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COVID-19 breakthrough infections in rheumatic diseases patients after vaccination

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Abstract

Background: Rheumatic diseases patients receiving Rituximab had severe COVID-19 disease. Although they had impaired humoral immune responses following COVID-19 vaccine, they had preserved cellular immune responses. Waning of COVID-19 antibody responses was observed within six months post vaccination among immunocompromised patients. Recent reports showed fatal outcome of breakthrough SARS-CoV-2 infections among vaccinated high-risk rheumatic diseases patients receiving Rituximab. SAR-CoV-2 serological tests were not performed.

Objective: Evaluation of COVID-19 vaccine humoral responses and breakthrough infections among low risk fully vaccinated rheumatic patients during the Delta Variant Era.

Methods: A case series of 19 fully vaccinated patients with rheumatic diseases were followed to determine post vaccine SARS-CoV-2 neutralizing antibody titers and to monitor the development of breakthrough infections up to eight months post vaccine at our tertiary care center in Jeddah, Saudi Arabia from 1st April until 30th November 2021.

Results: The mean age of patients was 49 years old. 10% of patients were receiving Rituximab. 73% of patients had positive SARS-CoV-2 serological testing post second vaccine. Two mild breakthrough COVID-19 infections were diagnosed six months post second dose of vaccine. Patients were less than 65 years, did not receive Rituximab, did not have interstitial lung diseases and had positive post vaccine serological testing.

Conclusions: We demonstrated high SARS-CoV-2 neutralizing antibodies seroprevalence and self-limiting breakthrough infections in low risk rheumatic diseases patients during the Delta Era. Future studies are needed to study the outcome of rheumatic diseases patients in the Era of Omicron in view of viral immune escape responses.

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Introduction

The effect of COVID-19 on patients with rheumatic diseases is complex; while age and comorbidities are associated with severe COVID-19 disease, the effect of biological therapies on COVID outcome remains unclear [1]. The use of Rituximab is associated with severe disease and prolonged hospitalization in patients with...
rheumatic diseases infected with COVID-19 [2]. COVID-19 vaccine is recommended for patients with autoimmune inflammatory diseases being safe and immunogenic; even in patients receiving B cell depleting agents, they had impaired humoral but preserved cellular immune responses to COVID-19 vaccine [3]. As more cases of SARS-CoV-2 variants of concerns are being detected worldwide, more cases of post COVID-19 vaccine breakthrough infections are being diagnosed in patients with variable disease severity depending on the extent of immunosuppression [4]. Cook et al. described the clinical characteristics and outcomes of 16 breakthrough COVID-19 infections in BioNTech BNT162b2 vaccinated patients with high risk rheumatic diseases (31% of patients received Rituximab). 6 (38%) patients required hospitalization, 1 (6%) patient required ventilation and 2 (13%) patients died. SARS-CoV-2 serological status was not described [5].

Our objective was to determine post vaccine SARS-CoV-2 neutralizing antibody titers in a case series of patients with low risk rheumatic diseases and follow them for breakthrough COVID-19 infection, 8 months post second dose of vaccine, during the Delta variant Era.

Methods

Study type

Our study was a prospective descriptive analysis of a case series of 19 patients in a single center, a tertiary care hospital, King Faisal specialist hospital and research center in Jeddah, Saudi Arabia, from 1st April 2021 until 30th November 2021.

Inclusion criteria

Patients with systemic rheumatic diseases who completed two doses of BioNTech BNT162b2, those who agreed to participate in the study and patients who provided blood for SARS-CoV-2 antibody testing at least 14 days following the second dose of BioNTech BNT162b2 were included. Patients who were fully immunized were followed for the development of breakthrough infections, eight months post second dose of vaccine.

Exclusion criteria

We excluded patients who received non BioNTech BNT162b2COVID-19 vaccines, those who received one dose BioNTech BNT162b2COVID-19 of and patients who presented for blood collection less than 14 days post second dose of vaccine.

It was difficult to have the same time frame for post vaccine serological tests as patients were vaccinated at different time points in different hospitals and most of them were not available in town for blood collection.

Laboratory tests

5 ml of whole blood were collected from the patients, transported directly to our CAP accredited lab, where the blood centrifuged for 5 min by 3500 rpm using Eppendorf centrifuge, Hamburg/Germany. Sera were manually pipetted each in 1.5 ml Eppendorf tube and stored immediately on − 30 °C for serology testing.

SARS-CoV-2 neutralizing antibodies were detected using in house ELISA and Micro-neutralization (MN) assay as previously described [6].

In-house ELISA

Antigen coating of flat bottom microtiter plates (Immulon® 2 HB, USA) was performed overnight at 4 °C with 100 ng per well of SARS-CoV-2 (2019-nCoV) spike S1 +S2 ECD-His recombinant protein (Sino Biological, China). Subsequently, the plates were subjected to three washes with PBS containing 0.1% Tween 20 (PBST) prior to blocking in PBST containing 5% skimmed milk for 1 h at room temperature. This step was followed by three washes with PBST. Sera were diluted at 1:100 dilutions in PBST containing 5% skimmed milk, added at 100 μl volume, and incubated for an hour at 37 °C. Following three washes with PBST, 100 μl of secondary antibody (goat KPL peroxidase-labeled antibodies to human IgG; Seracare, USA) were added for 5 min for color development prior to addition of 100 μl of N hydrochloric acid (HCL) to stop the reaction. Using Elx 880 bioelisa Reader (Biohit, Spain), the optical density was measured at 450 nm (OD_450). OD_450 values of > 0.27 were considered positive. Negative and positive controls utilized in this assay were sera of a healthy blood donor and a recovered COVID-19 patient known to have neutralizing IgG antibodies, respectively. The in-house ELISA provides 100% sensitivity, 98.4% specificity, 98.8% agreement, and high overall accuracy.

Micro-neutralization (MN) assay

MN assay was used as a confirmatory test to determine the presence of SARS-CoV-2-specific neutralizing antibodies. The local SARS-CoV-2 clinical isolate (SARS-CoV-2/human/SAU/85791 C/2020) (Genbank accession number MT630432.1) was utilized in this assay. The virus stock was propagated and titrated by Median Tissue Culture Infectious Dose (TCID_50) on African green monkey kidney cells Vero E6 (ATCC® CRL-1586™). Sera were subjected to heat inactivation at 56 °C for 30 min. Then, samples were serially diluted in DMEM containing 2% fetal calf serum (DMEM-FCS) and added with equal volume of DMEM-FCS containing 100 TCID_50 of SARS-CoV-2 on confluent cells. The cells were incubated at 37 °C in 5% CO2 until extensive cytopathic effect was observed (typically for 3–4 days). Uninfected cells and SARS-CoV-2 infected cells in the absence of human serum were utilized as controls. MN titers of ≥ 1:20 were considered positive.

Data sources

Electronic charts were reviewed for patients’ demographics and clinical characteristics. Ministry of health data base was reviewed for details of vaccination, type of vaccine, timing of vaccine and breakthrough COVID-19 infections. Patients with breakthrough COVID-19 infections were called by phone to determine disease severity and outcome.

Statistical analysis and variables

Categorical data were presented as frequencies and percentages while continuous data were presented as mean and standard deviation (SD). Mann Whitney test was used to examine differences in continuous variables between patients with positive and negative serological testing. SPSS (Version 25.0. Armonk, NY: IBM Corp) was used for all statistical analyses.

Results

A total of 19 patients was included in our study. The mean age was 49.1 ± 11.1 years and 89.5% were females. 37% of patients had
Breakthrough SARS-CoV-2 infection after BioNTech BNT162b2 vaccine

| Days from the second dose of BioNTech BNT162b2 vaccine to SARS-CoV-2 antibody tests | Titer of SARS-CoV-2 neutralizing antibodies |
|---|---|
| No | 37.0 ± 23.8 |
| Yes | 462.9 ± 483.0 |

## Discussion

In our case series of low risk rheumatic diseases, we demonstrated 73% seroprevalence of SARS-CoV-2 and mild breakthrough infections in the Era of Delta variant.

A prospective cohort study in Netherlands showed that 92% of patients with auto immune diseases had positive SARS-CoV-2 serological testing after two doses of vaccine [7]. Our results showed that 10% of patients developed breakthrough COVID-19 infections six months following the second dose of vaccine. Levin et al. described waning of antibody response post BioNTech BNT162b2 vaccination over six months especially in immunocompromised patients [8]. A third booster dose of COVID-19 vaccine is currently recommended for immunocompromised patients, 28 days post second dose vaccine [9].

In our study, self-limiting breakthrough infections were observed in patients who were less than 65 years old, did not have interstitial lung diseases, did not receive B cell depleting agents and had positive SARS-CoV-2 serological testing post second vaccine dose. On the other hand, Cook et al. described fatal breakthrough infections in patients who received rituximab and had chronic lung diseases [5].

We did not observe any breakthrough infections among patients with negative SARS-CoV-2 serological testing. We postulated that the adherence of patients to the use of universal face masks

Table 2

| Number | Age | Gender | Days between vaccine and serology | Neutralizing SARS-CoV-2 Antibody results | Titer | Post-vaccine COVID 19 infection | Pre-vaccine COVID 19 infection | Diagnosis | Medication |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 42 | Female | 21 | Positive | 1280 | No | No | RA | MXT, HQ |
| 2 | 31 | Female | 21 | Positive | 1280 | No | No | SA | ADL |
| 3 | 50 | Female | 16 | Positive | 1280 | No | Yes | SLE | PRD |
| 4 | 53 | Female | 20 | Positive | 640 | No | No | RA | PRD, TCZ |
| 5 | 54 | Female | 20 | Positive | 640 | No | No | SLE | HQ |
| 6 | 52 | Male | 55 | Positive | 320 | Yes | No | PA | SKB |
| 7 | 53 | Male | 47 | Positive | 320 | Yes | No | SLE | HQ |
| 8 | 36 | Female | 25 | Positive | 160 | No | No | SLE | MXT |
| 9 | 55 | Female | 47 | Positive | 160 | No | No | GCA | PRD, MXT |
| 10 | 47 | Female | 25 | Positive | 160 | No | No | RA | ETC |
| 11 | 55 | Female | 52 | Positive | 80 | No | No | SLE | None |
| 12 | 41 | Female | 56 | Positive | 80 | No | No | BD | PRD, AZN |
| 13 | 25 | Female | 19 | Positive | 40 | No | No | SLE | PRD, MXT, RXB |
| 14 | 60 | Female | 92 | Positive | 40 | No | No | RA | TCZ |
| 15 | 60 | Female | 34 | Negative | NA | No | No | RA | RXB |
| 16 | 49 | Female | 14 | Negative | NA | No | No | RP | MMF |
| 17 | 60 | Female | 24 | Negative | NA | No | No | PA | MXT |
| 18 | 39 | Female | 22 | Negative | NA | No | No | RA | PRD, TCZ |
| 19 | 70 | Female | 92 | Negative | NA | No | No | RA | PRD, MXT, ADL |

| Abbreviation: Days between vaccine and serology: days from second dose of COVID-19 vaccine to SARS-CoV-2 serology, NA: not applicable, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, PA: psoriatic arthritis, BD: Behcet disease, GCA: giant cell arteritis, PRD: prednisolone, MXT: methotrexate, TCZ: tocilizumab, HQ: hydroxychloroquine, ADL: adalimumab, MMF: mycophenolate mofetil, AZN: azathioprine, ETC: Etanercept, RXM: Rituximab, SKB: Secukinumab |
contributed to the prevention of COVID-19 transmission in Saudi Arabia [10].

Our study was limited by small sample size, lack of a control group, lack of serial serological testing for each patient and unavailability of results of whole genome sequence for COVID-19 isolates of the breakthrough infections. The sample size was small to determine risk factors for breakthrough COVID-19 infections such as age, gender, type of rheumatological diseases, comorbidities, medications, and SARS-CoV-2 neutralizing antibodies.

Delta variant was the predominant COVID-19 strain in Saudi Arabia at the time of our study [11]. Although breakthrough infections were mild in our study of low risk rheumatic patients during the Delta variant Era, future studies are needed to evaluate the outcome of breakthrough infections in rheumatic disease patients during the Omicron variant in view of viral immune escape responses [12].

Ethical approval

This research was approved by the institutional research board, King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia: IRB-2020-54. Patients were consented and agreed to participate in the study.

Funding

The research was funded by Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia, grant number (8-3) 2120191.

Conflict of interest

None.

Data Availability

Details of data can be provided when requested.

Acknowledgment

We would like to thank our patients who contributed to this research. The authors acknowledge the generous charitable donation from the Late Sheikh Ibraheem Ahmed Azhar in the form of reagents and supplies as a contribution to the scientific research community. The authors would like to thank the funding support of Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia, grant number (8-3)2120191. We appreciate the technical support of King Abdulaziz University and King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia. Special thanks for Dr Edward Cupler and Dr Ashraf Dada for their tremendous efforts to support our research projects during COVID-19 pandemic at King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia. We would like to thank Lama Hefni, research coordinator, department of Medicine, King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia for her outstanding care to supervise the progress of our research projects.

Contribution

All authors contributed equally to this research; study concept, data acquisition, data analysis, performing the experiment, writing and reviewing the manuscript.

Patient consent for publication

Not applicable.

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