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COVID-19 breakthrough infections in vaccinated individuals at BPKIHS, Nepal

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Abstract

Background Although there have been reports of COVID-19 breakthrough infections in vaccinated individuals, the vaccines have demonstrated a high efficacy in preventing severe illness and death. Nepal has reported fewer studies of COVID-19 breakthrough infections. Hence, this study has objective to assess the prevalence, and to describe clinical characteristics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) breakthrough infection.

Methods This descriptive study was conducted from January to December 2022. The study enrolled 200 individuals who had received the recommended doses of the COVID-19 vaccine and they were RT-PCR positive diagnosed with vaccine breakthrough infections after 14 days of completing the vaccination course. The patient's demographic and clinical profiles, as well as their outcomes in terms of severity, length of hospital stay, and mortality were recorded.

Results The prevalence of SARS-CoV2 infection was 6.3% (547/8682). Among fully vaccinated personnel, the prevalence of breakthrough infections was 6.2% (200/3175). This study found the Omicron variants in respondents. The mean age of the patients was 38.28 years, and 41.5% (83/200) of the breakthrough cases were healthcare workers. The mean time gap between the second dose of vaccination and a positive RT-PCR test was 354.68 days. Of the 200 breakthrough cases, 89% (178) had mild symptoms, 9% (17) had moderate symptoms requiring hospitalization, and 2% (4) were severe cases that required intensive care facility. Among the severe cases, 3 out 4 were above 60 years old. Furthermore, the patients greater than 60 years had longer hospital stays ($p < 0.0001$) however no deaths were recorded.

Conclusion Fully vaccinated individuals can experience COVID-19 breakthrough infections and the majority of cases present with mild symptoms. Elderly patients have a higher likelihood of severe disease and longer hospital stay compared to younger patients. The results of this study emphasize the importance of vaccination in mitigating the severity of the disease.

Keywords Breakthrough infection, Variants, RT-PCR, Vaccine effectiveness, Clinical outcomes, Nepal

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Background

COVID-19 is the spectrum of clinical manifestations caused by the infection with severe acute respiratory syndrome coronavirus 2(SARS-CoV2). The virus was first isolated and identified in patients who were exposed at a seafood market in Wuhan City, China in December 2019 [1]. Typical symptoms of COVID-19 are fever, dry cough, fatigue, and in severe cases dyspnea often with pulmonary involvement. Many infections in particular children and young adults are asymptomatic or cause mild symptoms while other older people or people with comorbidities are at higher risk of severe respiratory failure or death [2]. In general, natural infection/vaccination is the most effective method for a long-term strategy for prevention and control of COVID-19 but reinfection by the same or different variants in the previously infected individuals and the vaccinated population was reported frequently [3]. Vaccines are still regarded as essential, reasonably priced tools to contain the COVID-19 pandemic, even in the face of the advent of SARS-CoV2 mutations that pose a threat to mass immunization campaigns.

Various types of vaccines like mRNA vaccines, vector vaccines, killed vaccines, etc. are available but the duration of immunity is not known yet. In this context, the term breakthrough infection was coined which means the detection of SARS-CoV2 RNA or antigen in a respiratory specimen collected from a person ≥ 14 days after they have completed recommended dose of a U.S. Foods and Drug Administration (FDA)-authorized vaccine [4].

Breakthrough infections are caused by a variant of concern (VOC) [5]. VOC is a variety for which there is evidence of an increase in transmissibility, severity of the illness, a significant decline in neutralization by antibodies developed during previous contact or immunization, a reduction in the efficacy of therapies or vaccines, or a failure in diagnostic identification [6]. One indication of selection for increased transmission in humans is that several new variants have spread globally and rapidly displaced pre-existing strains. This was first documented for the D614G substitution, which spread around the world in the spring of 2020 and displaced most strains lacking this substitution [7]. More recently, variants first identified in the UK (B.1.1.7 or alpha), South Africa (B.1.351 or beta), Brazil (P.1 or gamma), California (B.1.427 and B.1.429 or epsilon), New York (B.1.526 or iota), India (B.1.617.2 or delta) and South Africa (B.1.1.529 or Omicron) have been believed to be displacing already-existing viral strains as they spread [6]. In light of this, there is a great deal of interest in finding out which variations are more effective in infecting people who have had vaccinations [7].

Globally, the World health organization (WHO) has reported 760 million SARS-CoV2 infections and 6.8 million deaths. With the development and rapid rollout of COVID-19 vaccines, 13 billion people have been vaccinated. The prevalence of breakthrough infection in the United States has been reported within the range of 2–12% [8, 9]. Studies are conducted in the neighboring countries India and Pakistan but our country lacks reports on COVID-19 breakthrough infections. Currently, the vaccines approved by the government of Nepal for emergency use are Oxford-AstraZeneca (including COVISHIELD), Janssen Sinopharm BIBP, Pfizer-BioNTech, COVAXIN, Sputnik V, CoronaVac [10]. According to WHO Nepal, over 40% of the 31 million population have been fully vaccinated till 16th January 2022, and still vaccination programs are ongoing [11].

The local reports of breakthrough infections are of utmost importance and hold significant value in multiple domains, including the development of booster vaccines, assessment of vaccine efficacy against infection, evaluation of the impact of viral variants, and the formulation of recommendations for masking and social distancing for vaccinated individuals. Therefore, this study was conducted to determine the prevalence of SARS-CoV2 infection and to detect the prevalence of COVID-19 breakthrough infection with their socio-demographic, clinical details, and outcomes among individuals tested in this tertiary center.

Materials and methods

We adopted a descriptive study during the period of 1 year (January 2022 -December 2022) to carry out this study. The study has been approved by the Institute Research Committee B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal (Ref no-403/079/080-IRC).

Study population, inclusion, and exclusion

Individuals coming to BPKIHS for SARS-CoV2 testing were eligible for this study. Further, all SARS-CoV2 positive patients tested by RT-PCR and who had received either two doses or a booster dose of vaccine, and had completed a 14-day post-vaccination period as on the date of interview by telephonic method. Also the admitted individuals in the COVID ward were included. Verbal inform consent were taken from the respondents before interview. Individuals who had received only a single dose of vaccine or did not answer the phone calls and who did not give their consent to participate in the interview were excluded from the study. A proforma was utilized that incorporated breakthrough COVID-19 health status details including socio-demographics, vaccination, date of PCR, clinical symptoms, and clinical outcome.

Clinical outcome measures.

a. Severity (mild /moderate /severe).

mild -cold, cough, headache, and associated symptoms.

moderate- symptoms lead to hospital admission.

severe- ICU admission required or not.

Study definition

Vaccine breakthrough infection is considered when detection of SARS-CoV2 by RT-PCR in a person after 14 days of completion of recommended doses by the United States Food and Drug Administration.

Sample size

Based on the prevalence of breakthrough infection of 11.3% reported from Maulana Azad medical college of Delhi, India, considering a 95% confidence interval and 80% power, the calculated sample size using the proportion method was 154. We included all the participants 200 fulfilling the inclusion criteria within the study period of 1 year. Inlusion criteria includes all individualswith SARS CoV2 positive and Exclusion criteria were those individuals who did not gave consent to take part in this study.

Technical details

The nasopharyngeal and oropharyngeal swab was collected in Viral Transport Medium (VTM) CITOSWAB (Citotest Labware Manufacturing Co. LTD, China). The nucleic acid extraction was carried out by a full-Automatic Nucleic Acid Extraction Instrument, 96 Channel, ZK-96 manufactured by Nanjing Zongkebio Medical Technology.

PCR

A real-time reverse transcription PCR was conducted containing specific primers and fluorescent probes targeting ORF1a, E, and N gene SARS-CoV2(Maccura Covid-19 PCR Test kit). The PCR reaction was carried out in 30 µL (MACCURA, CHINA) that includes 12.8 µL of qRT-PCR RXN Mix, qRT Enzyme mix 2.2 µL, internal control 0.5 µL, PCR water 4.5 µL and RNA template 10 µL. The PCR reaction was conducted in Rotor-Gene Q (QIAGEN) Sequncing technology Nanophore MinION. Cycling conditions were Reverse transcription at 55 ^{o}C for 15 min, followed by 95 ^{o}C for 2 min, and 40 cycle of 95 ^{o}C for 15 s, 60^{o}C for 30 s. Each PCR run was validated with positive, negative and internal control. Out of 200 breakthrough cases, virus genome sequencing were done for 3 samples in National public health laboratory (NPHL), Kathmandu and published in GISAID [12] due to resource constraints.

Statistical analysis

All data collected was entered in MS Excel Office 16 and analyzed in IBM Statistical Package for Social Sciences (SPSS) ver. 22. For descriptive analysis mean, median, standard deviation, and percentage proportion were calculated. The Chi-Square test was used to assess the association between two categorical variables. T-test was used for the comparison of two mean values of two independent samples value less than was considered statically significant. In addition, survival analysis of variables were also analysed in R (Version 4.1.2) using "Survival" and "Survminer" package.(https://cran.r-project.org/web/packages/survival/survival).

Results

Altogether 8682 individuals were included in the study. Among them, 6.3% (547/8682) were PCR positive and 3175 were fully vaccinated. The prevalence of breakthrough infection among fully vaccinated personnel was 6.2% (200/3175). Out of 200 breakthrough cases, virus genome sequencing was done for 3 samples in the National public health laboratory (NPHL) Kathmandu and published in Global Initiative on Sharing All Influenza Data (GISAID) [Accession ID: EPI_ISL_9388265, EPI_ISL_9388288, EPI_ISL_9388287] of the breakthrough cases were found to be Omicron variants.

Total 8682 individuals came to BPKIHS for RT PCR, where 3175 were vaccinated and out of these 200 individuals were positive breakthrough infection with where 24 individuals were more than 60 years and 176 individuals were less than 60 as shown in Fig. 1.

Among 200 breakthrough cases, 53.5% were females. The mean age was 38.5±15. A total of 83(41.5%) of the breakthrough cases were healthcare workers. The mean time gap between the second dose of vaccination and RT-PCR positive was found to be 354.68 days. During the study period, a total of 102 (51.0%) individuals had received two doses of vaccine, and 98 (49%) received a booster dose. The majority of the population was vaccinated with COVISHIELD 122 (61%) followed by VEROCELL 60 (30.0%), and PFIZER 18 (9.0%), (Demonstrated in Table 1). Medical comorbidities were present in 12.0% of the breakthrough cases where hypertension was the most common comorbidity accounting for 21 (10.5%) (Described in Table 1).

The study revealed that COVID-19 infection occurred at 310 days among non-healthcare workers and 420 days among healthcare workers after receiving a second dose of vaccination (Fig. 2).

This report showed two peaks in COVID-19-positive cases; the first peak occurred at around 300 days after vaccination, and the second at around 425 days after vaccination.(Fig. 3).

Days of COVID-19 PCR positive after 2nd dose of vaccine was 410 days with COVISHIELD, 340 days with Verocell, and 300 days with the Pfizer vaccine (Figure-4).

A comparison of breakthrough infection events was performed among three different vaccines in the Kaplan-Meier curve. Age is a significant factor in predicting survival probabilities and individuals less than 60 have a

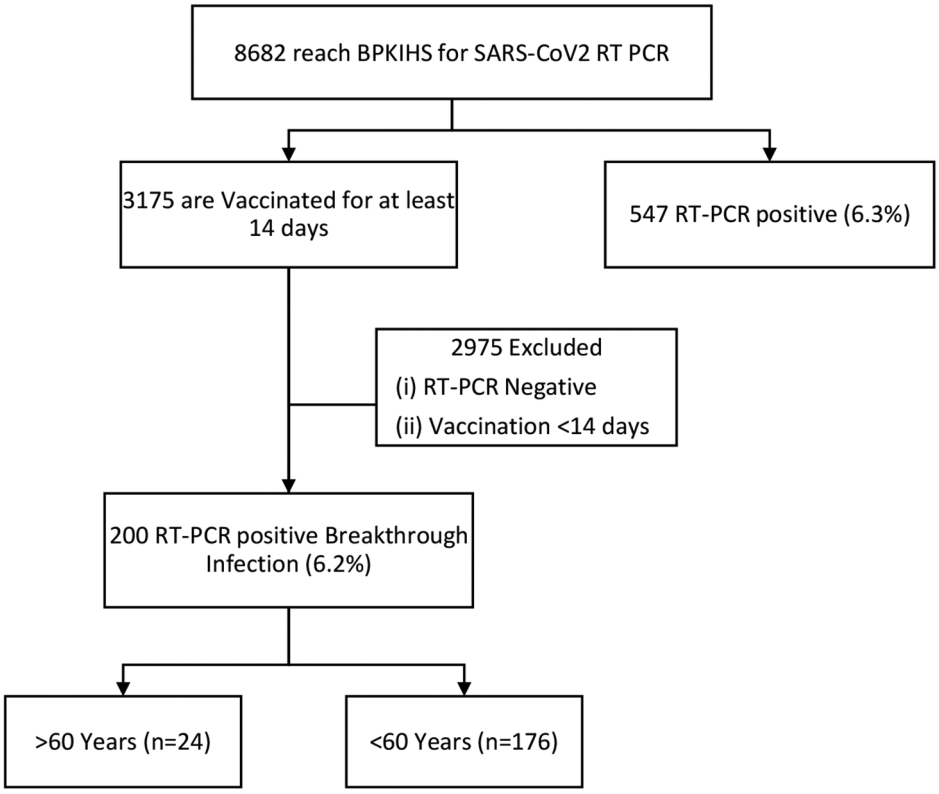

Fig. 1 Flow chart of the studied population

better chance of surviving over time compared to those above 60 years old. By using the Kaplan-Meier curve, our result showed that patients less than 60 years old had constant events and events started in more than 450 days but in above 60 years old had events started in 200 days which were constantly increasing over time (Fig. 5).

Out of 200 COVID-19 breakthrough cases, fever was the most common clinical presentation accounting for 174 (87.0%). Cough was the second leading clinical presentation accounting for 128 individuals (64.5%) followed by common cold 121 (60.5%), sore throat 55 (27.5%), myalgia 79 (39.5%), headache 56 (28.0%) and shortness of breath accounting for 20 (12.3%). We found only two individuals 2 (1.0%) ageusia. Regarding the impact of breakthrough COVID-19 infections, it was observed that individuals who have been fully vaccinated and still contracted the virus were less likely to suffer from severe illness or mortality. Among the total number of cases studied, 89% experienced mild symptoms, 9% developed a moderate form of illness and 2% experienced severe symptoms that required hospitalization. Out of the 178 cases that were self-isolating at home, none were reported to have acquired secondary infections that required hospitalization and were treated as outpatients.

Our investigation further unveiled that hospitalization duration is positively correlated with increasing age; wherein advanced age is associated with an extended period of hospital stay. (Figure- 6)

Discussion

This study was carried out during the period of the COVID-19 third-wave outbreak which swept through Nepal from January to December of 2022. The prevalence of breakthrough infection was 6.3%. The gene sequence analysis identified the SARS-CoV2 omicron variant during this period. Omicron is the most highly mutated variant compared to other variants of SARS-COV2 which is highly infectious and spreads rapidly and causes less severe disease than the other variants [13].Specifically, Omicron was estimated to be 36.5% more transmissible than Delta in a South African study. Additionally, research from South Africa, the UK, and Denmark indicates that Omicron has a reproduction number (R0) of 7 or higher, compared to 5 for Delta and 2.8 for the wild SARS-CoV-2, and a three to four times higher risk of infection than Delta [13].

The overall prevalence of COVID-19 breakthrough infections documented in this study is slightly lower than another report from the central part of the country by

Table 1 Socio-demographic details of the individuals with breakthrough infections

Parameter		*N* = 200 (%)	Mean ± SD (*N* = 200)
Age (mean ± SD)			38.28 ± 15.13
Age Group Distribution	< 15 years	1(0.5%)	
	15–30	79(39.5%)	
	31–45	71(35.5%)	
	45–60	25(12.5%)	
	Above 60	24(12.0%)	
Duration of a gap (days) between the second dose vaccination and positive RT-PCR			354.68 ± 86.21
Gender, no (%)	Male	93 (46.5%)	
	Female	107 (53.5%)	
Healthcare worker, n (%)		83 (41.5%)	
Name of Vaccine	COVISHIELD, n (%)	122 (61.0%)	
	VEROCELL, n (%)	60 (30.0%)	
	PFIZER, n (%)	18 (9.0%)	
Two doses of Vaccine, n (%)		102 (51%)	
Booster Dose, n (%)		98 (49%)	
Comorbidities	HTN	21 (10.50%)	
	Diabetes Mellitus	11 (5.5%)	
	COPD	3 (1.5%)	

The value is expressed as n (%) or mean ± SD, for normally distributed and median, IQR for not normally distributed.

Pokharel K et al. [14] and a neighboring country India by Tyagi K et al. [15] and Maulana Azad Medical College of Delhi by Sharma P et al. [16] However, other studies from India by Arora G et al. [17], and USA by Kerwin H et al. [18], reported a prevalence of 7.91% and 5.5% were similar to our result. Most strikingly, less than 1% of people who have received all recommended vaccinations have been recorded to get a breakthrough infection in August 2021, according to the Centers for Disease Control and Prevention (CDC).

This study depicted the majority of breakthrough infections were encountered in the middle-aged group which was 38(SD-38.28 ± 15.13) with 12 years in children and 82 years in older age. A finding similar to other studies that also have observed a higher rate of breakthrough among the middle-aged group in the United Arab Emirates by Dash NR et al. [19] This increased risk may be attributed to various social behaviors, such as increased contact with unvaccinated minors, higher frequency of social and work-related interactions, and potentially a lower perception of risk compared to the older age group.

According to our study, the overall ratio of female to male breakthrough infections was 1.15:1.Women may be more susceptible to infection because of their responsibilities as caregivers, both at home and in the healthcare system.In addition, women are more likely to provide care for sick relatives or children. Also Physiological, genetic, and behavioral variables can all be linked to the reasoning behind it [20, 21]. Males are less likely than females to experience a breakthrough infection, according to comparable research by Almufty HB et al. [22]

In our study, 200 people had three immunizations out of the many vaccines available. People who attend BPKIHS frequently receive the vaccinations of COVISHIELD, VEROCELL, and Pfizer. Most people received COVISHIELD (n = 122), followed by VEROCELL (n = 60) and Pfizer (n = 18). According to our study, people with COPD, diabetes, and hypertension are the three most common co-morbid conditions. Hypertension was the most prevalent comorbidity among breakthrough infections in research of a similar nature by KAMAL et al. [23].A weakened immune response and increased rates of morbidity are associated those with co-morbidities, putting them at increased risk for the severity of SARS-CoV-2 [24].

In this study, we found that non-healthcare workers contracted COVID-19 infections within a shorter period (310 days) in compared to healthcare workers (420 days) after the vaccination. This may be due to differences in adherence to preventive measures, such as social distancing, mask-wearing, and other precautions, between healthcare workers and non-healthcare workers, which may explain the observed discrepancy in the timing of COVID-19 infection [19].

Our study showed that there were two peak periods where individuals may be at higher risk of contracting COVID-19 after receiving a vaccine. This may be due to changes in individual behavior, changes in testing practices, and other factors such as changes in the population demographic, emergence of other co-infections, or environmental factors [25]. Based on published results from various vaccine trials and other data sources, it has been estimated that people immunized against COVID-19 would lose about half of their defensive antibodies every 108 days [26]. As a result, vaccines that initially offered 90% protection against mild cases of the disease may be only 70% effective after 6 to 7 months [26]. The period between complete COVID vaccination and COVID PCR positive was different among individuals receiving various types of vaccine. This suggests that vaccine type may play a vital role in the effectiveness of protection against COVID-19 infection. Our report showed that COVISHIELD recipients have the longest period of 410 days for breakthrough infection after vaccination. This may be attributed to multiple factors such as varying levels of efficacy exhibited by each vaccine, as well as individual differences, including age and underlying health conditions [27].

Our findings demonstrated that patients under the age of 60 had consistent breakthrough events that began more than 450 days of vaccination, while those above 60

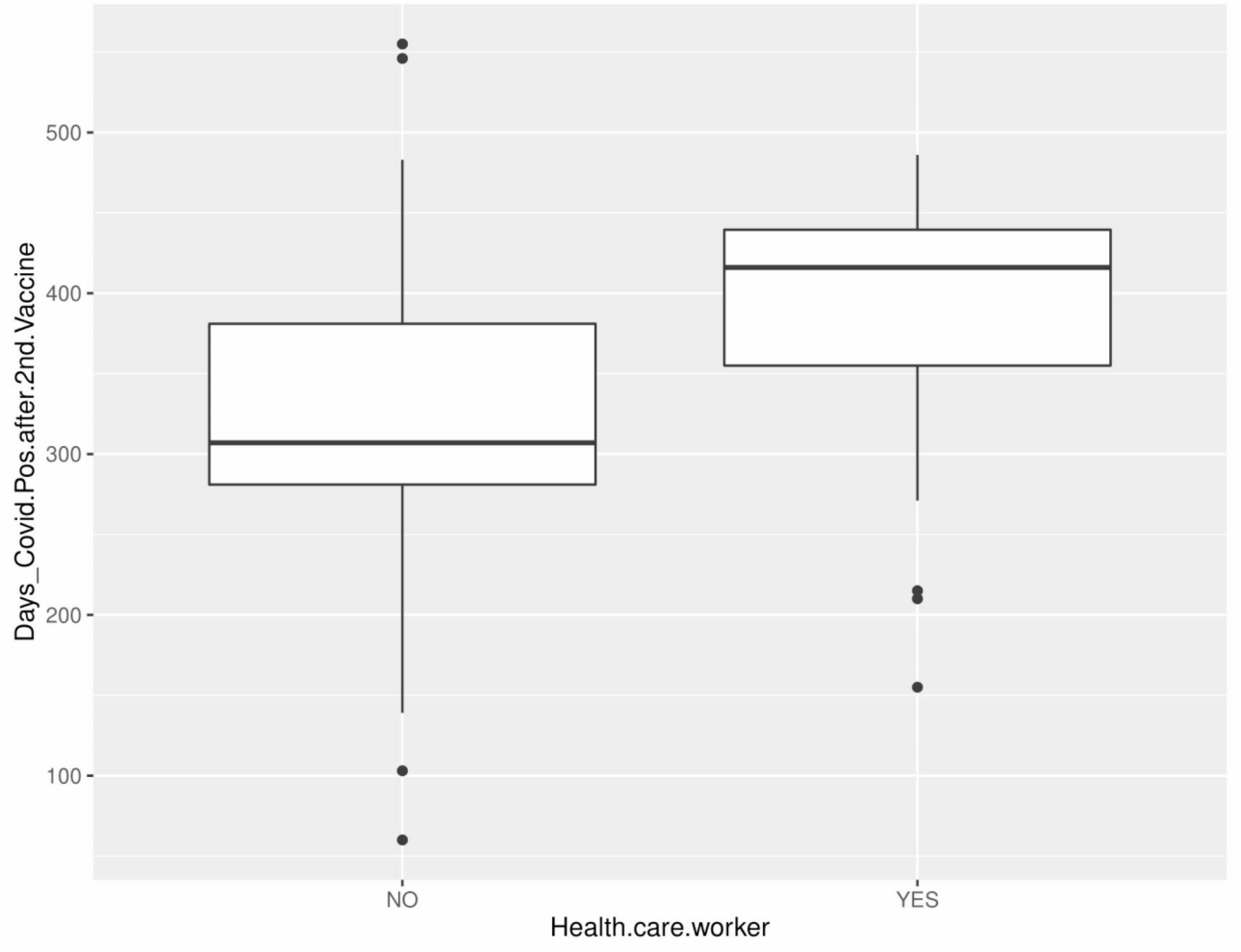

Fig. 2 Comparison among healthcare workers and non-healthcare workers after the second dose of vaccination

experienced similar events earlier beginning from 200 days which increased with time. Age has a vital role in forecasting the likelihood of survival, and those under the age of 60 have a higher probability of surviving through time than people above the age of 60.

Fever was the most frequent clinical manifestation, accounting for 174 (87.0%) of the 200 COVID-19 breakthrough cases. The second most frequent clinical symptom, cough, was reported by 128 people (64.5%). Our findings align with a study conducted by Kamal et al. [23] which reported similar results.

Our study demonstrated that the vaccines COVISHIELD, VEROCELL, and Pfizer reduced the severity of breakthrough illness in terms of hospitalization, ICU admission, and mortality with the majority of the mild presentations. Our finding parallels the results from different countries including Pakistan by Mahroof S et al. (94%) [28], studies from India by Dash GC et al. (83%) [3] and (94%) by Sharma P et al. [16] in New Delhi, and the USA by Uschner D et al. (85%) [29] of asymptomatic COVID-19 breakthrough cases. The frequency of hospitalization in this study due to moderate to severe symptoms was similar to reports from the eastern state of India by Dash GC et al. [3] 9.9%, centers in Bihar, India by Singh CM et al. [30] 10%, and Spain by Turabian JL et al. [31] 11%. But the cases of hospitalization were slightly lower than a report from India by Niyas VK et al. [32] which was 5.5%. These reports from different regions mirror the impact of vaccination in limiting disease severity. There was no mortality reported in our study. A similar clinical outcome of no mortality was observed in studies from many regions [14, 33, 34].

Our study also elucidated that the younger age group showed higher survival probabilities than the older group above 60 years. Older age may be impacted by various determinants, such as age-related factors and pre-existing medical conditions, the intensity of breakthrough infections, viral load, emerging strains of the virus, and immunization status [31]. Among the severe cases with ICU admission, 75% of breakthrough cases were above 60

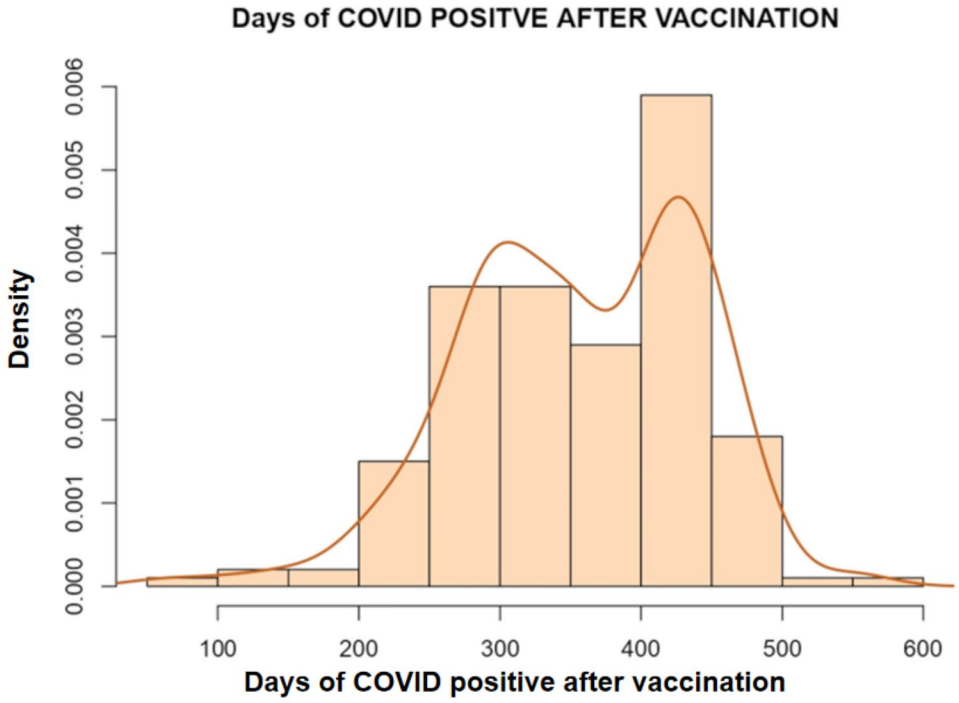

Fig. 3 Days of PCR positive among individuals after vaccination. (Density = Frequency/total population)

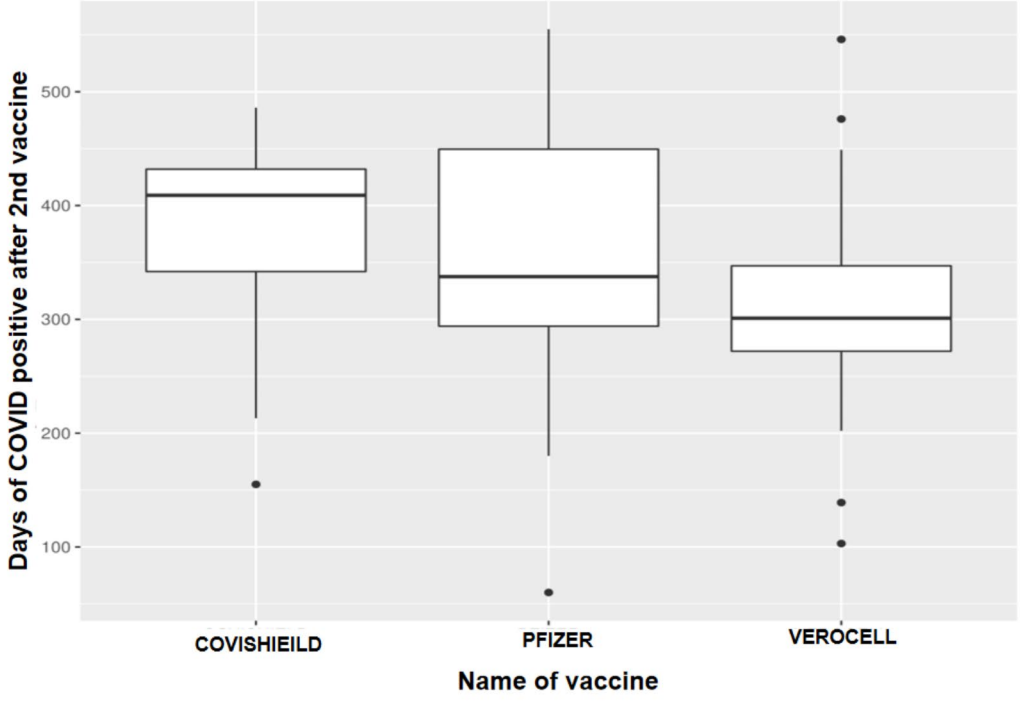

Fig. 4 Comparison between three vaccination and the result of PCR positive among individuals

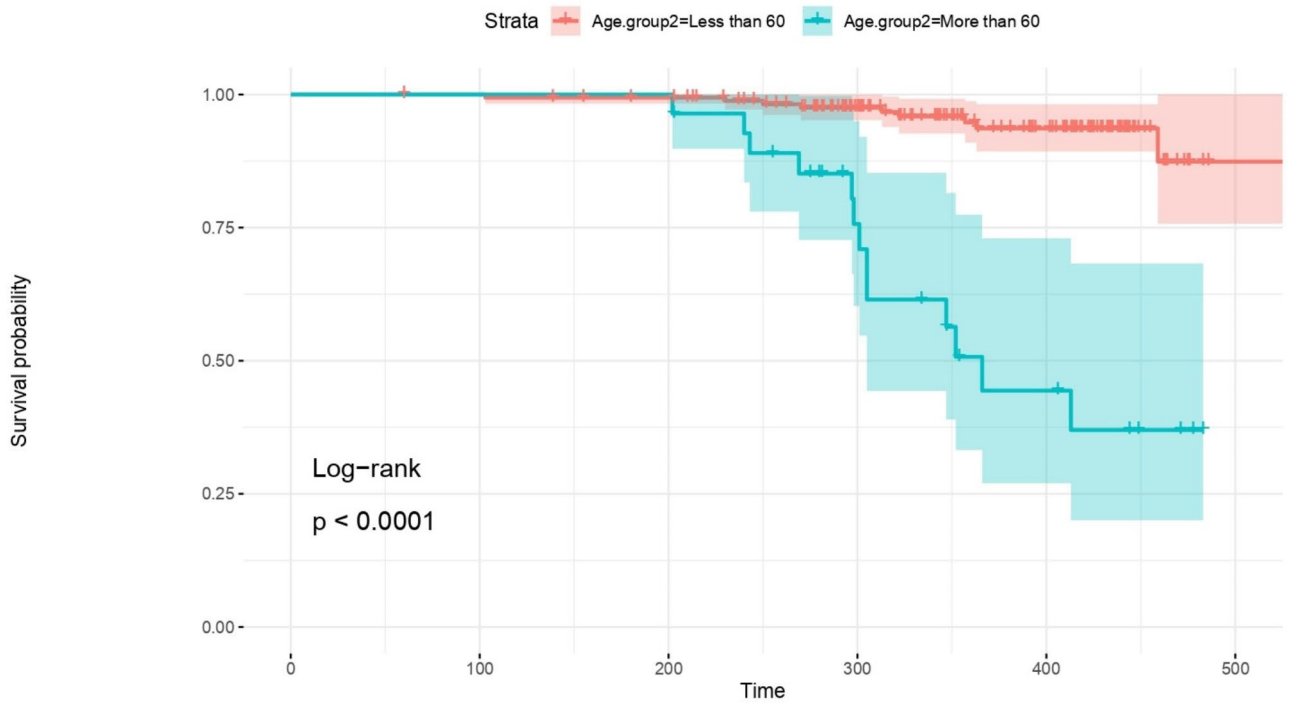

Fig. 5 Comparison of survival probability among the age > 60 years and < 60 years

Table 2 Prognosis and outcomes of COVID-19 infection > 14 days after vaccination

Parameter	*N* = 200 (%)	< 60 year	> 60 year	Test statistics	*P*-value
Severity of Disease					0.000
Mild, n (%)	178(89.0%)	165(92.69%)	13(7.30%)	$X^2 = 33.80$	
Moderate, n (%)	18 (9.0%)	10(55.55%)	8(44.44%)	$X^2 = 19.71$	0.000
Sever, n (%)	4 (2.0%)	1(25%)	3(75%)	$X^2 = 15.34$	0.006
Hospitalization, n (%)	22(11.0%)				
Length of stay, (mean ± SD)	4.6 ± 2.53	3.909 ± 2.38	5.363 ± 2.57	t = 1.37	0.185
Improvement					
Home Isolation	178 (89.0%)				
Discharge on request					
Death, n (%)	0				

The values are expressed as n (%), mean ± SD.

years. Further analysis also revealed a positive correlation was observed between age and the duration of hospital stay, indicating that as age increased, the length of hospitalization increased. This suggested that older individuals tended to have longer hospital stays compared to younger individuals. Similar studies conducted in Korea by Jang SY et al. [35] have reported that the average length of hospital stays is longer in the older age group, indicating that advanced age is a significant factor contributing to an extended duration of hospitalization.

Limitations

Despite documentation of breakthrough infection within the short period in our setup, this study had a few limitations including a small sample size and the absence of a control group for comparison, restricting the assessment and comparison of risk factors and outcomes in vaccinated versus unvaccinated individuals. However, our study findings indicate that getting vaccinated against COVID-19 is a useful method for reducing the chance of developing severe disease and the risk of hospitalization. Therefore, we recommend the maximum coverage of COVID-19 vaccination for the public as the benefits of vaccination are greater but their efficacy should be thoroughly observed with a large number of individuals concerning various types of vaccines to explore their explicit efficacy for future use.

Conclusion

SARS-CoV2 infection rates at this hospital are 6.3%. According to our data, 6.2% of COVID-19 infections were breakthrough infections, the majority of which were moderate cases with few hospitalizations and no reported morbidity. This implies that COVID-19 breakthrough infection can happen in people who have had

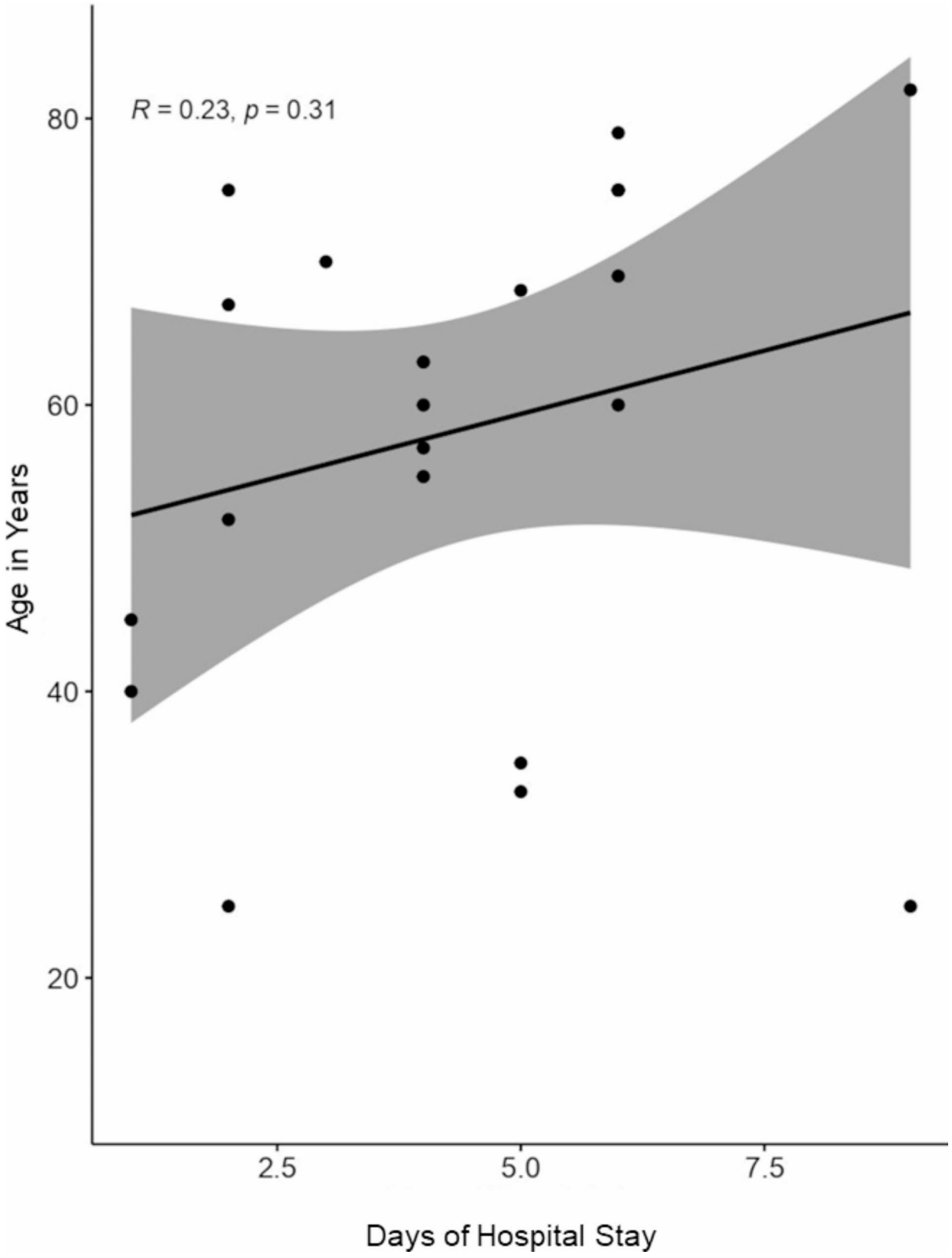

Fig. 6 Length of hospitalization according to age

vaccinations but with less severe infection. The older age group of those over 60 had a larger proportion of serious infections and a longer hospital stay as compared to the younger age group. Overall, our results point to the effectiveness of vaccination in reducing severe COVID-19 clinical outcomes, although it is still possible for infections to emerge and care should be taken, especially among susceptible groups.Measures include maintaining the public's compliance with preventive measures, ensuring adequate healthcare infrastructure and education regarding such cases, and expanding the programs to promote booster immunization of the susceptible groups. Some of the challenges that can be resolved, for instance, by appealing to the importance of its continuation, to get more help, and to explain more include fatigue, lack of funds, and vaccine reluctance. These tactics will enable BPKIHS to effectively manage and reduce some impact of breakthrough infections particularly among vulnerable groups.

Author contributions
All authors reviewed the manuscript.

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None.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethics approval from Institutional Review Committee(IRC), B.P. Koirala Institute of Health Sciences, Dharan, Nepal. Code-IRC/2303/022. Consent were obtained by written consent and verbal consent with Telephonic interview.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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