Vaccine 41 (2023) 3137–3140

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immune responses in children after vaccination with a typhoid Vi-tetanus toxoid conjugate vaccine in Bangladesh

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ARTICLE INFO

Article history:
Received 24 February 2022
Received in revised form 15 April 2022
Accepted 4 April 2023
Available online 13 April 2023

Keywords:
Bangladeshi children
CRT-TCV
Long-term immune response

ABSTRACT

A cluster-randomized trial of Vi-TT was conducted in Dhaka, Bangladesh, using JE vaccine as the control. A subset of 1,500 children were randomly selected on 2:1 basis (Vi-TT vs JE) to assess immune response. Blood was collected before vaccination, and on days 28, 545 and 730 post-vaccination and plasma anti-Vi-IgG response was measured. A robust, persistent antibody response was induced after single dose of Vi-TT, even after 2 years of vaccination. While there is no accepted serological antibody threshold of protection, analyzing the antibodies of children who received Vi-TT provides evidence that may later be useful in predicting population protection.

1. Introdcution

Salmonella enterica serovar Typhi (*S.* Typhi) causes typhoid fever, and is transmitted through food and water contaminated with human feces. Typhoid fever is a public health concern in areas where clean water and sanitation infrastructure are not well established. It is estimated that more than 9 million cases and 110,000 deaths due to typhoid fever occur each year with an especially high incidence rate in school-aged and pre-school-aged children residing in urban slums of developing countries [1,2]. Increased levels of antimicrobial resistance in *S.* Typhi strains and emergence of multidrug-resistant and extensively drug-resistant strains in different geographical location of Asia and Africa is worsening the situation [3,4].

Typhoid fever also remains a major public health problem in Bangladesh. A prospective population-based surveillance was carried out in densely populated area of Mirpur (wards 3 and 5) in Dhaka city, Bangladesh to measure the age-stratified burden of typhoid and paratyphoid fever. The highest disease burden was seen in children 5–9 years of age with overall incidence of 161 (95 % CI: 145–179) per 100,000 person-years of observation; increased levels of antimicrobial resistance in *S.* Typhi strains has also been reported [5].

Both short and long-term control strategies including vaccination and water, sanitation, and hygiene (WASH) interventions are important to contain the spread of typhoid fever in urban slums, where the burden of the disease is very high. However, large financial investments for WASH intervention are required to ensure proper sanitation and safe water, likely not implementable in most low-income countries in the near future. The World Health Organization (WHO) prequalified the Vi capsular polysaccharide vaccine conjugated to tetanus toxoid carrier protein (Vi-TT) (Typbar® TCV, Bharat Biotech International Limited) in December 2017 [6] and recommended the typhoid conjugate vaccine (TCV) for routine immunization programmes for children above 6 months in countries with high typhoid burden and encouraged generation of efficacy data by conducting clinical trials including co-administration studies [6]. The Typhoid Vaccine Acceleration Consortium (TyVAC) has been conducting large randomized controlled trials of Vi-TT in different urban settings of Asia and Africa and the studies revealed very encouraging efficacy data against clinical typhoid [7–9]. In

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https://doi.org/10.1016/j.vaccine.2023.04.014
0264-410X/

addition, a randomized controlled trial performed in Burkina Faso has revealed that the Vi-TT vaccine is safe when co-administered with routine group A meningococcal conjugate vaccine (MCV-A) and measles– rubella (MR) vaccine and a robust immunogenicity profile has also been documented with co-administration of TCV and MCV-A [10].

A large-scale cluster randomized-controlled trial (CRT) of Vi-TT vaccine, conducted in a geographically defined area of Dhaka city, Bangladesh, using SA 14–14-2, live attenuated Japanese encephalitis (JE) vaccine (Chengdu Institute of Biological Products) as the control. An interim analysis of 18 months data of this trial has reported 85 % total protection including 81 %, 80 % and 88 % vaccine effectiveness for the children aged 9 months to < 2 years, 2–4 years and 5 to < 16 years, respectively [7]. As part of this study, blood specimens were collected from a subset of vaccinees of both Vi-TT and JE clusters to assess the immune responses induced after vaccination and plasma anti-Vi-IgG antibody responses were measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit [7]. To assess the long-term immunogenicity and the decay of levels of antibody responses in vaccinees over time, the vaccinees were followed-up until two years post-vaccination. Here, we report the plasma anti-Vi-IgG antibody responses in vaccinees of all age groups (<2 years, 2–4 years and 5 to < 16 years) on day 0 before vaccination and on day 28, at 18 months and two years.

2. Methodology

2.1. The Vi-TT trial and immunogenicity study

A cluster randomized-controlled trial (CRT) of a single-dose Vi-TT vaccine was conducted in Mirpur (wards 2, 3 and 5), Dhaka city of Bangladesh between April 2018 and March 2020 to evaluate the protective effectiveness of the vaccine. A total of 205,760 population was enumerated in the study area during baseline census, conducted between 14th February and 25th March 2018. The study area was divided into 150 clusters. Clusters were demarcated by natural borders wherever possible and included contiguous households with approximately equal numbers of populations. A baseline vaccination campaign was conducted between 15th April and 15th May 2018 where 41,344 children were vaccinated. The trial is registered at https://www.isrctn.com as ISRCTN11643110.

For the immunogenicity study, 18 of 150 clusters were randomly selected to either Vi-TT or JE vaccine, and a subset of approximately 1,500 participants were selected on a 2:1 basis (Vi-TT vs JE) to blood specimens at baseline (day 0) before vaccination, and then at day 28, at 18 months (day 545), at two years (day 730). Selection of participants was also age-stratified (<5 years vs ≥ 5 years of age on 1:1 basis). Participants for the immunogenicity study were randomly selected manner by an independent, unblinded statistician. Blood specimens (3–5 ml) were collected in sodium heparin tubes and transported to the Mucosal Immunology and Vaccinology Laboratory, icddr,b for processing. Before blood collection and vaccination, written informed consent was obtained from parents/guardians of all participants and participant assent was also obtained from participants aged 11 to < 16 years.

2.2. Laboratory analysis

Plasma was collected from blood specimens by centrifugation method and anti-Vi-IgG antibody titres in plasma were measured using the VaccZyme commercial enzyme-linked immunosorbent assay (ELISA) kit (The Binding Site Group ltd, Birmingham, UK) [7,11].

2.3. Statistical analysis

Pearson's chi-squared test was used to compare the percentages of dichotomous variables among participants immunized with Vi-TT versus JE vaccines. Two-sample *t*-test with equal variances was used to estimate the difference in mean age of the two vaccine groups. Antibody titres were log-transformed before all statistical processes. Seroconversion rate was defined as ≥ 4-fold increase in plasma antibody titres over preimmunization titre and seroconversion rates between two groups were compared by using Pearson's chi-squared and Fisher's exact tests. 2-sided P-values < 0.05 were considered statistically significant. Paired *t*-test was performed for comparison of antibody titres within Vi-TT recipients between different time points.

3. Results

3.1. Baseline characteristics of immunogenicity participants at enrollment

For the immunogenicity study, blood specimens were collected from 1,009 recipients of Vi-TT vaccine and 503 recipients of JE vaccine at baseline before vaccination. Due to losses to follow-up of vaccinees at different time points, 954 Vi-TT recipients and 479 JE recipients on day 28, 829 Vi-TT recipients and 427 JE recipients on day 545, and 820 Vi-TT recipients and 374 JE recipients on day 730 were included in the analysis. An analysis of baseline characteristics of vaccinees demonstrated no significant differences between Vi-TT and JE recipients with respect to age at enrollment, number of participants of different age groups, gender, temperature before vaccination and previous history of typhoid fever (Table 1).

3.2. Anti-Vi-IgG antibody responses in Vi-TT and JE vaccine recipients

For Vi-TT recipients, the anti-Vi-IgG antibody geometric mean titre (GMT) before vaccination on day 0 was 3.9, 4.8 and 5.5 ELISA units (EU)/mL for children aged < 2 years, 2–4 years and ≥ 5 years of age, respectively. After vaccination on day 28, the responses rose to 3053, 3047 and 2899 EU/mL; on day 545, the responses were 55.9, 105.3 and 199.4 EU/mL; on day 730, the responses were 40.7, 74.8 and 161.6 EU/mL for the Vi-TT vaccinees at < 2 years, 2–4 years and ≥ 5 years, respectively (Table 2). Before vaccination, the anti-Vi-IgG GMT in JE recipients aged < 2 years, 2–4 years and ≥ 5 years were 4.3, 4.8 and 5.6 EU/mL respectively and no sig-

Table 1
Baseline characteristics of immunogenicity participants by vaccine groups.

Characteristics	Vi-TT (N = 1009)	JE (N = 503)	*P* value
Age in years at enrollment (Mean ± SD)	6.1 ± 4.3	6.0 ± 4.3	0.683[‖]
Number and percent of vaccinees of different age groups			
<2 years	132 (13.1 %)	63 (12.5 %)	0.954[¶]
2–4 years	372 (36.9 %)	187 (37.2 %)	
≥5 years	505 (50.0 %)	253 (50.3 %)	
Gender			
Female n (%)	517 (51.2 %)	250 (49.7 %)	0.573[¶]
Body temperature (°C) (Mean ± SD)	36.30 ± 0.35	36.28 ± 0.36	0.327[‖]
Previous history of typhoid			
Yes n (%)	57 (5.7 %)	25 (5.0 %)	0.583[¶,a]

[‖] Two-sample *t*-test with equal variances.
[¶] Pearson's chi-squared test.
[a] Response of nine participants were "Unknown" and categorized them as "No" to perform the statistical test.

Table 2
Plasma anti-Vi-IgG responses among vaccinees at different time points of post-vaccination compared with pre-vaccination.

Response	Time point	Participants aged < 2 years			Participants aged 2–4 years			Participants aged ≥ 5 years		
		Vi-TT recipients	JE recipients	*P* value	Vi-TT recipients	JE recipients	*P* value	Vi-TT recipients	JE recipients	*P* value
GMT EU/mL (95 % CI)	D0	3.9 (3.75–4.10)	4.3 (3.6–5.2)	0.45[#]	4.8 (4.5–5.2)	4.8 (4.3–5.30)	0.816[#]	5.5 (5.1–5.8)	5.6 (5.1–6.2)	0.9118[#]
	D28	3053 (2686–3471)	4.3 (3.5–5.4)	< 0.0001[#]	3047 (2766–3357)	4.7 (4.2–5.3)	< 0.0001[#]	2899 (2649–3173)	6.1 (5.5–6.8)	< 0.0001[#]
	D545	55.9 (46.8–66.8)	4.7 (3.6–6.0)	< 0.0001[#]	105.3 (94.0–118)	5.1 (4.5–5.8)	< 0.0001[#]	199.4 (180.7–219.9)	6.1 (5.4–6.9)	< 0.0001[#]
	D730	40.7 (34.1–48.6)	4.6 (3.5–6.0)	< 0.0001[#]	74.8 (66.8–83.9)	5.4 (4.7–5.2)	< 0.0001[#]	161.6 (145.9–178.9)	6.0 (5.4–6.8)	< 0.0001[#]
Fold-rise over baseline GMT	D28	782.8	1.0	–	634.8	0.98	–	527.0	1.08	–
	D545	14.3	1.09	–	21.9	1.06	–	36.2	1.08	–
	D730	10.4	1.07	–	15.6	1.12	–	29.4	1.07	–
Seroconversion rate (≥4-fold increase in plasma antibody titers over preimmunization titer)	D28/D0	120/120 (100)	1/55 (1.82)	< 0.0001[‖]	345/347 (99.9)	2/177 (1.13)	< 0.0001[‖]	485/487 (99.5)	2/247 (0.81)	< 0.0001[‖]
	D545/D0	86/94 (91.5)	3/46 (6.52)	< 0.0001[‖]	273/291 (93.8)	5/157 (3.18)	< 0.0001[‖]	429/444 (96.6)	9/224 (4.02)	<0.0001[¶]
	D730/D0	77/92 (83.6)	3/39 (7.69)	< 0.0001[‖]	253/287 (88.1)	7/127 (5.51)	< 0.0001[¶]	418/441 (94.7)	8/208 (3.84)	<0.0001[¶]

nificant difference was seen post-vaccination in those vaccinated with JE (Table 2).

An analysis of the anti-Vi-IgG antibody GMT among three age groups of Vi-TT recipients has revealed no significant difference on day 28. However, the differences were statistically significant in vaccinees between age groups on days 545 and 730. The response in vaccinees aged 2–4 years was significantly higher ($P < 0.0001$) than the vaccinees of < 2 years of age and significantly lower ($P < 0.0001$) than in vaccinees aged ≥ 5 years on days 545 and 730 post-vaccination (Supplementary Table 1).

Fold-rise of geometric mean titres (GMTs) of anti-Vi-IgG responses, relative to baseline, in Vi-TT recipients aged < 2 years were 777.6, 14.24 and 10.18 times higher on 28, 545 and 730 post-vaccination days, respectively. For children aged 2–4 years, fold-rise of antibody titres were 627.7, 21.7 and 15.4 times higher on days 28, 545 and 730 respectively than the baseline GMTs. For older children, aged 5 to < 16 years, 525.3-, 36.1- and 29.3-fold higher antibody responses were observed on days 28, 545 and 730 respectively. For JE recipients in all age groups the fold-rise of GMT anti-Vi-IgG responses were not >1-fold on follow-up days after vaccination (Table 2).

On day 28, all recipients of Vi-TT showed seroconversions (≥4-fold rise of antibody responses compared to baseline), except for two participants vaccinated at 2–4 years and two participants vaccinated at ≥ 5 years of age. On day 545, seroconversion rates were 91.5 %, 93.8 %, 96.6 % for recipients of Vi-TT at < 2 years, 2–4 years and ≥ 5 years of age respectively, and on day 730, the rates were 83.6 %, 88.1 %, 94.7 % for children vaccinated in these three age groups, respectively. For JE recipients, the seroconversion rates were < 2 % for all on day 28; the rates were 6.52 %, 3.18 %, 4.02 % for the children at < 2 years, 2–4 years and ≥ 5 years of age respectively on day 545 and on day 730, the rates were 7.69 %, 5.51 %, 3.84 % (Table 2).

We have carried out an exploratory analysis of anti-Vi-IgG titre in 766 Vi-TT recipients for whom blood specimens were available at all time points (days 0, 28, 545 and 730) to better understand the decline in antibody titre over time. A significantly higher anti-Vi-IgG titre was seen on days 28, 545 and 730 when compared to day 0 before vaccination. anti-Vi-IgG antibody titre on days 545 and 730 were significantly lower compared to day 28 and the response was significantly lower ($P = 0.0133$) on day 730 compared to day 545 (Supplementary Fig. 1).

3.3. Antibody responses induced in Vi-TT recipients with breakthrough infection

One Vi-TT recipient had breakthrough infection with *S.* Typhi positive bacteremia who was enrolled on day 543 post-vaccination in the passive surveillance for typhoid fever which was carried out in the TyVAC study. The anti-Vi-IgG antibody response was 103.93-fold on day 28 compared to the baseline titer; however, the response had decreased significantly to 1.38 and 1.16 folds on days 545 and 730 respectively.

4. Discussion

In this study we demonstrate the longitudinal changes in IgG antibody levels against *S.* Typhi specific Vi-antigen (anti-Vi-IgG) in Vi-TT and JE vaccine recipients among Bangladeshi children over the period of two years after vaccination. A single dose regimen of Vi-TT, given to Bangladeshi children aged 9 months to < 16 years induces a robust anti-Vi-IgG antibody responses. The responses remained relatively unchanged in the JE recipients during the entire study period.

There are no established assays for measuring functional antibodies that are considered true immunological correlates of protection against typhoid fever. However, according to the WHO, estimation of anti-Vi-IgG antibody responses by enzyme-linked immunosorbent assay is the key assessment tool to measure immunogenicity of the new typhoid conjugate vaccines [12]. In this study we have used the same enzyme-linked immunosorbent assay kit (VaccZyme, Binding Site) to measure the antibody titre that has been used in different efficacy trials of TCV, conducted in Kolkata, Nepal, and Burkina Faso [8,10,12].

A significantly higher anti-Vi-IgG antibody responses were seen in Vi-TT recipients of all age groups after day 28, at 18 months and two years post-vaccination compared to baseline antibody response. The finding is consistent with other immunogenicity studies of TCV trials, conducted in typhoid endemic countries [8,10,12]. A decline in antibody levels was observed over time in Vi-TT recipients of this trial; the responses were declined on days 545 and 730 compared to day 28 in all age groups and the anti-Vi-IgG titre on day 730 was significantly lower than day 545, however, the response was not disappeared on day 730. We found 10.4-, 15.6- and 29.4-fold in GMT antibody response in children at < 2 years, 2–4 years and 5–15 years, respectively on day 730 over baseline GMT. Similarly, a decline of antibody response was observed (7.5-and 8.8-fold in GMT in children aged 2–4 years and 5–15 years, respectively) in Kolkata study after two years of vaccination with Vi-TT [12].

Age dependent decline of antibody titres were observed over the study period, the highest decline was seen in lower age group (<2 years of age) compared to children of older age groups (2–4 years and $\geq$ 5 years) at 18 months and two years post-vaccination. This could have occurred due to subclinical exposure to *S.* Typhi that usually begins in early school age due to consumption of contaminated food and water outside of the home and develop natural immunity in pre-school and school-going children. A study conducted in Nepal has also demonstrated that the natural, humoral immune response to *S.* Typhi is acquired with age and related to subclinical exposure to the organism [13].

Since typhoid fever is caused by an enteric pathogen, and IgA antibody shows a critical role in inducing mucosal immune response, it is important to determine if IgA provides any protection at intestinal mucosa or IgA is a surrogate marker of protection against typhoid fever. A human challenge model study has revealed higher anti-Vi-IgA responses and its association with protection against typhoid fever in participants who were immunized with TCV [14]. IgA response was also significantly higher in individuals who received TCV in Nepal efficacy trial [15]. Therefore, we are also planning to measure anti-Vi-IgA titres in plasma specimens used for IgG ELISA to reveal the magnitude of IgA responses in Bangladeshi children.

This study confirms that Vi-TT vaccine is highly immunogenic in Bangladeshi children aged between 9 months and < 16 years and induced a persistent anti-Vi-IgG plasma antibody response after a single dose of immunization with Vi-TT, even after 2 years of vaccination. Our findings also suggest that children who will receive the vaccine at young age (<2 years) will need booster doses for protection against typhoid fever.

The findings of this trial will help for consideration by policy makers to integrate TCV to the routine immunization programme in Bangladesh and globally and to help guide the need for and timing of booster doses of the vaccine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the study participants and the large number of study workers. The icddr,b is grateful to the Government of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support. TyVAC acknowledges Bharat Biotech International Limited for supplying the investigational vaccine and members of the Data and Safety Monitoring Board for their oversight of the clinical trials.

Financial support

This work was funded by the Bill & Melinda Gates Foundation (grant number OPP1151153).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.04.014.

References

[1] Global Burden of Disease Collaborative Network. GBD 2019 Cause and Risk Summaries: Typhoid fever — Level 4 cause. Seattle, United States: Institute for Health Metrics and Evaluation (IHME); 2020. http://wwwhealthdataorg/results/gbd_summaries/2019.
[2] Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, et al. Typhoid fever in children aged less than 5 years. Lancet 1999;354:734–7.
[3] Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins. MBio 2018:9.
[4] Qamar FN, Yousafzai MT, Khalid M, Kazi AM, Lohana H, Karim S, et al. Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. Lancet Infect Dis 2018;18:1368–76.
[5] Meiring JE, Shakya M, Khanam F, Voysey M, Phillips MT, Tonks S, et al. Burden of enteric fever at three urban sites in Africa and Asia: a multicentre population-based study. Lancet Glob Health 2021;9:e1688–96.
[6] World Health O. Typhoid vaccines: WHO position paper, March 2018 - Recommendations. Vaccine 2019;37:214–6.
[7] Qadri F, Khanam F, Liu X, Theiss-Nyland K, Biswas PK, Bhuiyan AI, et al. Protection by vaccination of children against typhoid fever with a Vi-tetanus toxoid conjugate vaccine in urban Bangladesh: a cluster-randomised trial. Lancet 2021;398:675–84.
[8] Shakya M, Colin-Jones R, Theiss-Nyland K, Voysey M, Pant D, Smith N, et al. Phase 3 Efficacy Analysis of a Typhoid Conjugate Vaccine Trial in Nepal. N Engl J Med 2019;381:2209–18.
[9] Patel PD, Patel P, Liang Y, Meiring JE, Misiri T, Mwakiseghile F, et al. Safety and Efficacy of a Typhoid Conjugate Vaccine in Malawian Children. N Engl J Med 2021;385:1104–15.
[10] Sirima SB, Ouedraogo A, Barry N, Siribie M, Tiono AB, Nébié I, et al. Safety and immunogenicity of co-administration of meningococcal type A and measles-rubella vaccines with typhoid conjugate vaccine in children aged 15–23 months in Burkina Faso. Int J Infect Dis 2021;102:517–23.
[11] Ferry BL, Misbah SA, Stephens P, Sherrell Z, Lythgoe H, Bateman E, et al. Development of an anti-Salmonella typhi Vi ELISA: assessment of immunocompetence in healthy donors. Clin Exp Immunol 2004;136:297–303.
[12] Mohan VK, Varanasi V, Singh A, Pasetti MF, Levine MM, Venkatesan R, et al. Safety and immunogenicity of a Vi polysaccharide-tetanus toxoid conjugate vaccine (Typbar-TCV) in healthy infants, children, and adults in typhoid endemic areas: a multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study. Clin Infect Dis 2015;61:393–402.
[13] Pulickal AS, Gautam S, Clutterbuck EA, Thorson S, Basynat B, Adhikari N, et al. Kinetics of the natural, humoral immune response to Salmonella enterica serovar Typhi in Kathmandu. Nepal Clin Vaccine Immunol: CVI 2009;16:1413–9.
[14] Dahora LC, Jin C, Spreng RL, Feely F, Mathura R, Seaton KE, et al. IgA and IgG1 Specific to Vi Polysaccharide of Salmonella Typhi Correlate With Protection Status in a Typhoid Fever Controlled Human Infection Model. Front Immunol 2019;10:2582.
[15] Shakya M, Voysey M, Theiss-Nyland K, Colin-Jones R, Pant D, Adhikari A, et al. Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. Lancet Glob Health 2021;9:e1561–8.