Research letter

Immunogenicity and safety of a heterologous prime-boost COVID-19 vaccine schedule: ChAdOx1 vaccine Covishield followed by BBV152 Covaxin

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Highlights

The evidence for effectiveness of heterologous priming of COVID-19 vaccine is very limited. Here, we studied eighteen participants who received heterologous vaccination regimen of AstraZeneca's ChAdOx1-nCov-19 followed by inactivated whole virion BBV152. Heterologous group participant doesn't report any adverse event following immunization and demonstrated high humoral and neutralizing antibody response.

Text

COVID-19 vaccination program was started in India on January 16th 2021.[1] Whole virion inactivated BBV152 (Covaxin) and AstraZeneca's ChAdOx1-nCov-19 (Covishield) were the two vaccines initially rolled out under this program.[2,3,4,5] Frontline workers including healthcare professionals, elderly above the age of 60 and adults with co-morbidity were prioritized for vaccination before reaching out to all the adults. During expansion of the aforementioned vaccination program, a group of participants in the northern state, Uttar Pradesh received Covishield (CS) as the first dose followed by inadvertent administration of Covaxin (CV) as the second dose. Considering this, we studied the safety and immunogenicity of a heterologous prime-boost COVID-19 vaccination. A total of 98 vaccine recipients who have completed two weeks or more after the second dose of vaccine were included in this study.. Participants were recruited in three cohorts during May to June 2021: heterologous group (n=18, first dose CS, second dose CV administered at an interval of six weeks), among them, 11 were male (M/F:11/7) with a median age of 62 years (IQR 54.25-69.75). A comorbid condition (hypertension) was reported in one participant. CS group (n=40, 22 were males (M/F:22/18) with a median age of 65.5 years (IQR 62-69) and co-morbidity was observed among 4 participants who received two vaccine doses at six weeks interval. CV group (n=40, two vaccine doses administered at four weeks interval including 23 females (M/F:17/23) with a median age of 56 years (IQR 45.5-63) and 3 with co-morbidities (Supplementary table-1) (Supplementary figure-S1).

Information on the demographic profile, vaccination history and clinical history including recent illness, co-morbidities and details of adverse events following

immunization (AEFIs) were recorded by trained investigators of the ongoing national vaccination program. The data was collected manually using paper-based forms, home visits, personal interviews and telephonic interactions and further recorded in the predesigned questionnaire.

The most common local AEFI reported after first and second vaccine dose was pain at injection site; CS (5%; 5%), CV (7.5%; 7.5%) and heterologous group (11.1%; nil). No other local AEFI such as erythema, induration, pruritis or pustule formation was recorded by any of the participants. Most commonly reported systemic AEFI were pyrexia and malaise with frequency of pyrexia was reported among CS group (20%, 15%), CV group (30%, 15%) and heterologous group (27.77%, 11.1%) after 1st dose and 2nd vaccine dose. Malaise was also observed in CS group (5%, 5%), CV group (32.5%, 15%) and heterologous group (33.3%, 5.5%) after 1st dose and 2nd vaccine dose respectively (Supplementary figure S2 a-d). Overall, the frequency of AEFI in the heterologous group was similar to the participants of the CV/CS groups establishing the safety of heterologous vaccination. The frequency of local and systemic AEFI was lower after the second dose in all three groups.

Five millilitre of blood was collected from each of the participants post-immunization to test the S1-RBD, Nucleocapsid (N) IgG, neutralizing antibody (NAb) response and phenotyping of lymphoid cells.

The sera of the participants from the CS, CV and heterologous groups demonstrated geometric mean titre (GMT) of 2260 (95%CI: 1881-2716), 710 (95%CI: 461-1092) and 1866 (95%CI: 1003-3472) respectively with S1-RBD ELISA. The GMT with N-protein ELISA in CS, CV and heterologous groups were 353.7 (95%CI: 219.9-568.9), 742.4 (95%CI: 485.8-1134) and 1145 (95%CI: 520.7-2520) respectively. The sera of the

participants of CV, CS and heterologous groups shown GMT of 111 (95%CI: 98.59-124.9), 86 (95% CI: 138.2-252.0), 171.4 (95%CI: 121.3-242.3) respectively with inactivated SARS-CoV-2 (whole virus) ELISA (Figure 1 a-c). The ELISA titres were analysed using the Kruskal-Wallis test along with Dunns' multiple comparisons.

Plaque reduction neutralization test (PRNT50) demonstrated neutralizing antibodies (NAb) geometric mean titre (GMT) of 162 (95%CI:76.74-342), 122.7 (95%CI:59.36-253.7), 48.43 (95% CI:19.71-119) and 51.99 (95%CI:19.65-137.6) respectively in the participants sera of the CS group against B.1, Alpha, Beta, Delta variants. The GMT for the sera of participants from the CV group against B.1, Alpha, Beta and Delta variants was 156.6 (95%CI: 105.2-233.1), 112.4(95% CI: 76.56-164.9), 52.09 (95%CI: 34.9-77.73) and 54.37 (95%CI: 27.26-108.4) respectively. The sera of the heterologous group participants had GMT of 539.4 (95%CI: 263.9-1103), 396.1 (95%CI: 199.1-788), 151 (95%CI: 80.21-284.3) and 241.2 (95%CI: 74.99-775.9) respectively against B.1, Alpha, Beta and Delta variants. Wilcoxon signed-ranks test was performed to analyse the statistical significance of the neutralization titre for the heterologous group.

The GMT ratio was higher for Beta relative to B.1 variant [3.35 (95%CI: 2.87-3.89; p-value=0.0062)] in comparison to Alpha [1.32 (95%CI: 1.29-1.35); p-value>0.999] and Delta variant [3.12 (95% CI: 2.49-3.91); p-value=0.0820] in the CS group (Figure 1 d). Similarly, GMT ratio was higher for the Beta relative to B.1 variant [3.01 (95%CI: 3.00-3.01; p-value=0.0001)] in comparison to Alpha [1.39(95%CI: 1.37-1.41); p-value>0.999] and Delta variant [2.88 (95%CI: 2.15-3.86); p-value=0.0196] in the CV group (Figure 1 e). The GMT ratio was higher for the Beta relative to B.1 variant [3.57(95%CI: 3.29-3.88; p-value =0.0125)] in comparison to Alpha [1.36(95%CI: 1.33-1.40); p-value >0.999] and Delta variant [GMT ratio: 2.24(95% CI: 1.42-3.52); p-value >0.999] in the heterologous

group (Figure 1 f). The heterologous group had ~3-fold higher titre in comparison to CS and CV groups (Figure 1 g-j). Kruskal-Wallis test along with Dunns' multiple comparisons was performed to assess the significance in NAb titres of the sera against different SARS-CoV-2 variants.

Vaccinated individuals demonstrated higher IgG titres for the S1-RBD protein followed by N protein (Figure 1 k-m). Neutralization of participants' sera, with average age ranging approximately between 62.0-63.0 yrs, administered with different vaccines against B.1, Alpha, Beta and Delta variants were compared to observe the effect of age on NAb titres. It is observed that NAbs were 1.25, 3.95 and 1.30 fold reduced in heterologous group for VOCs compared to B.1. Similarly the NAb were reduced with homologous Covishield [1.33, 3.9, and 2.74], homologous Covaxin [1.4, 2.45, and 2.08] vaccination for respective VOCs compared to B.1 (Figure-1 n-p).

Assessment based on the surface marker of the gated lymphocytes from lysed whole blood (Supplementary Figure S3) was found to be comparable among all the study groups i.e., heterologous group and CS or CV groups in terms of the percentage of cells expressing CD3, CD4, CD16+56 or CD19. However, % CD8+ T cells in the CS group (median (IQR): 47.66 (40.03–55.02), $p < 0.05$) was found significantly high compared to CV group (median (IQR): 39.40 (33.87–49.27) (Supplementary Figure S4 a-f).

In our study, no major systemic AEFIs were reported and reactogenicity profile of the participants of heterologous group demonstrated that mixing of the two vaccines derived from different platforms is safe. The humoral immune response and NAb titres in the heterologous group were significantly high in neutralizing VOCs; Alpha, Beta and Delta variants. The limitations of study include low sample size; short follow up period and unavailability of baseline serological and immunological data of the participants.

Heterologous vaccination will pave the way for induction of improved and better protection against the VOCs and to overcome the challenges of shortfall of particular vaccines. It will also remove hesitancy about the vaccines in people's mind that could have genesis in programmatic 'errors. However, multicentre randomized clinical trials needs to be carried out to conclusively prove these findings.

Author contributions

PDY, RKS and BB conceived the idea. PDY, RK and KZ designed the study. GD, KZ, RRS, RS and SC contributed patient recruitment and data acquisition. GS, HK, ASA, DAN, GRD carried out the experiment. PDY, RKS supervised all parts of the study, PDY, SK, RRS, HK performed analysis and PDY, NG, SK, PA, SP wrote the manuscript. All the authors have approved the manuscript.

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Legend to figure

Figure 1: Anti-SARS-CoV-2 IgG titres of participants sera vaccinated with the different vaccines. a) Anti-SARS-CoV-2 IgG titres of vaccinated participants sera for S1-RBD protein for homologous Covishield [n=40, (blue)], homologous Covaxin [n=40, (green)] and heterologous Covishield/Covaxin [n=18, (pink)]. **b)** N-specific IgG level in serum in the three groups. **c)** Inactivated SARS-CoV-2 IgG levels in serum of the three groups. The statistical significance was assessed using a two-tailed Kruskal Wallis test with Dunn's test of multiple comparisons; p-value less than 0.05 were considered to be statistically significant. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD). The normality of the data was compared using the Shapiro-Wilk test for normality using two-tailed test in GraphPad Prism v 9.2.0. The ELISA titres against S1-RBD, N protein and inactivated SARS-CoV-2 (whole virus) was compared in sera of the three groups and analysed statistically using the Kruskal-Wallis test along with Dunns' multiple comparisons in GraphPad Prism.

Neutralization of participants sera vaccinated with different vaccines against B.1, alpha, beta and delta variants: The plaque reduction neutralization test (PRNT50) was performed against the B.1 (NIV2020-770, GISAID accession number: EPI_ISL_420545), Alpha [B.1.1.7, hCoV-19/India/20203522 SARS-CoV-2 (VOC) 202 012/01], Beta (B.1.351, NIV2021-893, GISAID accession number: EPI_ISL_2036294) and Delta (B.1.617.2, NIV2021-1916, GISAID accession number: EPI_ISL_2400521) variant. NAb titre of participants sera against the B.1, beta, alpha and delta variant administered with 2 doses of Covishield (n=40) (d), 2 doses of Covaxin (n=40) e) and a single dose of Covishield followed by Covaxin (n=18) (f). A matched pair two-tailed pair-wise comparison was performed using the Wilcoxon signed-rank test to analyze

the statistical significance. Neutralization titre of participants' sera vaccinated with different vaccines against different variants; Alpha (g), Beta (h), Delta (i), and B.1 (j). The statistical significance was assessed using a two-tailed Kruskal Wallis test with Dunn's test of multiple comparisons was performed to analyze the statistical significance. A p-value less than 0.05 were considered to be statistically significant for the comparison. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD). Wilcoxon signed-ranks test was performed to analyse the statistical significance of the neutralization titre of the each participants sera vaccinated with different vaccines against the different variants. Kruskal-Wallis test along with Dunns' multiple comparisons was performed to assess the significance in NAb titres of the sera against each variant.

Anti-SARS-CoV-2 IgG and the neutralizing antibody titres of participants sera, with average age ranging approximately between 62.0-63.0 yrs, administered with different vaccines against B.1, Alpha, Beta and Delta variants: k) Anti-SARS-CoV-2 IgG titres of vaccinated individual's sera for S1-RBD protein **l)** N-specific IgG level and **m)** Inactivated SARS-CoV-2 IgG levels in serum for 2 doses of Covishield (n=14), 2 doses of Covaxin (n=9) and a single dose of Covishield followed by Covaxin (n=6). The statistical significance was assessed using a two-tailed Kruskal Wallis test with Dunn's test of multiple comparisons. NAb titres of participants sera against the B.1, Beta, Alpha and Delta variant administered with 2 doses of Covishield (n), 2 doses of Covaxin (o) and a single dose of Covishield followed by Covaxin (p). A matched pair two-tailed pair-wise comparison was performed for the comparing the participants sera administered with 2 doses of Covaxin while the rest were compared using the Wilcoxon signed-rank test to analyse the statistical significance. A p-value less than 0.05 were considered to be statistically significant for the comparison. The dotted line on the figures indicates the

limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD).