

Immunogenicity and Safety of Typhoid Conjugate Vaccine in Healthy Indian Subjects: A Randomized, Active-controlled, Comparative Clinical Trial

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Objective: To compare the immunogenicity and safety of an investigational typhoid Vi conjugate vaccine (Test TCV) with a marketed typhoid Vi conjugate vaccine (Comparator TCV).

Design: Randomized, controlled trial.

Setting: Tertiary care and multispecialty hospitals.

Participants: 240 healthy subjects of 6 months to 45 years. Pediatric (<18 years) subjects were enrolled after day 21 safety assessment of adult subjects.

Intervention: Participants received a single-dose of test TCV or comparator TCV at baseline and were followed up for 6 weeks post-vaccination.

Main outcome measure(s): Primary variable was to demonstrate non-inferiority of the test TCV with the comparator TCV for seroconversion post-vaccination (≥ 4 -fold rise in antibody titre). Secondary variables were seroconversion in the adult and pediatric cohorts, and geometric mean titre of antibodies while the safety was based on reported adverse events.

Results: A total of 117 subjects (Adult-58, Pediatric-59) and 119 subjects (Adult-60, Pediatric-59) in test and comparator group, respectively completed the study. The seroconversion rate with test TCV (overall-94.8%, adult-96.6% and pediatric-93.1%) was non-inferior to comparator TCV (overall-91.6%, adult-91.7% and pediatric-91.5%). The geometric mean titres of antibodies (EU/mL) at baseline (test TCV: overall-7.6, adult-10.0, and pediatric-5.7; and comparator TCV: overall-8.0, adult-12.0, and pediatric-5.3) and at end of study (test TCV: overall-1121.0, adult-1411.0 and pediatric-891.1; and comparator TCV: overall-1104.0, adult-1199.0 and pediatric-1014.0) were also comparable between the groups ($P > 0.05$ for all). The most common adverse event was injection-site pain followed by fever in both the groups.

Conclusion: The immunogenicity and safety of test TCV is comparable to already marketed comparator TCV.

Keywords: Conjugate vaccine, Polysaccharide protein conjugate, Tetanus toxoid, Typhoid vaccine.

Trial Registration : CTRI/ 2016/05/006975

Typhoid (enteric) fever is a major public health disorder worldwide including India [1]. Several typhoid conjugate vaccines (TCVs) in which Vi capsular polysaccharide of *Salmonella typhi* is conjugated to the various carrier proteins have been developed to overcome the immunological drawbacks of the conventional typhoid polysaccharide vaccines [2]. Two such TCVs containing tetanus toxoid as the carrier protein have already been approved and marketed in India viz, Tybar-TCV (Bharat Biotech International Ltd.) [3] and Pedatyph (Bio-Med Pvt. Ltd.) [4]. The former contains 25 mcg of the Vi

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polysaccharide while the latter contains only 5 mcg of the Vi polysaccharide. TCVs can also be administered to infants and toddlers. The current study was conducted to evaluate the immunogenicity and safety of a new investigational indigenously developed TCV (Test TCV) in the target population.

METHODS

This was a pre-licensure, randomized, multicentre, single-blind, non-inferiority, phase II/III clinical study

conducted at 8 centres (tertiary care or multispecialty hospitals) during June to November, 2016. The study was approved by the Regulatory Authority and the respective Institutional Ethics Committees of all the study sites. The study was registered prospectively with Clinical Trial Registry of India.

Prior to screening, a written informed consent with a prior consent for audio-video recording of the consent process was obtained from the adult subjects and guardians of the pediatric subjects; an assent was also obtained from the pediatric subjects aged ≥ 7 years. Healthy subjects of either gender aged 6 months to 45 years were considered eligible if the adult subjects or guardians of the pediatric subjects were literate enough to fill the adverse event (AE) details in the diary cards. The subjects were excluded if they had a history of hypersensitivity to any component of the vaccine, typhoid fever or vaccination against typhoid fever within the past 3 years, fever or infectious disorder of any origin of >3 days in the past month, any vaccination within the past 7 days; any febrile illness ($\geq 37.5^\circ\text{C}$) at the time of enrollment; any clinically significant systemic disorder, immunological disorder, coagulation disorder or thrombocytopenia; any anticoagulant, immunosuppressive or immunostimulant therapy; administered blood, blood products or immunoglobulins within the past 3 months or planned administration during the study; pregnant and lactating women and female subjects not using acceptable contraceptive measures; participation in another clinical trial in the past 3 months; or history of alcohol or drug abuse in the past one year. Urine pregnancy test was done for adult females during the screening. The subjects were equally divided in the adult (18-45 years) and the pediatric (6 months to <18 years) cohorts; the enrolment in the pediatric cohort commenced after review of day 21 safety data of all the subjects enrolled in the adult cohort by an independent data and safety monitoring board. Pediatric cohort was stratified according to age into 6 month to less than 2 year, 2 to less than 5 year and 5 to less than 18 year.

A centralized block randomization plan of block size four was generated from www.randomization.com and a unique sequence of randomization numbers from this plan was provided to each study site. Eligible subjects were randomized (1:1) to receive a 0.5 mL single-dose of either the test TCV (Cadilla Healthcare Ltd., Ahmedabad, India) (Batch No. BO09S03) or the comparator TCV (Batch No. 76DL15026) which contained 25 mcg purified Vi capsular polysaccharide of *S. typhi* conjugated to tetanus toxoid. As the antigenic composition of the test TCV mimics that of Typbar-TCV, it was selected as the comparator (Comparator TCV). Comparator TCV has also been

prequalified by the World Health Organization (WHO) in December, 2017 and it is indicated for active immunization against *S. typhi* infection in 6 months to 45 years age group [5]. Comparator TCV was procured from the market for this study. The vaccine was administered in the upper arm or in the anterolateral aspect of the upper thigh for younger children, at baseline (day 0) following which the subjects were closely observed for at least 30 minutes for any immediate AEs. Loading of the injection for vaccination was done out of sight of the subjects/guardians to maintain single-blinding. The subjects were later followed up on an outpatient basis on day 7 ± 3 , 21 ± 7 and 42 ± 14 .

Diary cards were given to the adult subjects or the guardians of the pediatric subjects to record solicited local (pain, redness, swelling and induration) and systemic (fever, headache, nausea, vomiting, malaise, arthralgia and myalgia) AEs for 7 days post-vaccination and unsolicited AEs till the end of the study. Any abnormality in the vitals or physical examination was also to be reported as an AE. The severity of AEs was graded as mild, moderate or severe as per the defined criteria (supplementary table) and causality was assessed as per the WHO's criteria for AEs following immunization [6]. In addition, the investigators also graded the tolerability to the vaccine based on the reported AEs.

Two mL blood samples were collected at baseline and 6 weeks post-vaccination for assessment of anti-Vi IgG antibody titre by the commercial Vacczyme ELISA kits (Binding Site Group Ltd., UK) at the central accredited laboratory. The primary outcome was seroconversion rate which was defined as four-fold or higher rise in anti-Vi antibody titre post-vaccination as per the WHO recommendations [7,8]. The secondary outcomes were geometric mean titre (GMT) of antibodies and seroconversion rate and GMT of antibodies in both age cohorts. The safety variables were local or systemic AEs, serious AEs (SAEs) reported, if any, and overall tolerability evaluation by the investigators based on the reported AEs as follows: Excellent - no AE, Good - mild AE(s), Fair - moderate AE(s) and Poor - severe or serious AE(s).

Assuming the seroconversion rate of at least 95% based on the published results of the comparator vaccine [9], a sample size of 238 subjects (1:1 allocation) was calculated to demonstrate the non-inferiority of the test TCV as compared to the comparator TCV considering 90% power, 2.5% level of significance and dropout rate of 15%.

Statistical analysis: The test TCV was considered non-inferior to the comparator TCV if the lower limit of 95%

CI for the difference between their seroconversion rates was above the pre-defined non-inferiority limit of -10% [8]. The GMTs between the groups were compared using the unpaired *t*-test while the GMTs within the groups were compared using the paired *t*-test after log transformation of antibody titres. The seroconversion rate and the incidence of AEs was compared using Chi-square or Fisher's exact test. Immunogenicity was assessed for both per-protocol and modified intention-to-treat analysis (defined as all randomized subjects who completed the study including the subjects with protocol violations) while all the vaccinated subjects were considered for the safety assessment.

RESULTS

In this study, 240 subjects (120 pediatric, 123 females) were randomized (**Fig. 1**). The mean (SD) age, height, weight and body mass index of the subjects were 16.1 (12.5) years, 130.2 (35.8) cm, 37.1 (22.9) kg and 19.0 (4.8) kg/m² respectively. The baseline characteristics of the subjects are as mentioned in **Table I**.

The seroconversion rates for the overall population, and the adult and the pediatric cohorts were 94.8%, 96.6% and 93.1% in the test group and 91.6%, 91.7% and 91.5% in the comparator group, respectively. The difference between proportions (95% CI) were 3.2% (-3.2%, 9.7%), 4.9% (-3.5%, 13.3%) and 1.6% (-8.1%,

11.2%) for the overall population, and the adult and the pediatric cohorts, respectively. The seroconversion rates for the age groups of 6 m to <2 y, 2 to <5 y and 5 to <18 y were 100%, 90% and 90% in the test group and 81.8%, 100% and 94.4% in the comparator group respectively; ($P>0.5$).

A significant rise in the GMTs of anti-Vi antibodies was reported post-vaccination in both the study groups and in various age groups ($P<0.0001$). The GMTs of antibodies at the end of the study were comparable between the groups ($P>0.05$) (**Table II**). The results of seroconversion and GMTs of antibodies were similar when analyzed by modified intention-to-treat analysis (data not shown).

In this study, 33.6% and 43.8% subjects in the test and comparator group respectively had reported AEs ($P=0.11$). The characteristics of AEs reported in the overall population and the adult and the pediatric cohorts are given in **Table III**. All the AEs resolved within 7 days of their occurrence with 91.5% AEs in the test group and 93.6% AEs in the comparator group getting resolved within 3 days of occurrence. There was no SAE reported during the study in either of the two study groups. Most of the AEs were mild in intensity, 93.2% in the test group and 81.7% in the comparator group. A certain, probable or possible association of AEs with the vaccine was seen in 88.1% and 85% of test and comparator group respectively. The overall tolerability assessment was excellent, good, fair and poor in 66.4%, 31.1%, 2.5% and 0% subjects in the test group, and 56.2%, 33.9%, 9.1% and 0.8% subjects in the comparator group, respectively ($P=0.09$).

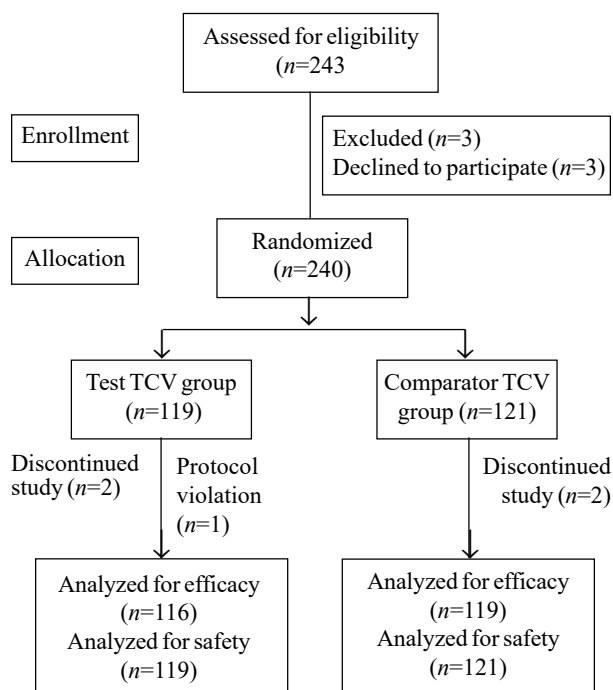


Fig. 1. Study flow chart.

Table I Baseline Characteristics of the Study Population

Characteristic	Test group		Comparator group	
	Adult n=59	Pediatric n=60	Adult n=61	Pediatric n=60
Median Age (y)	26.4	3.1	24.8	3.2
Males, n	21	35	26	35
Baseline titer*	10.1 (7.8, 13.8)	5.6 (4.5, 7.1)	12.0 (8.4, 17.0)	5.2 (4.1, 6.7)
6 mo to <2 y	–	4.0 (3.6, 4.5)	–	4.5 (3, 6.7)
2 y to <5 y	–	5.1 (3.5, 7.4)	–	4 (3.4, 4.6)
5 y to <18 y	–	8.3 (5, 13.9)	–	8.2 (4.3, 15.5)

*Data presented as geometric mean titer (95% CI) in ELISA units/mL; $P>0.05$ for all inter-group comparisons.

Table II Geometric Mean Titre of Anti-Vi Antibodies at Study Completion

Population type	Test group		Comparator group	
	n	Geometric mean (95% CI)	n	Geometric mean (95% CI)
Overall	116	1121 (857.2, 1467)	119	1104 (845.8, 1441)
Adult	58	1411 (1104, 1803)	60	1199 (960.2, 1498)
Pediatric	58	891.1 (551.7, 1439)	59	1014 (618.3, 1664)
6 mo to <2 y	18	1115 (628.4, 1979)	22	637.1 (201.5, 2014)
2 y to <5 y	20	709.3 (262.3, 1918)	19	1307 (739, 2313)
5 y to <18 y	20	914.8 (352.1, 2377)	18	1370 (672, 2793)

P>0.05 for all inter-group comparisons.

Table III Local and Systemic Adverse Events Reported in Study Subjects Receiving Typhoid Conjugate Vaccine

Parameter	Test group			Comparator group		
	Overall (n=119)	Adult (n=59)	Pediatric (n=60)	Overall (n=121)	Adult (n=61)	Pediatric (n=60)
Subjects with AEs	40 (33.6)	27 (45.8)	13 (21.7)	53 (43.8)	30 (49.2)	23 (38.3)
AEs reported	59	38	21	93	45	48
Local AEs	39	29	10	49	30	19
Pain	30 (25.2)	23 (39.0)	7 (11.7)	39 (32.2)	26 (42.6)	13 (21.7)
Swelling	5 (4.2)	3 (5.1)	2 (3.3)	6 (5.0)	1 (1.6)	5 (8.3)
Redness	4 (3.4)	3 (5.1)	1 (1.7)	3 (2.5)	2 (3.3)	1 (1.7)
Irritation	0	0	0	1 (0.8)	1 (1.6)	0
Systemic AEs	20	9	11	44	15	29
Fever	*7 (5.9)	5 (8.5)	#2 (3.3)	*17 (14.1)	5 (8.2)	#12 (20.0)
URTI	3 (2.5)	0	3 (5.0)	8 (6.6)	1 (1.6)	7 (11.7)
Diarrhea	3 (2.5)	1 (1.7)	2 (3.3)	1 (0.8)	1 (1.6)	0
Myalgia	2 (1.7)	2 (3.4)	0	6 (5.0)	4 (6.6)	2 (3.3)
Malaise	1 (0.8)	0	1 (1.7)	4 (3.3)	2 (3.3)	2 (3.3)
Headache	1 (0.8)	0	1 (1.7)	2 (1.7)	1 (1.6)	1 (1.7)
Arthralgia	1 (0.8)	1 (1.7)	0 (0.0)	2 (1.7)	1 (1.6)	1 (1.7)
Vomiting	1 (0.8)	0	1 (1.7)	2 (1.7)	0	2 (3.3)
Nausea	1 (0.8)	0	1 (1.7)	0	0	0
Urticaria	0	0	0	2 (1.7)	0	2 (3.3)

AE: Adverse events; URTI: Upper respiratory tract infection; Data presented as no. (%); *P*>0.05 for all inter-group comparisons except **P*=0.05 and #*P*<0.05.

DISCUSSION

In the present study, seroconversion rate post-vaccination with the test TCv was non-inferior to the comparator TCv, and GMT of antibodies post-vaccination were comparable for both the vaccines. The safety of the test TCv evaluated in terms of reported AEs was also comparable to the comparator TCv.

The seroconversion rate with the comparator TCv reported earlier varied from 91.9-100% in various age

groups [9,10]. The seroconversion rates reported with Pedatyph were 83% [11] and 100% [12] in pediatric subjects. The seroconversion rates with varying concentrations of another TCv, Vi-CRM₁₉₇ (1.25 mcg, 5 mcg, 12.5 mcg and 25 mcg) in adult subjects in phase I and II studies were also in the range of 95-100% [13]. The seroconversion rate reported for the test TCv in this study was also similar. Likewise, the GMT of antibodies post-vaccination in this study also correlated well with that reported for the comparator TCv in previous studies

WHAT IS ALREADY KNOWN?

- Typhoid conjugate vaccines are associated with a better immunological response as compared to the conventional unconjugated polysaccharide vaccines.

WHAT THIS STUDY ADDS?

- Immunogenicity and safety profile of the test typhoid conjugate vaccine is comparable to the already marketed vaccine in the target population of 6 months to 45 years of age.

in which similar antibody assessment method was used [9,10]; however, the GMT of antibodies post-vaccination with other TCVs could not be directly compared due to the difference in the antibody assessment method [11-16]. Further, the safety data reported in this study is also consistent with that reported for other TCVs [13,17] and typhoid polysaccharide vaccines [18-20].

The limitations included single-blind nature of the study as the difference in the physical characteristics and packaging of the test TCV and the comparator TCV precluded double-blinding. The study was conducted in a limited sample size (albeit sufficient to draw statistical conclusions) with a short-term follow-up. However, a long-term extension of this study is being conducted in which the persistence of antibodies around 3 years after primary vaccination will be evaluated.

Owing to the improved immunological properties, permission for use in younger children including infants and longevity of the immune response of TCV, WHO has recommended a single-dose of TCV from 6 months to 45 years of age in endemic regions to prevent typhoid fever [21]. The results of this study indicate that the immunogenicity and safety of the test TCV is comparable to that of the comparator TCV.

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Competing interests: RK, AKK, UN, SKJ, TRB, RV, SS, VKG: were the clinical trial investigators and they received honorarium from the sponsor for the conduct of the study. PD, PP, RM: are employees of M/s. Cadila Healthcare Ltd.

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