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Research Article

PERSISTENCE OF IMMUNOGENICITY RESPONSE AFTER SINGLE DOSE OF PEDA TYPH™, VI CONJUGATE TYPHOID VACCINE IN INFANTS AND CHILDREN: A 10 YEAR FOLLOW UP STUDY

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ABSTRACT

New Vi polysaccharide conjugate vaccines have emerged as a promising approach for the prevention of typhoid particularly in infants. The present follow up study was undertaken to demonstrate long term efficacy and persistence of immunogenicity and duration of antibody response in children who were immunized with *PedaTyph*™ (Typhoid vi conjugate vaccine), 10 years back. The measuring range of the assay was 7.4 - 600 U/ml. A p-value < 0.05 was considered as statistically significant. The mean antibody titre after 10 year post immunization was found to be 32.625 EU/ml by VaccZyme™ ELISA method and 19.883 EU/ml by NIH ELISA method. All the subjects (100%) showed high levels of anti-Vi IgG antibody titre even after 10 years of primary vaccination. None of the children reported typhoid fever during the last 10 years. It was thus concluded that Vi conjugate vaccine *PedaTyph* ™ is highly immunogenic and can provide long-term immunogenicity without booster vaccination.

Keywords: Typhoid, Vi conjugate vaccine, safety, immunogenicity.

INTRODUCTION

Salmonella enterica serotype *typhi* (*S. typhi*) is a Gram- negative bacterium and is the leading cause of systemic febrile illness, typhoid fever. The clinical manifestation of typhoid includes prolonged fever, headache, nausea, vomiting, loss of appetite, and constipation or diarrhea (Mitra *et al.,* 2016). According to the reports by World Health Organization, in 2017 typhoid fever resulted in an approximately 21 million cases, 128,000 to 161000 deaths, worldwide (WHO, 2018). It is an important public health issue in countries located in south of globe, e.g. India. The disease is endemic in almost all parts of India with periodic outbreaks of water borne or food borne diseases (Kanungo *et al.,* 2008).In India, overall seropositivity rates in children ≤15 years in 1998-2002 were 29.98 ± 11.16 % that increased to 65.54 ± 6.22 % in 2011 (Banerjee *et al.,* 2014). The typhoid fever is common in the age group of 0-4 years and the incidence of disease in this age group was 478/100,000 annually (Kossaczka *et al.,* 1999). The incidence of typhoid decreases within the first year of vaccination remains low for 5-10 years among 6-15 years old children but the reduction in the incidence depends on the efficacy of vaccine (Pitzer *et al.,* 2014). As per WHO report, vaccination against typhoid fever

is a key control measure in high-risk areas (WHO, 2018).

Three newer generation vaccines: oral Ty21a vaccine that is administered orally, Vi polysaccharide vaccine (Vi vaccine) that contains vi polysaccharide of *S.typhi* bacteria and is administered intramuscularly and vi conjugate polysaccharide vaccine in which vi polysaccharide is conjugated as a carrier protein have been licensed in India and is being considered useful in public health programs (Germanier and Fiirer,1975; Robbins and Robbins, 1984; Fraser *et al.,* 2007; Assaf-Casals and Dbaibo, 2016; Bröker *et al.,* 2017). Vi polysaccharide vaccines cannot be used in children less than two years of age because of their poor immunogenicity and T cell independent properties (Mitra *et al.,* 2016). Conjugation to a carrier protein converts T cells-independent antigens into T cells-dependent ones, thereby providing a long lasting protection in children less than two years (Mitra *et al.,* 2016) and thus overcoming the drawback of plain vi antigen vaccine .

Vi conjugate vaccines, where Vi polysaccharide is linked to a carrier protein, can overcome these drawbacks Vi conjugate vaccines, where Vi polysaccharide is linked to a carrier protein, can overcome these drawbacks Vi conjugate vaccines, where Vi polysaccharide is linked to a carrier protein, can overcome these drawback conjugate Vi capsular polysaccharide vaccines, where Vi polysaccharide is linked to a carrier protein, can overcome these drawbacks.(Mitra *et al.,* 2016).Efficacy of Vi Conjugate Vaccine against Typhoid Fever in Young Children have been established earlier and protective levels of IgG anti-Vi antibody for Vi conjugate vaccine was found 3.52 by ELISA Units/ml (Lanh *et al.,* 2003).

The first commercially available Vi antigen conjugated with tetanus toxoid (TT) was manufactured by Bio-Med (P) Ltd in India and is available by trade name *PedaTyph*™ licensed in 2008. Serological immune response in infants and young children after three weeks of vaccination with *PedaTyph*^{TM} is well established in various clinical trials (Chinnasami *et al.,* 2013; Garg *et al.,* 2014; Chinnasami *et al.,* 2015; Mitra *et al.,* 2016). Efficacy, safety and immunogenicity of this Vi-conjugate typhoid vaccine *PedaTyph*^{TM} was demonstrated in children aged 6 months to 12 years post vaccination for a period of one year (Mitra *et al.,* 2016). The present study further evaluated the persistence of immunogenicity response in children who were immunized with *PedaTyph*^{TM} 10 years back.

MATERIALS AND METHODS

Clearance and approval

The study was conducted in accordance with the ethical principles of Declaration of Helsinki developed by the World Medical Association (Kimmel, 2008; Tagliabue and Rappuoli, 2018), Schedule Y of the Drugs and Cosmetics Act (Nair *et al.,* 2009; John *et al.,* 2013) and good clinical practice (GCP). A written informed consent was obtained from each subject prior to the study. The follow up phase III clinical trial study was conducted as per protocol approved by the ethical committee of Lala Lajpat Rai Memorial Medical College (LLRM) Hospital; Meerut (U.P.) The clinical trial was also approved by the Clinical Trial Registration of India (CTRI) reference no. CTRI/2017 1061008844 dated 15/06/2017.

Initial clinical trial

A multi-centric, open, controlled and comparative phase III clinical trial was conducted in the year 2007 on 206 subjects at LLRM Medical College Meerut, Medical College Bhavnagar (Gujarat), Gandhi Medical College (Hyderabad, Andhra Pradesh) for the evaluation of safety and immunogenicity of *Peda Typh*^{TM} (Garg *et al.,* 2014).

Study Design

This was a follow up study of the phase III clinical trial for the evaluation of

persistence of immunogenicity of Vi conjugate vaccine (*PedaTyph*^{TM}). Subjects participated from LLRM medical college was followed after ten years. Volunteers were recruited as per inclusion/exclusion criteria as per the approved study protocol. Subjects of either gender (10-19 years of age) tracked from earlier trial (Garg *et al.,* 2014) were included in the study. All the subjects were vaccinated with a single dose of *PedaTyph*^{TM} in the year 2007.The subjects re-enrolled in the study were screened for the history of typhoid fever.

Evaluation of immunogenicity

Blood samples (1-2 ml) were collected aseptically by venipuncture. Sera were collected by centrifugation and samples were stored and calibrated at -20^{o}C deep freezer. Antibody response was evaluated by ELISA test kit (VaccZyme^{TM} Human Anti-S Typhi Vi IgG Enzyme Immunoassay kit (The Site Binding Group Ltd, Product code: MK091.U, Batch No.: 411159-3).

All the subjects included in the study were vaccinated with a single dose of *PedaTyph*^{TM} in 2007. The results of phase III clinical trial were reported earlier (Garg *et al.,* 2014). In the phase III clinical trial, Vi IgG antibody were evaluated by an in-house NIH ELISA method; whereas in the present follow up study, a commercial kit VaccZyme^{TM} was also used.

Statistical analysis

Anti-Typhi Vi IgG antibody levels were observed directly from calibration curve. The calibrator values were adjusted by a factor of 100 to account for a 1:100 sample dilution. During follow up study, in-house standard reference was also estimated with VaccZyme^{TM} human Anti-S Typhi Vi IgG Enzyme Immunoassay Kit. The antibody titers were analyzed by a validated program of ELISA for windows version 2.00 issued by Centres for Diseases Control and Prevention (CDC), U.S. Department of Health of Human Services. A *p*-value <0.05 was considered as statistically significant. The test vaccine was provided by M/s Biomed Private Limited

RESULTS

In 2007, 59 subjects were enrolled at LLRM College, Meerut. Of these, 21 subjects were willing to participate in the follow up study in 2017. The mean antibody titre after 10 year post immunization was found to be 32.635 EU/ml by VaccZyme^{TM} ELISA method and 19.883 EU/ml by NIH ELISA method (Table 1). It was observed that post vaccination, all the subjects (100%) showed high levels of anti-Vi IgG antibody titre even after 10 years of primary vaccination with 5 µg (0.005 mg) of Vi antigen conjugated to tetanus toxoid. None of the children reported typhoid fever during last 10 years.

DISCUSSION

Typhoid fever is a systemic infection and a major health problem in developing countries and the situation is worsened by multi drug resistance (Finn, 2004). It was reported earlier that children bear a substantial proportion of typhoid fever in endemic areas (Britto *et al.,* 2017). The disease can be controlled by implementation of appropriate vaccination strategies. Modern typhoid vaccines are available and considered safe and highly effective in preventing typhoid. Efficacy of Vi based vaccines was conventionally proven in various clinical trials and efforts have been made to define serum anti Vi IgG levels that could be considered as protective and more effective (Szu *et al.,* 2014).

Initially, Klugman *et al.,* (1996) reported that protection afforded by the vaccine could be correlated with Vi antibodies. Later, it became obvious in other studies done for conjugate polysaccharide vaccines (meningococcal and pneumococcal) that IgG was the principal mediator of protection (Lee *et al.,* 2003). An important aspect of a conjugate vaccine is its ability to activate T-

helper cells that enhance the magnitude (titre) and quality (avidity and subclass) of IgG Vi antibody. Szu *et al.,* (2014) reported a trial in children aged 2 to 5 years suggesting that Vi-rEPA vaccine (l0 µg/ml of anti Vi IgG) provided a long term protection with 89% efficacy after immunization. Besides antibody titre, the duration of antibody response is also considered to be critical to evaluate the duration of efficacy of vaccine (Ochiai *et al.,* 2014). However, antibodies to Vi polysaccharide vaccine and other vaccines can decrease with the time. Thus, antibody response needs to be boosted by repeated immunization (Finn, 2004). A vaccine that could provide long term immunogenicity without repeated booster doses could be highly beneficial and cost-effective for countries like India.

The results of the present study showed that Vi IgG antibody titre were maintained up to 10 years post-vaccination and thus offered continued protection against typhoid fever. There were no clinical symptoms of typhoid among the enrolled subjects during the follow up study period which proved the safety efficacy of *PedaTyph*^{TM} Vi conjugated Typhoid Vaccine. Earlier studies also showed that Vi antibodies persist for different duration of periods of which longest duration reported was of ten years (Lanh *et al.,* 2003, Chinnasami *et al.,* 2015; Klugman *et al.,* 1996; Froeschle and Decker, 2010) .This is important as subsequent dose after every three years may not be required, provided (Froeschle and Decker, 2010 ; Ochiai *et al.,* 2014) the subjects are vaccinated by *PedaTyph*^{TM} as recommended by global health guidelines.

Table 1. ELISA Vi Polysaccharide antibodies in human sera expressed in ELISA units, 1 ELISA unit =1U/ml

S.No.	VaccZyme ELISA	NIH ELISA
1	48.108	29.334
2	43.024	26.234
3	22.93	13.984
4	37.213	22.690
5	33.558	20.121
6	46.098	28.108
7	43.999	26.828
8	20.022	12.208
9	30.773	18.764
10	29.899	18.231
11	22.049	13.444
12	32.549	19.846
13	31.658	19.303
14	29.023	17.696
15	20.993	12.800
16	28.155	17.167
17	50.147	30.577
18	26.327	16.053
19	19.778	12.059
20	29.841	18.195
21	39.196	23.900
Mean	**32.635**	**19.883**

CONCLUSION

The present follow up study concluded that Vi conjugate vaccine (Bio-Med (P) Ltd.) was found to be highly immunogenic and Vi polysaccharide antibodies response persisted in all the subjects after 10 years post vaccination.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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