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Safety and Immunogenicity of Salmonella Typhi Vi conjugate vaccine (Peda Typh™) in children upto five years

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Abstract- Background: Conjugate typhoid vaccine can prevent increasing cases of typhoid in younger children. Commercially available conjugate vaccine has limited studies to support its use. Aims: To study the safety and immunogenicity of Vi conjugate typhoid vaccine (PedaTyph™). Materials and Methods: Four hundred children with age <5 years were randomised to receive either one dose (Group A) or two doses (Group B) of vaccine. Adverse effects were monitored and paired blood samples were collected for immunogenicity. Due to inadequate funding analyses of group B could not be done. Results: Adverse events occurred in 17% and 21% of children after first and second doses respectively. These events were not severe and recovered within 48 hours. Single dose of vaccine increased mean antibody titers by nine fold and seroconversion was seen in 83% of children. An 11-fold rise in mean antibody titers and seroconversion rate of 89% were noted in children below two years while six fold rise in titers and 73% seroconversion rate were recorded in children above two years. Conclusions: Vi conjugate typhoid vaccine is safe and immunogenic. Immunogenicity is better in children below two years. Single dose is not enough for seroconversion in all individuals.

Index Terms- Typhoid vaccine, Seroconversion, Peda Typh™, Antibody titers

I. INTRODUCTION

Typhoid fever remains a serious public health problem in several Asian regions. According to a prospective disease-surveillance study, rates of blood culture confirmed typhoid fever, among children five to fifteen years of age ranged from 180 to 494 cases per 100,000 [1]. Kalkaji study in India documented that, 25% of *salmonella enterica* serovar Typhi culture positive cases occurred in children less than three years of age [2]. Also treating them is becoming difficult with emergence of multiresistant strains of *S*.Typhi [3]. Vaccination can be highly beneficial in reducing the disease burden. Currently available Vi polysaccharide typhoid vaccine like other polysaccharide vaccines is poorly immunogenic in infants and cannot be used in children less than two years of age. They require *adhoc* immunization visits, which may cause low vaccination coverage and increased delivery costs. Hence, Vi conjugate vaccine is required to address this increasing incidence of typhoid fever in younger children. Field trials with the US National Institutes of Health (NIH) Vi conjugate vaccine provide strong experimental evidence of safety and immunogenicity in children less than two years of age [4]. The only conjugate typhoid vaccine available commercially in India (Peda Typh™ manufactured by BIO MED pvt ltd, India) is biochemically not the same as NIH prototype vaccine. Its safety and immunogenicity data is limited to a single trial sponsored by the manufacturer and published in their website [5]. We conducted this trial to add more evidence regarding the safety, immunogenicity of this Vi-conjugate typhoid vaccine.

II. SUBJECTS AND METHODS

The study was approved by SRM medical college institutional ethical committee and was registered in Clinical Trials Registry of India (CTRI/2010/091/003031). This prospective study was conducted at our immunization clinic in the department of paediatrics from January 2011 to November 2011. The study was conducted in compliance with Good Clinical Practice Guidelines of International Conference on Harmonization (ICH-GCP). Signed informed consent was obtained from parents before enrolling their children.

III. STUDY PROTOCOL

The study was a randomized comparative trial with blinding done at the level of analyses of serum samples. Healthy children between three months to five years of age were randomly allocated to two groups. The following were excluded, children with past history of documented typhoid fever (blood culture positive/Widal test titre>1:160), those vaccinated with typhoid vaccine, those who received blood products/immunoglobulin (less than four weeks duration) and those on prolonged steroid therapy (greater than four weeks). Two hundred children in group A received one dose and two hundred children in group B received two doses (eight weeks apart) of Vi conjugate typhoid vaccine (Peda Typh™). The vaccine (0.5ml) was given intramuscularly with preferred site being anterolateral aspect of thigh. After vaccination, children were observed for at least 30 minutes. Parents maintained dairy recording systemic reactions (fever with temperature >37.5 °C, lethargy, inconsolable crying, poor appetite) and local reactions (erythema/ swelling) during the first seven days after vaccination. Serious adverse events were monitored throughout the study period. Serum samples were collected before and after eight weeks of final vaccination for estimation of antibody titers.

IV. VACCINE

Vaccine used for the study was Pedatyph™ manufactured by Bio-Med private limited, India. Each 0.5ml of the vaccine contains five microgram Vi polysaccharide of S.typhi conjugated to five microgram of tetanus toxoid protein in isotonic saline. The vaccine has been licensed by Drugs Controller General of India (DCGI) for marketing and currently post marketing studies are being undertaken for optimal use. NIH prototype vaccine used in Vietnam field trials was different from Pedatyph™ as it contained 25microgram of Vi polysaccharide conjugated to recombinant exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA)[4].

V. SEROLOGIC TESTING

Vi antibody titers were measured by ELISA (Enzyme Linked Immunosorbent Assay) modified from ELISA technique of NIH (USA) [6].Each well was coated with 0.4µg/well of Vi polysaccharide covalently conjugated to bovine serum albumin. Dilutions of serum to be tested (1:100 for pre-immune serum and 1:100, 1:400 for post-immune serum) were made and added to the wells. NIH standard reference anti Vi polysaccharide human serum with assigned unitage 118 ELISA unit (One ELISA unit is approximately equivalent to 0.1 µg of IgG anti Vi/ml) was used as reference. Anti human IgG-HRP conjugate and substrate for peroxidase were added sequentially. The optical density was measured in ELISA reader at 492 nm wavelength. A standard curve was generated using a four parameter fit. Antibody titers in test sera were calculated from this curve.

VI. STATISTICAL ANALYSIS

Sample size was based on Acharya et al[7] study in Nepal. Taking power of study 80%, β=0.08, α=0.05 sample size came to 149. Assuming 30% drop out rate we decided to include 200 children in each group for the study. Anti-Vi antibody levels were expressed as Geometric Mean Titers (GMT) with two sided 95% confidence intervals (CI). Pre-immunization and post-immunization titers were compared using paired Student's *t*-test and p values <0.05 were considered statistically significant. Greater than fourfold rise in antibody titers over pre-immunization levels was taken as seroconversion.

VII. PROTOCOL MODIFICATIONS

We were able to vaccinate both groups (A & B) and analyse samples from group A with available funding. Due to inadequate funding, analyses of samples from group B could not be done. We have modified the study to follow up both groups after a period of one year (2013) and persistence of antibody titers will be compared then. Safety results of both groups and immunogenicity results of Group A are being presented here.

VIII. RESULTS

A. Safety of conjugate typhoid vaccine

A total of four hundred children (group A & B) were monitored for adverse events. Out of two hundred children in group B 168 children came for second dose of vaccine. Systemic reactions (fever, lethargy, loss of appetite, unconsolable crying) and local reactions (erythema or swelling) were monitored. No serious adverse effects were noted. No adverse effects were not noted in 83% of children after first dose and 79% of children after second dose. Most commonly reported adverse reaction was fever and was noted in 13%, 18% of children after first and second doses respectively. Other adverse effects occurred in less than 2% of children. All the adverse reactions recovered within 48 hours. Adverse effect results are summarized in Table1.

B. Immunogenicity

Out of 200 children who received one dose of conjugate vaccine 163 children came for follow up after 8weeks. 101 children were less than two years and 24 children were less than one year. The ratio of girls to boys was 1.1:1. The Geometric mean titres of anti-Vi antibodies before and eight weeks after receiving Vi conjugate typhoid vaccine was 0.22 µg/ml and 2.08µg/ml respectively (P<0.001 compared to previous immunization level). Children showed a nine fold rise in serum anti-Vi antibody titers. Seroconversion rates (≥4fold increase over pre-immunization anti-Vi antibody level) following one dose of conjugate typhoid vaccine was 83%. Either two fold or three fold rise in antibody titers were seen in 94% of children.

GMT of anti-Vi antibodies after eight weeks of vaccination for children ≤2 years of vaccination was 2.72µg/ml. In contrast, GMT for children >2years was 1.35 µg/ml. This corresponds to 11 fold rise in antibody titers for ≤2years as compared to six fold rise for >2years. Seroconversion rates for children ≤2years was 89% and 73% for children >2years.Children <1year documented even better seroconversion rate of 96% (n=24).Thus children <2years recorded higher antibody titers and seroconversion rates than others.

IX. DISCUSSION

Enteric fever is a serious health problem with a global burden estimated to be 21 million new cases and 200,000 deaths every year [8]. Clean water and adequate sanitation has decreased incidence in developed countries but still remains impractical in developing countries. Drugs are available for treating typhoid fever but antibiotic resistant strains are on the rise [9]. Resistance to fluoroquinolones was present in 44% to 57% of cases in Karachi, Kolkata and Vietnam [1]. Vaccination against typhoid remains the only practical solution to this health problem.

Typhoid fever is no longer a disease of school children and adolescents. Study conducted in Kalkaji, India showed that 25% of culture positive cases were from children under three years and 44% from children under five years [3]. Prospective disease surveillance studies conducted by diseases of most impoverished program (DOMI) showed that incidence of typhoid among children aged two to five years was similar to older children and adolescents [1]. Study recommends vaccine that can be given in the Expanded Programme on Immunization (EPI) schedule for infants.

But currently available Vi polysaccharide vaccine cannot be used in less than two years as it does not induce immunological memory and repeated vaccination cannot boost immunogenicity [10]. Newer Vi conjugate vaccines can overcome this limitation of Vi polysaccharide vaccines [11]. Prototype Vi conjugate vaccine developed by National Institutes of Health (NIH) is highly efficacious but not available commercially [4, 6, 12, 13]. Results from

Phase I and Phase II clinical trials with Vi-CRM_{197} developed by Novartis Vaccines Institute for Global Health (NVGH) are promising[14].

Biomed Pvt. Ltd., Ghaziabad, India has developed Vi conjugate typhoid vaccine (PedaTyph™) and is commercially available in Indian market. However safety and immunogenicity data is limited to a single clinical trial (Phase III) sponsored by the company and published in their website [5]. We conducted this trial to study safety and immunogenicity of this Vi-conjugate typhoid vaccine.

Vi conjugate typhoid vaccine was observed to be safe. 83% of children receiving first dose and 79% of children receving second dose didn't experience any adverse effects. Adverse events which occurred in remaining children were not severe and recovered within 48 hrs. This incidence was comparable with results recorded by phase III clinical trial (13%) of Pedatyph™[5]. Fever was the most commonly observed adverse reaction. Fever occurred more frequently in children less than two years (Table 1).

Regarding immunogenicity, single dose of PedaTyph™ increased mean antibody titers by nine fold and seroconversion was seen in 83% of children. This was in contrast to 100% seroconversion and more than 25 fold rise in titers claimed by the manufacturer in their phase III clinical trial results. Considering the fact that either three or two fold rise in titers were seen in 94% of the vaccinated children and additional doses may increase seroconversion rate, 83% for a single dose is acceptable.

Variability in immunogenicity of this vaccine with age is worth mentioning. Eleven fold rise in mean antibody titers and seroconversion rate of 89% were noted in children less than two years while six fold rise in titers and 73% seroconversion were recorded in greater than two years. Seroconversion was even better for less than one year old children (96%). Phase III clinical trial of PedaTyph™[5] has demonstrated similar inverse correlation of immunogenicity with age.

Since available results are for single dose, PedaTyph™cannot be compared with Vi-rEPA vaccine whose results are based on two doses [4,6,12,13]. Follow up study for persistence of immunogenicity in both groups (single dose & two doses) will answer whether protective efficacy reaches close to 89% of Vi-rEPA or not [15].

Study findings suggest that PedaTyph™ is safe and immunogenic. However, single dose is not enough for seroconversion in all individuals. Immunogenicity is inversely correlating with age and children less than two years are mounting better immune response compared to older ones.

X. TABLES

Table 1. Number (%) of children reporting with Local and Systemic reactions*

		First dose typhoid vaccine			Second dose typhoid vaccine		
		All Children	**<2 years**	**>2 years**	**All Children**	**<2 years**	**>2 years**
		n= 400	n= 228	n= 172	n= 168	n= 99	n=69
Local reactions	**Severity**						
Erythema	Any	6 (1.5)†	2(1)	4(2)	3(2)	1(1)	2(3)
	>5cm	0	0	0	0	0	0
Induration	Any	4(1)	1(0.5)	3(2)	1(0.5)	0	1(1.5)
	>5cm	0	0	0	0	0	0
Systemic reactions	**Severity**						
Fever	>38.0˚C	51(13)	36(16)	15(9)	30(18)	22(22)	8(12)
	>39.5˚C	0	0	0	0	0	0

Inconsolable crying	< 4hrs	2(0.5)	2(1)	0	1(0.5)	1(1)	0
	>4hrs	0	0	0	0	0	0
Lethargy	Any	5(1.5)	3(1.5)	2(1)	2(1)	2(2)	0
Poor Appetite	Any	2(0.5)	1(0.5)	1(0.5)	0	0	0

* Adverse events recorded by parents during first seven days after vaccination
† Number in parantheses represent percentage values

Table 2. Geometric Mean Titers (GMT) of anti Vi antibodies and seroconversion rates after one dose of Vi-conjugate vaccine

Group	n	GMT of anti-Vi antibody (µg/ml)		Magnitude of increase
		Pre-immunization	8 weeks	
All Children	163	0.22(0.20-0.25) *	2.08† (1.76-2.48) *83%***	9-fold
≤2yrs	101	0.24(0.20-0.27)	2.72† (2.22-3.31) *89%*	11-fold
2-5yrs	62	0.21(0.18-0.24)	1.35† (1.02-1.79) *73%*	6-fold

* Figures in parentheses are 95% confidence intervals.
** Figures in italics represent percent seroconversion (≥ 4-fold increase in antibody titer)
†Significantly different from pre-immunization level ($p<0.001$) by Student's t-test

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