentavalent vaccine administration.

METHODS

This cohort study was conducted in the Departments of Pediatrics and Microbiology at University College of Medical Sciences and Guru Tegh Bahadur Hospital, Delhi over a period of 17 months (December 2013-April 2015) after approval from the Ethical Committee of the institute and written informed consent from the parents.

Clinically healthy eligible neonates born consecutively at term gestation were allocated in LBW (1800 to 2499 grams) and normal birth weight (2500 to 4000 grams) groups within 24 hours of birth till desired sample size was reached. Infants born to hepatitis B positive mothers, neonates suffering from sepsis, birth asphyxia, meconium aspiration syndrome, gross congenital anomalies, requiring exchange

transfusion and whose families were planning to leave the area before the period of completion of study were excluded.

Sample size was calculated based on the study by Sharma, *et al*. [7] Wherein indigenous pentavalent vaccine produced anti HBs geometric mean titers (GMT) of 616.7 mIU/mL in healthy term infants . Expecting a difference of 15% in anti HBs titers between LBW and normal birth weight babies, at 80% power and 5% level of significance, 476 infants were required, equally divided between LBW (birth weight 1800-2499g) and normal birth weight (birth weight 2500-4000g) groups. Considering an estimated attrition rate of 10%, we planned to recruit 265 infants in each group (total 530 infants).

Pentavalent vaccine consisting of Diphtheria, Tetanus, Pertussis, Hepatitis B, and Haemophilus influenzae type B Conjugate vaccine adsorbed (Serum Institute of India Ltd, Pune) was used. Each dose of 0.5 mL contained Diphtheria Toxoid 25 Lf (30 IU), Tetanus Toxoid 2.5 Lf (40 IU), *B. pertussis* (whole cell) 16 OU (4.0 IU), HBsAg (rDNA) 10 mcg and Purified capsular HiB Polysaccharide (PRP) conjugated to Tetanus Toxoid (carrier protein) 10 mcg.

We collected 2 mL of cord blood in plain sterile vial from placental end and stored at -20˚C after separation of serum. Breastfeeding was initiated within 1 hr after normal delivery; and within 2 hrs in babies delivered through caesarean section. Monovalent recombinant Hepatitis B vaccine (dose 0.5 mL, 10 mcg purified HBsAg), manufactured by Biological E. Ltd, India, was administered in the anterolateral aspect of thigh within 24 hours of birth by a trained staff nurse. BCG and OPV zero dose were also administered at the same time. Birth weight, length and head circumference were recorded at birth by standard methods.

Infants were called at 6 weeks (+2 weeks), 10 weeks (+2 weeks) and 14 weeks (+2 weeks) and 0.5 mL pentavalent vaccine (DTwP-HBV-Hib) was administered by intramuscular injection into the anterolateral aspect of thigh with all aseptic precautions by trained staff nurses. Trivalent OPV was also administered simultaneously. All infants were monitored for 1 hour following immunization for development of any adverse event. Mother/guardian was given a proforma to record the adverse events at home, and was advised to contact telephonically or return back on occurrence of any serious adverse event. The proforma for adverse events was checked at each follow-up visit and minor adverse events such as fever and local tenderness were managed symptomatically. Mothers were counselled to practice exclusive breastfeeding at each follow-up. Weight, length and head circumference were recorded at each visit by standard methods. Mothers were counselled to bring the infants 6 weeks after the third dose of pentavalent vaccine and 2 mL venous sample was collected in plain vial; serum was stored at -20˚C.

Serum samples were thawed and anti HBs titers were estimated using enzyme linked immunosorbent assay (ELISA) based kits (DIA.PRO, Diagnostic Bioprobes Srl, Italy). The calibrators

and samples were tested as per the protocol provided with the kit. Validation check was carried out on the controls. The GMTs were calculated by taking the antilog of the mean of the logarithmic transformation of the titers.

Statistical analysis: Mean, standard deviation, interquartile range and GMT of antibody titers were calculated. Antibody titers between the two groups were compared using unpaired student t-test. Proportion of infants developing adverse event following immunization to be compared was assessed using chi-square test. The analysis was carried out using SPSS software (version 20.0).

RESULTS

A total of 93.1% (*n*=443) infants completed follow-up (LBW =225; 94.5%; normal birth weight=218; 91.6%) (***Fig.* 1**). ***Table* I** depicts the baseline characteristics of participants in both groups.

The median (IQR) cord blood anti HBs levels of 443 infants was 0 (0,0). Minimum level of anti HBs titers observed after 6 weeks of primary immunization with pentavalent vaccine was 40 mIU/mL in both the groups. Maximum anti HBs titers attained were 280 mIU/mL and 282 mIU/mL, respectively in LBW and normal birth weight groups. Mean (SD) anti HBs titers were 206.76 (60) mIU/mL and 214 (55.46) mIU/mL, respectively for LBW and normal birth weight infants (*P*=0.17). Anti HBs GMTs were 194.76 mIU/mL and 204.2 mIU/mL in LBW and normal birth weight infants, respectively and the difference was not significant. (*P*=0.17).

***Table* II** shows the adverse events observed in the infants of the two groups. Whilst 484 babies returned for follow-up after 1st dose, the AEFI reported here are of 443 babies who completed 3 doses of Pentavalent vaccine. Common adverse events were fever, tenderness and induration. All the adverse events resolved with symptomatic management. These adverse events decreased with subsequent doses of immunization. There were no episodes of persistent cry, hypoxic hyporesponsive events, anaphylaxis or seizures.

DISCUSSION

We evaluated the immunogenicity of the hepatitis B vaccine component of pentavalent vaccine in term low birth weight (birth weight 1800- 2499 grams) and normal birth weight (2500-4000 grams) babies. In this study we found that all infants irrespective of their birth weight, attained seroprotective titres (anti HBs ≥10U/mL). Baseline anti HBs titers observed in the cord blood samples were negligible, and after four doses of hepatitis B vaccine, given as monovalent Hepatitis B vaccine at birth followed by three primary doses of pentavalent vaccine, all infants achieved seroprotective levels of anti HBs titers and Anti HBs GMTs were comparable in LBW and normal birth weight infants delivered at term gestation.

The assumed difference of 15% in the anti HBs titres between LBW and normal birth weight infants was arbitrary and this could have affected the actual sample size. The other limitation of the study was that the number of infants studied might not address safety and tolerability of the vaccine. A good (93%) follow-up of the infants at 6 weeks after immunization is the strength of the study.

Earlier studies have shown concerns that LBW infants have low levels of T and B lymphocytes and lower vaccine specific IgG responses as compared to normal birth weight babies [8-10]. Studies evaluating the immunogenicity of hepatitis B component of pentavalent vaccine in term infants enrolled at 6 weeks of age have concluded the vaccine to be immunogenic with seroprotection rates ranging from 97% to 100% [11-14]. Studies evaluating different brands of pentavalent (DTwP-HBV- Hib) vaccine reported comparable anti HBs GMT in all infants [7,14,15]. Our study reiterates that pentavalent vaccine is highly immunogenic in infants immunized with monovalent hepatitis B vaccine at birth. Although, all infants in our study achieved seroprotective titers, the anti HBs GMTs observed in our trial were lower than those reported in the above studies in both normal birth weight and LBW infants. Different pharmacological preparations and physical properties of the vaccine; characteristics of ELISA testing kits and ethnicity may be the possible reasons for different immunogenicity and levels of GMTs. However, this difference does not seem to be clinically significant because anti HBs seroprotective titre (>10mIU/mL) is attained in all children.

There are studies documenting the good immunogenicity of monovalent hepatitis B vaccine in both low birth weight and normal birth weight infants [5, 6, 16, 17]. These results correlate well with our results where term LBW infants attained good immune response and reiterate the fact that pentavalent vaccine is as immunogenic as the separately administered monovalent vaccine. A retrospective cohort study in Nigeria analyzing the immunization records from June 2011 to May 2013 revealed the significant improvement in uptake of vaccines and completion of the schedule when pentavalent vaccine was used as compared to separately administered DPT and Hepatitis B vaccine [18]. The vaccine was also safe and tolerable in these studies.

We conclude that three primary doses of pentavalent vaccine administered along with zero dose of Hepatitis B vaccine at birth achieved comparable seroprotective anti HBs GMT in LBW and normal birth weight infants and that the immunization with pentavalent vaccine appears to be safe.

Contributors: MMAF: envisaged and conceptualized the study, supervised the work, and critically reviewed the manuscript and will stand guarantor; CV: collected the data, searched literature, carried out estimation of anti HBs titers, drafted and analyzed the results. MN: helped in search of the literature and reviewed the manuscript; IK: critically analyzed the results and reviewed the manuscript. All authors approved the final draft.

Funding: Directorate of Family Welfare, Govt. of NCT of Delhi.

Competing Interest: None stated

WHAT THIS STUDY ADDS?

- Hepatitis B component of pentavalent vaccine is equally immunogenic in term low birth weight (1800-2499g) infants as in normal weight babies.

WHAT IS ALREADY KNOWN?

- Monovalent hepatitis B vaccine produces adequate immunity in term low birth infants.

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TABLE I BASELINE PARAMETERS OF ENROLLED INFANTS AT BIRTH

Parameters	*Low Birth Weight Group (n=225)*	*Normal Birth Weight Group (n = 218)*
Gestational age (weeks) [#]	37.8 (0.7)	39.4 (1.4)
Birth weight (g)*	2119 (187.9)	2781.6 (269)
Length (cm)*	47.5 (0.9)	49.7 (1.4)
Head circumference (cm)*	32.5(0.8)	33.9 (0.6)
Cord blood Anti HBs titres (mIU/mL)*	0.0(0.0-0.0)	0.0(0.0-0.0)

*Values expressed as mean (SD) [#]Values expressed as median (inter quartile range, IQR).

TABLE II INCIDENCE OF ADVERSE EVENTS IN BOTH GROUPS

Adverse event	**After 1st dose (%)**		***P* value**	**After 2nd dose (%)**		***P* value**	**After 3rd dose (%)**		***P* value**
	LBW (*n*=225)	NBW (*n*=218)		LBW (*n*=225)	NBW (*n*=218)		LBW (*n*=225)	NBW (*n*=218)	
Tenderness	73 (32.4)	62(28.4)	0.4	55 (24.4)	49 (22.5)	0.6	37 (16.4)	34 (15.6)	0.8
Redness									
Any	54 (24)	44 (20.2)	0.5	24 (10.7)	28 (12.8)	0.2	12 (5.3)	19 (8.7)	0.1
Mild (<5 mm)	10 (4.4)	7 (3.2)		8 (3.6)	4 (1.8)		0 (0)	0 (0)	
Moderate (5-20 mm)	40 (17.8)	31 (14.2)		16 (7.1)	24 (11)		12 (5.3)	19 (8.7)	
Severe (>20mm)	4 (1.8)	6 (2.8)		0 (0)	0 (0)		0	0	
Induration									
Any	72 (32)	73 (33.5)	0.6	42 (19.1)	43 (19.7)	0.9	19 (8.4)	20 (9.1)	0.4
Mild (<5 mm)	12 (5.4)	10 (4.6)		0 (0)	0 (0)		0	0	
Moderate (5-20 mm)	57 (25.3)	56 (25.7)		43 (19.1)	43 (19.7)		19 (8.4)	20 (9.1)	
Severe (>20 mm)	3 (1.3)	7 (3.2)		0 (0)	0 (0)		0	0	
Fever									
Any	103 (45.8)	105 (48.1)	0.6	105 (46.7)	98 (44.9)	0.3	50 (22.2)	48 (22)	0.7
100.1-101°F	43 (19.1)	49 (22.5)		50 (22.2)	41 (18.8)		31 (13.8)	26 (12)	
101.1-102°F	40 (17.8)	42 (19.2)		50 (22.2)	46 (21.1)		19 (8.4)	22 (10)	
>102°F	20 (8.9)	14 (6.4)		5 (2.2)	11 (5)		0 (0)	0 (0)	
Restlessness	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Excessive sleepiness	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Vomiting	16 (7)	13 (5.6)	0.7	9 (4)	10 (4.2)	0.8	5 (2.2)	5 (2.3)	1.0
Diarrhoea	12 (5.3)	9 (4.13)	0.6	5 (2.2)	7 (3.2)	0.5	0 (0)	0 (0)	
Persistent cry	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Seizures	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Anaphylaxis	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	

Values expressed as n (%)

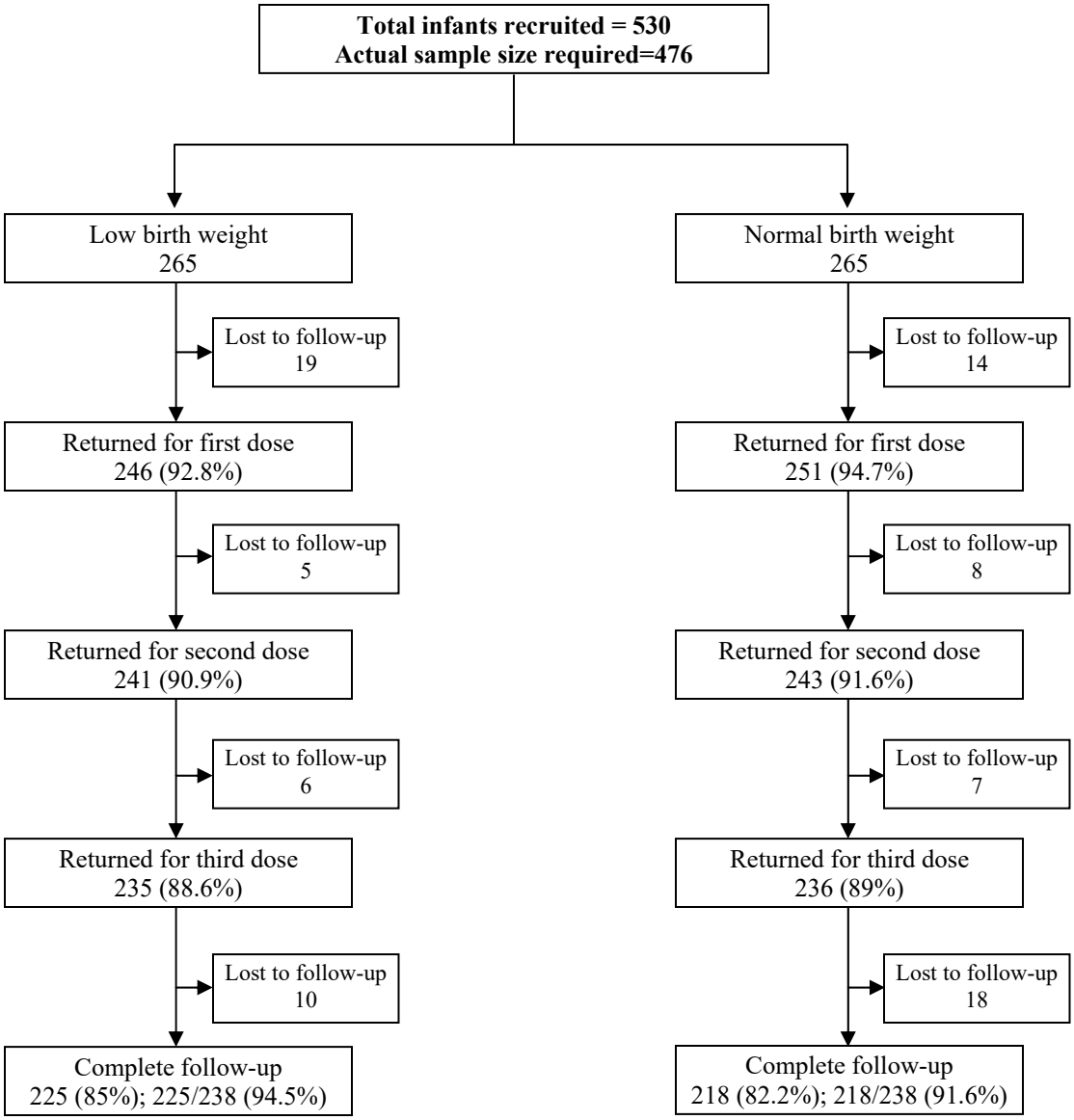

***Fig.* 1:** *Distribution of Participants in the study.*