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Research article

The severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) omicron sub-variants in Bangladesh cause mild COVID-19 and associate with similar antibody responses irrespective of natural infection or vaccination history

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ABSTRACT

Objective: Genomic surveillance and seroprevalence of severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) in Bangladesh is paramount for COVID-19 pandemic preparedness yet lagging the high-income countries due to limited resources.
Methods: SARS-CoV-2 variants, COVID-19 symptoms, and serology were prospectively evaluated in a cross-sectional study of Bangladeshi adults testing RT-PCR positive in 2021 and 2022.
Results: SARS CoV-2 Omicron variants of asymptomatic or mild COVID-19 in 2022 replaced Delta variant infections requiring hospitalization and oxygen support. The omicron XBB became predominant in July 2022 and associated with cough, headache or body ache and loss of smell; 47 of 68 (69 %), 30 of 68 (44 %) and 27 of 68 (40 %) respectively at higher frequency than BA.1/BA.2; 16 of 88 (18 %), 13 of 88 (15 %) and 0 of 88 (0 %) $p < 0.01$, $p < 0.01$ and $p < 0.0001$. Linear regression analysis reveals no associations between the number of previous infections and the number of symptoms, $r = -0.084$, $p = 0.68$. The anti-nucleoprotein (N)-protein IgG post COVID-19 and anti-Spike (S) protein IgG post-COVID-19 vaccination were similar between BA.2, BA.4/BA.5 and XBB and significantly lower than the levels in delta variant infections ($p < 0.001$).
Conclusions: Omicron XBB subvariants emerged in Bangladesh two months prior to previous reports and include unique patterns of S-protein mutations not assigned in PANGO lineage. The SARS CoV-2 omicron break-through infections persist in the presence of sustained antibody responses and vaccinations, underscoring the importance of molecular surveillance in low-income countries.

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1. Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) first reported December 2019 in Wuhan China (Wuhan-Hu-1) has caused over 800 million 2019 coronavirus infectious disease (COVID-19) cases and 7,000,000 reported COVID-19 deaths worldwide [1]. Bangladesh, among the most densely populated countries in the world with 1270 people per square kilometer, reported the first cases of SARS CoV-2 infection in March 2020. Since then, over 2,000,000 confirmed COVID-19 cases and 30,000 COVID-19 related deaths are reported according to the Bangladeshi government official statistics [2].

The virus mutation rate and the recombination of genetically distinct viruses' drives the emergence and spread of SARS CoV-2 variants having distinct phenotypes of transmissibility, COVID-19 severity, and immune evasion [3,4]. While deleterious mutations are rapidly purged, the complex process of adaption, fitness and selection of advantageous variants is intimately tied to the immunity (vaccination and/or prior infection) and behavior within the human host population [5].

COVID-19 in Bangladesh has been driven by successive waves of SARS-CoV-2 infection having clinical severity, viral loads and antibody responses associated with distinct variants of concern (VOC) [6,7]. Bangladeshi delta variants between July 28, 2021, and January 25, 2022, gave case fatality ratio (CFR) of 2 % and hospitalization rates of 47–59 % estimated from intensive care unit (ICU) bed occupancy [8–10]. The outbreak of delta variants in Dhaka occurred approximately 6 months after the highly transmissible delta variant in India which was associated with 400–600 daily mortality, 400,000 total deaths, widespread scarcity of hospital beds, lack of oxygen supply and opportunistic fungal infection [11]. The heightened anxiety and fear in Bangladesh of India's delta variant, led to sealing of its land border with India, implementation of lockdowns, strict quarantine rules and ramped up nation-wide vaccination [8]. These measures helped to delay the spread of delta variant and to reduce the mortality in Bangladesh's COVID-19 third wave. Interestingly, the slow start in roll out of vaccines to the Bangladeshi general population after introduction of Covishield to Dhaka health care workers in February 2021, did not translate into increased nation-wide COVID-19 related mortality during the delta variant surge [12–14].

In the period between January 1 and February 31, 2022, SARS CoV-2 BA.1 and BA.2 omicron variants were predominant in Bangladesh [15]. The COVID-19 clinical presentation among 90 study subjects, 94 % receiving 2-vaccine doses, had 45 % without symptoms and 55 % either rhinitis, fever and/or mild symptoms, [16]. In April and May 2022, little or no sequence information on SARS CoV-2 variants was available because of COVID-19 cases having low positivity rate and Ct values greater than 30. In the period between June 4, 2022, and July 1, 2022, 53 out of 65 RT-PCR confirmed COVID-19 cases were identified as omicron BA.4/BA.5 subvariant and 2 cases of BA.2 [15].

This study focuses on enhanced surveillance of SARS-CoV-2 in Bangladesh in 2022 by identifying omicron subvariants and comparing their symptoms and antibody responses to delta variant COVID-19 cases from 2021. The findings underscore the remarkable capacity of a low-income country with strained health care resources and limited laboratory services to identify novel patterns of spike protein mutations and to assess the association of omicron subvariant infections with COVID-19 symptoms and antibody responses.

2. Materials and methods

2.1. Study design and sample collections

Cross-sectional and prospective study of three groups of RT-PCR SARS-CoV-2 positive Bangladeshi COVID-19. Group 1: 40 cases of symptomatic COVID-19. Recruitment began on the September 23, 2021 and ended on the October 13, 2021; 28 with no vaccine, and 12 with either 1 or 2 doses of AstraZeneca vaccine [16]. Group 2: 88 cases; recruitment began on January 9, 2022 and ended on February 10, 2022 at Dhaka Medical College; 82 received two doses of AstraZeneca COVISHIELD vaccine and 6 were non-vaccinated. Group 3: 78 cases, recruitment began on July 26, 2022, and ended on October 22, 2022, in Dhaka and Chittagong; 77 received two or more doses of either AstraZeneca COVIDSHIELD and/or Pfizer BNT162b2 and one non-vaccinated.

The following inclusion criteria was applied to all three groups included the random selection of SARS-CoV-2 RT-PCR-positive COVID-19 cases aged between 15 and 85 years, with informed consent. Cases were excluded with RT-PCR Ct values $\geq$ 38, ages below 15 or above 85 years, and/or absence of informed consent. Each participant at the time of their nasopharyngeal swab, were given a questionnaire in Bengali and English to complete [supplement]. The questionnaire requested information on the patient age, gender, residence, contact details, name and date of hospital entry and answers to the following queries: dates of swab collection and blood sampling, date of first, second and subsequent vaccine doses, blood pressure, co-morbidities of diabetes, cardiovascular, lung and/or internal organs, date(s) of previous coronavirus infection, COVID-19 symptoms, whether or not the patient was admitted to a hospital after being diagnosed or identified with COVID-19 symptoms, whether or not they were given supplemental oxygen during their hospital stay.

2.2. Quantification of viral RNA

Nasopharyngeal specimens were collected and stored at −80 °C in a virus collection and preservation medium (Khang Jian Medical Apparatus Ltd., Taizhou, China) and transported on dry ice to Germany There were 3 cohorts: cohort 1 (n = 25), cohort 2 (n = 23), and cohort 3 (n = 78). Cohort 1 serum samples were collected between day 19 and day 27 post onset of COVID-19 symptoms (POCS), cohort 2 serum samples with BA.2 omicron variant were collected between day 186 and day 237 POCS. Cohort 3 serum samples were

collected between day 33 and day 113 POCS. All samples were preserved in screw-tight cryopreservation vials (Greiner-bio) at a temperature of −20 °C.

The viral RNA of nasopharyngeal specimens was extracted with a Magnapure 96 instrument (Roche, Mannheim, Germany) using the DNA and viral nucleic acid Kit (Roche, Penzberg, Germany). The RNA concentration in each sample was determined by reverse transcriptase polymerase chain reaction (RT-PCR) using the LightMix® Modular SARS-CE assay (40–0770 and 60–0770, TIB MolBiol, Berlin, Germany) and programming on a 480II light cycler (Roche, Penzberg, Germany). The point at which the amplification curve of the E gene crossed the vertical threshold line in the RT PCR cycle (Ct) was reported as positive if the sample Ct was less than 40.

2.3. Screening of spike protein mutations and sequencing

The amino acid mutations in the spike protein RBD region 319 to 541 were identified by post-RT-PCR melting curve analysis targeting amino acid mutations R346T, S371 F/L, S373P, K444T, V445P, N460K, E484A, F486S. The VirSNiP SARS CoV-2 typing assays (TibMolBiol, Cat. No. 53-0844-96, 53-0831-96, 53-0846-96, 53-0845-96, 53-0847-96, 53-0848-96) and LightCycler® Multiplex RNA Virus Master (Roche Cat. No. 06,754,155 001) were performed following the manufacturer's instructions. The pairs of forward (F) and reverse (R) sequencing primers were designed according to the SARS-CoV-2 reference strain NC_045512, resulting in overlapping fragments covering 2.1 kb of the spike-gene [17]. The amplification conditions were as follows: RT at 55 °C for 5 min, activation for 2 min at 95 °C, 45 cycles with 10 s at 95 °C, 20 s at 58 °C, and 30 s at 72 °C for product amplification. The amplified products with 411–501 bp were purified with NucleoSpin Gel and PCR clean-up (Macherey-Nagel, Düren, Germany), sequenced using the BigDye version 3.1 cycle sequencing kit (Thermo Fisher Scientific Life Technologies GmbH, Waltham, MA, USA), and subjected to an automated sequence analysis using the Sanger method on SeqStudio Genetic Analyser (Thermo Fisher Scientific Inc., Waltham, MA, USA). Sequence alignments and analysis were made using the molecular evolutionary genetic analysis (MEGA) software.

2.4. Assessment of Anti-SARS CoV-2 antibodies

The positive anti-nucleocapsid (N)-protein IgG and anti-spike (S)-protein IgG reactivity were according to the recommended cut-off value of NovaTec units (NTU) > 11 for positive results Novalisa SARS CoV-2 (COVID-19) Cat Nr. COVG0940 and GSD Novalisa SARS CoV-2 quantitative IgG Cat Nr. CVGQ0970 respectively (Novatec Diagnostics, Dietzenbach, Germany); NTU = X * 11/QC, where X = OD450nm − OD620nm of the test sample and QC = OD450nm − OD620nm of the quality control equivocal serum sample. E > 11 NTU. All ELISA measurements were performed on a Multiskan 96-well reader (Lot 357–706872, Thermofisher Scientific Life Technologies, Darmstadt, Germany).

Table 1
Characteristics of the study population. The study populations in Dhaka (Group 1 and Group 2) and in Dhaka and Chittagong (Group 3).

Characteristics	SARS-CoV-2 rtpcr POS				
	Group 1 Sept/Oct 2021	Group2 Jan/Feb 2022	Group 3 May 29 to Oct 26, 2022 Dhaka & Chittagong, Dhaka only	p-value	
				1 vs 2	2 vs 3
Number of cases enrolled	40	88	78		
Age Median (Range) years	50 (23–85)	37 (7–95)	31 (18–75)	<0.001	0.16
Gender M/F	20/20	75/15	60/18	<0.001	0.9
Specimen Collection Intervals rowhead					
Swab Days POCS median (range)	5 (2–7)	2 (1–4)	2 (0–6), 2 (0–4)	<0.001	0.02
Serum Days POCS median (range)	18 (15–23)	215 (186–238)	66 (33–139), 76 (35–117)	<0.0001	<0.0001
Serum Days after last vaccine median (range)	NA	489 (155–540)	258 (11–633), 271 (7–410)	NA	<0.0001
COVID-19 Severity rowhead					
Hospitalized n (%)	36 (90)	0 (0)	0 (0)	<0.0001	ND
Difficulty breathing n (%)	29 (70)	7 (8)	5 (6.4), 5 (11)	<0.0001	0.14
Oxygen support n (%)	29 (70)	0 (0)	5 (6.4), 5 (11)	<0.0001	0.14
Variant n (%) rowhead					
Delta	40 (100)	0 (0)	0 (0)	NA	NA
Omicron BA.1	0 (0)	23 (26)	0 (0)	NA	<0.0001
Omicron BA.2	0 (0)	65 (74)	1 (1.2), 0 (0)	NA	<0.0001
Omicron BA.4 or BA.5	0 (0)	0 (0)	7 (9), 3 (6)	NA	0.07
Omicron XBB	0 (0)	0 (0)	70 (86), 44 (85)	NA	<0.0001
Vaccination status rowhead					
Not vaccinated	28 (70)	6 (7)	1 (1.3) 1 (2)	NA	0.54
1-dose	7 (17.5)	0 (0)	0 (0)	NA	NA
2-dose	5 (12.5)	82 (93)	19 (24.4), 30 (64)	NA	<0.0001
Booster	0 (0)	0 (0)	58 (74.3), 16 (34)	NA	<0.0001
Previous SARS-CoV-2 Infection rowhead					
No	NA	8 (9)	40 (51), 15 (32)	NA	0.08
Yes	NA	46 (52)	35 (45), 32 (62)	NA	0.07
Not known	NA	34 (39)	3 (4), 0 (0)	NA	0.8

2.5. Statistical analysis

The Ct values, the levels of the anti-SARS CoV-2 S-proteins IgA and IgG, and the levels of the anti-N-protein IgG are presented as the mean with a standard deviation and as medians with ranges. Comparisons of the median values between patient groups were assessed by a nonparametric Mann–Whitney sum rank test, the mean of values by unpaired *t*-test and proportions by Chi-square analysis. A p-value of ≤ 0.05 was considered statistically significant. Correlation analyses were calculated with the Spearman's rank correlation coefficients. The correlation coefficients $r > 0.4$ or $r < -0.4$ with significance at $p < 0.05$ were considered strong positive or strong negative associations, respectively. Statistical analysis was performed with MedCalc version 14 for Windows (MedCalc Software, Mariakerke, Belgium).

2.6. Institutional Review Board Statement

The study was carried out under the approval by the local Institutional Review Board of the National Institutes of Laboratory Medicine Referral Center No. NILMRC/IRB/2021/07 Dhaka, Bangladesh. In addition, our present study was conducted in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. Informed written consent was obtained for blood draws and collection of nasopharyngeal swabs from the participants.

3. Results

3.1. Study participants

Demographic information was collected from the participants in all three groups. The subjects with the delta variant infections of group 1 were previously reported [16] and included in this study to compare their symptoms and antibody responses with that of the subjects in group 2 and group 3. Significant differences were observed in the median age and gender distribution between subjects in group 1 compared to those in groups 2 and 3. Group 1 shows a bias towards symptomatic COVID-19 cases, whereas Groups 2 and 3 show a higher proportion of younger males and asymptomatic cases (refer to Table 1). Participants from Groups 1 and 2 lived in Dhaka neighborhoods, while Group 3 included participants from Dhaka and Chittagong. The higher frequency of hospitalization and severe COVID-19 cases in group 1 compared to groups 2 and 3 was significant which was expected, given known severity of SARS-CoV-2 delta variant infections in September and October 2021.

3.2. Identification of SARS-CoV-2 variants

Distinct SARS CoV-2 variants were identified in the three COVID-19 groups pursuant with the timeline for transition from delta variant to omicron variants. Group 1 were exclusively delta variant [16]. Group 2 had ten omicron BA.1 and twenty-three BA.2. Group 3 had one BA.1, seven BA.4/BA5 and seventy omicron XBB subvariants. Four of the seven BA.4/BA.5 were in Chittagong between June

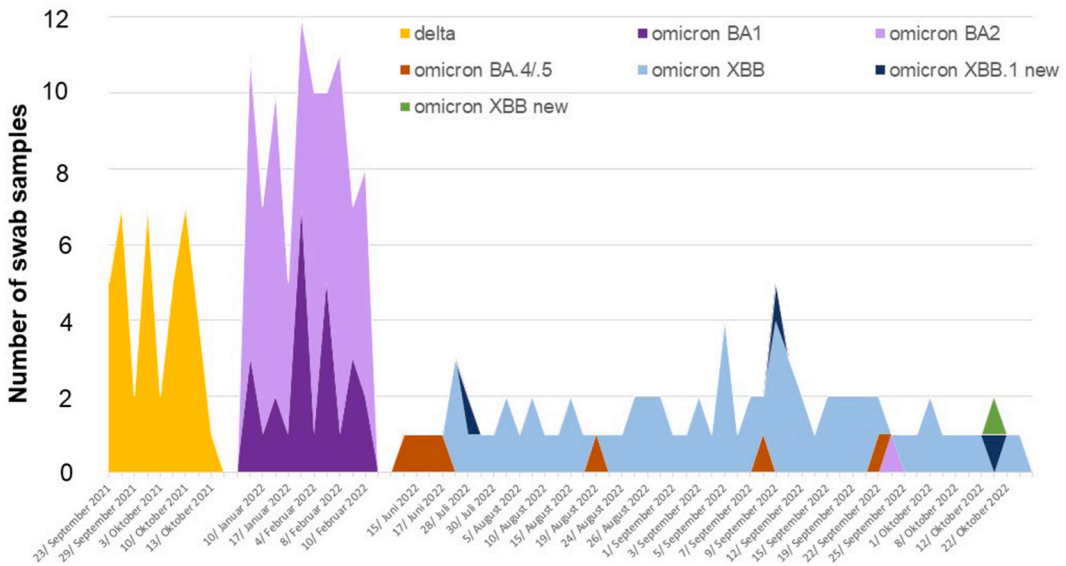

Fig. 1. Timeline for transition from SARS CoV-2 delta to SARS CoV-2 omicron sub variants. The delta variant (orange) n = 40 and omicron variants BA.1 (dark purple), n = 10 and BA.2 (light purple) n = 23 previously reported in Dhaka [16]. The omicron BA.4/BA.5 (brown) and omicron XBB (light blue) and novel sub-variants of XBB (dark blue) and BA2.75 (green) from Chittagong n = 31 and Dhaka n = 47.

15, 2022, and July 28, 2022, and three in Dhaka between August 19, 2022, and September 22, 2022. Two isolates in Chittagong were not clearly identified using the VirSNiP-Test and their sequence patterns were submitted to GISAID; one rare BA.2.75.1 on September 25, 2022, accession ID EPI ISL 17268140 and BA.5.2.6 on June 15, 2022, accession ID EPI ISL 17268139. Group 3 XBB omicron variants between July 26, 2022, and October 28, 2022 (Table 1, Fig. 1) carry L3681 and 48SP mutations and included three isolates not clearly identified using the VirSNiP-Test. The S-protein sequence patterns of two XBB mutants from Dhaka were submitted to the GSIAD with accession ID numbers XXB.1 EPI ISL 17268,137 and CJ.1 EPI ISL 17268,138.

3.3. Comparison of COVID-19 symptoms and frequency between SARS CoV-2 variants

90 % of the Group 1 delta variant COVID-19 cases were hospitalized of which 70 % reported difficulty breathing and required oxygen support. No hospitalizations were reported among the Group 2 and the Group 3. Difficulty of breathing was reported with supplementary oxygen administration in 8 % of Group 2 and in 6–11 % of Group 3 (Table 1, Fig. 2). Asymptomatic COVID-19 had a significantly higher frequency among omicron BA.1 and BA.2 than among the omicron XBB cases (Fig. 2A). Fever, cough, and headache were the most frequent symptoms in all three groups of SARS CoV-2 infections. The symptoms of headache or body ache, cough and loss of smell were reported at higher frequency among infections with the omicron subvariants variants BA.4/BA.5 and XBB compared to infections with omicron BA.1 and BA.2 (Fig. 2A). The number of symptoms show no associations; neither with the number of SARS-CoV-2 infections (Fig. 2B) nor with the number of SARS CoV-2 vaccinations (Fig. 2C). The number of infections in the Group 3 Dhaka patients were higher after 2-vaccine or 3-vaccine doses compared to 2 cases of none or 1- vaccine dose. The trend was without significance due to the low sample size (Fig. 2D).

3.4. Antibody responses following SARS CoV-2 infection and COVID-19 vaccination

Antibody responses to the nucleocapsid (N) protein, a structural component of coronavirus-2 crucial for the RNA genome packaging, are a reliable indicator for the recovery from natural SARS CoV-2 infection. Anti-N-protein IgG levels differentiate between subjects with previous natural infection from those which received S-protein vaccine especially in human populations having high S-protein vaccination rates. The anti-N-protein IgG seropositivity rates were delta 100 %, omicron BA.2 74 %, omicron BA.4/BA.5100 % and omicron XBB 93 % (Fig. 3A). Anti-N protein IgG levels negatively correlated to the number of days POCS by linear regression analysis (Fig. 3B) and show no association with the number of SARS CoV-2 infections (Fig. 3C).

All SARS-CoV-2 omicron infections gave anti-S protein IgG greater than two log units above the 11 NTU/mL cut-off (Fig. 4A) Sera of the BA.1 omicron infections were not available for evaluation. No association was found between anti-S protein IgG levels and the period of post-COVID-19 vaccination between day 11 and day 620 (Fig. 4B). In the group 3 Dhaka patients, the anti-S-protein IgG levels

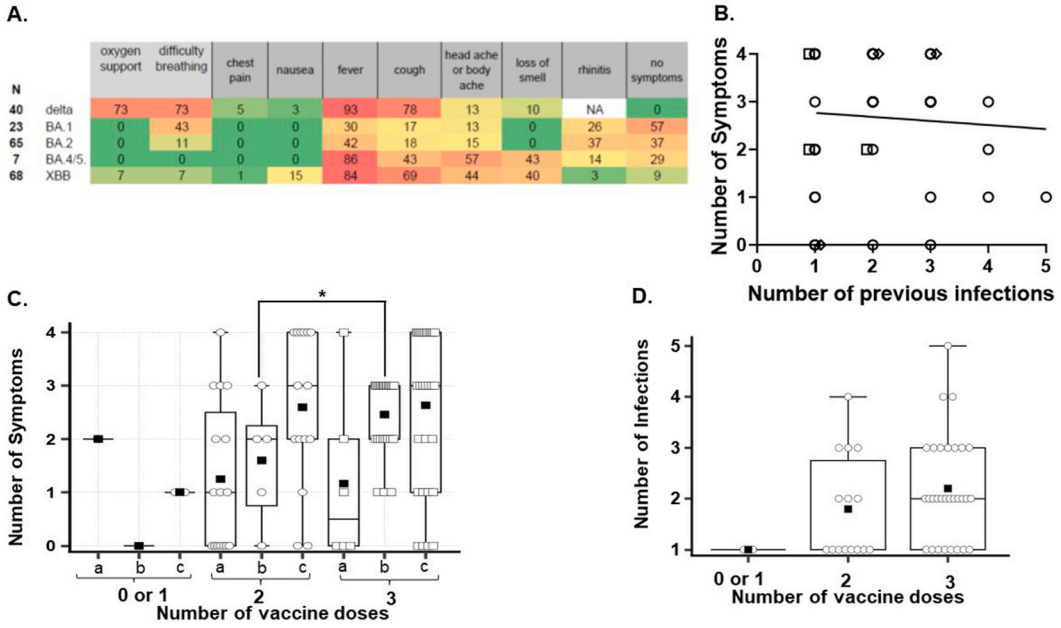

Fig. 2. COVID-19 symptoms in SARS CoV-2 variant infections. A. The type of symptom and the percentage of cases reporting each symptom are compared between SARS CoV-2 variants. The color-coded frequency of symptoms. The proportion of XBB omicron having symptoms were significantly higher than that of BA.1 or BA.2 $p < 0.01$ and $p < 0.001$ Chi Square test. **B.** Linear regression analysis of the number of symptoms in omicron subvariants reported in Dhaka district. BA.4/BA.5 (open squares), XBB (open circles), XBB mutants (open diamonds). **C.** Box-whisker plot of number of vaccine doses versus number of symptoms. Group 2 (a), Group 3 Chittagong (b) and Group 3 Dhaka (c). Comparison of median number of symptoms by rank independent Mann-Whitney test; *$p = 0.06$. **D.** Number of vaccine doses versus number of infections for Group 3 Dhaka.

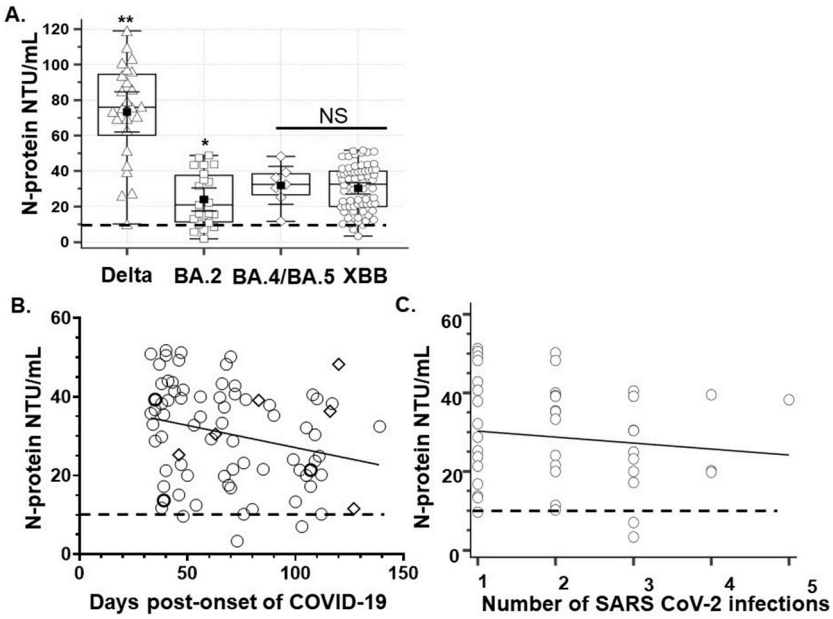

Fig. 3. Distribution and time course of anti-N protein IgG. (**A**) Anti-N-Protein IgG activity; mean delta 73 NTU/mL (95 % CI 62–85), BA.2 24 NTU/mL (95 % CI 17–30), BA.4/BA.5 32 NTU/mL (95 % CI 21–46), XBB 30 NTU/mL (95 % CI 27–33), XBB 31.5 NTU/mL (95 % CI 26–37) >35 < 76 days interval between infection and serum sampling. **B**) Anti-N-protein IgG levels in different SARS CoV-2 variants post-onset of COVID-19 symptoms (POCS). **C**) Number of infections versus anti-N-protein IgG levels. Delta (open triangles) BA.2 omicron (open square), BA4/BA.5 omicron (open diamond), XBB omicron (open circles) and XBB novel mutants (bold open circle); linear regression analysis r = −0,113 (95 % CI -0,2097 to −0,01572, p = 0.02). **p < 0.001, *p < 0.05 mean differences unpaired *t*-test.

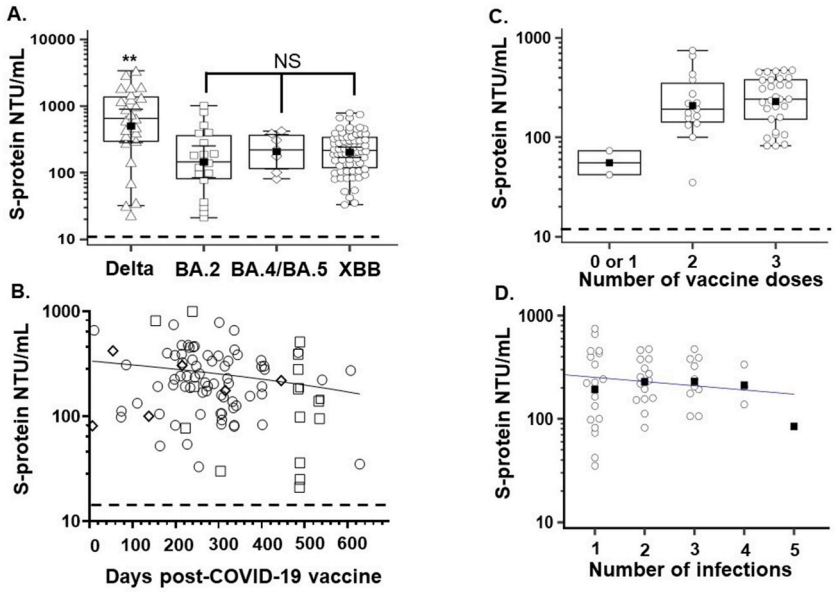

Fig. 4. Distribution and time course of anti-S protein IgG. (A) Anti-S protein IgG activity delta 949 NTU/mL (95 % CI 578–1319), BA.2250 NTU/mL (95 % CI 119–382), BA.4/BA.5241 NTU/mL (95 % CI 117–364), XBB 254 NTU/mL (95 % CI 213–294), XBB 275 NTU/mL (95 % CI 203–346). **(B)** Vaccine coverage versus anti-S-protein IgG levels in Group 3 Dhaka; none, 1-dose vaccine, 2-dose vaccine or 3-dose vaccine. (**C**) Anti-S-protein IgG levels post COVID-19 vaccination. **D.** Number of infections versus anti-S-protein IgG levels. **p < 0.001 mean differences of anti-S-protein IgG levels between delta variant versus the omicron variants in independent unpaired *t*-test.

were at similar levels after 2-dose or 3-dose SARS CoV-2 vaccine (Fig. 4C) and did not increase after repeated SARS CoV-2 infections (Fig. 4D).

4. Discussion

Molecular surveillance of SARS CoV-2 variants in Bangladesh during COVID-19 vaccine break-through infections are important for public health policy and pandemic preparedness [18]. SARS CoV-2 variants carrying lineage specific mutations, particularly in the S-protein receptor binding domain (RBD) give insights into SARS CoV-2 evolution. Their timely identification is crucial for assessment of potential pathogenic and transmissible variants and decisions on proactive measures to improve diagnostic tests and vaccines.

The first 2 years of the COVID-19 pandemic were characterized by convergent SARS-CoV-2 S-protein mutations at residues K417, L452, E484, N501 and P681 across different variants of concern (Alpha, Beta, Gamma, and Delta). Between February 2020 and May 2020, the variants carrying the amino acid mutation of aspartate to glutamate at the spike protein position 614 (D614G variant) spread throughout the world and increased the transmissibility of SARS-CoV-2 by conferring higher viral loads in young hosts without an apparent increase in the severity of the disease [19]. In September 2020, new genetic variants, carrying E484K and N501Y mutations such as B.1.1.7 (also known as the UK variant) and B.1.351 (also known as the South African variant) showed greater transmissibility and the capacity to escape antibody detection [20]. The enhanced transmissibility of Delta variant has been associated with critical S-protein mutations D614G, L452R, P681R, and T478K.

In November 2021, the omicron (B.1.1.529) with spike protein RBD mutations K417 N, S477 N, T478K, E484A and N501Y were identified. In the Spring 2022 and throughout the third year of the pandemic, the Omicron, and its sub-lineages, acquired additional 30 to 45 mutations at different amino acid residues, namely the R346, K444, N450, N460, F486, F490, Q493, and S494 which are associated with immune escape and an increased risk of reinfection [21,22]. Unfortunately, the information on Bangladeshi omicron subvariants has been lagging that of North America, Europe, China, and Asia.

The XBB omicron is a recombinant of the BA.2.10.1 and BA.2.75 sub-lineages which was first documented on samples collected on August 13, 2022 [23,24]. The rapid global spread of XBB variants has been attributed to the F486P mutation in the S1 protein which confers increased binding affinity to the human cell receptor for SARS-CoV-2, ACE-2 and multiple substitutions in the spike protein which cooperatively contribute to the resistance of XBB variants to humoral immunity [24–26].

The EpiCoV GISAID database contains 7701 entries of SARS CoV-2 sequences from Bangladesh of which 2000 entries belong to the omicron VOC, B.1.1.529 plus BA as of March 15, 2023 [27]. The entries of 194 XBB and 11 of XBB.1 omicron, show earliest collection date on September 12, 2022, and September 19, 2022, respectively [27]. Our sampling of SARS CoV-2 positive nasopharyngeal swabs from Chittagong and Dhaka, detected the omicron XBB on July 28, 2022, and show that XBB omicron is predominant throughout August 2022. Previous SARS CoV-2 genomic surveillance in Bangladesh found BA.5. omicron predominant in August 2022 with a few cases of BA.2.75 [15].

Although there are gaps in our survey between June 17 and July 28, 2022, we assume that XBB emerged in Bangladesh during July 2022, indicating approximately two months prior to the XBB omicron infections reported in India and Singapore [23] and the 40 SARS CoV-2 omicron XBB variants isolated between September and October 2022 in Bangladesh [28]. The XBB.1 mutant EPI_ISL_17268137 carrying L368I and missing F486P mutation and the rare omicron sequence patterns of CJ.1 EPI_ISL_17268138, BL.1/BA.2.75.1 EPI_ISL_17268140 and the BA.5.2.26 EPI_ISL_17268139 all associated with mild COVID-19 symptoms, predominantly body ache and loss of smell. Complete genome sequencing and analysis were not performed for these GISAID submissions. Assignment to the most probable omicron subtype was based on presence of rare mutations and/or missing amino acid mutations in the spike gene. On this basis, no evidence is presented that these omicron subvariants pose a tangible threat to the Bangladeshi population.

To our knowledge the serological study represents the longest follow-up period of SARS CoV-2 antibody responses in RT-PCR confirmed Bangladeshi COVID-19. The anti-N protein IgG positivity, 74–100 %, and anti-S protein IgG positivity 100 % throughout the 238-day observation period all represent antibody responses of vaccine break through infections. The significantly higher anti-N-protein IgG and anti-S-protein IgG levels of delta variant group 1 versus omicron groups 2 and 3 confirm our previous findings in Bangladesh [14,16]. The results are also consistent with the report in a European cohort showing higher antibody responses in delta variant; 440 U/mL to 717 U/mL anti-N-protein IgG and 790–949 U/mL anti-S-protein IgG versus omicron variants BA.1 and BA.2; 391 U/mL to 485 U/mL anti-N-protein IgG and 346 U/mL to 456 U/mL anti-S-protein IgG 60 days after infection [29].

In the current study, antibody measurements of delta variant 15–23 days post-infection were made at a significantly shorter interval between POCS and serum sampling than that of omicron BA.1 and BA.2 median 215 days and omicron XBB median 66 and 76 days. The validity of comparing antibody responses of delta variant infection versus antibody responses of omicron variant infections in this study is supported by data on the delta variant infection in India. Stable titres of anti-N-protein IgG, 3-fold above cut-off were found up to 70 days post-infection and anti-S protein IgG levels, 78 U/mL (IQR 40–163) at 7 days increased to 213 U/mL (IQR 89–258) at 112 days post–SARS CoV-2 infection by quantitative automated Roche immunoassay [30]. Based on this evidence, higher anti-SARS CoV-2 antibody levels in the delta variant infection of Group 1 versus the omicron variants of Group 2 and Group 3 likely indicate the propensity of delta variant to elicit stronger antibody responses than omicron variants rather than being attributed to the shorter time intervals between the dates of infection onset and the dates of serum sampling.

The SARS CoV-2 BA.1. and BA.2 omicron in January and February 2022 and the BA.4/BA.5 and XBB omicron subvariants in July to October 2022 all represent breakthrough infections. Although, the anti-S-protein IgG levels by Novalisa correlate to neutralizing activity in cell-based assays of original SARS CoV-2 strain, the Novalisa measurements in this study can not distinguish between the anti-S-protein IgG originating from natural omicron infections versus the vaccinations.

Interestingly, anti-S-protein RBD monoclonal antibodies generated in volunteers having SARS CoV-2 omicron BA-2 breakthrough

infections after BNT162b2 vaccination have strong neutralizing activity against BA.2 and wild type SARS CoV-2 but show dramatic reduction in their capacity to neutralize BA.4/BA.5 and XBB in pseudoviral cell assays [26]. This data provides compelling evidence that omicron BA.4/BA.5 and omicron XBB variants rapidly escape antibody responses directed against BA.2 variant and original SARS CoV-2. The anti-S-protein IgG of Group 3 did not protect against XBB break-through infections and are likely a complex mixture that include non-neutralizing fractions that bind S-protein epitopes of BA.2, BA.4/BA.5 and XBB.

Anti-S-protein seropositivity of Bangladeshi adults in the period between April 16 and October 15, 2020, was 20–40 %, by non-commercial ELISA, 14–28 days after SARS-CoV-2 RT-PCR confirmed COVID-19 [31,32]. Between May 2020 and November 2020, we found 14–29 % anti-N-protein IgG positivity in Dhaka compared to 73 % anti-N-protein positivity in Narayanganj, the adjacent district to Dhaka. The anti-N-protein levels were independent of endemic Dengue infection [14]. In the period between May 26, 2021, and June 6, 2021, SARS CoV-2 infections of 5 alpha or beta VOCs and 31 delta variant VOCs gave anti-N-protein IgG seropositivity of 70–80 % and anti-S-protein IgG seropositivity of 92 % 12–16 days post-SARS CoV-2 infection [17]. These cases early in the pandemic with less than 4 % receiving 2-vaccine doses, and the current data on 2022 omicron infections with 100 % either 2-vaccine or 3-vaccine doses reveal that Bangladeshis attained a high rate of seroprevalence from repeated SARS CoV-2 infections prior to vaccination.

A major limitation of this study is the heterogeneity between the three groups with respect to SARS CoV-2 variants and vaccine coverage. The issue is partially resolved in Group 3 presenting predominantly omicron XBB from two different Bangladeshi provinces both having extensive vaccination. Our analysis reveals that the number of symptoms in this group was affected neither by the number of infections nor by the number of vaccine doses. Furthermore, the anti-S-protein IgG levels were without significant differences after 2-vaccine versus 3-vaccine doses and after one to five repeated infections. These findings indicate that the anti-S-protein IgG reached peak levels early in the Bangladeshi COVID-19 pandemic when the SARS-CoV-2 delta variant infections were predominant and associated with either moderate or severe COVID-19. The B-lymphocyte and T-lymphocyte responses to S-protein vaccine overlap with omicron break-through infections causing mild or asymptomatic COVID-19 and did not necessarily lead to increased antibody production. The booster vaccine without a third vaccine dose was sufficient to sustain the anti-S-protein IgG levels and likely prevented COVID-19 related mortality. Since the XBB omicron break-through infections are without hospitalizations, we can not discern whether the increased number of symptoms in Group 3 compared to BA.1 and BA.2 of Group 2, indicate a worsening clinical presentation attributed to XBB variants per se. To what extent the anti-S-protein IgG levels persisting beyond 1-year post-COVID-19 vaccination are correlated to cellular immune responses and cytokines of lingering post-COVID-19 symptoms or broadly associate with autoreactivity is beyond the scope of this study.

The SARS CoV-2 omicron variant infections in 2022 having ostensibly mild COVID-19 reinforce evidence that the COVID-19 pandemic is no longer a public emergency of major international concern. The detection of omicron XBB and novel omicron sub-variants without hospitalization, oxygen support or severe COVID-19 symptoms does not preclude the risk new variants will emerge to pose a health threat in the future. Complacency and popular perception that the "COVID-19 pandemic is over" should not undermine the need to continue molecular surveillance of SARS-CoV-2 variants in animal reservoirs and in diverse human populations including low-income countries.

Data availability statement

Data associated with this study has been deposited in the public available repository Global Initiative on Sharing All Influenza Data (GISAID). GISAID submissions EPI_ISL_17268137 carry L368I and missing F486P mutation. EPI_ISL_17268138, BL.1/BA.2.75.1 EPI_ISL_17268140 rare omicron sequence patterns of CJ.1 and EPI_ISL_17268139 rare BA.5.2.26 sequence.

Institutional Review Board and informed consent

The study was carried out under the approval by the local Institutional Review Board of the National Institutes of Laboratory Medicine Referral Center No. NILMRC/IRB/2021/07 Dhaka, Bangladesh. In addition, our present study was conducted in compliance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. Informed consent was obtained for blood draws and collection of nasopharyngeal swabs from the participants.

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CRediT authorship contribution statement

Simon D. Lytton: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Formal analysis, Conceptualization. **Asish Kumar Ghosh:** Supervision, Resources, Investigation, Conceptualization. **Rakibul Hassan Bulbul:** Methodology, Investigation. **Tasnim Nasifa:** Visualization. **Rashid Mamunur:** Supervision, Project administration, Methodology. **Christian Meier:** Methodology, Investigation. **Olfert Landt:** Supervision, Resources, Project administration. **Marco Kaiser:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestsMarco Kaiser (M.K), Christian Meyer (C.M.) and Olfert Landt (O.L.) are affiliated with TIB-MOLBIOL GmbH. The analyses and molecular tools were performed at TIB-MOLBIOL. There is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31011.

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