ORIGINAL ARTICLE

ANNALS OF PUNJAB MEDICAL COLLEGE

Long-Term Antibody Response to SARS-CoV-2 in Healthcare Workers of Allied Hospital Faisalabad: A Post-Infection Analysis

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Submitted for Publication: 22-03-2024
Accepted for Publication 20-06-2024

How to Cite: Shamsi FB, Azam A, Ghumman NA, Mujahid T, Hayder R, Owais M, Raza HA, Kalim Z, Shafique M. Long-Term Antibody Response to Sars-Cov-2 in Healthcare Workers of Allied Hospital Faisalabad: A Post-Infection Analysis. APMC 2024;18(2):111-115. **DOI: 10.29054/APMC/2024.1582**

ABSTRACT

Background: Analyzing SARS-CoV-2 antibody persistence is crucial for optimizing public health and deepening our understanding of immunity. Addressing geographic gaps is necessary for comprehensive insights into antibody persistence post-infection. **Objective:** To evaluate the persistence of SARS-CoV-2 antibodies post-infection among healthcare workers (HCWs) at Allied Hospital, Faisalabad Pakistan. **Study Design:** Longitudinal study. **Settings:** BSL-III Laboratory/Department of Pathology, Allied Hospital, Faisalabad Pakistan. **Duration:** July, 2020 to June, 2021. **Methods:** Blood samples were obtained from naturally infected individuals two weeks after their PCR-positive diagnosis providing informed consent. The detection and quantification of SARS-CoV-2 IgG antibodies were conducted utilizing the Enzyme-Linked Immunosorbent Assay (ELISA) to ascertain the IgG antibody levels. **Results:** A total of 94 HCWs were included with a mean age of 30 years. The antibody persistence was observed in 39% of participants with a mean age of 35 years. The study also highlighted a decrease in antibody reactivity across all age brackets over time particularly noticeable in young adults, with all groups demonstrating a decrease to approximately 60% reactivity by the end of the twelfth month. **Conclusion:** The study revealed a similar distribution among both genders. A notable finding is an overall decrease in antibody reactivity over time in all age groups, particularly in young adults, with a general decline to about 60% by the twelfth month. This study provides vital insights for public health policies and clinical management of SARS-CoV-2 among healthcare professionals.

Keywords: *COVID-19, SARS-CoV-2, antibody persistence, respiratory infections, healthcare workers, Enzyme-Linked Immunosorbent Assay.*

INTRODUCTION

COVID-19, attributable to the SARS-CoV-2, proliferated worldwide at an accelerated pace as a highly transmissible zoonotic disease, predominantly through respiratory droplets. This pandemic significantly disrupted the global economy, healthcare systems, and societal norms, leading to economic hardships and public health emergencies.[1,2] Healthcare workers (HCWs) face a heightened risk of contracting COVID-19 due to direct patient care and exposure to contaminated environments.[3,4] The pandemic also severely impacted the mental and emotional well-being of HCWs,

underscoring the complex challenges faced by these professionals.[5-8] The generation of immune response to SARS-CoV-2, especially antibody production, is crucial in combating the virus. Antibodies play a vital role in vaccine development, therapeutic interventions, and understanding post-infection immunity. The body generates specific antibodies (IgM, IgA, followed by IgG) offering extended immunity and crucial defense against reinfection.[9] However, antibody longevity varies among individuals, with some maintaining high levels for longer periods.[10,11]

Research on antibody response duration and efficacy against SARS-CoV-2 variants is ongoing. Targeted studies are needed, particularly for HCWs due to their high virus exposure. Current geographic-specific antibody studies are limited, necessitating further investigation to inform HCW protection strategies and vaccination policies.

Allied Hospital Faisalabad, Punjab Pakistan served as a crucial COVID-19 response center during the COVID-19 pandemic as a main testing, treatment, and vaccination hub. The region's distinct epidemiological trends, impacting healthcare services and workers, highlight the need to study the effects of viruses on healthcare systems and personnel. This research holds critical value for enhancing the preparedness and response of healthcare systems in similar settings, especially during pandemic situations.

So, the main objective of the current study includes assessing the duration of SARS-CoV-2 antibodies' presence post-infection among HCWs.

METHODS

This longitudinal study was conducted at the Biosafety Level III (BSL-III) Laboratory/Department of Pathology, Allied Hospital, Faisalabad Pakistan. Duration of the study was one year from July 2020 to June 2021. Non-probability purposive sampling technique was used to select 94 naturally infected SARS-CoV-2 healthcare workers.

HCW's at Allied Hospital who had naturally acquired and confirmed RT-PCR COVID-19 results, consented to participate, and were available for follow-up between July 2020 and June 2021 were included in the study.

Vaccinated individuals, those who did not provide informed consent, participants with negative COVID-19 RT-PCR results, and those unavailable for follow-up were excluded.

Blood collection utilized the VACUETTE® system (Gel separating vial), followed by centrifugation at 5000 g for 5 min and serum was stored at -80°C. Subsequent collections occurred monthly, with six additional collections for participants displaying an antibody response.

Aliquot were thawed before analysis. Omega Diagnostics® ELISA kit was used with 97% specificity and 96% sensitivity for SARS-CoV-2 IgG antibody status. The Indirect ELISA process involved preparing a microtiter plate coated with SARS-CoV-2 antigens, adding participant's serum samples, incubating, washing, and introducing a secondary antibody for detection.[12] Colorimetric measurement was followed at 450 nm and 630 nm using an ELISA reader (BioTek Instrument Inc., Winooski, VT, USA).[12]

Data analysis expressed variables in frequencies, means, and standard deviations, assessing gender and age influences using the natural logarithm of antibody levels as a dependent variable using IBM® SPSS Statistics V25.

RESULTS

ELISA Results Analysis

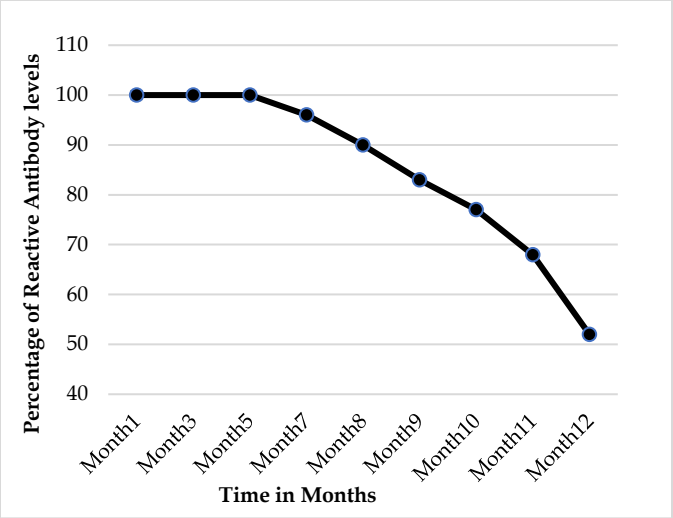
Throughout a year-long period, a decreasing trend in antibody levels was noted among participants. The percentage of reactivity, indicative of the proportion of participants showing a reactive antibody response, remained at 100% for the first five months. However, a gradual decline was noted from Month 7 (96%) onwards, reaching as low as 39% by Month 12.

The following table and graph shows the trend in antibody levels over the months (Table 1 & Figure 1):

Table 1: Longitudinal Analysis of SARS-CoV-2 Antibody Levels in HCWs over 12 months

Months	Antibody Levels Mean ± Standard Deviation	Percentage Reactive (%)
1	2.23 ± 0.83	100%
3	1.83 ± 0.75	100%
5	1.52 ± 0.74	100%
7	1.35 ± 0.70	96%
8	1.22 ± 0.67	90%
9	1.14 ± 0.65	83%
10	1.09 ± 0.62	77%
11	0.91 ± 0.60	68%
12	0.72 ± 0.56	52%

Figure 1: Decline in the Percentage of Reactive Antibody Levels among HCWs over 12 months



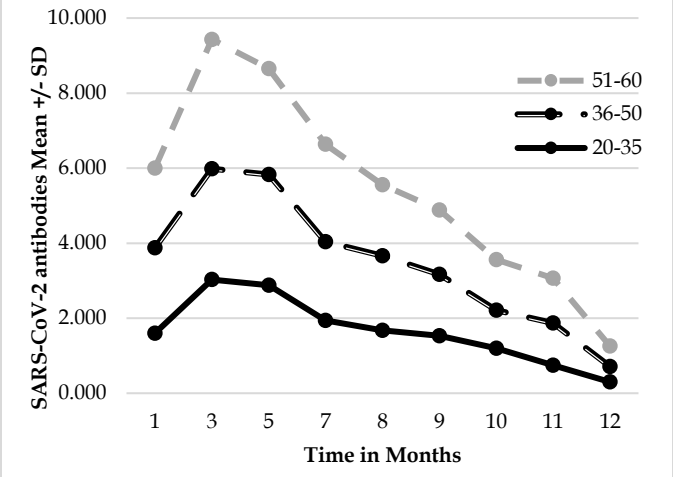
Analysis of ANTIBODY Levels among Different Age Groups: The mean antibody levels and the percentage of reactive individuals within three age groups following PCR testing over a year were determined. The results are shown in Table II.

Table 2: Comparative SARS-CoV-2 Antibody Responses across Age Groups Post-PCR Confirmation

Age groups	Months	Antibody Levels Mean ± Standard Deviation	Percentage Reactive
Young Adults (20-35 years)	1	2.028 ± 0.838	100%
	3	1.597 ± 0.706	100%
	5	1.302 ± 0.661	100%
	7	1.140 ± 0.590	97%
	8	1.008 ± 0.534	90%
	9	0.932 ± 0.512	81%
	10	0.902 ± .495	73%
	11	0.744 ± 0.517	63%
	12	0.600 ± 0.484	46%
Middle-Aged (36-50 years)	1	2.655 ± 0.739	100%
	3	2.280 ± 0.653	100%
	5	1.946 ± 0.680	100%
	7	1.699 ± 0734	95%
	8	1.584 ± 0.721	89%
	9	1.534 ± 0667	84%
	10	1.439 ± 0.605	84%
	11	1.130 ± 0.608	79%
	12	0.913 ± 0595	63%
Old Age (51-60 years)	1	2.450 ± 0.667	100%
	3	2.126 ± 0.723	100%
	5	1.825 ± 0.782	100%
	7	1.696 ± 0.792	94%
	8	1.592 ± 0.736	94%
	9	1.420 ± 0.791	88%
	10	1.351 ± 0.768	81%
	11	1.194 ± 0.671	75%
	12	0.843 ± 0.693	62%

In all age groups, the trend was consistent: mean antibody levels diminished over time, and the percentage of reactive individuals decreased as the months after PCR increased. This indicated a decline in detectable antibodies and possibly a decrease in immunity over time. The trend is depicted graphically in Figure 2.

Figure 2: Mean SARS-CoV-2 Antibody Levels by Age Group over 12 Months Post-PCR Confirmation

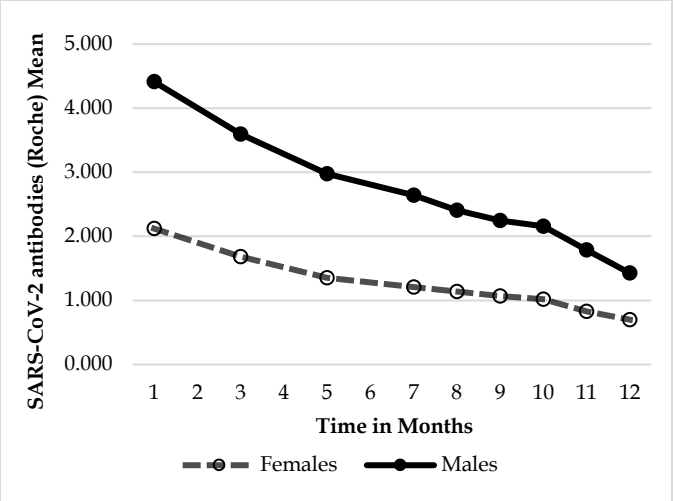


Gender-wise Analysis of Antibody Levels: Data tracked changes in mean antibody levels, standard deviation, and reactivity percentages over 12 months post-PCR for females and males. Both genders exhibited decreasing mean levels and reactivity, with males initially higher but joining by the twelfth month. Standard deviation suggested decreasing variability over time as shown below in Table 3 and Figure 3.

Table 3: Gender-Based SARS-CoV-2 Antibody Reactivity and Levels Over 12 Months Post-PCR Confirmation

Gender	*Months after PCR*	*Antibody Levels Mean ± SD*	*Percentage Reactive*
Females	1	2.121 ± 0.798	100%
	3	1.683 ± 0.643	100%
	5	1.353 ± 0.647	100%
	7	1.211 ± 0.612	92%
	8	1.140 ± 0.604	86%
	9	1.070 ± 0.611	81%
	10	1.019 ± 0.618	72%
	11	0.829 ± 0.653	61%
	12	0.699 ± 0.598	50%
Males	1	2.292 ± 0.848	100%
	3	1.914 ± 0.809	100%
	5	1.625 ± 0.775	100%
	7	1.432 ± 0.749	98%
	8	1.267 ± 0.699	93%
	9	1.175 ± 0.675	84%
	10	1.136 ± 0.618	79%
	11	0.958 ± 0.563	72%
	12	0.727 ± 0.548	53%

Figure 3: Mean SARS-CoV-2 Antibody Levels by Gender over 12 Months Post-PCR Confirmation



DISCUSSION

HCWs, due to their front-line roles in the COVID-19 pandemic response, are recognized as a demographic with an elevated risk of exposure to SARS-CoV-2. This risk underscored the need for a deep understanding of their long-term antibody responses. This was critical for crafting safety guidelines and setting booster vaccination schedules. The present study aimed to fill key gaps in this knowledge, specifically addressing how immunity persisted after infection. The findings guide managing healthcare staff to prevent operational challenges and inform additional vaccine doses for HCWs.

Literature supports the immunological model showing antibody synthesis phases. Our study used ELISA to measure total antibody levels. The research findings supported by previous literature describing IgG levels reach their highest point approximately seven to eight weeks after the onset of the disease. Subsequently, following a period of stability in their production, these levels gradually decrease, with the rate of decline varying from person to person.[13-15]

The observed decrease in antibody levels and reactivity among the study participants aligns with findings from the existing literature on the waning of immune responses over time.[16,17] Following an initial increase, the peak in antibody production occurred in the Fifth month after the first positive PCR test. Subsequently, in the majority of individuals, antibody production gradually declined, as illustrated in Figure 1. The duration of our study's observation aligned with the confirmed presence of antibodies in the monitored patients. The longest observed period and the most extended detected antibody response were both 12 months. This pattern is consistent with studies suggesting that after an initial immune response to a natural infection, antibody titers tend to decrease. For instance, Alzaabi *et al*., (2021) found that the IgG levels in patients varied across different visits: first to second, second to third, and first to third. Graphs show trends over 90+ days and 120+ days. Sample sizes were 113 for the first visit, 63 for the second, and 27 for the third. Each line represents an individual's IgG level trend between visits.[18]

Swartz *et al*., (2022) in their work on COVID-19 patients indicated that while antibody levels peak within the first few weeks after infection, there is a significant decline in the following months, which is in concordance with the trends observed in our study.[19]

The variation in antibody levels at the beginning of the study could be due to individual differences in the immune response, which is supported by the literature that acknowledges the heterogeneity in how different immune systems react to pathogens.[20] The early robust reactivity, with 100% of participants showing a reactive antibody response initially, demonstrates an effective acute immune response commonly seen following infection or vaccination, as documented by various immunological studies.

The gradual decline in reactivity from Month 5 onwards, reaching a low by Month 12, is also reflected in the literature, including research by Wajnberg *et al*,[21] which has implications for potential vulnerability to reinfection and the duration of protection conferred by vaccination.

Furthermore, the current study findings about the age-related differences in antibody levels and reactivity support research suggesting that immune senescence affects the longevity of antibody responses.[22] The slower decline in antibody levels among middle-aged individuals compared to young adults, and the more pronounced decline in older adults after seven months post-PCR, may reflect age-related changes in immune function.

The detailed trends in antibody levels and percentage reactivity among females and males provide important insights into potential sex-based differences in immune response dynamics. The literature acknowledges these differences, noting that hormonal influences and genetic factors might contribute to variability in immune responses.

CONCLUSION

The presence of SARS-CoV-2-specific IgG antibodies for up to a year post-infection is significant. Peak production at five months indicated a strong initial immune response, crucial for defense against reinfection. Antibodies persisting for over 10 months suggested nearly a year of potential protection, aiding in public

health planning and assessing reinfection risk for booster vaccinations.

LIMITATIONS

The limitations of this study include a narrow time frame, focus on a single institution, and exclusive examination of IgG antibodies.

SUGGESTIONS / RECOMMENDATIONS

Future research should investigate the long-term clinical implications of declining antibodies to reinfection and, the effects of vaccination on the longevity and efficacy of natural antibody responses in HCWs. Additionally, studying demographic and occupational factors contributing to antibody persistence variability could guide policies on booster vaccinations and infection control for frontline medical staff.

CONFLICT OF INTEREST / DISCLOSURE

There is no conflict of interest involved.

ACKNOWLEDGEMENTS

We express our gratitude to the BSL-III Lab/Department of Pathology team at Allied Hospital Faisalabad, encompassing the BSL-III Lab Coordinator (2020-2021), Pathology Technologists, and all assisting staff for their instrumental support and successful execution of this research.

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