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HALF LIFE DETERMINATION OF COVID-19 ANTIBODIES AMONG VACCINATED AND NON-VACCINATED PEOPLE

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had a profound impact on global health, economy, and society. Serological investigations, play a pivotal role in understanding the immune response generated in these individuals, including the production of specific antibodies. This retrospective cross-sectional study was conducted in Faisalabad, Pakistan, aimed to investigate the prevalence of COVID-19 and the dynamics of SARS-CoV-2 antibodies among vaccinated and non-vaccinated people. Total (N=300) participants from three different medical institutes were enrolled and the participants were divided into two groups based on their vaccination status: non-vaccinated (N=150) and vaccinated (N=150). ELISA and PCR were performed on blood tests for the detection of antibodies and reactive cases. Based on gender showed that in the non-vaccinated group, 85 individuals (56.6%) were males, while 65 individuals (43.3%) were females. In the vaccinated group, there were 80 males (53.3%) and 70 females (46.6%). Regarding age groups, the participants were categorized into four groups. In the non-vaccinated group, 50 individuals (33.3%) were in the age range of 21-30 years, as were 50 individuals (33.3%) in the vaccinated group. The 31-40 year age group had 30 participants (20.0%) in both the non-vaccinated and vaccinated groups. In the non-vaccinated group, 30 individuals (20.0%) were in the 41-50 year age range, while 40 individuals (26.6%) fell in the same age group in the vaccinated group. For individuals above 50 years of age, there were 40 participants (26.6%) in the non-vaccinated group and 30 participants (20.0%) in the vaccinated group. At the end of the study, 22 of the participants were non-reactive against SARS-CoV-2 IgG antibodies out of 150 that were non-vaccinated while total 29 individuals were non-reactive against IgG antibodies that were vaccinated. This study emphasizes the urgent need for enhanced genomic surveillance in Pakistan to monitor the emergence and spread of SARS-CoV-2 variants, highlighting the importance of timely detection, and understanding their implications for public health interventions and vaccine development.

Keywords: Covid-19, Antibodies, RT-PCR, SARS-CoV-2, ELISA

INTRODUCTION

The coronavirus disease (COVID-19) pandemic, initiated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has catalyzed a profound impact on global health, economy, and social norms (Acter et al., 2020). As the global tally of COVID-19 cases continues to escalate, unraveling the intricate molecular characteristics of the virus and scrutinizing the immune response in infected individuals becomes imperative for efficacious disease management and control (Bikdeli et al., 2022). Serological investigations play an instrumental role in deciphering the immune response elicited in these individuals, particularly concerning the production of specific antibodies (Cao et al., 2022).

For over a year, the Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) has remained at the forefront of public consciousness (Badulak et al., 2021). This disease, originated by SARS-CoV-2, poses a formidable global health challenge. Originating in Wuhan, China, it swiftly disseminated worldwide (Mohan and Nambiar, 2020). The World Health Organization (WHO) officially designated the disease as COVID-19 on February 11, 2020, symbolizing "Corona Virus Disease" and "19" for its emergence in 2019. Subsequently, in March 2020, WHO declared COVID-19 a pandemic (Cucinotta and Vanelli, 2020). The first documented case of COVID-19 in Pakistan was reported in Karachi on February 26, 2020, with the infection initially traced to individuals returning from Iran. As of December 17, 2021, Pakistan has confirmed 1,290,848 COVID-19 cases, with a 2.23% mortality rate (28,870 deaths), as reported by the National Command and Operation Center (NCOC, 2021). Globally, WHO reported 271,963,258 confirmed COVID-19 cases, including 5,331,019 deaths (WHO, 2021).

SARS-CoV-2 belongs to the Coronaviridae family, characterized as a single-stranded, enveloped, positive-sense RNA virus (Guan et al., 2020). Within the Coronaviridae family, classification into four genera is based on differences in protein sequences: α-coronavirus (α-CoV), β-coronavirus (β-CoV), γ-coronavirus (γ-CoV), and δ-coronavirus (δ-CoV) (Ahamad et al., 2021). The viral genome comprises two large overlapping polyproteins, open reading frame (ORF) 1a and ORF1b, from which the viral polymerase (RdRp) and other nonstructural proteins (Nsps) crucial for RNA synthesis or host response regulation are processed (Wang et al., 2020). The roles of the envelope and membrane proteins in viral pathogenesis and immune evasion are subjects of ongoing investigation, with the former facilitating virus assembly and the latter mediating viral entry into host cells via the angiotensin-converting enzyme-2 (ACE2) receptor (Shanmugaraj et al., 2020, Yan et al., 2020).

Clinical manifestations of SARS-CoV-2 infection typically include fever, dry cough, fatigue, and dyspnea, often accompanied by respiratory symptoms such as nasal congestion or runny nose (Huang et al., 2020). Severe cases may necessitate mechanical ventilation due to respiratory compromise (Yan et al., 2020). Detection of specific antibodies, including IgM and IgG, via serological assays, aids in diagnosing past or recent infections and assessing population prevalence (Hartnack et al., 2021). Various diagnostic methods, including RT-PCR, rapid antigen tests, and CRISPR-based assays, contribute to the arsenal for COVID-19 detection, continually evolving to enhance speed, accuracy, and accessibility (Hernández-Huerta et al., 2021, Böger et al., 2021, Sidiq et al., 2020) .

The presence of antibodies, while indicative of immune response, does not guarantee immunity against reinfection or determine its duration. To ascertain infection status comprehensively, a combination of RT-PCR for active infection and serological tests for antibody detection is recommended (Khoshkam et al., 2021). Notably, IgG antibodies, produced later in infection, confer long-term immunity, whereas IgM antibodies signify early immune response (Mathur et al., 2021). Understanding the dynamics and roles of different antibody classes, alongside cellular immune responses involving CD4+ and CD8+ T cells, contributes to deciphering COVID-19 immunity and informs vaccine development (Tretyn et al., 2021, Hall et al., 2021).

Vaccination against SARS-CoV-2 plays a pivotal role in inducing protective immune responses and curbing disease transmission (Chan et al., 2021). Messenger RNA (mRNA) and inactivated virus vaccines represent major vaccine platforms, demonstrating efficacy and safety in preventing COVID-19 (Wu et al., 2021). Studies suggest a period of reduced reinfection risk following natural infection, indicating the potential for acquired immunity (Gazit et al., 2022). However, ongoing research is essential to elucidate the durability and effectiveness of natural and vaccine-induced immunity against

SARS-CoV-2 (Pooley et al., 2023). Understanding the immune responses to SARS-CoV-2 infection, including antibody dynamics and cellular immunity, is crucial for effective disease management and vaccine development. This research aims to explore the prevalence of COVID-19 and investigate antibody levels among healthy individuals and COVID-19 patients, contributing to our understanding of COVID-19 epidemiology and immunity.

MATERIALS AND METHODS

The primary objectives of the study were to ascertain the prevalence of COVID-19 cases, identify SARS-CoV-2 variants, and assess antibody levels in both healthy individuals and COVID-19 positive patients. The subsequent subsections delineate the specific materials, procedures, and statistical analyses conducted.

Ethical approval

Ethical clearance was obtained from Government College University Faisalabad, Medical Superintendent of Allied Hospital Faisalabad and the Ethical Review Committee of Faisalabad Medical University before sample collection and processing.

Participants

A total (N=300) individuals from three medical institutes were recruited, including those who had tested positive for COVID-19 and those who were fully vaccinated but not infected. Participants were divided into two categories, the individuals without COVID-19 vaccination (COVID +ve), this group comprised individuals with laboratory-confirmed COVID-19, confirmed via reverse transcriptase-polymerase chain reaction (RT-PCR). Only individuals who provided their consent were enrolled in the study. The participants were divided into two groups based on their vaccination status: non-vaccinated (n=150) and vaccinated (n=150). Blood samples were collected post-recovery for antibody detection, with follow-up assessments conducted monthly and the individuals with COVID-19 vaccination (COVID –ve), this group included individuals vaccinated against COVID-19, without prior infection. Blood samples were collected approximately 10 day's post-second vaccination dose to evaluate the immune response.

Blood sample collection

Five milliliters of blood was collected from each participant in serum separator tubes, centrifuged, and stored at -20°C until further analysis.

Detection of SARS-CoV-2 IgG antibodies

IgG antibodies against SARS-CoV-2 were assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Omega Diagnostics) with a specificity and sensitivity of 95.2% and 95.4%, respectively. ELISA was performed for qualitative detection of IgG antibodies against SARS-CoV-2, following the kit protocol. The necessary materials, including vortex mixer, micro centrifuge, pipettes, tubes, and ELISA reader, were utilized. All reagents were equilibrated to room temperature and appropriately mixed before use. Wash buffer was diluted 1:10 with distilled water, and samples were diluted in sample diluent.

ELISA protocol

Diluted samples were added to antigen-coated microplates, along with controls. The plates were sealed and incubated, followed by washing and addition of conjugate. After incubation, substrate and stop solution were added, and the optical density was measured using an ELISA reader.

RESULTS

The results were presented, analyzed, and interpreted to provide insights into the prevalence of COVID-19, the genetic diversity of the virus, and the immune response in individuals. Additionally, the implications and significance of the findings was discussed, contributing to the existing knowledge on SARS-CoV-2 and its impact on hospitalized patients.

Demographic characteristics of participants

Based on gender showed that in the non-vaccinated group, 85 individuals (56.6%) were males, while 65 individuals (43.3%) were females. In the vaccinated group, there were 80 males (53.3%) and 70 females (46.6%). Regarding age groups, the participants were categorized into four groups. In the non-vaccinated group, 50 individuals (33.3%) were in the age range of 21-30 years, as were 50 individuals (33.3%) in the vaccinated group. The 31-40 year age group had 30 participants (20.0%) in both the non-vaccinated and vaccinated groups. In the non-vaccinated group, 30 individuals (20.0%) were in the 41-50 year age range, while 40 individuals (26.6%) fell in the same age group in the vaccinated group. For individuals above 50 years of age, there were 40 participants (26.6%) in the non-vaccinated group and 30 participants (20.0%) in the vaccinated group. These demographic characteristics provide a snapshot of the study participants and will help in analyzing the data and drawing conclusions related to the prevalence of COVID-19 and the impact of vaccination on different age groups and genders.

Duration of SARS-CoV-2 antibodies after natural infection

Those individuals who were truly positive for SARS-CoV-2 infection by RT-PCR, recruited in this study. We were following up the individuals after every three months till 1 year. In the first month of the study, all serological samples of the participants were positive for IgG antibodies. At the end of the study, 22 of the participants were non-reactive against SARS-CoV-2 IgG antibodies out of 150.

Waning of SARS-CoV-2 antibodies from M1 - M3

After three months all participants were reactive for IgG antibodies except one.

Waning of SARS-CoV-2 antibodies from M4 - M6

After 6 months total 5 of the participants were non-reactive for IgG antibodies.

Waning of SARS-CoV-2 antibodies from M7 - M9

After 9 months total 8 of the participants were non-reactive for IgG antibodies

Waning of SARS-CoV-2 antibodies from M10 - M12

After 1 year total 22 participants out of 150 were non-reactive for IgG antibodies. It apparently shows that antibodies began to rapidly drop after a year.

Out of the 150 cases, 128 individuals remained reactive for SARS-CoV-2 antibodies, while 22 individuals became non-reactive during the course of the study. These findings provide an overview of the serological response to natural SARS-CoV-2 infection among the study participants. The results of the 1-year follow-up of individuals included in the study, conducted every three months.

Table 1: One year follow-up study data of non-vaccinated individuals (N=150) after every three months.

Sr. No.	Time (Months)	Reactive cases (N)	Non-reactive cases (N)
1	1M	149	1
2	2M	149	1
3	3M	149	1
4	4M	149	1
5	5M	148	2
6	6M	145	5
7	7M	144	6
8	8M	144	6
9	9M	142	8
10	10M	139	11
11	11M	132	18
12	12M	128	22

Table 1 shows the number of cases that remained reactive and non-reactive for SARS-CoV-2 antibodies at each time point. As the study progressed, a gradual decrease in the number of reactive cases and an increase in non-reactive cases can be observed. These findings indicate the waning of SARS-CoV-2 antibodies over time among the study participants as shown in Figure 1.

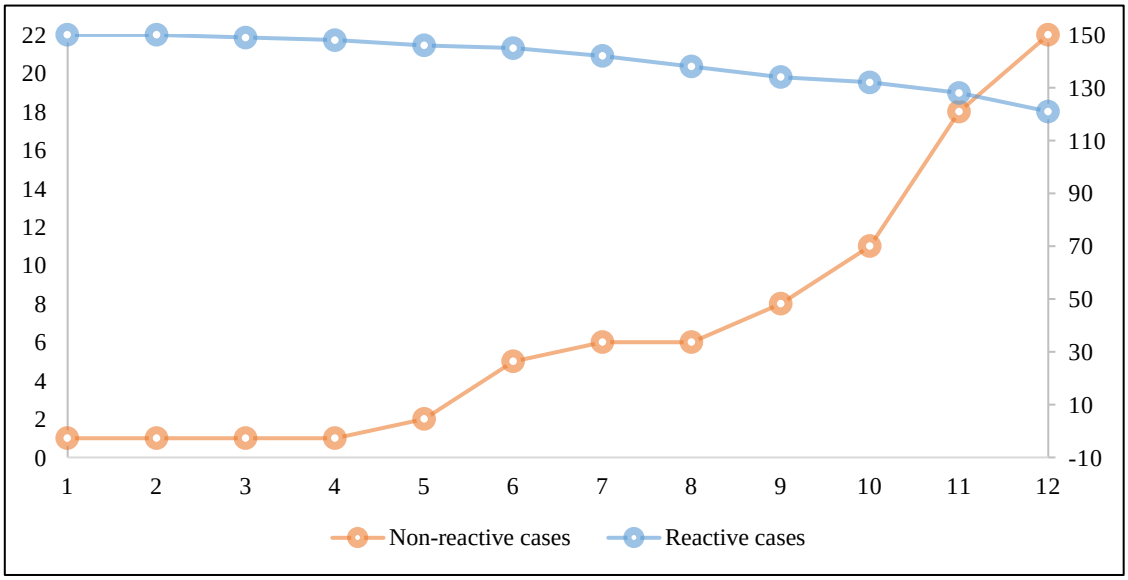

Figure 1: Represents the results of the 1-year follow-up of individuals included in the study, X-Axis represents the months while Y-axis represents the number of individuals.

Among the male participants, 71 individuals tested reactive while 14 individuals tested non-reactive, making a total of 85 participants. Among the female participants, 57 individuals tested reactive while 8 individuals tested non-reactive, making a total of 65 participants. These findings provide insights into the gender-based distribution of SARS-CoV-2 antibodies following natural infection.
Among individuals aged 21-30 years, 42 tested reactive and 8 tested non-reactive, making a total of 50 participants. In the 31-40 years age group, 27 tested reactive and 3 tested non-reactive, making a total of 30 participants. For the 41-50 years age group, 25 tested reactive and 5 tested non-reactive, making a total of 30 participants. Lastly, among individuals aged over 50 years, 34 tested reactive and 6 tested non-reactive, making a total of 40 participants. These findings provide insights into the age-wise distribution of SARS-CoV-2 antibodies following natural infection.

Duration of SARS-CoV-2 antibodies after vaccination
Those individuals who received two doses of COVID-19 vaccines were recruited for this study. Those individuals who had already immune from natural infection were excluded from this study. We were following up the individuals for the collection of blood samples after every three months. In the first month of the study all individuals were positive for IgG antibodies in serum samples. And, at the end of the study, total 29 individuals were non-reactive against IgG antibodies.

Waning of SARS-CoV-2 antibodies from M1 - M3
After three months all serological samples were reactive for IgG antibodies except one.

Waning of SARS-CoV-2 antibodies from M4 - M6
After 6 months only 5 samples were non-reactive for SARS-Cov-2 IgG antibodies.

Waning of SARS-CoV-2 antibodies from M7 - M9
After 9 months total 16 samples were non-reactive for SARS-Cov-2 IgG antibodies

Waning of SARS-CoV-2 antibodies from M10 - M12

At the end of the study total 29 samples of vaccinated individuals were non-reactive for SARS-CoV-2 IgG antibodies.

Out of the 150 vaccinated participants, 121 tested reactive for SARS-CoV-2 antibodies, indicating a positive immune response to the vaccine. On the other hand, 29 individuals showed a non-reactive result, suggesting a lack of detectable antibodies. These findings highlight the distribution of reactive and non-reactive cases following vaccination in the study population**.**

Table 2: One year follow-up study data of vaccinated individuals (N=150) after every three months.

Sr. No.	Time (Months)	Reactive Cases (N)	Non-Reactive Cases (N)
1	1M	150	0
2	2M	150	0
3	3M	149	1
4	4M	148	2
5	5M	146	4
6	6M	145	5
7	7M	142	8
8	8M	138	12
9	9M	134	16
10	10M	132	18
11	11M	128	22
12	12M	121	29

Table 2 represents the number of cases observed after vaccination over a period of 12 months. Each row corresponds to a specific time point, indicating the duration after vaccination, while the columns display the number of reactive and non-reactive cases at each respective time point. The data shows the changing trend in the immune response of individuals post-vaccination. Initially, at 1 month and 2 months, all cases were reactive. However, as time progressed, a small number of individuals started showing non-reactive results, with the number gradually increasing over the course of the study.

The "Total" column represents the total number of individuals in each gender category. The "Reactive" column indicates the number of individuals who tested positive for SARS-CoV-2 antibodies, while the "non-reactive" column represents the number of individuals who tested negative for SARS-CoV-2 antibodies. The data shows that among the male participants, 61 individuals were reactive, while 19 individuals were non-reactive. In the female group, 60 individuals were reactive, and 10 individuals were non-reactive as shown in Figure 2.

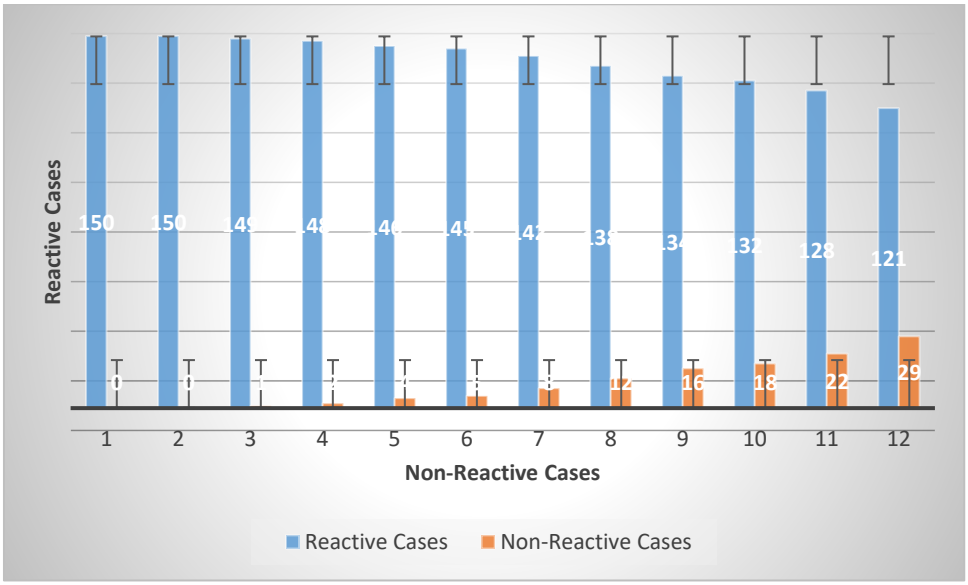

Figure 2. Represents the reactive and non-reactive cases among vaccinated people after one year.

The "Total" column represents the total number of individuals in each age group. The "Reactive" column indicates the number of individuals who tested positive for SARS-CoV-2 antibodies, while the "non-reactive" column represents the number of individuals who tested negative for SARS-CoV-2 antibodies. The data shows that among individuals aged 21-30 years, 48 individuals were reactive, and 12 individuals were non-reactive. In the age group of 31-40 years, 26 individuals were reactive, and 4 individuals were non-reactive. Among individuals aged 41-50 years, 34 individuals were reactive, and 6 individuals were non-reactive. Lastly, among individuals older than 50 years, 23 individuals were reactive, and 7 individuals were non-reactive.

DISCUSSION

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has profoundly impacted global health and economies, necessitating ongoing surveillance and research to comprehend its genetic diversity, transmission dynamics, and the emergence of new variants (Chow et al., 2023). Our study aimed to compare results with previous research on SARS-CoV-2 variants, focusing on molecular characterization and serological investigations among hospitalized patients, and analyzing genetic diversity and transmission patterns in our region. This aligns with global studies emphasizing stringent infection control measures in healthcare settings (Wang et al., 2020). Gender-wise distribution showed a slightly higher proportion of positive cases among females, suggesting potential variations in susceptibility and healthcare-seeking behavior. Similar prevalence rates have been reported in other regions, supporting the notion of low COVID-19 prevalence among hospitalized populations (Sheikh et al., 2021, Goyal et al., 2020). However, limitations include data collection from a single healthcare facility, possibly limiting generalize-ability, and inclusion criteria that may underestimate overall prevalence. Our study included 300 individuals from Faisalabad, categorized by vaccination status and gender distribution. We observed a gradual decline in SARS-CoV-2 antibodies over time following both natural infection and vaccination, consistent with prior research (Ibarrondo et al., 2021, Lumley et al., 2021). This underscores the need for continuous monitoring and potential booster vaccinations. Gender

and age-wise analyses revealed higher antibody levels among males and individuals aged 21-30 years, possibly due to higher exposure rates. Older age groups also exhibited significant immune responses, indicating vaccination efficacy across diverse age ranges (Bwire and Paulo, 2020, Takahashi et al., 2020).

Our findings contribute to understanding COVID-19 prevalence and antibody dynamics after natural infection and vaccination, informing public health measures and vaccination strategies. However, further research is essential to comprehensively grasp long-term antibody dynamics, correlate them with protection against reinfection and severe disease, and explore T cell responses' role (Grifoni et al., 2020). Additionally, studying antibody levels in symptomatic versus asymptomatic individuals and comparing them with healthy populations can provide insights into immune protection and susceptibility to reinfection (Ng et al., 2021).

CONCLUSION

This study emphasizes the urgent need for enhanced genomic surveillance in Pakistan to monitor the emergence and spread of SARS-CoV-2 variants, highlighting the importance of timely detection, and understanding their implications for public health interventions and vaccine development. Strengthening genomic surveillance capabilities is crucial for effective pandemic management, enabling early detection and tracking of viral variants through expanded surveillance scope and functional characterization. Implementing robust genomic surveillance strategies empowers policymakers to make informed decisions regarding targeted interventions, while ongoing research and collaboration are essential for adapting public health strategies based on emerging scientific evidence. Continued vigilance in monitoring and understanding SARS-CoV-2 variants will aid in the development of effective public health measures, ultimately contributing to global efforts in controlling the spread of COVID-19.

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