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ORIGINAL RESEARCH

Efficacy of Bacillus Calmette–Guérin (BCG) Vaccination in Reducing the Incidence and Severity of COVID-19 in High-Risk Population (BRIC): a Phase III, Multi-centre, Quadruple-Blind Randomised Control Trial

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ABSTRACT

Introduction: Universal coverage of vaccines alone cannot be relied upon to protect at-risk populations in lower- and middle-income countries against the impact of the coronavirus disease 2019 (COVID-19) pandemic and newer variants. Live vaccines, including Bacillus Calmette–Guérin (BCG), are being studied for their effectiveness in reducing the incidence and severity of COVID-19 infection.

Methods: In this multi-centre quadruple-blind, parallel assignment randomised control trial, 495 high-risk group adults (aged 18–60 years) were randomised into BCG and placebo arms and followed up for 9 months from the date of vaccination. The primary outcome was the difference in the incidence of COVID-19 infection

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at the end of 9 months. Secondary outcomes included the difference in the incidence of severe COVID-19 infections, hospitalisation rates, intensive care unit stay, oxygen requirement and mortality at the end of 9 months. The primary analysis was done on an intention-to-treat basis, while safety analysis was done per protocol.

Results: There was no significant difference in the incidence rates of cartridge-based nucleic acid amplification test (CB-NAAT) positive COVID-19 infection [odds ratio (OR) 1.08, 95% confidence interval (CI) 0.54–2.14] in the two groups, but the BCG arm showed a statistically significant decrease in clinically diagnosed (symptomatic) probable COVID-19 infections (OR 0.38, 95% CI 0.20–0.72). Compared with the BCG arm, significantly more patients developed severe COVID-19 pneumonia (CB-NAAT positive) and required hospitalisation and oxygen in the placebo arm (six versus none; $p = 0.03$). One patient belonging to the placebo arm required intensive care unit (ICU) stay and died. BCG had a protective efficacy of 62% (95% CI 28–80%) for likely symptomatic COVID-19 infection.

Conclusions: BCG is protective in reducing the incidence of acute respiratory illness (probable symptomatic COVID-19 infection) and severity of the disease, including hospitalisation, in patients belonging to the high-risk group of COVID-19 infection, and the antibody response persists for quite a long time. A multi-centre study with a larger sample size will help to confirm the findings in this study.

Clinical Trials Registry: Clinical Trials Registry India (CTRI/2020/07/026668).

PLAIN LANGUAGE SUMMARY

The Bacillus Calmette–Guérin (BCG) vaccine has been studied previously in several settings, including reducing childhood mortalities due to viral infections and induction of trained immunity and reducing upper respiratory tract infections and pneumonia in older adults. This multi-centre trial has tried to evaluate the efficacy of BCG revaccination in reducing the incidence and severity of COVID-19 infections in adults between 18 and 60 years of age belonging to the high-risk group owing to the presence of comorbidities including diabetes, chronic kidney disease, chronic liver disease

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and chronic lung diseases. A single dose of BCG vaccine produced significantly high titres of BCG antibodies lasting for six months. While there was no significant reduction in the incidence of COVID-19 infection, there was an 8.4% reduction in the incidence of symptomatic COVID-19 disease at the end of 9 months of follow-up. In addition, there were significantly fewer severe COVID-19 infections requiring hospital stay and oxygen support. However, the overall numbers of severe COVID-19 infections were low. Thus, the study shows that BCG can protect against symptomatic and severe COVID-19 disease. However, it might not reduce the incidence of new infections. The study results are significant for low- and middle-income countries without adequate coverage of primary doses of COVID-19 vaccination, let alone the booster doses. Future studies should evaluate the BCG vaccine's efficacy as a booster compared with routine COVID-19 vaccine boosters.

Keywords: BCG vaccination; Incidence of COVID-19; Severe COVID-19; Symptomatic COVID-19; Vaccine efficacy

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Key Summary Points

The use of easily accessible live vaccines like Bacillus Calmette–Guérin (BCG), which has shown protective efficacy for other infections, was studied to supplement routine COVID-19 vaccinations, especially in low- and middle-income countries without adequate coverage and access to COVID-19 vaccines

We hypothesised that vaccination with BCG will reduce the incidence and severity of COVID-19 infections in high-risk groups.

This multi-centre quadruple-blind randomised controlled trial showed a significant reduction in the incidence of microbiologically proven (CB-NAAT-positive) severe COVID-19 infections. In addition, it also showed a reduction in the incidence of acute respiratory illness (probable symptomatic COVID-19 infection based on seroconversion rates). However, there was no significant difference in the overall incidence of microbiologically proven (CB-NAAT positive) COVID-19 infection. However, there was a higher COVID-19 seroconversion in the placebo arm compared with the BCG arm at the end of 9 months of follow-up

This study shows that BCG protects high-risk adults between 18 and 60 years of age from symptomatic acute respiratory illness (probable symptomatic COVID-19 infection) and severe disease, like most available COVID-19 vaccines in low- and middle-income countries. However, it does not necessarily reduce the overall incidence of COVID-19 infection.

INTRODUCTION

Despite the significant impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on world health and the economy, there is a significant deficit in the delivery of protective measures for at-risk populations worldwide due to cost, administrative issues, availability and acceptability. This is particularly true for lower- and middle-income countries with large populations at higher risk of acquiring COVID-19 infections. Almost one in two people in this region has not received complete vaccination, with virtually minuscule coverage for booster doses [1].

Epidemiological studies have shown that vaccination with Bacillus Calmette–Guérin (BCG) at birth reduces childhood mortality through protection against neonatal sepsis and respiratory infections, most often caused by viral infections [2–4]. This association was further strengthened by the non-targeted protective efficacy of BCG vaccination on acute lower respiratory tract and respiratory syncytial virus infections in children [5]. In a previous randomised placebo-controlled trial involving healthy human volunteers, those who received the BCG vaccine had significantly lower viraemia and sturdier anti-viral responses to an attenuated yellow fever vaccine strain [6]. A similar induction of trained immunity with accelerated natural killer cell and monocyte activation resulting in reduced parasitaemia was seen after controlled malaria infection in another randomised control trial [7]. The utility of BCG vaccination in reducing the incidence of acute upper respiratory tract infections and pneumonia in the elderly was shown in two previous trials [8, 9]. While preliminary epidemiological studies have shown a correlation between coverage of BCG vaccination and incidence and severity of COVID-19 infection, potential confounding factors were not accounted for in these analyses [10, 11]. Several ongoing trials are assessing the efficacy of BCG vaccination in reducing the incidence and severity of COVID-19 infection, results of which are awaited [12].

Relying solely on universal vaccine coverage to prevent new infections and reduce the severity of the disease might not be enough. In this context, BCG can be considered a cost-effective and easily accessible means of reducing morbidity and mortality due to COVID-19 infections. The effect on innate immunity primarily mediates the protection through the epigenetic, transcriptional and functional programming of natural killer (NK) cells or monocytes or through heterologous T-cell immunity [12].

In this study, we have evaluated the efficacy of BCG vaccination in reducing the incidence and severity of COVID-19 infection in high-risk younger adults between 18 and 60 years of age, which has not been studied in previous trials and who constitute a significant proportion of the affected population in low- and middle-income countries around the world.

METHODS

Study Design and Setting

This was a phase III multi-centre quadruple-blind, parallel assignment randomised control trial with an allocation ratio of 1:1, conducted across three hospitals in India, namely All India Institute of Medical Sciences, New Delhi, School of Tropical Medicine, Kolkata and Calcutta Medical College, Kolkata, and Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. Ethics approval for the study was obtained from the ethics committee of the respective centres, and the study was registered prospectively in the Clinical Trials Registry-India (CTRI number CTRI/2020/07/026668) (Supplementary Appendix 1), which is the official trial registry for all trials conducted in India. A data safety monitoring board regularly assessed the data from all three sites. The trial was done in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization–Good Clinical Practice guidelines. In addition, this study has followed Consort guidelines for reporting randomised control trials. The Indian Council of Medical Research, Ministry of Health and

Family Welfare, Government of India funded this study. However, none of the funding agency members was involved in the design, conduct, analysis and reporting of the study at any stage.

Study Participants

The study population included adults (between 18 and 60 years of age) with underlying medical conditions, including subjects with poorly controlled diabetes or diabetes-related complications, chronic kidney disease dialysis-dependent and non-dependent, chronic lung disease including bronchial asthma, chronic obstructive airway disease (COPD), and non-cystic bronchiectasis and cardiovascular disease including a history of coronary artery disease (CAD) or hypertensive heart disease, who were thereby at higher risk of severe COVID-19 infection (Supplementary Appendix 1). The list of complete inclusion and exclusion criteria is provided in the study protocol in Supplementary Appendix 1. All participants gave informed written consent for participation in this study.

Study Interventions

Participants who fulfilled the inclusion and exclusion criteria were randomly assigned in a 1:1 ratio to BCG and placebo arm. Randomisation was done using a computer-generated random number list with variable block sizes ranging from three to six and was stratified on the basis of the presence of comorbidities. Individual random numbers were placed in sealed opaque envelopes, which were opened by medical professionals who were blinded to the participants who received the intervention at the time of allocation of participants. Separate healthcare professionals who were blinded to the contents of the syringe administered the injections to the participants. After randomisation, subjects received either a 0.1 ml intradermal injection of Bacillus Calmette–Guérin (BCG) vaccine [a freeze-dried powder containing a live attenuated strain of *Mycobacterium bovis* containing between 0.2 and 0.8 million colony-forming units (CFUs) procured from the Serum Institute of India] or 0.1 ml of normal saline intradermally. BCG is included as a part of the universal immunisation programme in India and has a coverage of over 70% since the later part of the twentieth century. However, ascertaining the immunisation on the basis of history alone or presence of BCG scar alone was not considered accurate enough for evaluating history of childhood immunisation. We have instead used BCG antibody titres to look for significant differences in the BCG antibody titres between the BCG and placebo groups during follow-up and ascertained whether this correlated with a reduction in incidence and severity of COVID-19 infection.

Assessments

All subjects underwent screening for symptoms of COVID-19 infection and testing for COVID-19 infection by using a CB-NAAT test (performed using commercially available cartridge-based nucleic acid amplification tests purchased from Cepheid) along with BCG and COVID-19 antibodies at baseline. All subjects were followed up at 1, 3, 6 and 9 months where they were screened for COVID-19-related symptoms by healthcare professionals who were blinded to the allocation of the participants. In addition, BCG antibody titres were measured at 1, 3 and 6 months using commercially available ELISA kits [Recombivirus Human Anti-Tuberculosis BCG IgG Enzyme Linked Immunosorbent Assay (ELISA) kit manufactured by Alpha Diagnostic International] and COVID-19 antibody levels (anti-spike antibody) at 3, 6 and 9 months of follow-up using commercially available COVID-19 IgG (COVID KAVACH) ELISA kits. Adverse events were monitored for 1 h post-vaccination and subsequently during each follow-up visit. All patients underwent blood testing, including haemogram, liver and renal function tests at follow-up visits, along with purified protein derivative (PPD)-specific IgG antibodies (BCG antibody) and COVID-19 antibodies using ELISA. The principal investigator and team maintained all data in a centralised database.

Outcomes

The study's primary outcome was the difference in the incidence of new COVID-19 infection in the two arms, either microbiologically by CB-NAAT testing from nasopharyngeal swabs or by symptom screening at follow-up visits. The secondary outcomes were the difference in the incidence of severe COVID-19 infections [which was defined as those with respiratory rate (RR) > 30/min, oxygen saturation (SpO_2) < 90% on room air, severe respiratory distress, or fulfilling criteria for acute respiratory distress syndrome], hospitalisation rates, oxygen requirement, ICU stay, mortality and vaccination-related severe adverse events (grade 3 and above) as per the US Food and Drug Administration (FDA) grading of adverse events between the two arms [13].

Statistical Analysis

Since there were no prior studies on the protective efficacy of BCG vaccination in preventing COVID-19 infection at the time of design of this study, a sample size of convenience was chosen for this study. We decided to enrol approximately 400 patients in each arm. However, as the study progressed, it became difficult to recruit subjects belonging to high-risk groups who had not received COVID-19 vaccination and who were COVID-19 antibody negative, owing to the national vaccination programme and multiple waves of COVID-19 infection. Hence, the funding agency and data safety monitoring board (DSMB) decided to analyse the data. All data were analysed using STATA software version 15.0. Baseline characteristics were compared between the two arms of the study. Categorical variables were summarised by frequency and percentage, while a test of proportions was used to compare the proportion between the two groups. Fisher's exact and chi-square tests were used to compare the difference between the two groups. Quantitative variables were summarised by mean [standard deviation (SD)] or median [interquartile range (IQR)] as appropriate, and Student's *t*-test/Wilcoxon rank-sum test was used to compare the distribution between the two groups. The primary analysis was done on an intention-to-treat (ITT) basis, and safety analysis was done on all those who received their allocated intervention. Primary outcomes and secondary outcomes were compared between the two groups using Fisher's exact test, and the odds ratio was calculated using logistic regression analysis. Only the first event of CB-NAAT positivity was considered, and subsequent positive events were not included in the calculation of incidence rates. A p value of < 0.05 was considered statistically significant. Vaccine effectiveness was calculated using the formula: effectiveness = $(1 - \text{OR}) \times 100\%$. Every effort was made to ensure that missing data, if any, were collected from the subjects. For COVID-19 CB-NAAT positivity and symptom analysis as well for severe COVID-19 infection (including hospital stay, oxygen requirement), missing data, if any, were taken as negative for COVID-19 or absence of severe infection, respectively, while for BCG and COVID-19 antibody analysis, the last value carried forward method was used.

RESULTS

Patient Characteristics

A total of 495 subjects were enrolled between October 2020 and December 2021 and randomly assigned to two arms: BCG vaccination group ($n = 246$) and placebo group ($n = 249$) (Fig. 1). Thirty-two subjects dropped out after randomisation; however, they were included in the final analysis as part of the intention-to-treat analysis. The baseline demographic and clinical profile of the patients, body mass index (BMI) and presence of comorbidities are presented in Table 1 and were similar in the two groups. The intervention arm saw a significant increase in BCG titres compared with the placebo arm following BCG vaccination, which was maintained throughout the follow-up at the end of the first, third and sixth months (p value < 0.001 at each interval; Table 2). There were no vaccination-related serious adverse events (grade 3 or grade 4) in either of the two arms.

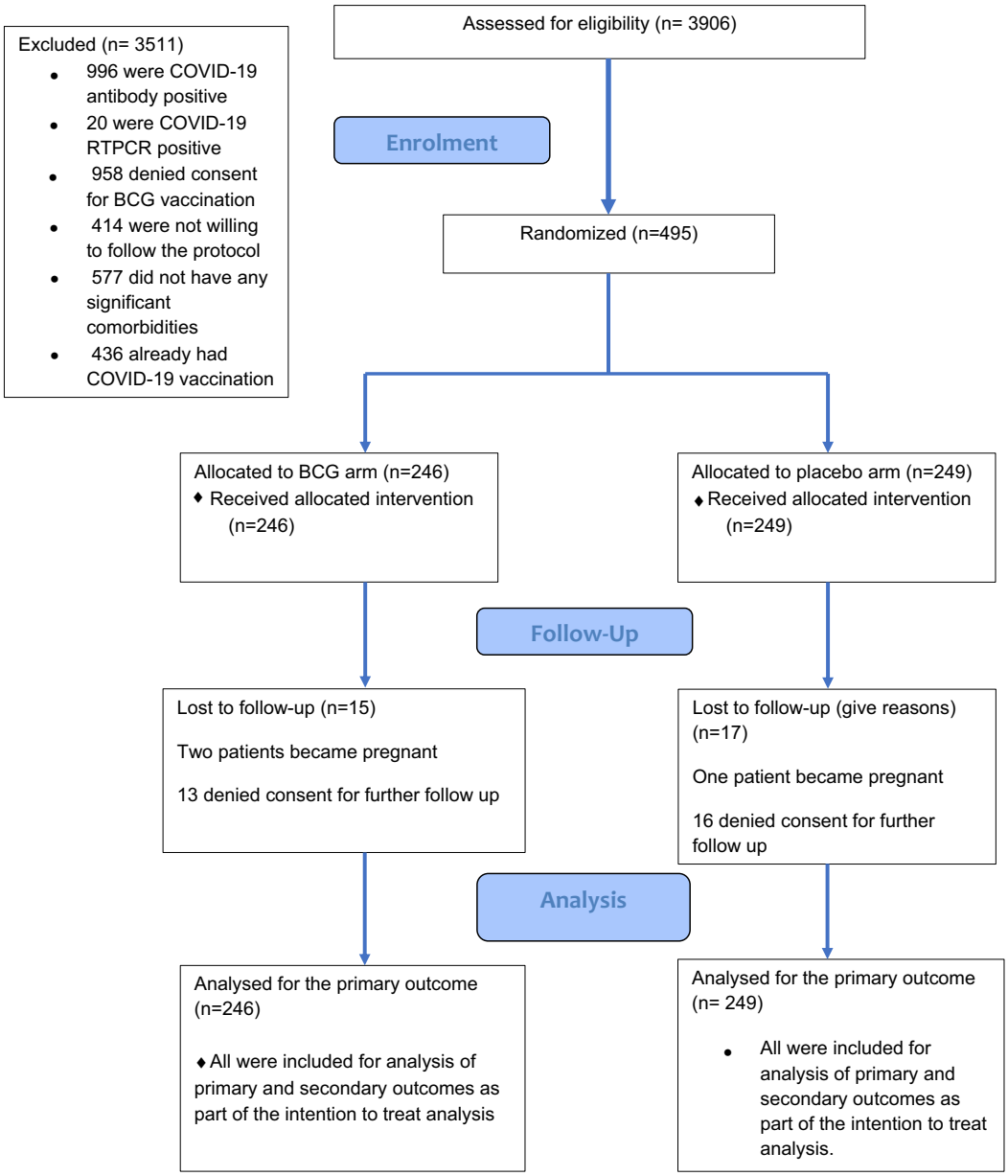

Fig. 1 Trial profile

Primary and Secondary Outcomes

The primary outcome of this study was the incidence of new COVID-19 infections detected by either CB-NAAT testing of subjects or using clinical symptom analysis during the follow-up of the study after vaccination with BCG or placebo at baseline. At the end of 9 months, there was no significant difference in the incidence of CB-NAAT-positive COVID-19 infections between the two arms (OR 1.08, 95% CI 0.54–2.14) (Table 3). However, when we screened patients on the basis of clinical symptoms of COVID-19 infection, there was a statistically significant 8.4% reduction in the incidence of probable COVID-19 infection in the BCG arm compared with placebo (Table 3). There was quite a high level of COVID-19

Table 1 Demographic profile combined for all centres ($n = 495$)

Characteristics at enrolment	BCG group (n = 246) 49.7%	Placebo group (n = 249) 50.3%	p value
Age (years) mean ± SD	43 ± 10	44 ± 10	0.23
Age (years), n (%)			0.56
18–30	33 (14%)	32 (13%)	
31–40	60 (24%)	48 (19%)	
41–50	86 (35%)	95 (38%)	
51–59	67 (27%)	74 (30%)	
Female, n (%)	124 (50%)	113 (46%)	0.26
Male, n (%)	122 (50%)	136 (54%)	
BMI (kg/m^2), mean ± SD	26 ± 4	26 ± 4	0.83
Comorbidity profiles			
Diabetes mellitus (DM)	125 (50%)	125 (50%)	0.89
Cardiovascular disease (CVD)	77 (32%)	86 (34%)	0.44
Chronic lung disease (CLD)	37 (15%)	39 (16%)	0.84
Chronic kidney disease (CKD)	35 (14%)	31 (13%)	0.56
More than one comorbidity	25 (11%)	32 (12%)	0.34
History of TB	14 (6%)	10 (4%)	0.38

antibody seroconversion overall at 9 months and a higher percentage of COVID-19 antibody positivity in the placebo arm as compared with the BCG arm at 6 months and 9 months, which is statistically significant (Table 4). Thus, one can conclude that there were more symptomatic COVID-19 infections in the placebo arm as compared with the BCG arm.

The secondary outcomes included a difference in the incidence of severe COVID-19 infections among the two groups. There was a significantly higher number of severe COVID-19 infections (CB-NAAT positive) as well as requirement for hospitalisation and oxygen support in the placebo arm compared with the BCG arm (Table 5). Owing to low event rates, ICU requirement and mortality could not be compared between the study arms as a single patient in the placebo arm required ICU admission and subsequently died, while there was none in the BCG arm. There were no vaccination-related severe adverse events (grade 3 or grade 4 adverse events) in either of the two arms. While there were minor adverse events in both arms (grades 1 and 2), they were not included in the outcome analysis as part of the study protocol.

We calculated the effectiveness of BCG vaccination for preventing symptomatic COVID-19 infection on the basis of the odds ratio using the formula (1 − odds ratio) × 100%, which came out to be 62% (95% CI 28–80%).

DISCUSSION

Our study showed that vaccination with BCG can protect high-risk individuals (those with comorbidities) less than 60 years of age against symptomatic acute respiratory illness probably due to COVID-19 infections, considering the

Table 2 BCG antibody titre level on follow-up in both groups ($n = 495$)

Serial number	Follow-up time	BCG Ab titre (IU) (BCG group) ($n = 246$)	BCG Ab titre (IU) (placebo group) ($n = 249$)	p value
1	Baseline	1.9 (0.1–9.9)	2.1 (0.01–19.0)	0.28
2	1 month	3.8 (0.1–17.7)	2.0 (0.1–14.2)	< 0.001
3	3 months	6.0 (0.1–27.3)	2.1 (0.2–27.2)	< 0.001
4	6 months	7.8 (0.2–26.2)	2.0 (0.2–26.4)	< 0.001

Table 3 Primary outcome: COVID-19-positive incidence rates during follow-up

Event	BCG group (incidence rates)	Placebo group (incidence rates)	p value	Odds ratio	95% CI
Definite COVID-19 infection (CB-NAAT-positive cases)	18 (7.3%)	17 (6.8%)	0.862	1.08	0.54–2.14
Probable COVID-19 infection (symptom screening)	15 (6.1%)	36 (14.5%)	0.003	0.38	0.20–0.72

context of the pandemic and seroconversion rates at follow-up, and reduce the severity of microbiologically proven disease in those who are infected. While there was no significant difference in the overall incidence of microbiologically proven COVID-19 infection, a significantly lower number of subjects developed symptomatic acute respiratory illness in the BCG arm compared with placebo. In addition, there was a significantly lower number of patients with microbiologically proven severe disease requiring oxygen support or hospitalisation. However, other severity parameters, including ICU admission and death, could not be compared owing to low event rates in both arms. If we track the COVID-19 circulation in the country during the course of the study, the study started at a time when the first wave of the pandemic was on a downward trend, and subsequently around March 2021, the delta wave had started, which peaked in the country around early May. This probably explains the high seroconversion rates in the first 3 months of follow-up for the study. We also did a retrospective power analysis for likely symptomatic COVID-19 infection (severe acute respiratory illness) based on the outcomes since we used convenience sampling at the beginning of the study. This showed a power of 83.5% for the study to detect a difference of 5% between the two arms for symptomatic COVID-19 infection.

A previous randomised control trial in elderly patients (ACTIVATE trial) showed a 45% reduction in the incidence of all new infections primarily driven by a reduction in the incidence of respiratory infections of probable viral origin and an increase in the time to the first infection compared with placebo [14]. A subsequent trial on elderly participants with comorbidities (ACTIVATE II) showed a statistically significant reduction in the incidence of a composite endpoint of possible/probable/definitive COVID-19 infection (by 68%) in individuals vaccinated with BCG compared with placebo [15]. In addition, there was a trend towards reduced incidence of severe infections in the BCG arm. A single-centre phase II randomised control trial involving healthcare workers at high risk of COVID-19 infection in Brazil has shown a trend towards a reduction in the incidence of COVID-19 infection in those vaccinated with BCG [16]. In a multi-centre randomised double-blind trial

Table 4 COVID-19 antibody on follow-up in both groups ($n = 495$)

Follow-up time	COVID antibody positivity (BCG group) (%)	COVID antibody positivity (placebo group) (%)	*p* value
Baseline	0	0	NC
3 months	54	62	0.10
6 months	72	84	0.003
9 months	81.2	89.5	0.04

NC not calculated owing to no events

Table 5 Secondary outcomes (COVID-19-positive patients)

Serial number	Symptoms	BCG group ($n = 246$)	Placebo group ($n = 249$)	*p* value
1	Severe COVID-19 infection	0	6 (2.4%)	0.03
2	Hospitalisation	0	6	0.03
3	Oxygen requirement	0	6	0.03
4	ICU admission	0	1	NC
5	Death	0	1	NC

NC not calculated owing to no events in the BCG arm

conducted across three facilities in West Cape South Africa which included 1000 healthcare workers who received either BCG vaccination or placebo, there was no significant difference between the two groups in terms of COVID-19 incidence or hospitalisation due to COVID-19. However, this study was limited by a lower-than-expected attack rate and a low hospitalisation rate due to COVID-19, which reduced the power to detect statistically significant effect due to BCG [17]. Another multi-centre double-blinded randomised control trial in Poland studied the effect of BCG vaccination on healthcare workers in Poland using an initial screening with tuberculin skin test to identify those who had prior BCG vaccination and subsequently randomised tuberculin-skin-test-negative patients to receive either BCG or placebo. No significant difference was noted between the three groups in terms of number of COVID-19 events, seroconversion rates or IgG antibody levels in those who showed seroconversion [18].

There are a few limitations to our study. Firstly, although symptom screening of both arms showed a higher incidence of likely symptomatic COVID-19 infection (severe acute respiratory illness) in the placebo arm compared with the BCG arm, the lack of microbiological testing in all patients at the time of probable symptoms of COVID-19 infection limits the complete generalisability of our findings. However, in the context of the pandemic and high rates of seroconversion at the end of 9 months, most of these cases can be presumptively diagnosed to be due to COVID-19 infection. Furthermore, severe COVID-19 infections in both arms were lower than anticipated at the beginning of the study, probably because the study included only adults below 60 years of age. Epidemiological data show that the highest proportion of patients with severe disease was older than 60 years [19, 20]. However, we had included younger adults with risk factors since there are very few data on the protective efficacy of BCG in this population and, in absolute numbers, they constitute quite a large proportion of the affected population in lower- and middle-income countries.

In addition, there was an absence of information regarding the strains of COVID-19 which might have caused infections in the study population. However, data from the national database would suggest that the delta (B.1.351) and omicron (B.1.1.529) variants were the predominant strains in circulation during the study period. The protective efficacy ChAdOx1 nCoV-19 vaccine (AZD1222) ranges from around 22% for mild to moderate COVID-19 infection to 67% for symptomatic COVID-19 infections at day 14 post-second dose. Similarly, BBV 152 shows approximately 78% efficacy for symptomatic COVID-19 infections at 14 days

post-vaccination and 56% at 42 days post-vaccination during a surge of infections due to the delta variant [21–24]. In our study, BCG vaccination had a protective efficacy of approximately 62% at 9 months for symptomatic COVID-19 infections. In addition, there was a significant reduction in the incidence of severe COVID-19 infections requiring hospitalisation.

CONCLUSION

Our study results suggest that BCG reduces severity of COVID-19 infection in those at higher risk of COVID-19 infections in addition to reducing the incidence of acute respiratory illness (probable symptomatic COVID-19). Furthermore, the BCG antibody levels persisted for a long duration. This might be significant since most of the protective efficacy of available vaccines has been shown to last for a much shorter duration, necessitating booster doses of vaccines to ensure a longer duration of protection from severe disease. In the context of inadequate coverage of the primary series of COVID-19 vaccination, BCG, which is cheaper and part of universal immunisation programmes in most countries, might provide a cost-effective alternative to protect against symptomatic and severe COVID-19 disease. Future studies can compare the protective efficacy of BCG vaccination with that of available COVID-19 vaccinations, including booster doses in resource-poor settings, to assess for non-inferiority. Future studies should also study the effect of BCG vaccination on the strength of antibody responses to COVID-19 vaccination, which might affect the need for and timing of booster doses.

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Conference on Harmonization–Good Clinical Practice guidelines.

Data Availability. Deidentified participant data, including statistical analysis plan and code, will be made available after publication upon request to the corresponding author (drsanjeevsinha@gmail.com).

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