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Original Article

Genomic analysis of SARS-CoV-2 variants of concern identified from the ChAdOx1 nCoV-19 immunized patients from Southwest part of Bangladesh

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ABSTRACT

Background: Bangladesh introduced ChAdOx1 nCoV-19 since February, 2021 and in six months, only a small population (12.8%) received either one or two dose of vaccination like other low-income countries. The COVID-19 infections were continued to roll all over the places although the information on genomic variations of SARS-CoV-2 between both immunized and unimmunized group was unavailable. The objective of this study was to compare the proportion of immune escaping variants between those groups.

Methods: A total of 4718 nasopharygeal samples were collected from March 1 until April 15, 2021, of which, 834 (18%) were SARS-CoV-2 positive. The minimum sample size was calculated as 108 who were randomly selected for telephone interview and provided consent. The prevalence of SARS-CoV-2 variants and disease severity among both immunized and unimmunized groups was measured. A total of 63 spike protein sequences and 14 whole-genome sequences were performed from both groups and phylogenetic reconstruction and mutation analysis were compared.

Results: A total of 40 respondents (37%, N = 108) received single-dose and 2 (2%) received both doses of ChAdOx1 nCoV-19 vaccine, which significantly reduce dry cough, loss of appetite and difficulties in breathing compared to none. There was no significant difference in hospitalization, duration of hospitalization or reduction of other symptoms like running nose, muscle pain, shortness of breathing or generalized weakness between immunized and unimmunized groups. Spike protein sequence assumed 21 (87.5%) B.1.351, one B.1.526 and two 20B variants in immunized group compared to 27 (69%) B.1.351, 5 (13%) B.1.1.7, 4 (10%) 20B, 2 B.1.526 and one B.1.427 variant in unimmunized group. Whole genome sequence analysis of 14 cases identified seven B.1.351 Beta V2, three B.1.1.7 Alpha V1, one B.1.526 Eta and the rest three 20B variants.

Conclusion: Our study observed that ChAdOx1 could not prevent the new infection or severe COVID-19 disease outcome with single dose while the infections were mostly caused by B.1.351 variants in Bangladesh.

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Introduction

COVID-19 pandemic continued at the cost of 4.5 million deaths from December 2019 until August 2021. Oxford-AstraZeneca (ChAdOx1 nCoV-19), Pfizer-BioNTech (BNT162b2), Johnson & Johnson (Ad26.COV2.S), Sputnik V (Gam-COVID-Vac), and few other COVID-19 vaccines were administered in different countries. Those vaccines claim 67%–100% efficacy in preventing COVID-19 clinical cases [1,2]. However, the continuous emergence of new variants introduced debates in preventing the disease. In Bangladesh, the Directorate General of Health Services announced a total of 3,227,598 samples were tested, of which 513,510 were positive and 7559 (1.5%) died [3] until December 2020. In 2021 until October, the number of tests increased into more than double (N = 7,150,144) with 1,055,039 SARS-CoV-2 positive cases and 20,292 deaths (1.9%) [4]. The increased death-case ratio is attributed due to B.1351 [5] and B1.617.2 [6] variants. Although, those numbers were substantially underestimated due to limited transportation towards the diagnostic facilities in many regional parts of Bangladesh. Before the second wave of the pandemic, Bangladesh has introduced ChAdOx1 nCoV-19 and until November 18, only 20.3% received two doses and 11.3% received one dose of vaccination [7]. Therefore, a large proportion of the population remains unimmunized or received only the first dose of ChAdOx1 nCoV-19 vaccine in Bangladesh. Almost 7.6 billion doses of different vaccines have been distributed among 52.4% of the global population, although only 4.7% population in low-income countries received at least one dose of a vaccine [7]. Several studies have been performed to evaluate the safety and efficacy of first doses vaccines [8]. The first dose of the ChAdOx1 nCoV-19 was found to be effective against 63.9% of symptomatic and asymptomatic cases [8]. In another randomized controlled trial, one standard dose showed 64.1% vaccine efficacy [1]. Bernal et al. reported that first dose ChAdOx1-S effectiveness reached 60% from 28 to 34 days with further increases to 73% from 35 days to onward for B.1.1.7 variant [9]. Although a large number of cases were being reported to be infected with SARS-CoV-2 during this interim period with those immune-escape variants. Continuous genetic surveillance would assist in revealing those new variants capable of escaping the current immunization. Our study group identified several immunized cases who were infected with SARS-CoV-2 during the national surveillance and hypothesize them as new immune escaping variants. This study aimed to identify the SARS-CoV-2 infected cases before or after the immunization with ChAdOx1 nCoV-19 and analyze the variants. This study additionally compared the COVID-19 disease severity and mutation analysis of SARS-CoV-2 between the immunized and unimmunized groups [10].

Method

Ethical approval

This study is approved by the ERC of Jashore University of Science and Technology (ERC no: ERC/FBST/JUST/2020-51). Verbal consent was taken from all participants after reading the purpose and objectives. Optimistic respondents were then asked for a telephone interview with the study questionnaire and enrolled in this study.

Study population

This study was conducted from March 1 until April 15, 2021 in South-West part of Bangladesh. The Genome Centre, Jashore University of Science and Technology, Jashore, Bangladesh, is conducting COVID-19 diagnosis as a part of the national surveillance system.

The inclusion criteria in this study were SARS-CoV-2 positive cases identified by the our study group using real-time PCR. The participants were excluded from the study who could not show the vaccine card or confirmation message after receiving the vaccine, did not receive our phone calls or had a history of immunological disorder.

A total of 4718 nasopharygeal samples were collected from 3 districts (Jashore, Narail and Magura) and tested for SARS CoV-2. The RNA was released from the samples using QuickExtract™ RNA extraction kit (Lucigen, Wisconsin, USA) following the manufacturer's instruction. Then, 10 μL of each viral RNA extract was amplified by one-step real-time reverse-transcriptase polymerase chain reaction (RT-PCR) using Novel Coronavirus Nucleic Acid Diagnostic Kit (Sansure Biotech Inc., China). A total of 834 (18%, N = 4718) samples were identified for the presence of N-gene or orf1b gene with a CT-value <40 and interpreted as SARS-CoV-2 positive according to the manufacturer's instruction.

Sample size

The survey was done by drawing a representative of 108 sample from 834 positive cases detected by real-time PCR. To produce statistically reliable estimates, sample size for the proportion was used to calculate the minimum required sample size [11]. We have considered a proportion of the indicators as 0.5 (50%) to give a conservative estimate of the sample size, 5% statistical level of significance, 10% margin of error, and 90% response rate; which results in a sample size of 108.

In this study, we selected a day-wise random number generated 135 cases (out of 834), of which only 108 were available for a telephone interview with prior verbal consent. All respondents (n = 108) were interviewed with a pre-tested structured questionnaire over the phone. In the case of unavailability of the respondents due to death, first kin attended the interview.

Vaccination status

All the participants were asked about their vaccination status, the number of doses, and the date of vaccination. The vaccination status was confirmed by the vaccine card provided by DGHS, Bangladesh [12] or the message delivered by the DGHS.

Spike protein sequencing

RNA was extracted from 140 μl of left-over samples using the QIAamp Viral RNA Mini Kits (QIAGEN, USA) according to the manufacturer's protocol. PCR was performed with 7 μl of RNA extract using the Luna® Universal One-Step RT-qPCR Kit (New England Biolabs Inc., USA) with the primers for RBD region of the spike portion of the genome. The details of the protocol are shown in the supplementary Table S1. The amplicons, confirmed in 1% (w/v) agarose, were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, ThemoFisher Scientific, Inc., USA). The sequenced FASTA files were initially cleaned with Chromas Pro [13] and aligned with the reference sequence (NC_045512.2/SARS-CoV-2/Wuhan-Hu-1) using Molecular Evolutionary Genetics Analysis (MEGA X) software [14] to detect the presence of any mutations.

Spatial distribution mapping

The spatial distribution of COVID-19 cases was mapped using the ArcGIS (version 10.6) software [15]. The nearest geolocations of the COVID-19 cases were approximated with the help of Google Earth Pro platform (a freely available google product, formerly

known as Keyhole Earth Viewer) and as per the description of the respondents.

Whole-genome sequencing

First-strand cDNAs were prepared from the extracted viral RNA using SuperScript™ III First-Strand Synthesis System (Invitrogen™, Thermo Fisher Scientific, USA). The concentrations of cDNA were determined using the dsDNA HS Assay Kit with Qubit 4 Fluorometer (Thermo Fisher Scientific, USA). The Ion AmpliSeq™ SARS-CoV-2 Research Panel (Thermo Fisher Scientific, USA) was used to prepare Ion AmpliSeq™ libraries. Two 5X primer pools that target 237 amplicons specific to the SARS-CoV-2 were used during target amplification PCR and the number of amplification cycles was determined based on their viral copy number. Each amplified sample were subjected to 2 μL FuPa reagents for partial digestion. Digested amplicons were ligated with P1 adapter and Barcode Adapters (Ion Torrent™, Thermo Fisher Scientific, USA). Magnetic bead cleanup was performed using Agencourt™ AMPure™ XP Reagent (Beckman Coulter, USA) to purify barcoded Ion AmpliSeq™ libraries. The library concentration was calculated using Ion Library TaqMan® Quantitation Kit and diluted into 100 picomolar (pM) according to the manufacturer's protocol. All equimolar libraries were pooled for the preparation of template-positive Ion Sphere™ Particles (ISPs) using the Ion 530™ Kit – OT2 (Thermo Fisher Scientific, USA) on the Ion One Touch™ 2 System. Template-positive ISPs were enriched on Ion One Touch™ ES system and loaded in Ion 530™ chip with control ISPs for sequencing into Ion S5™ System.

SARS CoV-2 phylogeny and mutation analysis

SARS CoV-2 genome consensus generation, phylogenetic reconstruction and mutation analysis were described in the Supplementary material.

Statistical analysis

Descriptive analysis was conducted using frequency distribution to explore the socio-demographic profile, COVID-19 related experience and information about hospitalization. A Chi-square test of independence was performed to explore the relationship between vaccination status and 'respondents' characteristics. In terms of smaller sample size, Fisher exact tests were used to determine the association between the outcome and disaggregated variable. All analysis were performed using STATA version 17.0.

Results

Description of the study population

This study reported information of 108 COVID-19 positive cases tested between March 1 to April 15, 2021. The socio-demographic profiles, comorbidity, vaccination and COVID-19 associated symptoms, diagnosis and treatment information of the studied population were presented in Table 1. Studied respondents were dominated by males (75%) and were mainly from aged group 50–60 years (34%) followed by 40–50 years (28%). The mean age of the respondents was 50 years (maximum 76.4, minimum 14.4 and median 50.5 years). Estimated Body Mass Index (BMI) suggests that half of the studied respondents can be characterized as either overweight (40.7%) or obese (10.2%). Majority of the respondents (66.4%) completed their secondary or higher education, whereas 14% of them did not complete their primary education. Occupationally, they were engaged in service or business (60.2%) homemaker (18.5%) and unemployed or retired (13.0%).

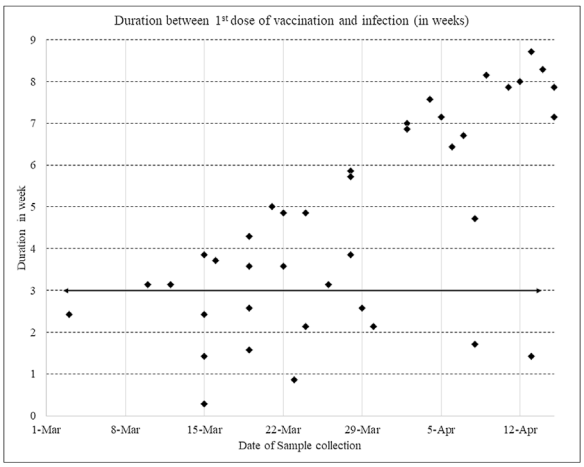

Fig. 1. Duration between SARS-CoV-2 infection and first dose of immunization. 86% (N = 36) of the cases were infected after 14 days and 71% (N = 30) of them were infected after 21 days of vaccination. Red dots represent the death cases and black dots represent the survived cases.

The study reported several comorbidities among half of the respondents. Most often, the respondents reported high blood pressure (31.5%) followed by diabetes (26%), cardiac diseases (9.3%) and asthma (4.6%) etc. Majority of the respondents suffered from fever (61%), dry cough (43.5%), loss of smell (26%), loss of taste (24%) and muscle pain (22%) during the infection. The respondents approached a COVID-19 test due to their generalized sickness (79.6%), exposure with COVID-19 patients (8.3%) and other reasons (12%). About 63% of respondents reported a complete recovery, while 30.6% were still suffering (generalized weakness, emotional changes, and dementia) and 6.5% died (Table S2). About 39% (n = 42) of the respondents were infected after the COVID-19 vaccination. The first dose of ChAdOx1 nCoV-19 vaccine was received by 40 (37.0%) cases and both doses were completed by only 2 (2%) cases. The average duration between vaccination (partially or completely immunized) and COVID 19 diagnosis was 32 (±17) days. Almost 86% and 69% of the respondents were infected minimum after 2 and 3 weeks of administration of first dose, respectively (Fig. 1). Among the respondents, 15.7% (n = 17) suffered from severe consequences and required hospitalization for 1–17 days (mean 7.72 days and median 6 days). Additional oxygen was supplemented to 13% (N = 14) of the respondents during their hospitalization; of them, seven respondents (6.5%) were shifted to the Intensive care unit (ICU), and four died. Three respondents died at home before rushing towards the hospital.

We performed the comparative analysis of vaccination status and 'respondents' characteristics (Table 1). We have disaggregated the analysis in two sections, section I considering all age groups, section II considering only 16–70 years old respondents. In all age groups, we observe that education status has significant relationship with vaccination status. Most of the respondents (83.3%) immunized group completed their secondary or higher education. The difficulty of breathing and loss of appetite were significantly higher in the unimmunized group (27% vs. 7%, and 23% vs. 7%, respectively, $p < 0.05$) which was also found among 16–70 years. Dry cough was also significantly high in all ages among unimmunized group ($p < 0.05$). Fever was more common among in that group although not significant ($p = 0.067$). Apparently, in our study we 'couldn't found any significant difference of hospitalization ($p = 0.383$) (Fig. S1) and duration of hospitalization ($p = 0.780$). The hospitalization rate of comorbid patients was also similar (24% both) between both groups (Table S3). Additionally, we compared the mean CT values of immunized and unimmunized patients,

Table 1
Statistical analysis of the study patients' profile.

Variables	Section I: All ages groups			Section II: 16–70 years		
	1st dose	Non immunized	P value	1st dose	Non immunized	P value
	% (n)	% (n)		% (n)	% (n)	
Sociodemographic profile						
Sex						
Male	83.3 (35)	69.7 (46)	0.111	82.5 (33)	70.5 (43)	0.171
Female	16.7 (7)	30.3 (20)		17.5 (7)	29.5 (18)	
BMI						
Normal	45.2 (19)	51.5 (34)		42.5 (17)	50.8 (31)	
Overweight	45.2 (19)	37.9 (25)	0.777	47.5 (19)	39.3 (24)	0.731
Obese	9.5 (4)	9.5 (7)		10.0 (4)	9.9 (6)	
Education						
No education or primary incomplete	4.8 (2)	20.0 (13)		5.0 (2)	18.0 (11)	
Secondary incomplete	11.9 (5)	24.6 (16)	0.009	12.5 (5)	24.6 (15)	0.027
Secondary or higher	83.3 (35)	55.4 (36)		82.5 (33)	57.4 (35)	
Occupation						
Unemployed	14.3 (6)	12.1 (8)		12.5 (5)	9.8 (6)	
Service/business	69.1 (29)	54.6 (36)	0.223	70.0 (28)	59.0 (36)	0.440
Homemaker	14.3 (6)	21.2 (14)		15.0 (6)	21.3 (13)	
Others	2.4 (1)	12.1 (8)		2.5 (1)	9.8 (6)	
Presence of any comorbidities						
Yes	59.5 (25)	43.9 (29)	0.114	60.0 (24)	44.3 (27)	0.122
No	40.5 (17)	56.1 (37)		40.0 (16)	55.7 (34)	
Comorbidities (Multiple response)						
Diabetes	19.1 (8)	30.3 (20)	0.193	17.5 (7)	29.5 (18)	0.171
High blood pressure	31.9 (13)	31.0 (21)	0.925	30.0 (12)	32.8 (20)	0.768
Clinical manifestation						
Symptoms (Multiple response)						
Fever	50.0 (21)	67.7 (44)	0.067	47.5 (19)	66.7 (40)	0.058
Dry cough	31.0 (13)	51.5 (34)	0.036	30.0 (12)	49.2 (30)	0.056
Running nose	7.1 (3)	13.6 (9)	0.361	7.5 (3)	14.8 (9)	0.355
Muscle pain	26.2 (11)	19.7 (13)	0.429	25.0 (10)	19.7 (12)	0.526
Difficulties in breathing	7.1 (3)	27.3 (18)	0.012	7.5 (3)	27.9 (17)	0.012
Loss of smell	19.1 (8)	30.3 (20)	0.193	17.5 (7)	31.2 (19)	0.125
Loss of taste	21.4 (9)	25.8 (17)	0.608	22.5 (9)	27.9 (17)	0.546
Loss of appetite	7.1 (3)	22.7 (15)	0.034	5.0 (2)	23.0 (14)	0.016
Complications after covid 19 positive (Multiple response)						
Generalized weakness	11.9 (5)	18.2 (12)	0.383	12.5 (5)	19.7 (12)	0.346
Emotional changes	7.1 (3)	7.6 (5)	1.000	7.5 (3)	8.2 (5)	1.000
Dementia	9.5 (4)	6.1 (4)	0.709	10.0 (4)	6.6 (4)	0.709
COVID-19 severity						
Hospitalized	11.9 (5)	18.2 (12)	0.383	10.0 (4)	14.8 (9)	0.485
Duration						
No hospitalization	88.1 (37)	81.5 (53)		90 (36)	85 (51)	
1 week	7.1 (3)	10.8 (7)	0.780	7.5 (3)	10 (6)	0.723
More than 1 week	4.8 (2)	7.7 (5)		2.5 (1)	5 (3)	
Oxygen	9.5 (4)	15.2 (10)	0.396	7.5 (3)	14.8 (9)	0.355
Spike protein variants						
Sanger variant						
B.1.351	52.4 (22)	39.4 (26)	0.185	52.5 (21)	39.3 (24)	0.193
B.1.1.7	0.0 (0)	7.6 (5)	0.154	0.0 (0)	4.9 (3)	0.275
B.1.526, B.1.427 and Conventional	7.1 (3)	10.6 (7)	0.737	7.5 (3)	9.8 (6)	1.000
Unknown	42.4 (17)	40.5 (28)	0.841	40.0 (16)	45.9 (28)	0.559

where we identified a significant increase of *N*-genes ((p = 0.026) and Orf-1b gene (p = 0.052) among unimmunized (Fig. S2).

Spatial distribution of identified COVID-19 variants

The spatial distribution of COVID-19 variants suggested an apparent clustering and localized proliferation (Fig. 2). South African variants (B.1.351) showed prominent clusters and, thus results in localized hotspots in the Jashore Municipality.

Spike protein variants

In this study, only 78 samples had the threshold cycle (CT value) below 35 in RT-PCR screening test and were considered as a potential candidate for partial or complete genome sequencing, of which, only 76 left-over samples were available. The concentration of spike protein RNA might be low in rest 13 samples and could not be visualized in gel electrophoresis. The template RNA provided 850bp spike protein amplicons in 63 'patients' samples, of which 24 patients were partially or completely immunized before the COVID-19 infection. Spike protein sequence mutation analysis revealed that 21 (87.5%) strains might be B.1.351 variant, one was B.1.526 variant and two were conventional without any mutation. Among the unimmunized 39 viral strains, spike protein sequences analysis suspected 27 (69%) B.1.351, 5 (13%) B.1.1.7, 4 (10%) conventional, 2 B.1.526 and one B.1.427 variant. Fig. 2 showed the spatial distribution of 63 SARS-CoV-2 variants among both groups, rest 45 out of 108 were marked as unknown in the map. Most cases (N = 95) were detected from the largest district, Jashore. Few other cases were located in Magura (N = 10) and Narail (N = 3). Mutation analysis revealed that 16% (10 out of 63) of the strains had non-

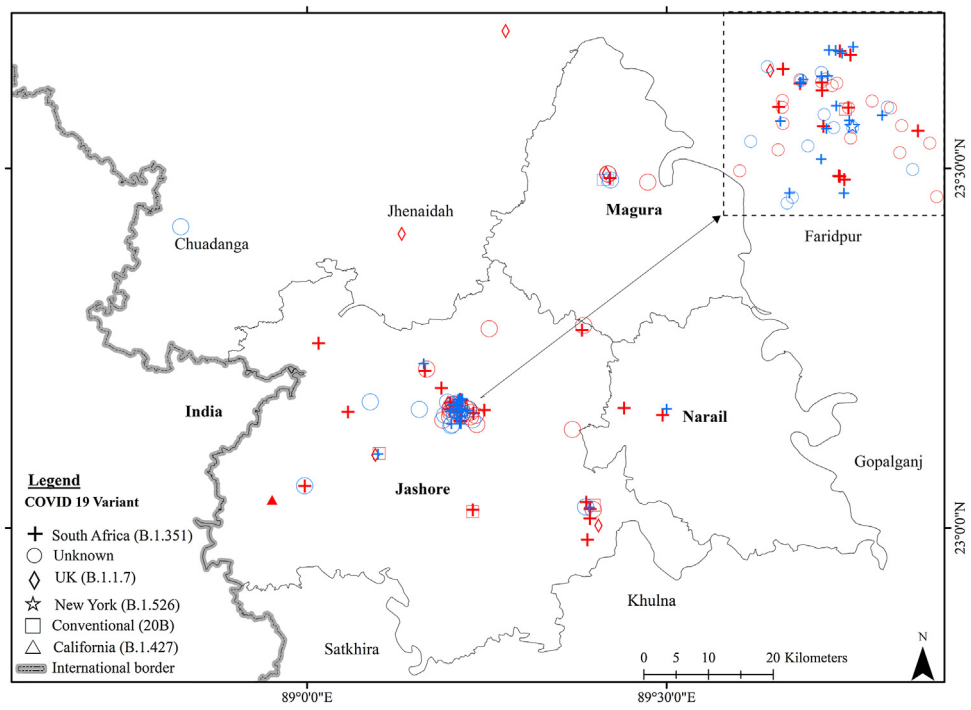

Fig. 2. Geospatial location of SARS-CoV-2 variants. Red marks indicate the unimmunized SARS-CoV-2 infected patients. Blue marks indicate the partial or complete immunized COVID-19 patients.

Table 2
Identification of SARS-CoV-2 variants and their non-synonymous unique mutations.

Spike protein sequences

Variant	Immunized (number of variants)	Unique mutations (no of strains)	Unimmunized (number of variants)	Unique mutations (no of strains)
B.1.351	21	F515S (2) W353R (1)	27	-I402N (1) -I402M (1) -G413W (1) -N394K, E406K, D405E, Q414E, S375F (1) -N394K, E406K, Q414E, S375F (1)
B.1.1.7	0		5	
Conventional	2		4	
B.1.427	0		1	
B.1.526	1		2	-F377I (1), -Q414E (1)

Whole genome sequences

Variant	Immunized (frequency of unique mutation in a single strain)	Average unique mutations	Unimmunized (frequency of unique mutation in a single strain)	Average unique mutations
B.1.351	11, 22, 19, 21	18.3	20, 22, 11	17.7
B.1.526	13		22	
B.1.1.7			34	
Conventional/unrecognized (20B)	7, 13	10	22, 12	17

synonymous unique mutations in their RBD region of the spike protein; 7 (18%, n = 39) of which belong to unimmunized group and 3 (12.5%, n = 24) belong to immunized group. Three B.1.351 variants and 2 B.1.526 variants each contain single unique mutation and two others B.1.351 variants have multiple novel mutations among the unimmunized group (Table 2). Compared to that, immunized group contains F515S mutation in two and W353R in one B.1.351 variant.

Complete genome and mutation analysis

The complete genomes of SARS-CoV-2 were analyzed to map a radial phylogenetic tree (Fig. 3) and it has been found that six different variants for both immunized and unimmunized groups (Table 2 and Table S4). Complete genome analysis (n = 14) included 7 B.1.351 Beta V2 variant, 3 B.1.1.7 Alpha V1 variant, one B.1.526 Eta variant and the rest three 20B variant (Fig. 4) from both groups. The t-test found a non-significant difference between the mean frequency of synonymous ($p = 0.396$) and non-synonymous ($p = 0.083$) mutations between the immunized and unimmunized group (Fig. 5). N501Y mutation was found in 9 strains; 3 harbored in partially immunized patients and 6 harbored in unimmunized patients. P681H mutations were possessed by 3 strains and all belonged to unimmunized patients. H69–V70 deletion found in 3 strains (2 unimmunized, 1 partially immunized patients) and Y144 deletion

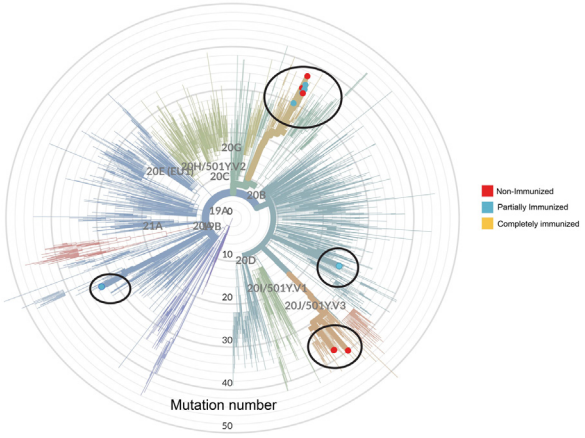

Fig. 3. Phylogenetic of tree with their existing clade with world reference genome. Red color represents unimmunized variants, sky-blue represents partially immunized and orange color represents completely immunized variants in the tree.

found in 2 strains (1 unimmunized, 1 partially immunized patients). E484K mutation was possessed by 7 (50%) strains among which 4 patients were partially immunized and 3 patients were unimmunized. K417N mutation was found in 4 partially immunized patients and 2 unimmunized patients. L452R substitution is found in 2 strains (Fig. 4).

Discussion

The emergence of SARS-CoV-2 variants is a global problem during COVID-19 pandemic [16–19]. Those variants have been designated as variants of interest (VOI) and variants of concern (VOC) by WHO [20] and CDC [21] depending on its genetic changes that affect transmissibility, disease severity, immune escape, diagnostic or therapeutic escape, increasing prevalence, increasing virulence, decreasing the effectiveness of vaccines etc.

This study found that the first or second dose of ChAdOx1 did not prevent the new infections by the variants of interests and variants of concern (B. 1.526, B.1.351, and 20B) in Bangladesh except the B.1.1.7 variant. Most of the viruses (87.5%) detected from the vaccinated patients were identified as B.1.351 variant, indicating that the ChAdOx1 could not prevent new infection by this variant of concern (Table 2). The SARS-CoV-2 variants and the efficacy of the different vaccines has been reviewed by Bian et al. [22] and

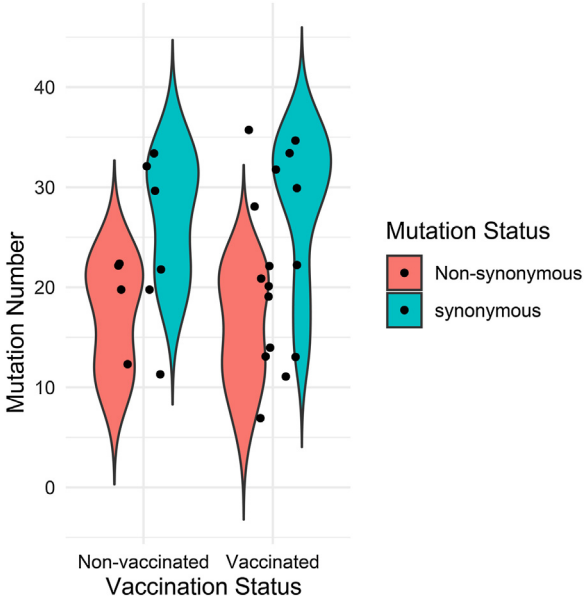

Fig. 5. Synonymous (p = 0.396) and non-synonymous (p = 0.083) mutation status between immunized and unimmunized patients.

demonstrated that efficacy was reduced from ranges 0.78 to 9. The highest reduction was 9.0 observed for B.1.351 variant of ChAdOx1 [23]. Madhi et al. also noted that ChAdOx1 vaccine efficacy was only 10.4% after two doses in South Africa, where B.1.351 was predominant [24]. Few studies found similar results that the ChAdOx1 vaccine is not effective against recent variant of concerns such as B.1.617.2 [9] and Delta 1 [25]. That raised a dubious situation about the efficacy COVID-19 vaccine among the population [26].

A study in Argentina, Brazil Chile, Colombia, Mexico, Peru, South Africa and the United States found that single-dose ChAdOx1 vaccine efficacy was 52–64% [27]. Few studies claimed that single-dose vaccination protects from infections including B.1.1.7 [9,28–30]. However, in our research, most of the COVID-19 infections (71%, N = 30) were identified after 3rd weeks of vaccine administration, with a mean duration of 32(±17) days (Fig. 1). Even after 4th week (52%, N = 22) of vaccination, the antibody couldn't prevent new infection by SARS-CoV-2 B.1.351 variant. A study by Tauzin et al. reported that BNT162b2 vaccine was 90% effective after three weeks by the production of g anti-receptor binding domain and

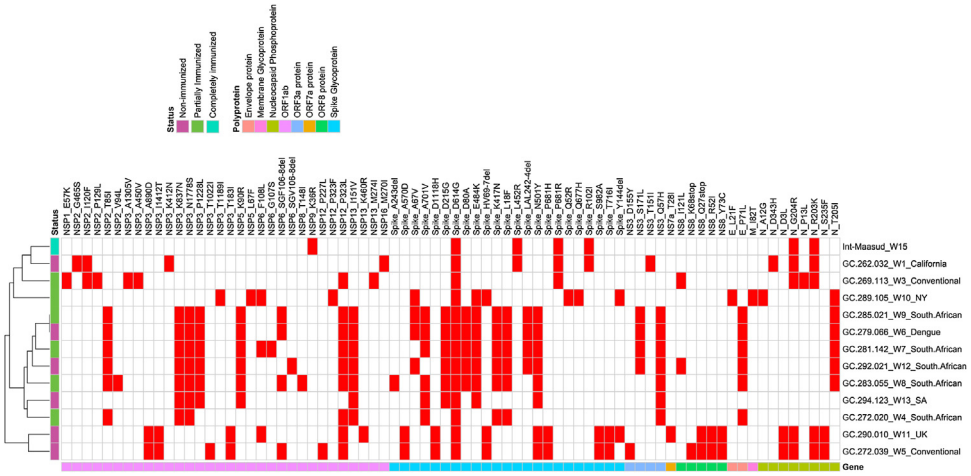

Fig. 4. Comparison of amino acid substitutions retrieved from genome sequences of SARS-CoV-2 viruses among immunized and unimmunized patients. Mutations and amino acid substitutions were retrieved from Nextclade (https://clades.nextstrain.org/).

spike antibodies with Fc-mediated effector functions and cellular CD4 + T cell responses [31].

However, Madhi et al. observed that ChAdOx1 vaccine couldn't offer any protection against low and mild infection of SARS-CoV-2 due to B.1.351 variant [24]. In our study, we found that the ChAdOx1 vaccine significantly reduce dry cough, loss of appetite and difficulties in breathing. However, there was no significant reduction of other symptoms like, running nose, muscle pain, shortness of breathing, generalized weakness, emotional changes etc. (Table 1). Madhi et al. could not include any hospitalized cases to observe the effect on severe cases. Our study found no significant differences in disease severity in terms of hospitalization ($p = 0.383$) (Figure S1) or the duration of hospitalization ($p = 0.780$) when compared between the immunized and unimmunized groups. Another previous study by Bernal et al. [9] reported 37% reduction of hospitalization in case of single-dose vaccination by ChAdOx1, although we found only 6.3% reduction (Table 1). SARS-CoV-2 infected patients comorbidity affected by diabetes, asthma or high blood pressure were facing the severe consequences throughout the pandemic. Previous studies found 28–50% of hospitalization among comorbid patients from January to March 2020 [32]. The hospitalization rate of comorbid patients in our study was 23.5% among the immunized and 24.1% among the nonimmunized groups (Table S3). In another study observed that one dose vaccination with either BNT162b2 or ChAdOx1 nCoV-19 could reduce the hospitalization rate by 51% among the elderly group aged above 80 [33].

Although there was no significant difference in hospitalized and comorbid groups, the RT-PCR results found a significant difference in threshold cycles values (Figure S2) of immunized and unimmunized groups ($p = 0.026$ for N-gene, $p = 0.052$ for ORF1b gene). A previous study also found similar results [34] without any correlation of the vaccine efficacy or disease severity and outcomes. However, Pritchard et al. showed a significant difference in Ct values with reduction of symptomatic infections, hospitalization and death [29].

There were no significant differences found in the number of the synonymous and non-synonymous mutations on the whole genome sequences of viruses collected from immunized and unimmunized patients, even though higher numbers of mutations were observed for unimmunized groups (Fig. 5). The unimmunized groups showed more unique mutations in spike protein as well as in other positions. Important mutations that can play vital roles in evading immune responses are the characteristic of variant of concerns. In our study, we found those mutations in both immunized and unimmunized groups. Asparagine to tyrosine conversion at 501 amino acid position (N501Y) of spike glycoprotein helps the virus latch on more tightly to human cells. But the mutation is not likely to help the virus evade current vaccines [35]. This mutation was harbored in 3 partially immunized patients and 6 unimmunized patients. Proline to Histidine substitution (P681H) at spike glycoprotein may help infected cells create new spike proteins more efficiently [35], which was found 3 unimmunized patients in our study. The histidine and valine deletion from 69 and 70 positions (H69–V70) and tyrosine deletion from 144/145 positions (Y144/145) in spike glycoprotein alter the shape of the spike and may help it evade some antibodies [35]. H69–V70 deletion was found among three patients (2 unimmunized, 1 partially immunized) and Y144 deletion was found among two patients (one in each group). Lysine to asparagine alteration at 417 positions (K417N) of spike glycoprotein helps the virus bind more tightly to human cells [35]. This mutation was found in 6 study patients (4 in immunized and 2 in unimmunized patients). E484K substitution may help the virus evade antibodies [35] which was found 4 immunized and 3 unimmunized patients. L452R substitution is common in B.1.427, but not yet shown to be more infectious (Fig. 4).

Bangladesh had started a pilot vaccination program with ChAdOx1 on January 27, 2021 and the nationwide vaccination program began on February 7. This study investigated to observe the variants after the selective pressure of vaccination in the population (Fig. 2), and the samples were collected from March 1 to April 15, 2021. During that time, Bangladesh was facing the second wave of SARS-CoV-2 infection in which Variant B.1.351 was found predominant [17]. The study was conducted before the first case of B.1.617.2 variant was reported in Bangladesh by our research group [36]. Whole-genome sequencing is an expensive and time-consuming process; thus, it was not suitable for large-scale variant surveillance in countries with limited research funding like Bangladesh. The possibility and necessity of detecting important mutations by partial sequencing of the viral genome was mentioned by our research group [37,38]. Detection of the 'signature mutations' in spike proteins by Sanger sequencing can be helpful in the initial screening of the variants. This initial data can be verified by performing whole-genome sequences considering the virus type, pathology and itinerary of the infected patient. In this study, we applied both partial and whole-genome sequencing for variant screening (Table 2 and Fig. 3).

This study has a limitation that 14% of respondents ($N = 6$) become infected within 14 days after the first dose of vaccination; thus, they can barely add an update on SARS-CoV2 variant profile of immunized group (Fig. 1). However, the rest 86% immunized patients were infected after 14 days to 45 days with an average of 32 days. The SARS-CoV-2 variants were identified by sequencing of RBD region, which was further strengthening by whole-genome sequencing. Our surveillance data and mutation analysis strongly indicated that continuous monitoring of variants is necessary during the COVID-19 vaccination program in Bangladesh.

Conclusion

This study demonstrated the first report from Bangladesh about the post-vaccination variants during the second wave of the pandemic by B.1.351 that provided the impact of selective pressure in the community after the first dose of vaccination. The continuous monitoring of variants is essential considering the large number of populations might receive only single dose for a while and there are limited supplies of this most demanding vaccine. The impact of vaccination shall be carefully measured because a large number of populations are getting infected during the interim period between two doses. Those, who get infected with COVID-19 after the first dose of vaccination, may be kept in the least priority list, rather than offering new personnel who neither receive any dose nor was recently infected. WHO shall also make a suggestion or guideline for the recently infected personnel regarding the minimum time gap between active infection and next dose of vaccine.

Authors contributions

HMA: Conceptualization, methodology, validation, formal analysis, investigation, visualization, writing original draft, funding aquisition.

MSH: Methodology, investigation, resources, validation, writing original draft.

MAAS: Investigation, analysis, data curation.

SR: Sortware, Formal analysis, data curation.

ASMRA: writing review and editing, resources

SLS: Investigation, analysis, data curation.

MTI: writing review and editing.

MRI: Data analysis, writing review and editing.

MMR: GPS mapping, data analysis, writing review and editing.

OKI: Sortware, formal analysis, writing review and editing, resources.

IKJ: formal analysis, validation, writing review and editing, resources, supervision, project administration.

MAH: writing review and editing, supervision, project administration.

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Conflicts of interest

All authors declare no competing interests in this study.

Data availability statement

The whole genome sequence data analysed in this study were deposited in GISAID (https://www.gisaid.org/) with accession no. mentioned in Table S4.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2021.12.002.

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