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Correlation of Sars-Cov-2 IGG Antibody Levels with Viral Load Among Vaccine Breakthrough Infections: A Study From Rawalpindi

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ABSTRACT

Objective: To determine the correlation between SARS-CoV-2 antibody titers and viral load in vaccine breakthrough infections.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Virology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Nov 2021 to May 2022.

Methodology: Three hundred and thirty-seven patients admitted at the Pak Emirates Military Hospital, who had completed the entire course of the inactivated SARS-CoV-2 vaccination and developed COVID-19, at least 14 days from the second dose were selected. Specimens for the viral load of SARS-CoV-2 were taken from posterior nasopharyngeal swabs and serum for anti-Spike antibodies. Viral load in the specimens were estimated using reverse transcriptase polymerase chain reaction. Meanwhile, antibodies level against the SARS-CoV-2 surface spike protein receptor binding domain were assessed using COBAS e411 Electrochemiluminescence.

Results: Two hundred and thirty-two (69%) patients were male, while 104(31%) were female. Age, Cycle Threshold value and antibody titers data were normally distributed. Mean age (in years) of participants was 41.83±15.35 (range 18-65). Mean Cycle Threshold value was 23.79±5.49 (range 13.3–35), while mean anti-Spike IgG titers was 165.29±84.62 (range 12–250). Pearson's correlation coefficient (r) was 0.023 with a 95% CI [-0.08, 0.13] and *p*-value >0.05, indicating no correlation between Cycle Threshold values and antibody titers.

Conclusion: No correlation was found between viral load and anti-spike antibody among patients presenting with breakthrough infections following vaccination with an inactivated vaccine.

Keywords: Breakthrough Infections, COVID-19, COVID-19 Vaccines, Viral Load.

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INTRODUCTION

The SARS-CoV-2 virus has caused a global pandemic, the development of a safe and effective vaccine against which gave a ray of hope in fighting its spread.[1] Inactivated SARS-CoV-2 vaccine trial conducted in multi-country demonstrated an efficacy of 79% against hospitalization. However, rapid mutations in the virus soon raised concerns about vaccine effectiveness and it was also shown that different spike protein mutations found in SARS-CoV-2 subtypes made the virus less susceptible to anti-Spike antibodies.[2,3] Anti-spike antibodies are produced after vaccination or natural infection. Re-infection of COVID-19 patients has put forward the concern of vaccine escape potential.[4] The number of breakthrough infections is increasing exponentially worldwide.[5,6] Breakthrough infections have a significant impact on undisputed public health security and raised speculation on the prospect of vaccine effectiveness.[7]

Experts have been focusing on the association between SARS-CoV-2 IgG antibodies and viral load in vaccinated individuals who have contracted the infection. A reduction in viral load caused by the vaccine not only improves patient outcomes but also suppresses further transmission.[8]

Data is not available on correlation of anti-Spike antibodies level with viral load among individuals affected with COVID-19 in Pakistan. Hence our study aims to determine the correlation between SARS-CoV-2 antibody titers and viral load in vaccine breakthrough infections.

METHODOLOGY

The study was conducted at Armed Forces Institute of Pathology in Rawalpindi from November

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2021 to May 2022, after approval from the Institutional Ethical Review Board (IRB No. FC-VIR21-7/READ-IRBI21/1281). Sample size of was calculated using WHO calculator keeping expected correlation coefficient of 0.15.[9]

Inclusion Criteria: Cases of either gender aged 18-65 years with PCR-confirmed diagnosis of SARS-CoV-2 who gave history of receiving two doses of the inactivated SARS-CoV-2 vaccine 2 weeks prior to onset of symptoms were selected.

Exclusion Criteria: Unvaccinated, partially vaccinated, pregnant and immunocompromised individuals were excluded.

Non-probability consecutive sampling was used to recruit patients, and informed consent was sought. A serum specimen from each selected individual was obtained within 3 days of onset of symptoms according to the date documented on the history sheet. Nasopharyngeal samples of all patients were routinely sent directly to the Armed Forces Institute of Pathology for SARS-CoV-2 PCR testing. The time of sample collection was critical to prevent confounding by secondary immune response due to infection.

To take a nasopharyngeal sample, the patient's head was inclined 70 degrees back. A sterile swab with a flexible shaft was carefully and gently inserted into the nostril parallel to the palate till resistance was observed. The swab was gently rolled and held in place for a few seconds to absorb secretions. Swab was rotated and slowly withdrawn, placed into viral transport medium and transported to virology department. Reverse transcriptase polymerase chain reaction was used to assess the viral load in the samples (RT-PCR). In a serum separator tube, whole blood was drawn. The blood was kept undisturbed at room temperature allowing it to clot. Antibodies against the SARS-CoV-2 surface spike protein receptor binding domain (RBD) were measured using COBAS e4.[11] Electrochemiluminescence with a positive cut-off of >0.8 units/mL. Neutralizing antibody assay e.g. Plaque Reduction Neutralization Assay could not be done due to non-availability. Viral genome sequencing was also not carried out.

Data was analyzed in Microsoft Excel version 2019 and Statistical Package for Social Sciences (SPSS) version 23.0. Mean and SD was reported for normally distributed variables for continuous. To assess correlation between CT values and antibody titers, scatter plot was made and Pearson's correlation coefficient was applied. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

There were 232(69%) male and 104(31%) female patients. Age distribution was skewed whereas CT value and antibody titers data were normally distributed. Median age (in years) of participants was 41.83±15.35 (range=18-65). Mean CT value was 23.79±5.49 (range 13.3 – 35) while mean anti-Spike IgG titers was 165.29±84.62 (range 12-250). Pearson's correlation coefficient (r) was 0.023 with a 95% CI [-0.08, 0.13] and a *p*-value of 0.67 (>0.05) indicating no correlation between CT values and antibody titers shown in Figure-1. Thirty-seven participants gave history of having some co-morbid condition (37/337, 10.97%). Hypertension (18/337, 5.34%) and Diabetes mellitus (14/337, 4.15%) were the most frequently mentioned co-morbidities as shown in Figure-2.

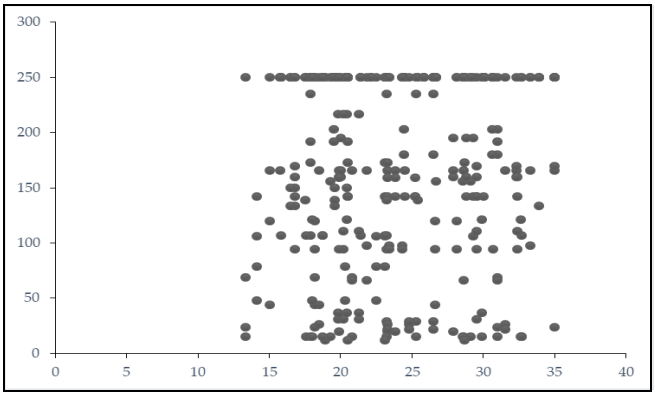

Figure-1: Scatter Diagram For Sars- Cov Viral Load (Ct Values) & Anti-Spike Antibody Titers (n=337)

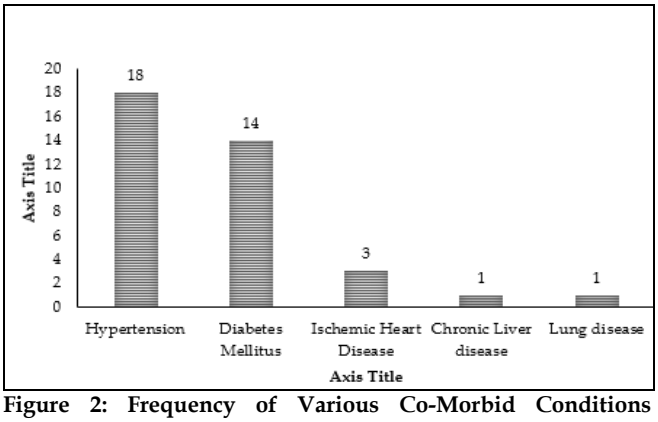

Figure 2: Frequency of Various Co-Morbid Conditions Reported (n=337)

DISCUSSION

Receptor binding domain (RBD) immunoglobulin G (IgG) levels commonly corresponded with balance titers. The quantitation of balance has uncovered high

intensity as an indicator of endurance. Notwithstanding the balance of wild-type SARS-CoV-2, patient sera have also been shown to kill the newly arisen SARS-CoV-2 mutation, recommending cross-security from reinfection by one or the other strain.[10] As the quantification of these antibodies has made it possible to analyze the immunity established against SARS-CoV-2, either by spontaneous infection or by specific vaccines, neutralizing antibodies have assumed relevance during the COVID-19 pandemic. They also seem a promising COVID-19 control strategy due to their capacity to stop or lessen the virus's infectivity.[11]

An international study also indicated the possible role of anti-Spike IgG titers in determining the severity of the disease as the median anybody titer was lower for patients with poor outcome.[11] However, the small number of patients with available antibody levels (n=69) in that study precluded any conclusive relationship to be drawn. Moreover, the study is limited to patients receiving mRNA vaccine. Another study conducted in Vietnam found no correlation between peak viral loads and neutralizing antibody concentrations, which raises the possibility that immunization may not lower the risk of transmission in situations involving breakthrough infections. The results of that study have also been drawn on a relatively small sample size (n=62) and after AstraZeneca vaccine.[12]

Our study is unique as it analyses the correlation between anti-spike antibodies and viral load in a large sample size (n=337) of people who received the inactivated vaccine. The results are consistent with studies on other types of vaccines as no correlation was found between viral load and anti-spike anti-bodies. The anti-Spike IgG titers were measured within 3 days after infection in order to prevent confounding by antibodies being produced in response to the breakthrough infection which usually takes 4-5 days to appear.[13-15]

A study conducted in Geneva indicated that vaccination was associated with lower infectious titers and faster clearance for Delta variant, showing that vaccination would also lower transmission risk.[16] Based on our study, it is not possible to comment on different COVID variants as our samples did not undergo next-generation sequencing, hence circulating variations were not discovered. It's possible that some of the variants may have vaccine-escape potential.[17] The absence of a relationship between viral load and anti-spike antibodies in our study can also be attributed to few elements.[18] For instance, unique immunization types and doses might induce various levels and sorts of antibodies. Furthermore, other safe factors, for example, lymphocyte reactions, may likewise add to protection against SARS-CoV-2 infection and diminish viral load.

LIMITATIONS OF THE STUDY

Due to funding limitations, next-generation sequencing (NGS) was not carried out. As a plague neutralization assay was not available in this setup, neutralizing antibody titers were not determined. Anti-spike antibody titers' initial results were also not obtained. Information provided by participants was used to confirm the vaccination status and hence the possibility of reporter bias remains.

CONCLUSION

No correlation was found between viral load and anti-spike antibody among patients presenting with breakthrough infections following vaccination with an inactivated vaccine.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

AS & EG: Conception, study design, drafting the manuscript, approval of the final version to be published.

EM & MAR: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

SKN & FA: Data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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