Original Article

SARS-CoV-2 Reinfections among Fully Vaccinated and Unvaccinated individuals: 7 months surveillance data

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ABSTRACT

The emergence of SARS-CoV-2 variants with a high degree of transmissibility and spread globally, even in areas with high vaccinations rate, spot concern about vaccine breakthrough infections by newly emerging variants worldwide. Data regarding SARS-CoV-2 vaccine breakthrough infections are scarce in Iraq. We aimed to evaluate SARS-CoV-2 breakthrough infection in our population following five-month since the first administration of COVID-19 vaccines in March 2021. We prospectively studied breakthrough infections among referred patients at the Erbil central public health laboratory, from August 1, 2021, to February 28, 2022. Relevant demographic and clinical information were collected alongside PCR testing, measuring Ct value and variant detection by Real-Time RT-PCR. The study comprised 30759 eligible participants, 22682 (73.7%) unvaccinated and 8067 (26.2%) vaccinated. Totally 2133 participants confirmed positive for SARS-CoV-2, with a 6.9% overall positivity rate. Among vaccinated participants, 933 (11.6%) vaccine breakthrough infections have been identified. A significant difference ($p < 0.01$) in PCR cycle threshold values was observed between vaccinated and non-vaccinated people. Vaccine breakthrough infection was detected with all three current authorized vaccines in Iraq. A variant study revealed Delta B.1.617.2 and Omicron (BA1, BA2) as causes of vaccine breakthrough infections, with dominance at different time points. Study results conclude that as population immunity levels increase by vaccination, vaccine breakthrough infections increase in parallel. Most of the breakthrough infections in Iraq are produced by current circulating variants of concerns Omicron and Delta variants.

Keywords: SARS-CoV-2, COVID-19, Variants of Concern (VOCs), Vaccine Breakthrough Infections, PCR Positivity Rate

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused COVID-19 since its emergence in December 2019, inducing a worldwide pandemic in a short time that has not previously affected the world since 1918 (1,2). Despite the significant progress in the global COVID-19 response for prevention and treatment approaches to this new disease, SARS-CoV-2 remains circulating and does not seem to disappear easily, even with multiple successful vaccines developed. SARS-CoV-2 is evolving, and newly emerging variants significantly change the virus properties, which can cause rapid spread into the population, increasing cases that require hospitalization. Worryingly, the current vaccines or therapies may become less effective for some emerging variants (2,3).

Emergent SARS-CoV-2 variants of concerns (VOCs), such as B.1.1.7 (Alpha), B.1.617.2 (Delta), and B.1.1.529 (Omicron) with advantageous mutations that resulted in higher infectivity and transmissibility of the virus, led to broadening questions regarding the effectiveness of available vaccines. Furthermore, VOCs' reporting of vaccine breakthrough infections is increasing globally (4, 5, 6).

Following vaccination began around January 2021; vaccine breakthrough infections were rare (<0.1 of COVID-19 cases). However, over more than a year of the vaccination process, the pattern changed (7). Reports of a substantial increase in vaccine breakthrough infection in many countries worldwide (e.g., France, the UK, Israel, Qatar, and the USA) have been recorded (7,8,9,10).

The vaccination campaign in Iraq started on March 10, 2021. Three vaccines were used: one mRNA vaccine (Pfizer-BioNTech

mRNA BNT162b2, USA), one recombinant viral vector adenoviruses (AstraZeneca ChAdOx1-S, Oxford University, UK), and one inactivated virus vaccine, SARS-CoV-2 Vaccine (Vero Cell), Inactivated (lnCoV), Sinopharm (Beijing, Wuhan)/BIBP. After that, Iraq was hit by the three waves of the COVID-19 epidemic: the second wave (March and April 2021), dominated by the Alpha variant; the third wave (from July and August), overwhelmed by the Delta variant; and the fourth-wave (January and February 2022) with a surge of the Omicron variant (11,12).

According to the World Health Organization (WHO), up to March 15, 2022, around 455 million recorded COVID-19 cases, and more than 6 million fatalities had been recorded worldwide. In Iraq, as of March 21, 2022, 2,316,044 recorded cases of SARS-CoV-2 (13), including 435,865 patients in the Kurdistan region. Studies are scarce regarding the vaccine breakthrough infections in Iraq; therefore, this prospective study aims to asses the incidence of SARS-CoV-2 infection after a vaccination campaign in the Erbil province /Kurdistan region of Iraq for a period of 7 months (August 2021– February 2022) to characterize the likely incidence of breakthrough infections in our population.

Materials and Methods

Study Design and Participants

This study was initiated on August 1, 2021, in the Molecular diagnostic unit at the Central Public Health Laboratory (CPHL), the main lab provider for COVID-19 testing in Erbil City. Data were collected until February 28, 2022. The study aimed to identify any breakthrough infection among all suspected cases of Covid-19 referred to the CPHL during the study period. Participants were considered unvaccinated if they had not received any COVID-19 vaccine dose at least seven days prior to participation, and partially or fully vaccinated if they had received one or two doses of COVID-19 vaccine respectively at least seven days before study enrolment (10) Vaccine breakthrough infections were defined as a positive SARS-CoV-2 PCR ≥14 days after receiving one or two doses of the vaccine. Relevant clinical information was collected upon recruitment, including patients' vaccination status (type and number of doses), age, and sex. Patients who were uncooperative or non-consenting were not included in this study. The someones who were not interested were excluded from the study. Individuals who accepted or did not accept the vaccine were eligible to participate in this study**.**

SARS-CoV-2 RT-PCR

Following World Health Organization guidelines, trained laboratory technicians collected nasopharyngeal and/or throat swab samples from the enrolled patients and examined them for SARS-CoV-2 by real-time RT-PCR. Viral RNA was extracted from the transport medium using the Viral Nucleic Acid Extraction kit (B-200-32), Zybio Nucleic Acid (DNA/RNA) Isolation System EXM3000 (Zybio Inc), or SphaeraMag DNA/RNA Isolation Kit on automating Phoenix-Pure96 system (Procomcure Biotech GmbH com.) per instructions of the manufacturer, and subjected to RT-PCR on "Rotor-Gene Q (QIAGEN) Real-Time PCR Detection System". Real-time RT-PCR was done utilizing LightMix. ®. SarbecoV E-gene plus EAV control and LightMix Modular SARS-CoV-2 (COVID-19) RdRP-gene (TIB Molbiol/Roche Diagnostics, Germany, 5 µL aliquots), or SARS-CoV-2 Nucleic Acid Detection Kit (Zybio Inc) targeting ORF1ab and N gene. To reduce real-time RT-PCR false-positive and false-negative results multiple controls (no template control, no extraction control, positive template control) were included in each RT-PCR reaction.

Variant Detection by Real-Time RT-PCR

To characterize variants of concern, 280 positive samples with cycle thresholds less than 30 were randomly selected and retested using PowerChek SARS-CoV-2 S-gene Mutation Detection Kit Ver.3.0 (Kogen Biotech, Korea). The test is based on the search for (K417N, L452R, E484A / K, N501Y, T547K, P681R) key mutations in SARS-CoV-2 S-gene, which allows the detection of all the variant of concerns (VOCs); Omicron (BA.1, BA.2), Delta, Alpha, Beta, and Gamma.

Statistical analysis

Statistical analyses were achieved using descriptive statistics, and a chi-square test was used to measure statistical significance for the entire work. They were considering a $P \leq 0.05$ or $P \leq 0.01$ as a significant level.

Results and Discussion

During the study period, from August 1, 2021, to February 28, 2022: A total of 30759 eligible participants were prospectively enrolled in the study and underwent RT-PCR tests for SARS-CoV-2. Of those, 2133 participants tested positive for SARS-CoV-2 by RT-PCR assay, with a (6.9%) overall positivity rate. Totally the ratio of vaccinated persons (26.2%) in the study was lesser than un-vaccinated; 22682 (73.7%) individuals were unvaccinated, 6149 (20.0%) received two-dose, 1896 (6.2%) had received one dose, and 0.1% (22) participates received booster dose. A monthly positivity rate of SARS-CoV-2 infection revealed a higher detection rate in August (9.9%), with a decline in the following months reaching (2%) in December 2021, followed by a sharply increasing rate in January 2022 (8.9). **(Figure 1)**

In accordance with the national vaccination campaign, we restricted the analyses to those aged ≥18 years. Among the 2133 positive cases, 1170 (54.9%) were male, 963 (45.1%) were female, with median age (range) of 47.5 (2 – 93) years, 1190 (55.8%), 691 (32.4%), and 220 (10.3%) were unvaccinated, fully vaccinated, partially vaccinated respectively. Collectively participants aged between 18 and 49 years represented 69.0% of infected individuals in our study population. The highest infection rate, 647 (30.3%), was detected in the age group 30 –

39, and the lower rate, 31 (1.5%), was in the age group ≥80. **(Table 1)**

Table 2 shows the comparison of PCR positivity incidence between vaccinated and non-vaccinated individuals (controls). The higher percentage of PCR positivity incidence was in the vaccinated group (11.6%), which is significantly different statistically ($p < 0.05$) from the non-vaccinated group in the distribution of the PCR positivity rate.

Of the 691 fully vaccinated breakthrough cases, 426 had obtained the Pfizer/BioNTech (BNT162b2 mRNA) vaccine, 164 the ChAdOx1 nCoV-19 adenovirus vector vaccine (Oxford/AstraZeneca), and 101 the Sinopharm inactivated virus COVID-19 (BBIBP-CorV). While in 220 partially vaccinated infected contributors, 115 had obtained the Pfizer/BioNTech mRNA vaccine (BNT162b2), 59 the ChAdOx1 nCoV-19 adenovirus vector vaccine (Oxford/AstraZeneca), and 46 the Sinopharm inactivated virus COVID-19 vaccine **(Figure 2)**.

To analyze the relationship between vaccination and reducing viral load in the frame of the SARS-CoV-2 vaccine breakthrough, the study compares the threshold cycle (Ct) value of positive results among 676 unvaccinated and 454 vaccinated individuals. A significant difference (P=0.002) in Ct values was observed between unvaccinated and fully vaccinated individuals. Low Ct values (<25) were identified in 530 of 676 unvaccinated (78.4%) and 329 of 454 fully vaccinated individuals (72.5%). The lowest Ct values of (12.3 and 12.7) were detected in unvaccinated and vaccinated individuals, respectively. Moreover, in fully vaccinated individuals, 14 (3.1%) of breakthrough infections had too low Ct values <15, compatible with extremely high viral loads **(Table 3)**.

Analysis of 280 isolates for variant distributions revealed the circulation of the Omicron and Delta variants with the predominate of Delta B.1.617.2 over August until near the end of December 2021 when replaced by the Omicron BA1 in January 2022. Totally 140 (50%) were identified as the Delta B.1.617.2 variant, 94 (33.6%) as Omicron BA1, and 19 (6.8%) as Omicron BA2, while 27 (9.6%) were Inconclusive **(Figure 3)**.

In this prospective study, we characterized all Covid-19 vaccine breakthrough infections among a representative population of Erbil city during the seven months after began of the vaccination program by five months in Iraq. Among the 2133 participants who confirmed positive for Covid-19, most were non-vaccinated (73.7%), yet (26.2%) were vaccinated, suggesting a good level of protection by vaccination against Covid-19 infections. However, we found a high rate of breakthrough infection (11.6%) among the vaccinated group, which differs significantly from the non-vaccinated group (5.2%).

A recent study conducted in Qatar (8) estimated a 0.15% cumulative infection incidence among BNT162b2-vaccinated individuals. Another study by P. Elliott *et al*. (14), released on November 2, 2021, reported a ratio of 0.4% among those who had received two vaccine doses. While a study achieved by Singanayagam A. *et al*. (10) declared that the incidence was 25% in fully vaccinated people compared with 38% in non-vaccinated people among exposed household contacts. This ratio variation could be because no vaccine is perfectly effective; vaccine type, time since vaccination, and some other reasons can affect vaccine effectiveness and, thus, the possibility of breakthrough infections (15,16). It is obvious that various levels of immunity are induced following immunization by different types of COVID-19 vaccines. Also, the level of protection provided by vaccine-induced immune responses may also be affected by the variant types of SARS-CoV-2 to which one is exposed (17).

Evidence suggests the impact of SARS-CoV-2 variants of concern in increasing vaccine breakthrough Infections. Mutations in the variants of SARS-CoV-2, particularly in spike protein, caused an increase in infectivity, transmissibility, and immune escape of the virus (7,17,18). Our study identified currently circulating VOCs (WHO) Delta and Omicron (BA1 & BA2) in our population with a similar prevalence in the vaccinated and non-vaccinated cases. We found a rising prevalence of infection associated with these two VOCs in two windows of time; first in August 2021, related to the altering of Alpha by Delta variant, and second in January 2022 with the predominant Omicron variant. This finding is in line with multiple reports around the world that showed a surge in cases due to vaccine breakthrough infections with the emergence of these two variants of concern (4,10,19).

A significant difference in PCR cycle threshold (Ct) values between fully vaccinated and unvaccinated individuals were observed in this study at the time of the test. Our results are consistent with previous studies that found a higher Ct value in vaccinated individuals than in unvaccinated, with the majority of infections being caused by the Delta or Omicron variant (9,14,21). In contrast, several studies have revealed no significant difference in cycle threshold values between vaccinated and non-vaccinated people with delta variant infections (22,23,24). Ct is a common quantitative measure of viral load (higher numbers indicate smaller levels of viral RNA) that is often used as a measure of infectiousness. However, in consideration of other infectiousness measures like antigen tests or viral culture, studies found that the probability of a test positivity for a given Ct value is lower in vaccinated than in unvaccinated individuals (17,25,26). The greater Ct values within vaccinated individuals may mean lower infectiousness, but as Ct values are a proxy measure of viral load and shedding, the interpretation of Ct values is further complicated by other factors related to host and viral, like the variant, vaccination status, age, and viral clearance, with considerable variation between individuals (9,10,17,27).

It is obvious that various levels of immunity are induced following immunization by different COVID-19 vaccines. Thus they provide varying effectiveness (18,28). We observed associated vaccine breakthroughs with all three current authorized vaccines in Iraq. The more significant percentage of infection was among individuals vaccinated with the Pfizer/BioNTech mRNA vaccine (BNT162b2). This is broadly due to the predominance of the Pfizer/BioNTech vaccine over the two other vaccines (AstraZeneca and Sinopharm). We noted

a 1% proportion of infections among persons who received vaccine boosters, here a question rising about whether administering a third dose will provide enhanced long-term immunity against breakthroughs or whether the immunity will wane over several months of time (17,21, 29).

As a matter of fact, subsequently the start of the SARS-CoV-2 pandemic has been difficult to control, and in the light of the recent identification of the B.1.1.529 (Omicron) variant, which rapidly spread worldwide and the discovery of Omicron XE (a recombinant of the Omicron BA.1 and BA.2 strains) is a reminder of the continuation of this challenge. To conclude, several factors may contribute to the increase in breakthrough cases; SARS-CoV-2 variants circulating at a given time, an increase in vaccination rate, possible immunity waning with time, and losing non-pharmacological interventions, such as mask-wearing in the community. Finally, our study describes vaccine breakthrough COVID-19 infections in Erbil province of Iraq after five-month of applying vaccination camping. The finding of the study provides somewhat an understanding of the current epidemiology and disease course of the SARS-CoV-2 virus in Iraq, which may fill some existing knowledge gaps.

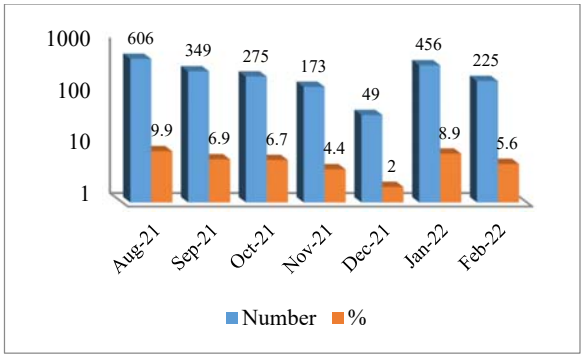

Figure 1. Monthly distribution of totally SARS-CoV-2 PCR positivity rate

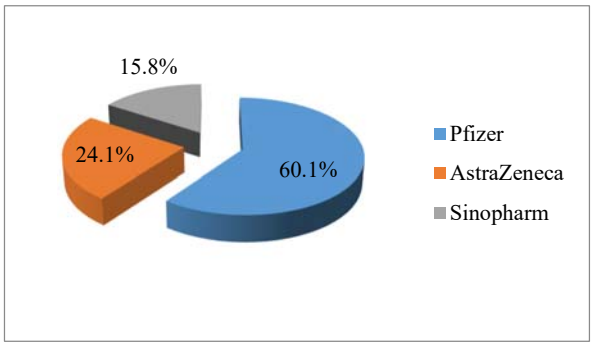

Figure 2. The proportion of vaccine types in 933 SARS-CoV-2 positive samples

Table 1. Characteristics of SARS-CoV-2 cases according to gender, age, and vaccine status (n=2133)

		Months								
		Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Total	
Character									No.	%
Gender	Male	362	198	140	95	23	241	111	1170	54.9
	Female	244	151	135	78	26	215	114	963	45.1
Age group (years)	< 10	21	12	11	4	1	3	6	58	2.7
	10 – 17	36	23	19	21	3	12	8	122	5.7
	18 – 29	123	77	49	24	10	64	42	389	18.2
	30 – 39	198	107	65	44	15	155	63	647	30.3
	40 – 49	106	54	55	41	9	113	58	436	20.4
	50 – 59	61	32	34	25	4	54	24	234	11
	60 – 69	32	23	29	4	1	37	14	140	6.6
	70 – 79	21	11	8	5	2	16	3	66	3.1
	≥80	8	10	5	5	1	0	2	31	1.5
	Unknown	0	0	0	0	3	2	5	10	0.5
Vaccine status										
	Unvaccinated	445	222	174	102	24	158	65	1190	55.8
	Full vaccinate	92	70	68	53	17	251	140	691	32.4
	Partial Vaccinate	69	57	33	18	8	27	8	220	10.3
	Booster	0	0	0	0	0	12	10	22	1
	Unknown	0	0	0	0	0	8	2	10	0.5
Total		606	349	275	173	49	456	225	2133	100

Table 2. Incidence of SARS-CoV-2 PCR positivity between vaccinated and non-vaccinated subjects

Parameter	Vaccinate	%	Unvaccinated	%

PCR (+)	933	11.6	1190	5.2
PCR (-)	7134	88.4	21492	94.8
Total	8067	100	22682	100

Table 3. Distribution of Ct values, according to vaccine status

CT value	Unvaccinated		Fully Vaccinated	
	Number	%	Number	%
< 15	37	5.5	14	3.1
15 - 19	200	29.6	101	22.3
20 - 25	293	43.4	214	47.1
26 - 30	126	18.6	115	25.3
> 30	20	2.9	10	2.2
Total	676	100	454	100

X^2: 16.08578 P: 0.0029

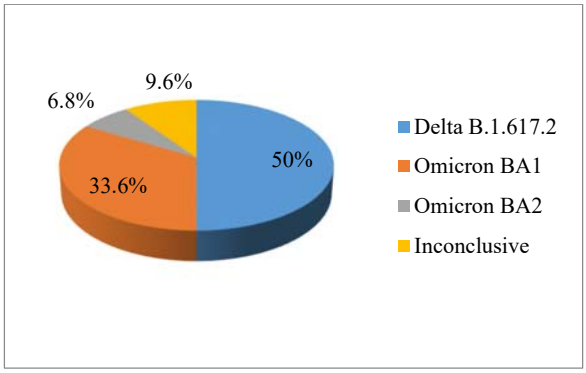

Figure 3. Frequency of SARS-CoV-2 variant of concerns identified across the time of the study

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Conflict of interest: None

Financial support: None

Ethics statement: Permission was obtained from the Erbil Health directorate authority to conduct this study and collect specimens and data from patients hospitalized at CPHL-Erbil in the context of national COVID-19 surveillance. All participants were aware of the study's aims and could refuse to participate if they wished to do so. Upon enrolment, verbal permission was acquired from all participants. Parents gave permission to children.

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