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Research Article

Comparison of the Clinico-Demographic and Molecular Profile of SARS-CoV-2 Omicron Variants BA.1 and BA.2 from North-East India

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Abstract

Purpose: The present study investigated the clinical and demographic characteristics of SARS-CoV-2 infected patients in North-East India during the SARS-CoV-2 Omicron wave from December 2021 to January 2022.

Methods: An in-house real-time RT-PCR assay was used to screen the Omicron sub-variants BA.1 and BA.2. The Omicron positive samples underwent whole genome sequencing to confirm the presence of sub-variants BA.1 and BA.2. Further, a retrospective study based on questionnaire survey was conducted to compare the clinico-demographic characteristics of patients infected with SARS-CoV-2 Omicron variants BA.1 and BA.2.

Results: Out of a total of 349 participants, 26 were positive for sub-lineage BA.1, while 323 were positive for sub-lineage BA.2. Overall, 77.90% of cases (272/349) exhibited symptoms, with fever being the most common. Patients infected with BA.2 sub-lineage experienced higher symptoms compared to BA.1 sub-lineage (79.87%, p=0.002). Among SARS-CoV-2 vaccinated individuals, 92.83% were infected with the Omicron variant and of those, 79.32% experienced symptoms (p=0.025). Of the individuals with a prior COVID-19 history, 37 out of 349 were re-infected with the Omicron variant, with only one case requiring hospitalization and none needing ICU care. Furthermore, all 37 SARS-CoV-2 re-infection cases reported in this study were positive for the BA.2 variant suggesting that BA.2 can evade and circumvent the herd immunity established through prior infections.

Conclusion: The predominant SARS-CoV-2 Omicron variant observed in the region during the period was BA.2. The lower number of hospitalizations including the need for ICU indicated a milder severity of the Omicron variant, potentially attributed to the protective immunity developed during pre-Omicron waves. Notably, re-infection cases demonstrated less severe infections indicating the active role of hybrid immunity derived from a combination of immunity from past infections and vaccination.

Keywords: BA.1; BA.2; Omicron; Re-infection; SARS-CoV-2; Vaccine

Abbreviations

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19: Coronavirus Disease 2019; ICU: Intensive Care Unit; WHO: World Health Organization; RBD: Receptor Binding Domain

Introduction

Since its emergence in 2019, SARS-CoV-2 has continuously evolved, leading to multiple waves of the COVID-19 pandemic. The ongoing expansion of the SARS-CoV-2 viral family, accompanied by significant genetic changes, has increased its ability to evade host immune responses, posing a threat to disease management and patient care. As of June 2025, the World Health Organization's COVID-19 dashboard reported approximately 778 million confirmed cases of SARS-CoV-2 worldwide, resulting in 7.1 million deaths. Despite the implementation of COVID-19 safety guidelines and widespread administration of vaccines, the novel coronavirus has continued to spread, leading to the emergence of the Omicron (B.1.1.529) variant in November 2021.

The highly transmissible Omicron variant is characterized by more than 50 mutations, including 26-32 amino acid substitutions and 15 mutations in the RBD domain of the spike protein [1-3]. Initially, the BA.1 sub-variant predominated over other sub-lineages (BA.1.1 and BA.2) worldwide [4]. However, BA.2 subsequently gained dominance due to its rapid transmission rate, leading to millions of Omicron-mediated SARS-CoV-2 positive cases within a month. Notably, BA.2 became prevalent in several countries, including India, indicating its selective advantage over the BA.1 variant [5]. The BA.1 sub-variant is distinguished by S-gene target failure (SGTF), which is absent in BA.2 [5]. Structurally, the BA.1 sub-lineage of Omicron differs from its sister variant, BA.2, with approximately 40 distinct mutations and a deletion at position 69-70 in the spike protein region [6-7].

Given this context, it is crucial to examine and compare the clinical parameters of individuals testing positive for Omicron, including their vaccination history, sub-lineage detection, prior history of SARS-CoV-2 infection and duration of illness. Accordingly, this study was designed as a questionnaire-based survey to investigate the clinical and demographic characteristics of Omicron-positive individuals diagnosed using whole genome sequencing.

Material and Method

Study Design, Study Site and Participants

A retrospective study based on telephonic interviews was conducted to analyze the clinico-demographic characteristics of patients infected with SARS-CoV-2 Omicron variants BA.1 and BA.2. The study utilized a questionnaire survey. It was carried out at the Regional Viral Research and Diagnostic Laboratory (Regional VRDL), ICMR-Regional Medical Research Centre (ICMR-RMRC), located in Dibrugarh, Assam, India, between February and May 2022. Participants included individuals who tested positive for Omicron variants BA.1 and BA.2. The selection of participants was random and based on testing records from the laboratory between December 2021 and January 2022. The study was approved by the Institutional Ethics Committee of ICMR-RMRC, NER, Dibrugarh, Assam, India and all procedures adhered to ethical standards set by the committee.

Questionnaire-Based Survey

A questionnaire was designed as an interview guide for the study. A team of investigators conducted telephonic interviews with the participants on a one-to-one basis. The average duration of each interview ranged from 10 to 15 minutes. The questionnaire covered various aspects, including vaccination history, previous SARS-CoV-2 infections, symptomatic or asymptomatic status, symptoms experienced, duration of illness, hospitalization status and more. Any missing information from participants was noted as missing data.

Laboratory Tests

An in-house real-time RT-PCR assay was used to screen the Omicron variant as well as to distinguish the sub-variant BA.1 and BA.2. Further, to confirm the sub-variant BA.1 and BA.2, the screened samples underwent whole genome sequencing in an Illumina MiSeq system using the CoviSeq/Miseq assay ver3 kit as per manufacturer’s protocol. The NGS data analysis was conducted using the Linux Command Line Interface (CLI), with the reads being aligned to the reference genome NC_045512.2. Additionally, variant analysis was performed using Nextclade online tool (https://clades.nextstrain.org/).

Phylogenetic and Pairwise Distance Analysis

Representative whole genome sequences of Omicron subvariant BA.1 and BA.2 detected in the study were subjected to the evolutionary phylogenetic analysis of the whole genome (~ 29,907 bp) with reference and nearest sequences. The tree-building approach considered factors such as general time reversal, gamma distribution and invariants (after the best model test) to calculate rate differences among sites. To enhance the robustness of the analysis, 500 bootstrap iterations were performed. To estimate the evolutionary divergence between sequences, pair-wise distance calculations were carried out using the Maximum Composite Likelihood model, while rate variation among sites was modelled using a gamma distribution. In total, 36 nucleotide sequences were included in these evolutionary analyses and all of these procedures were conducted within the MEGA X software. (GISAID Accession no of the representative sequences from the study are EPI ISL 16415608, EPI ISL 16415609, EPI ISL 17300697, EPI ISL 16415579, EPI ISL 16415610, EPI ISL 17300719, EPI ISL 17547225, EPI ISL 17627262, EPI ISL 17627263, EPI ISL 17627270).

Statistical Analysis

Data obtained were analyzed using SPSS Version 20 (IBM Corporation, Armonk, New York). Descriptive statistics, Odds ratio, Chi-square test were conducted for each category. A p-value ≤0.05 was considered statistically significant.

Results

Demographic Profile of the Study Participants

Initially, a total of 454 individuals who had previously tested positive for the Omicron variants BA.1 and BA.2 were randomly selected at the Regional VRDL, ICMR-RMRC in Dibrugarh, Assam, India (supplementary figure 1). Among the 454 Omicron-positive individuals, 349 individuals participated in the telephonic interview and provided complete data as per the questionnaire. The remaining participants were excluded due to reasons such as unavailability for phone contact, refusal to participate, lack of response or incomplete data.

The study consisted of 349 Omicron-positive individuals, with a mean age of 36.36 ± 15.2 years, as presented in Table 1. The age distribution showed a higher positivity rate for the Omicron variant in the young and middle-aged groups, with 117/349 individuals (16-30 years) and 119/349 individuals (31-45 years) testing positive.

Clinical Profile of the Study Participants

Symptomatic cases accounted for 77.90% (272/349), with fever being the most common symptom (185/349, 53%). Nineteen individuals required hospitalization and only two individuals required ICU care. Regarding the vaccination history, 92.83% (324/349) of individuals had been administered SARS-CoV-2 specific vaccines, either fully or partially. Among the vaccinated individuals, 96.60% (313/324) had received two doses, while 11/324 were partially vaccinated with one dose. Covishield was the most common vaccine administered (76.54%, 248/324), followed by Covaxin (20.67%, 67/324).

Demographic and Clinical Characteristics of Patients with Respect to Omicron Sub-Lineages BA.1 and BA.2

Among the 349 cases, 92.55% had sub-lineage BA.2, while only 7.44% had sub-lineage BA.1. Patients infected with BA.2 sub-lineage exhibited more symptoms (79.87%, 258/323) compared to patients infected with BA.1 (53.84%, 14/26). The Chi-square test revealed a statistically significant difference between the two groups (p-value = 0.002, Odds ratio-3.4, 95% CI of Odds ratio = 1.5-7.7).

Regarding hospitalization, three patients out of 26 BA.1 positive cases required admission, while 16 out of 323 BA.2 positive cases needed hospitalization, with two patients requiring ICU care.

Omicron Sub-Lineage Status in Relation to Previous COVID-19 Positivity History

It is important to highlight that among the 349 SARS-CoV-2 Omicron variant positive cases in this study, 37 out of 323 cases (11.45%) had a history of past SARS-CoV-2 infection during the pre-Omicron waves, as shown in Table 2. Our data revealed that all 37 individuals with a history of SARS-CoV-2 positivity were only positive for BA.2, which was an interesting finding (Table 2). Additionally, only one case of hospitalization was reported among the 37 re-infected cases and none of them required ICU care. It is worth noting that 34 out of 37 re-infected cases were fully vaccinated.

Comparative Analysis of Symptomatic and Asymptomatic Groups of Individuals Infected with the SARS-CoV-2 Omicron Variant

Symptomatic cases were more prevalent among female patients compared to male participants, with a p-value of 0.012, indicating statistical significance as presented in the Table 3 (Odds ratio-2.12, 95% CI odds ratio=1.17-3.82). In addition, 79.32% (257/324) of vaccinated individuals experienced symptoms. This distribution showed statistical significance, with a p-value of 0.025 and an Odds ratio of 2.56 (95% CI odds ratio=1.09-5.94) as shown in the Table 3. When comparing the symptomatic status with the age groups of the patients, no statistically significant difference was observed (Fig. 1).

NGS Based Phylogenetic Analysis

Sub lineage positivity of SARS-CoV-2 Omicron variants were further confirmed using whole genome sequencing followed by variant analysis. As per the NGS data, it is noteworthy to mention that, mutations at the positions N440K, K417N, R408S, D405N, T376A, S375F and S373P were only associated with BA.2 and not with BA.1 (supplementary figure 2). In addition, mutations at the positions G446S and G496S were only present in the samples infected with Omicron BA.1 variant (supplementary figure 1). Evolutionary analysis by Maximum Likelihood method revealed that the pair wise distance (p distance) between the BA.1 and BA.2 Omicron variants was observed to be 0.004%.

The evolutionary history of 36 whole genome sequences (~29.9kb) of SARS-COV-2 was inferred by using the Maximum Likelihood method and General Time Reversible model. The phylogenetic tree was rooted to the reference strain NC 045512.2 of Dec 2019. Representative whole genome sequences of SARS-CoV-2 strains from Dibrugarh, Assam are tagged in light blue dot, while Omicron variant BA.1 sub-lineage are colored in blue while BA.2 strains are colored in green (Fig. 2).

Study Population (N) = 349	
Mean age (Years ± SD)	36.36 ± 15.2
Gender N (%)	
Male	230 (65.9)
Female	119 (34.1)
Age Distribution N(%)	
0-15 YEARS	22 (6.3)
16-30 YEARS	117 (33.5)
31-45 YEARS	119 (34.1)
46-60 YEARS	66 (18.9)
61 and ABOVE	25 (7.2)
Omicron Sub lineage N (%)	
BA.1	26 (7.4)
BA.2	323 (92.6)
COVID-19 clinical status N (%)	
Asymptomatic	77 (22.1)
Symptomatic	272 (77.9)
Symptoms N (%)	
Fever	185 (53.0)
Running nose	113 (32.4)
Sore throat	68 (19.5)
Cough	139 (39.8)
Malaise and body ache	94 (26.9)
Head ache	63 (18.1)
Respiratory difficulties	9 (2.6)
Loss of appetite	17 (4.9)
Anosmia	11 (3.2)
Myalgia OR muscle pain	28 (8.0)

Diarrhoea	6 (1.7)
Hospitalization N (%)	19 (5.4)
ICU care N (%)	2 (0.6)
Covid-19 Vaccination N (%) Covaxin Covishield Others	324 (92.8) 67 (21) 248 (76.5) 4 (1.23)
Unknown	5 (1.54)

Table 1: Demographic and clinical profile of the SARS-CoV-2 Omicron positive cases based on telephonic interview.

Characteristics	BA.1 N (%)	BA.2 N (%)	P-value	Odds Ratio	95% CI of Odds Ratio
Gender					
Male	14 (53.8)	216 (66.9)			
Female	12 (46.2)	107 (33.1)			
Age distribution					
0-15 Years	1 (3.8)	21 (6.5)			
16-30 Years	7 (26.9)	110 (34.1)			
31-45 Years	7 (26.9)	112 (34.7)			
46-60 Years	7 (26.9)	59 (18.3)			
61 and Above	4 (15.4)	21 (6.5)			
COVID-19 clinical status					
Symptomatic	14 (53.8)	258 (79.9)	0.002	3.4	1.5 - 7.7
Asymptomatic	12 (46.2)	65 (20.1)			
Duration of symptoms					
1-7 Days	8 (30.8)	197 (61.0)			
8-15 Days	4 (15.4)	52 (16.1)			
16 and Above	2 (7.7)	9 (2.8)			
No symptoms	12 (46.2)	65 (20.1)			
Covid-19 Vaccination					
Yes	25 (96.2)	299 (92.6)			
No	1 (3.8)	24 (7.4)			
Hospital Admission					
Yes	3 (11.5)	16 (5.0)			
No	23 (88.5)	307 (95.0)			
ICU Requirement					
Yes	0 (0.0)	2 (0.6)			
No	26 (100.0)	321 (99.4)			
Prior COVID-19 infection					
Yes	0 (0.0)	37 (11.5)	0.07		
No	26 (100.0)	286 (88.5)			
Bold signifies statistically significant p-value					

Table 2: Demographic and clinical profile of the patients with respect to SARS-CoV-2Omicron sub-lineages.

Characteristics	Asymptomatic	Symptomatic	P-value	Odds Ratio	95% CI of Odds Ratio
	N (%)	N (%)			
Gender					
Male	60 (26.1)	170 (73.9)	0.012	2.12	1.17-3.82
Female	17 (14.3)	102 (85.7)			
Covid-19 Vaccination					
Yes	67 (20.7)	257 (79.3)	0.025	2.56	1.09-5.94
No	10 (40.0)	15 (60.0)			
Bold signifies statistically significant P-value					

Table 3: Comparison of the symptomatic and asymptomatic group of individuals infected with SARS-CoV-2 Omicron variant.

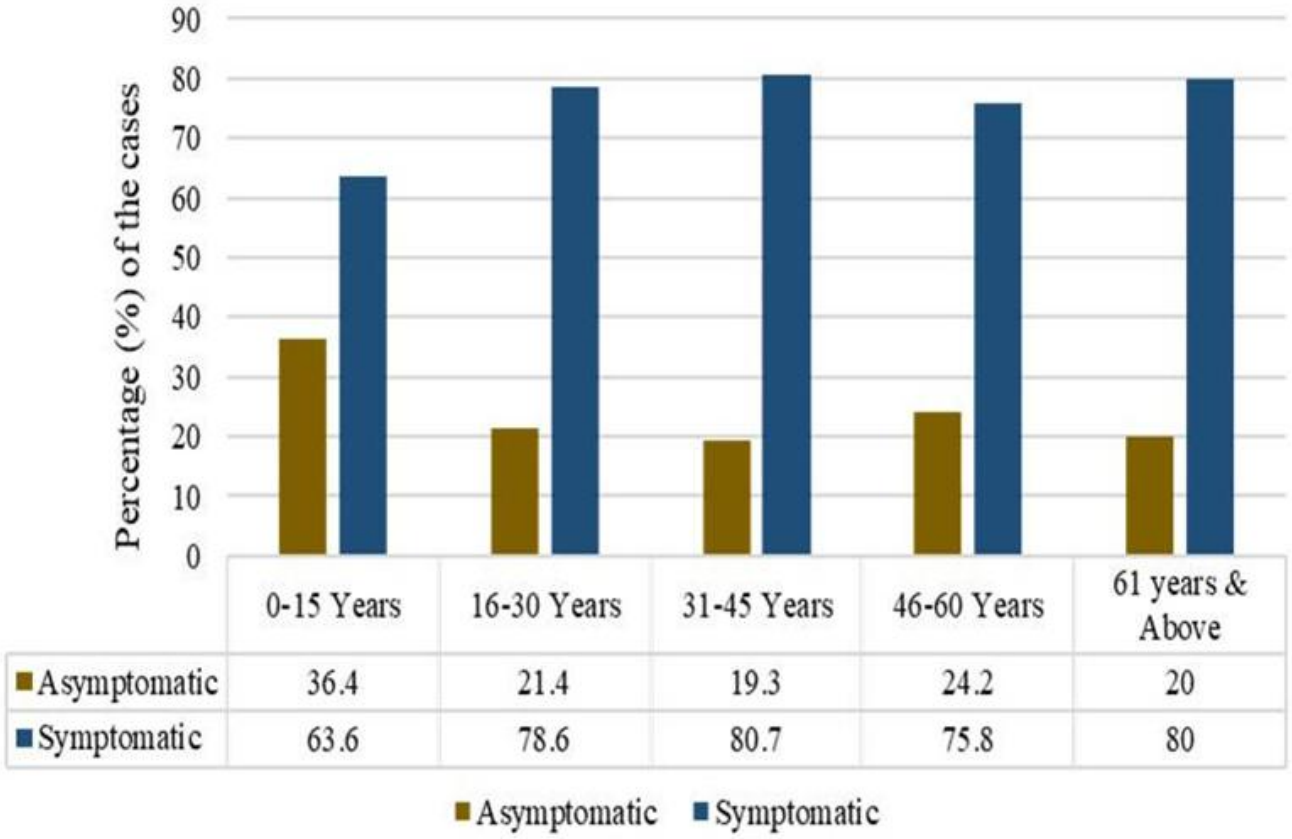

Figure 1: Percentage (%) of the symptomatic and asymptomatic SARS-CoV-2 Omicron positive cases with age.

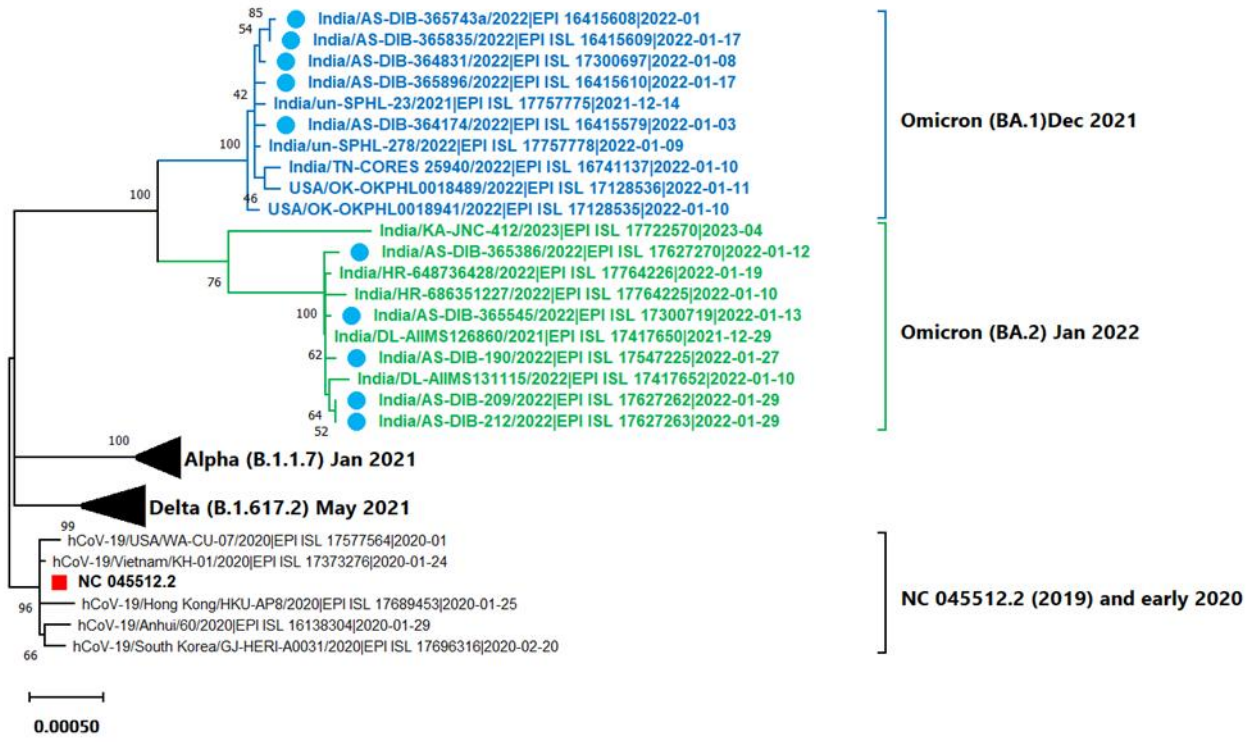

Figure 2: Evolutionary analysis of the SARS-CoV-2 Omicron variants by Maximum Likelihood method. The evolutionary history of 36 whole genome sequence (~29.9kb) of SARS-COV-2 was inferred by using the Maximum Likelihood method and General Time Reversible model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA X. The tree is rooted to the reference strain NC 045512.2 of Dec 2019. Representative WGS of SARS-CoV-2 strains from Dibrugarh, Assam are tagged in light blue dot, while Omicron variant BA.1 sub-lineage are coloured in blue while BA.2 strains are coloured in green.

Discussion

The current study aimed to compare the demographic and clinical characteristics of patients infected with SARS-CoV-2 Omicron BA.1 and BA.2 sub-lineages during December 2021 to January 2022. To the best of our knowledge, this is the first report to provide a clinical and demographic comparison of Omicron-positive patients from North-East India. Among the participants randomly selected for this study, a higher number of cases were infected with the BA.2 variant compared to BA.1, confirming BA.2 as the predominant circulating strain during the study period. The uneven distribution of cases between BA.1 and BA.2 is reasonable, considering the higher global prevalence of BA.2 compared to BA.1 [8]. Therefore, our data on the higher incidence of BA.2-associated SARS-CoV-2 infections is not surprising and is supported by previous studies reporting increased BA.2 cases in various regions [9-11]. NGS based validation of the samples showed mutations in the different positions which were either commonly or distinctly related with BA.1 and BA.2. Our findings were concomitant with the earlier studies where authors too reported about specific mutations associated with different SARS-CoV-2 variants [12-13]. Mutational analysis data further confirmed that mutations G446S and G496S are associated with only BA.1 variant and not with BA.2 as reported by a previously published study [14].

Our finding on a greater number of symptomatic caseswith SARS-CoV-2 Omicron infections is consistent with a previously published study from England conducted in 1,542,510 adults [15]. The severity in terms of hospital admissions during the Omicron wave was lower based on the data from our study and these findings align with a previous study by Webster, et al., which also reported a relatively lower or comparable risk of severe outcomes in BA.2 lineage infections compared to BA.1 [16]. Additionally, a study from Denmark demonstrated a lower risk of hospitalization for the Omicron variant compared to the Delta variant [17].

It is important to note that multiple waves of the pandemic have already passed, contributing to the development of protective immunity among regions exposed to SARS-CoV-2 globally. Therefore, the lower severity of the Omicron BA.2 variant may be attributed to the already acquired herd immunity from previous waves of SARS-CoV-2. However, the scenario could be different for populations with no prior exposure, where the Omicron wave may lead to more severe outcomes, as previously reported in China [18]. Furthermore, the absence of ICU requirements and only one hospitalization among the re-infected cases (37 out of 349) further indicates that previous infection with pre-Omicron waves provided some level of protection to individuals and reduced the risk of severe disease outcomes during re-infection, as discussed in a recently published review article [19]. This further supports the notion that hybrid immunity derived from past infection and vaccine administration may play a crucial role in reducing the severity of re-infection cases compared to previous infection.

Despite the development and widespread administration of effective vaccines against COVID-19 globally, various variants of SARS-CoV-2 with distinct structural changes have emerged, raising concerns about the effectiveness of these vaccines in eliciting sufficient protective immune responses. In light of this, the present study aimed to speculate on the SARS-CoV-2 Omicron variant's positivity rate in relation to the vaccination status of the study participants. The findings of the present study align with earlier reports indicating the limited effectiveness of vaccines against SARS-CoV-2 infection [20-22]. It is worth noting that most currently used vaccines were developed based on the original wild-type strain of SARS-CoV-2, while the virus has undergone rapid structural changes, giving rise to the circulation of multiple variants. Therefore, there is a pressing need for comprehensive investigations to understand the efficacy of administered vaccines in protecting against the currently circulating SARS-CoV-2 strains and the development of suitable vaccines targeting specific variants. One possible explanation for the higher proportion of symptomatic cases among the vaccinated individuals could be increased viral replication in these cases due to Antibody-Dependent Enhancement (ADE). This phenomenon involves elevated activation of immune cells, leading to severe inflammation or increased viral replication mediated by poorly neutralizing antibodies [23].

In the present study, the patients infected with the BA.2 sub-lineage exhibited a higher frequency of symptoms (79.87%, 258/323). This finding aligns with earlier research where the BA.2 sub-lineage was associated with a higher prevalence of symptomatic cases [15]. However, previous reports have indicated comparable severity between BA.1 and BA.2-induced SARS-CoV-2 positive cases [24-25]. Importantly, all 37 cases of SARS-CoV-2 reinfection reported in this study were positive for the BA.2 variant. A recently published study documented that protection obtained from previous infection with pre-Omicron variants against Omicron sub-lineages, including BA.2, was comparatively lower than protection obtained from past infection with the Omicron variant [26]. Thus, our finding further suggests that the BA.2 variant can bypass the herd immunity generated by previous SARS-CoV-2 infections.

The present study had certain limitations. The study group had only 25 unvaccinated individuals. Additionally, a higher number of BA.2 sub-lineage infections were detected compared to Omicron BA.1 sub-lineage infections, leading to an uneven distribution of cases between the two groups. Furthermore, the present study did not perform a comparative analysis of the clinical and demographic profiles of SARS-CoV-2 positive patients across different waves of the pandemic.

Conclusion

In conclusion, this study revealed that BA.2 was the predominant variant in the region during the study period and was associated with a higher frequency of symptomatic cases compared to the BA.1 variant. The lower number of hospitalizations and ICU admissions indicated a milder severity of the BA.2 variant, potentially attributable to the protective immunity developed during the pre-Omicron waves of the SARS-CoV-2 pandemic. Moreover, the occurrence of re-infection cases with the BA.2 variant suggests that BA.2 can evade and circumvent the herd immunity established through prior infections. However, the hybrid immunity resulting from a combination of past infection and vaccine administration may play a crucial role in reducing the severity of re-infection cases compared to primary infections.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential source of conflict of interest.

Source of Funding
The financial support for conducting the laboratory investigations is obtained under the scheme Regional VRDL, Dibrugarh which is funded by the Department of Health Research (DHR), Government of India.

Ethical Statement
The study was conducted under the Regional Viral Research and Diagnostic Laboratory (Regional VRDL), Dibrugarh which is approved by the institutional ethics committee of the Regional Medical Research Centre, N.E. Region (ICMR), Dibrugarh (letter No.RMRC/Dib/IEC (Human)/2019-20/135 date:12/08/2020). All the procedures in the study were in accordance with the ethical standards of the Committee.

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Author Contribution
BB performed the conceptualization, study design, data analysis, manuscript drafting, editing and critical review. NS performed thestudy design, telephonic interview, laboratory tests, data analysis, manuscript drafting and editing. NKB performed data analysis, manuscript editing and critical review.AIS and CKBperformed laboratory tests, telephonic interview, data analysis. RH performed laboratory tests, telephonic interview. PB and NK performed the telephonic interview.

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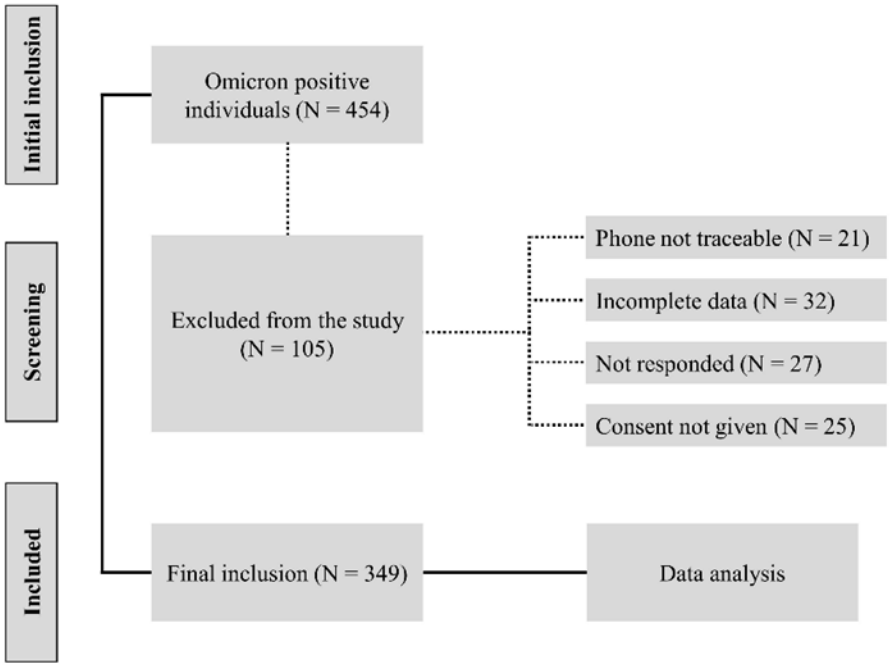
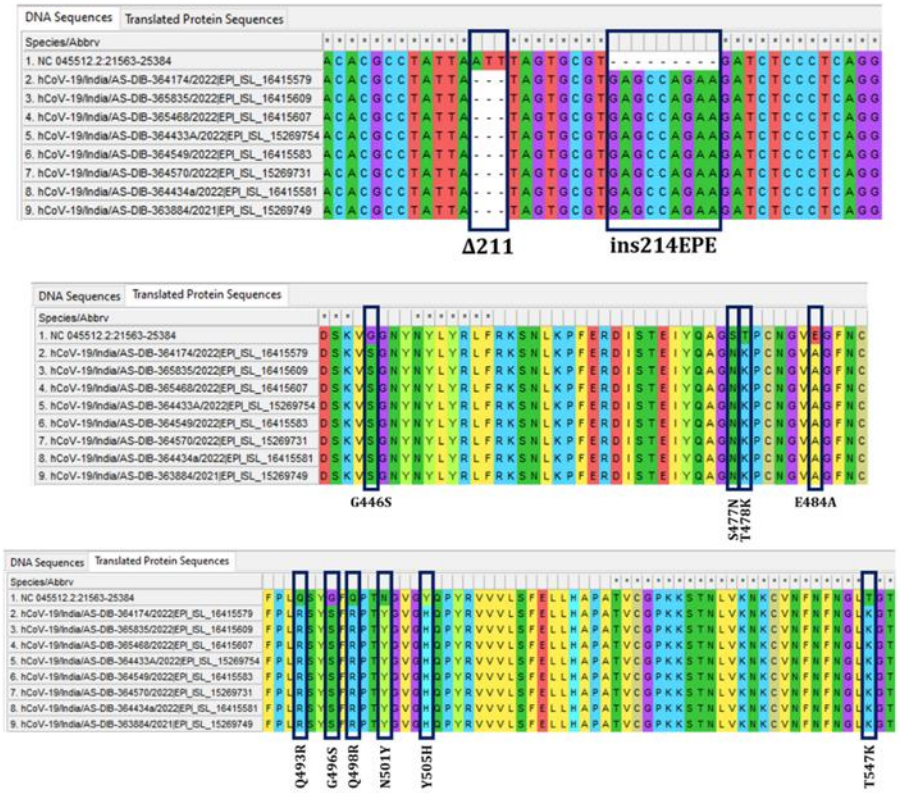
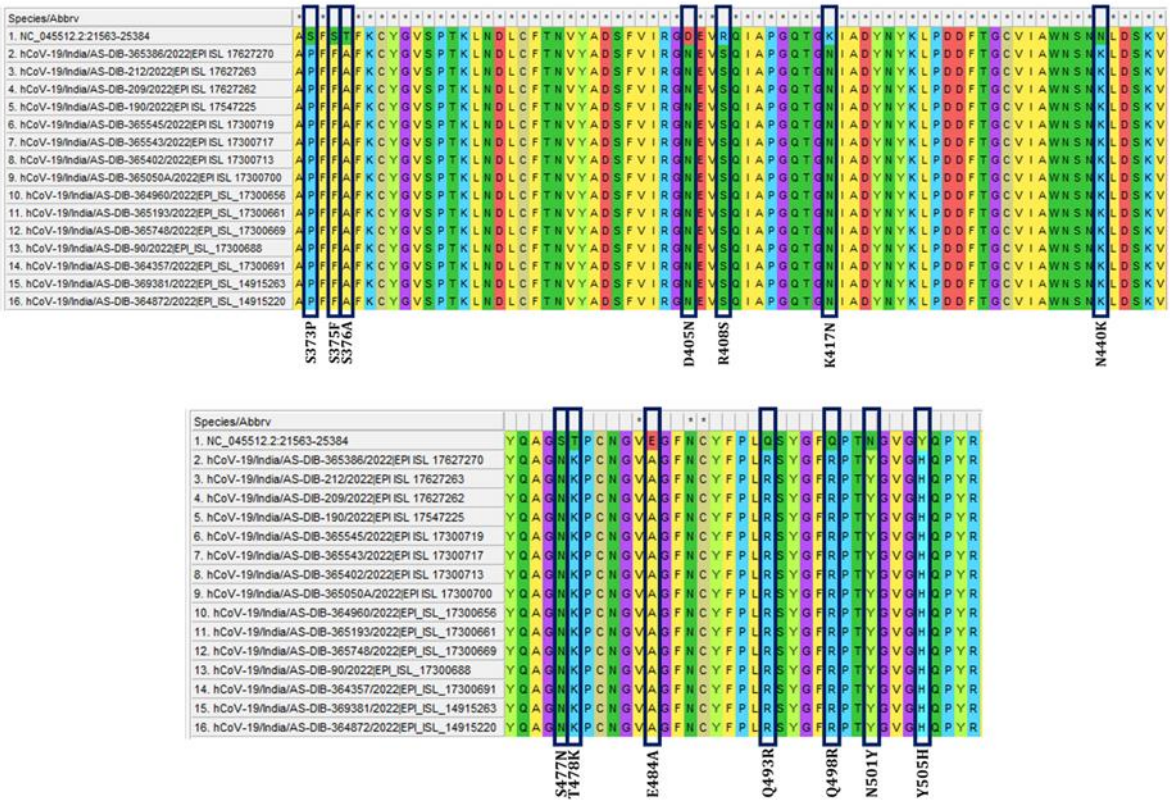
Supplementary Figures



Supplementary Fig. 1: Study design and screening of the participants for the comparative analysis.



Supplementary Fig. 2: Representative spike protein mutations of BA.1 SARS-CoV-2 Omicron variant.



Supplementary Fig. 3: Representative spike protein mutations of BA.2 SARS-CoV-2 Omicron variant.