

Study of Antibody Level in Vaccinated Healthcare Workers, both Infected and Noninfected by COVID-19 Over the Time Period 28 Days after 1st Vaccination and 6 Months or 1 Year after 2nd Vaccination

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Abstract

Background: The COVID-19 pandemic has proven how immunisation can avert illnesses, hospitalisations, and fatalities, affecting health systems worldwide. Medical staff are more likely to get infections due to their close contact with patients. Tracking antibody levels following immunisation, especially in people who have had infections, can help determine immune protection length and strength.

Methods: Researchers observed the Pathology Department of Patna Medical College and Hospital (PMCH). The study comprised 100 healthcare workers, 50 of whom were affected. Electrochemiluminescence Immunoassay (ECLIA) was used to measure serum antibody titers twice: 28 days after the first immunisation and 6 months or 1 year after the second.

Results: Previously infected participants demonstrated significantly higher antibody titers (109.77 ± 69.64 AU/mL) compared to non-infected individuals (13.31 ± 42.48 AU/mL) at 28 days. Although antibody levels declined over time in both groups, infected individuals maintained higher titers at 6 months.

Conclusion: A past COVID-19 infection boosts the immune response following vaccination, supporting hybrid immunity. Healthcare providers must offer booster doses and monitor serological levels to ensure long-term protection as antibody levels drop across all groups.

Keywords: Antibody levels, COVID-19, Healthcare workers, Hybrid immunity, PMCH Patna, Vaccination.

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Introduction

COVID-19, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, has plagued healthcare systems, economies, and society worldwide [1]. After originating in Wuhan, China, in December 2019, the virus spread swiftly. On March 11, 2020, the WHO labelled it a global pandemic. Moderate to severe infections usually affect the respiratory system. Common complications were ARDS and multi-organ failure. The epidemic tested the healthcare infrastructure and the healthcare personnel's mental and physical health. Frontline workers were especially vulnerable due to the absence of PPE, long hours in high-risk healthcare settings, and constant contact with infected patients [2]. This case showed the significance of prompt and effective immunisation to protect healthcare workers and prevent viral spread in hospitals. COVID-19 immunisations were a scientific breakthrough and a turning point in worldwide pandemic control. Vaccination programs reduced infections, illnesses,

hospitalisations, and fatalities in the general public and at-risk populations [3]. However, as new virus strains developed and immunity decreased, assessing antibody levels after vaccination became critical for estimating protection duration. Antibody testing shows the humoral immune response caused by vaccination or spontaneous infection by assessing the body's ability to recognise and neutralise the virus following re-exposure [4]. Tracking antibody titers over time can assist in spotting declining immunity and inform booster dose and re-vaccination decisions. Antibody surveillance helps evidence-based public health measures evaluate vaccine efficacy against emerging strains [5]. On January 16, 2021, India began the world's largest immunisation campaign with the vaccine campaign initially, Covishield and Covaxin were utilised [6]. Oxford-AstraZeneca developed Covishield, a viral vector vaccine made by the Serum Institute of India. A modified chimpanzee adenovirus encodes the SARS-CoV-2

spike protein. Bharat Biotech and the ICMR developed Covaxin, an adjuvant-formulated inactivated whole virion vaccine (BBV152), to increase immune response [7]. Each vaccine dosage was four to six weeks apart. Unlike Covaxin, which exposes the body to the complete inactivated virus, Covishield generates spike protein-neutralizing antibodies [8]. These vaccines reduced COVID-19 cases in India, saving lives and hospitalisations. Long-term implications of vaccine-induced immunity, especially in light of changing variability, were still explored.

Healthcare workers (HCWs) were prioritised in the vaccine schedule due to their increased exposure to the virus and vital role in pandemic healthcare. Vaccinating HCWs followed the WHO's strategic framework, which prioritised pandemic response personnel. According to several studies, healthcare workers are at risk of getting infectious diseases like COVID-19 due to their close contact with patients, high viral loads, and possible infection control breaches. Hundreds of Indian healthcare personnel contracted the pandemic initially, and many died. This group needed to be vaccinated to keep healthcare facilities working properly amid crises. According to this research, age, gender, length since vaccination, and history of infection may affect antibody responses in vaccinated people.

Short-term vaccination trials show antibody production, but longitudinal data on antibody persistence, especially after six months or a year, are lacking. Most studies on immunological dynamics have focused on shorter time intervals, usually weeks or months after immunisation. Over time, natural antibody fading lowers immune response and protection against reinfection. This emphasises the importance of healthcare workers and other high-risk populations evaluating their antibody titers to determine protection duration and additional doses. Furthermore, how the immune response differs between COVID-19-infected patients and those who were infection-free before immunisation is yet to be identified. Vaccination may increase antibody titers in sick people; many call this hybrid immunity.

This study examines antibody titers at two time points in previously infected and non-infected healthcare workers to determine the durability of immune responses post-vaccination in a high-risk group.

This study will influence booster dose and infection prevention programs and determine vaccine-induced immunity duration. This study helps us understand how vaccines function and how long healthcare personnel's immune systems survive, which improves pandemic preparedness and public health policies.

Objectives

1. To assess antibody levels in vaccinated healthcare workers infected and non-infected with COVID-19.
2. To compare antibody titers at two intervals: 28 days post-first dose and 6 months/1 year after the second dose.
3. To evaluate factors (age, gender, infection history) affecting antibody persistence.

Materials and Methods

Study Design: This hospital-based prospective and retrospective observational analysis included COVID-19-vaccinated HCWs with and without SARS-CoV-2 infection. The requirement to record the immediate and cumulative immune system effects of immunisation prompted the dual technique. A prospective component monitored vaccinated people, and a retrospective component verified vaccination and infection status using medical records and test results. Combining these methods allowed us to collect all essential data and create more reliable comparisons between infected and healthy people.

Study Setting: The research was conducted at PMCH, a major Bihar hospital. PMCH helped handle cases and immunise during the COVID-19 pandemic. This inquiry was conducted at the Department of Pathology due to its advanced diagnostic infrastructure, competent staff, and immunoassay analysers. The hospital's diverse healthcare staff was ideal for studying vaccine-induced immune responses in real life.

Study Population and Sample Size: Half of the healthcare workers in the study had been infected with the virus. Individuals were categorised by pre-immunization COVID-19 RT-PCR or antigen results. Doctors, nurses, laboratory technicians, and ancillary staff were sampled to represent occupational exposure levels. Immune responses varied by age and gender, and the study compared infection-induced and vaccine-induced immunity.

Study Duration: The study covered the first and subsequent stages of India's immunisation rollout and lasted for two years, from 2021 to 2023. This time frame allowed for the evaluation of both the initial and ongoing humoral immune responses by measuring antibody titers at two crucial points: 28 days following the initial dosage and 6 months or 1 year following the second treatment.

Data Collection and Laboratory Procedure: All participants submitted written informed consent before enrolment. The participant's age, gender, vaccination history (vaccine type and dose dates), occupation, and illnesses were noted. Two millilitres of venous blood were drawn aseptically with sterile syringes. They separated the serum by

centrifugation and stored it at a controlled temperature until testing.

The Roche Diagnostics Cobas e411 automated analyser with ECLIA technology quantified antibodies. This method was chosen for its high sensitivity and specificity in detecting anti-SARS-CoV-2 antibodies. ECLIA works because antigen-antibody complex formation causes light intensity to be proportional to antibody concentration during electrochemical reactions. Each person's results were expressed in arbitrary units per millilitre.

Inclusion Criteria: Healthcare workers who had received two doses of Covaxin or Covishield and consented to participate were included. Both previously infected and non-infected individuals were eligible.

Exclusion Criteria: Severely ill individuals, pregnant women, those who refused consent, participants with incomplete vaccination, or those who received booster doses were excluded to minimize confounding variables.

Statistical Analysis: All data were analysed using standard statistical methods. Antibody levels were shown as mean \pm SD. They compared infected and uninfected people, male and female, and age groups using Student's t-test. A p-value below 0.05 indicated statistical significance.

Ethical Considerations: The study followed ethical principles outlined by the Institutional

Ethics Committee of PMCH, Patna, which approved the research protocol. Participant confidentiality was ensured throughout the study, and data were used solely for research purposes.

Results

This study assessed the antibody levels of 100 vaccinated healthcare workers from PMCH, Patna, 50 infected, and 50 non-infected. To test immunological durability and compare responses between groups, antibody titers were measured 28 days and 6 months following the first and second vaccinations.

Antibody Levels in Infected and Non-Infected Healthcare Workers: A significant difference was observed in antibody titers between infected and non-infected healthcare workers. The mean antibody level 28 days after vaccination was 109.77 ± 69.64 AU/mL among previously infected participants compared to 13.31 ± 42.48 AU/mL in non-infected individuals. At 6 months post-vaccination, antibody levels decreased in both groups. Infected workers showed a mean value of 71.29 ± 63.03 AU/mL, while non-infected workers showed 50.19 ± 71.95 AU/mL. The difference between the two groups was found to be statistically significant ($p = 0.001$), indicating that prior infection substantially enhanced antibody response after vaccination.

Table 1: Comparison of Antibody Levels between Infected and Non-Infected Healthcare Workers

Group	Antibody Level (28 Days After 1st Dose)	Antibody Level (6 Months After 2nd Dose)	p-Value	Significance
Infected HCWs	109.77 ± 69.64	71.29 ± 63.03	0.001	Significant
Non-Infected HCWs	13.31 ± 42.48	50.19 ± 71.95	0.001	Significant

These findings suggest that hybrid immunity (natural infection plus vaccination) provides higher and more sustained antibody levels compared to vaccine-induced immunity alone.

Age-Wise Distribution of Antibody Levels: Antibody titers were found to vary across different age groups. The younger healthcare workers (23–32 years) demonstrated higher mean antibody

levels compared to older participants. After 28 days of vaccination, this age group recorded a mean value of 107.91 ± 72.56 AU/mL, which gradually decreased to 74.35 ± 72.23 AU/mL after 6 months. In contrast, individuals aged above 64 years showed markedly lower antibody titers (52.26 ± 0.00 AU/mL after 6 months). This age-related decline indicates a reduction in immunogenicity with advancing age.

Table 2: Antibody Levels by Age Group among All Participants

Age Group (Years)	Mean Antibody (28 Days)	Mean Antibody (6 Months)	Trend
23–32	107.91 ± 72.56	74.35 ± 72.23	Decline
33–43	90.88 ± 57.29	66.88 ± 59.46	Decline
44–53	124.73 ± 75.30	62.98 ± 62.49	Decline
54–64	128.61 ± 81.66	128.16 ± 81.66	Stable
>64	186.8 ± 0.00	52.26 ± 0.00	Sharp Decline

The younger age group had a stronger and longer-lasting immune response, consistent with prior

research demonstrating that younger people have more vaccine-induced immunity.

Gender-Wise Comparison of Antibody Levels: A gender-based study found significant antibody differences between male and female healthcare personnel. Males had a mean titer of 61.04 ± 76.39 AU/mL after 28 days, whereas females had 63.03 ± 72.68 AU/mL, indicating a slight difference.

However, at 6 months, females had higher titers (85.09 ± 66.22 AU/mL) than men (52.63 ± 67.23 AU/mL), with a p-value of 0.001. This shows females-maintained antibody levels better.

Table 3: Gender-Wise Comparison of Antibody Titers

Gender	Mean Antibody (28 Days)	Mean Antibody (6 Months)	p-Value	Significance
Male	61.04 ± 76.39	52.63 ± 67.23	0.001	Significant
Female	63.03 ± 72.68	85.09 ± 66.22	0.001	Significant

These results align with global observations suggesting that females often mount stronger humoral immune responses due to hormonal and immunogenetic factors.

Overall Trends and Decline over Time: After 28 days and 6 months, antibody levels decreased in all groups (infected, non-infected, male, female, and different ages). Even though previously infected patients had higher antibody titers, both groups declined in immunity over time, requiring booster vaccinations to prevent reinfection with SARS-CoV-2.

The study found that COVID-19-infected healthcare workers had a stronger and longer-lasting antibody response to immunisation. Immune systems were stronger in younger, female subjects. Reductions in antibody titers after 6 months emphasise the need for continual monitoring and booster doses for healthcare professionals to maintain immunity.

Discussion

This study measured antibody levels in COVID-19-infected and uninfected healthcare professionals after vaccination. People who had been infected with SARS-CoV-2 showed significantly higher antibody titers than those who had never been exposed. After six months, antibody levels often declined regardless of infection status. These results support the expanding corpus of studies showing that a hybrid immune response, combining vaccination with spontaneous infection, is more effective and long-lasting than immunisation unaccompanied.

At six months, females had stronger immunological persistence than males, while younger healthcare professionals had higher antibody titers. Gender and age-related immune system responsiveness differences may explain these variances.

The study concludes that COVID-19 immunity is not static, therefore high-risk populations like healthcare workers may need booster doses and long-term observation.

Comparison with Global Literature: For instance, [9] reported that six months after immunisation, healthcare personnel who had

contracted COVID-19 had far higher antibody titers than those who had not. [10] Revealed that SARS-CoV-2 infection before two doses of inactivated vaccines increased immune response, supporting hybrid immunity. [11] Found that healthcare professionals' antibody titers reduced six months after vaccination, notably in elderly adults.

According to [12], healthcare professionals' antibody levels dropped by 90-95% seven months after vaccination, indicating that immunity declines after immunisation. [13] Found that booster dosages raised antibody titers, especially in sick patients. These studies suggest that vaccination-induced immunity may not last forever and that booster shots are needed, especially in persons who have never had the virus.

Reasons for Higher Antibody Titers in Previously Infected and Younger Workers:

Healthcare workers who have been infected can attribute their increased antibody response to memory B cells created during natural infection. These cells linger in the body and promptly reawaken when exposed to the vaccine antigen, triggering a powerful secondary immune response. Antibody titers and neutralising capacity rise during anamnestic reaction. Vaccination generates an initial immune response in infection-naïve individuals, although it is often weaker and less prolonged.

Younger people have stronger humoral and cellular immune systems. In contrast, immunosenescence, characterised by decreased naïve T and B cell production and antibody affinity maturation, is associated with ageing. Younger healthcare personnel are more likely to have greater antibody titers for longer. Women had higher antibody persistence than males in this study may be because oestrogen boosts antibody manufacture and immune cell activity. Hormonal and metabolic changes may also cause these differences.

Biological Mechanisms: Hybrid Immunity and Memory Response:

Hybrid immunity, which combines infection-induced and vaccine-induced immunity, helps persons with previous infections have greater antibody responses. The immune system encounters spike, nucleocapsid, and

membrane proteins during spontaneous infection, increasing its immunological memory. After another shot, their dormant memory B and T cells awaken, triggering a stronger, faster antibody response that neutralises more targets.

Memory B-cell activation ensures long-term immunological surveillance, even when antibody levels decline. The immune system may protect itself without antibodies due to memory cells, which can persist for years and quickly change into plasma cells when exposed to the same antigen. This drop in antibody levels does not always mean immunity has fully worn off. Instead, it indicates that the body's humoral defences are waning, which can be reinforced by booster shots or reexposure to the pathogens.

Antibody Waning and Supporting Studies: This study adds to the mounting evidence that antibody levels naturally decline. [14] Found that antibody titers dropped substantially one month after immunisation with the BNT162b2 (Pfizer-BioNTech) vaccine. The humoral response diminished over six months. [15] Found that antibody levels peaked five weeks after the first dosage and rapidly decreased afterward in a UK population-based investigation. Both trials show that booster doses at regular intervals are needed to sustain vaccine-induced immunity, which is transitory. Immune system kinetics cause antibody-secreting plasma cells to decrease their activity when antigenic stimuli are absent, causing fading. Booster immunisation is important for restoring protective antibody levels and immunological memory, especially in high-risk populations like healthcare professionals.

Limitations: The first constraint is the small sample size (n=100), which may not apply to larger populations. Second, being a single-center study at PMCH, Patna, it may not represent healthcare institutions or regional differences. Thirdly, although this study only examined antibody titers, cellular immune responses like T-cell memory are also critical for long-term immunity.

Recommendations: Future research should focus on larger, multicentric, diverse group investigations to strengthen the evidence. Combining neutralising antibody and cell-mediated immunity testing would provide a more complete picture of immune protection. Healthcare workers and other susceptible populations should undertake antibody monitoring after booster doses to determine the greatest immunisation regimens and provide long-term protection.

Conclusion

Vaccinated healthcare professionals with a history of COVID-19 infection have stronger and longer-lasting antibody responses, according to a current

study. The results of this study support hybrid immunity, where spontaneous infection and vaccination strengthen and prolong the immune response. All patients' antibody titers decreased with time, regardless of infection status. To maximise protection, booster dosages must be given at the proper time. Younger healthcare workers and women showed higher antibody levels, suggesting immunogenicity is age and gender-dependent. These findings emphasise the importance of serological surveillance for antibody persistence and direct immunisation. Monitoring healthcare personnel's antibody responses is essential for long-term immunity and frontline protection against SARS-CoV-2 variants in high-risk clinical settings.

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