RESEARCH ARTICLE

Impact of Age, Gender, post infection and post vaccination status on antibody response in COVID 19 patients

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Abstract

Objective: To evaluate severe acute respiratory syndrome coronavirus-2 spike protein antibodies against coronavirus disease-2019 in post-infection and post-vaccinated individuals.

Method: The cross-sectional study was conducted from June, 1 to July 31, 2021 at the Rehman Medical Institute, Peshawar, Pakistan, and comprised subjects of either gender in whom immunogenicity was checked 35 days post-vaccination and 90 days post-infection. Correlation with age and gender was checked. Specimens were collected and investigated for severe acute respiratory syndrome coronavirus-2 spike protein antibodies by consuming electro-chemiluminescence immunoassay. Data was analysed using SPSS 23.

Results: Of the total 256 patients enrolled, 70(27.34%) were included; 49(69%) males and 21(29.6%) females. The overall mean age was 44±7.75 years. Among 30(42.8%) patients with positive polymerase chain reaction test, the mean time between the positive test and antibody screening was 90±30 days. Among the 40(57.2%) vaccinated individuals, the time between vaccination and antibody screening was 35±9.74 days. Overall, 68(97%) patients revealed robust positive findings to severe acute respiratory syndrome coronavirus-2 spike proteins antibodies >50IU/mL. Male subjects had significantly higher immunogenic response compared to females (p=0.001), and immunogenicity decreased with advancing age (p<0.001). Also, post-vaccinated patients' antibody response was significant compared to post-infection patients' response (p=0.001).

Conclusion: Majority of the patients had significantly higher antibody titers against severe acute respiratory syndrome coronavirus-2 post-infection and post-vaccination. Males and younger individuals developed a significant humoral immunity compared to females and the elderly.

Keywords: Antibodies, COVID-19, SARS-CoV-2, Vaccination. (JPMA 72: 1805; 2022)

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Introduction

The coronavirus diseased-2019 (COVID-19), a novel positive-strand ribonucleic acid (RNA) coronavirus of the Coronaviridae family was declared a pandemic by the World Health Organisation (WHO) on March 11, 2020.[1] It continued to spread despite repeated lockdowns and long-term control measures in most countries. The envelope (E), membrane (M), nucleocapsid (N) and spike (S) proteins in the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) genome are structural proteins. S1 and S2 are the two subunits of the S protein.[2]

The S1 subunit contains the receptor binding domain (RBD), which has a high affinity for the angiotensin converting enzyme 2 (ACE2) receptor on the cell surface membrane. The interaction of SARS-CoV-2 RBD with ACE2 viral receptor on the host cell is what causes the infection.[2,3]

The humoral immune response to infection or vaccination has two basic outcomes: antibody production by antibody secreting cells (ASCs), which can provide rapid serological immunity, and the development of long-lived memory B cells, which can mount recall responses.[4,5] Memory B cells drive the recall response by producing new antibodies by establishing new ASCs or re-entering germinal centres for subsequent rounds of somatic hyper-mutation if circulating antibodies fail to protect against a future exposure.[6,7] COVID-19-infected persons are expected to have antibodies and memory B cells for at least 6-8 months.[8-12] In SARS-CoV-2-exposed persons, no memory responses or effective vaccine dose protocols have been investigated.[13]

Control techniques, such as mask-wearing, physical separation, and the use of contact tracing, were beneficial to decrease the virus spread, but no significant advantages were observed, and it was realised that vaccinations were the only realistic way out of the pandemic.[14] Most COVID-19 vaccination approaches to date have aimed at generating neutralising antibodies against S protein, hence preventing SARS-CoV-2 infection in its early stages. Several vaccine candidates have been demonstrated to be safe and effective in clinical studies[15] and vaccination in mass

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numbers (when used in combination with other existing control measures) is recognised as one of the most important aspects of pandemic management. Although the results of clinical trials are encouraging, real-world evidence on vaccines is still lacking.

The current study was planned to evaluate SARS-CoV-2 S antibody levels among post-infected and post-vaccinated individuals in a tertiary care setting.

Materials and Methods

The cross-sectional study was conducted from June, 1 to July 31, 2021, at the Rehman Medical Institute, Peshawar, Pakistan. After approval from the institutional ethics approval board, the sample size was calculated using the WHO calculator[16] on the basis of literature.[17] The sample was raised using purposive sampling technique. Those included were individuals of either gender aged 18-80 years, who were selected retrospectively, while those with negative polymerase chain reaction (PCR) test and those not vaccinated were excluded. Also excluded were patients receiving immunosuppressive therapy or suffering from an immunosuppression-related disease, having no history of infection or vaccination, those aged <18 years and pregnant women.

Post-vaccinated patients had received two doses of either Sinopharm or Sinovac 21 days apart as validated by the vaccination certificate. Both the vaccines contained inactivated virus. The post-infection status was validated by PCR report. Post-infection patients were divided into three groups on the basis of mild, severe or carrier state, based on the Chinese Centre for Disease Control fuidelines.[18] Samples for immunoglobulin-G (IgG) quantitative antibodies were obtained by trained personnel on day 90 and day 35 in post-infection and post-vaccinated individuals, respectively. Written informed consent was obtained from all the subjects..

Standard protocols were used to collect a nasopharyngeal swab, and the presence of SARS-CoV-2 was identified by reverse transcriptase PCR (RT-PCR) (Light Mix Modular SARS-CoV-2 COVID-19 RdRP, Roche, Switzerland) testing. According to manufacturer's recommendations, a cycle threshold (CT) result of 15-30 was considered positive.

An electro-chemiluminescence immunoassay (ECLIA) (Abbot Architect, United States) based on double-antigen sandwich assay principle was tested (Abbot Advice Dx SARS-CoV-2 IgG II assay, US) for quantitatively determining antibody levels to the SARS-CoV-2 S protein. Blood samples were collected using acid citrate dextrose, sodium citrate, potassium ethylenediaminetetraacetic acid (EDTA), tri-potassium EDTA or lithium heparin tubes. This competitive serological assay simultaneously determined an individual's seropositivity against the SARS-CoV-2 S protein and estimated the neutralising capacity of anti-S antibodies to block interaction with the human ACE2 required for viral entry. Natively-folded viral S protein RBD-containing antigens via avidin-biotin interactions were noted. Sera were then supplemented with soluble ACE2-fragment crystallizable (Fc) fusion to compete for RBD-binding serum antibodies, and antibody binding was quantified. Comparison of signal from untreated serum and ACE2-Fc-treated serum revealed the presence of antibodies that compete with ACE2 for RBD-binding, as evidenced by loss of signal with ACE2-Fc treatment. The manufacturers recommended antibodies cutoff level of 50.0AU/mL, above which the value was considered positive. The diagnostic measuring interval of 22.0 to 25000.0 AU/mL was found to be the linearity.

Data was analysed using SPSS 23. Age, gender, time duration of sample collection were all subjected to descriptive statistics. Pearson correlation was used to establish the correlation of age, gender, post-infection and post-vaccination status with antibody levels. P<0.05 was taken as statistically significant.

Results

Of the total 256 patients enrolled, 70(27.34%) were included; 49(69%) males and 21(29.6%) females. The overall mean age was 44±7.75 years. Among 30(42.8%) patients with positive PCR test, the mean time between positive the test and antibody screening was 90±30 days. Among the 40(57.2%) vaccinated individuals, the time between vaccination and antibody screening was 35±9.74 days (Table 1).

Overall, 68(97%) patients revealed robust positive findings to SARS-CoV-2 S protein antibodies >50IU/mL. Male subjects had significantly higher immunogenic response compared to females (p=0.001), and immunogenicity decreased with advancing age (p<0.001). Also, post-vaccinated patients' antibody response was significant compared to post-infection patients' response (p=0.001) (Table2).

Table-1: Demographic characteristics (n=70).

Variables	n (%)
Gender	
Male	49 (69)
Female	21 (29.6)
Mean Age (years)	44±7.75
Post infection	30(42.8)
Mean Time of sample collection (post-infection) (days)	90±30 days
Post-vaccination	40(57)
Mean Time of sample collection (post-vaccination) (days)	35±9.74 days.

Table-2: Correlation of immunogenicity with age, gender and patient status.

Variables	Antibody level(AU/mL)	*p*-value
Gender		
Male	3589	0.001
Female	1968	0.236
Age group (years)		
<30	89	0.215
30-40	1200	0.003
40-49	3240	0.001
50-59	2365	0.272
>60	178	0.028
Post-infection	1305	0.46
Post-vaccination	1773	0.001

Table-3: Clinical status of post-infection individuals (n=30).

Status of Patient	n (%)
Mild symptoms	8(26.6)
Severe Symptoms	12 (40)
Carrier State	10 (33.3)

Table-4: Clinical diagnostic criteria of coronavirus disease-2019 (COVID-19) symptoms.

Clinical Diagnostic Criteria symptoms	Mild[8] n (%)	Clinical Diagnostic Criteria	Severe[12] n (%)
Fever	4(50)	Dyspnoea	3(25)
Cough	2(25)	Acute Respiratory distress	2(16.6)
Fatigue	1(12)	Pulmonary infiltrates	0
Diarrhoea	1(12)	Decrease in oxygen saturation	5(41.6)
		Multiple ground glass opacities	0

Among the 30(42.8%) post-infection patients 12(40%) had severe symptoms (Table 3), and among those with severe symptoms, the most common was decrease in oxygen saturation 5(41.6 %) (Table 4) None of the vaccinated individuals had previously encountered Covid-19 infection.

Discussion

In the current study, SARS CoV-2 post-infection or post-vaccination individuals were selected for investigation. A substantial portion of the enrolled patients in study presented with remarkably higher anti-SARS-CoV-2 antibody levels of >50 AU/mL, and the results are comparable with other clinical trials.[17,19]

People who had been previously exposed to COVID-19 had significant humoral immunogenic response compared to non-infected individuals which was consistent with earlier findings.[20]

Only a few studies have assessed gender differences in COVID-19 incidence and disease progression and an independent review of the responsible factors is still absent. Based on differences in innate and adaptive immunity, steroid hormone synthesis by the gonads, and sex chromosomal variables, it is interesting to note that males showed a greater immunological response than females in an Italian study.[21] In viral infections, sex hormones are known to regulate innate immune responses. Oestrogens influence receptor responsiveness and the creation of pro-inflammatory cytokines, which can be life threatening if released in excess. Oestrogens that bind to the oestrogen receptor alpha (ERa) or beta receptor (ERb) can thereby influence the immune system. All immune cells express ERa, which is essential in their maturation and regulation. It is also immunologically protective, since it is involved in the generation of interferon (IFN) type I and the activation of natural killer (NK) cells. ERb is engaged in pro-inflammatory events and has the opposite effects. The decrease of ERa in elderly women is linked with immunosuppression, indicating that oestrogen can protect against COVID-19.[22]

It has been reported that antibody levels decline with advancing age.[19,23] In one study on 34 individuals in the USA, peak antibody titers occurred between 30 and 152 days post-infection.[24] Similar findings have been reported from Israel.[19]

Protective correlations have already been established for a range of viral diseases. These associations are typically established on a certain titer of antibody obtained from vaccination or spontaneous infection, which considerably diminishes the chance of (re)infection, like for example, the Hem agglutination inhibition level for influenza virus, where a 1:40 level minimises the possibility of transmission by 50%.[25] It is uncertain whether human infection with SARS-CoV-2 prevents against reinfection[26] and if so, for how long. We know that neutralising antibodies are produced by common human coronaviruses and that these antibodies can last for decades, preventing against reinfection or attenuating symptoms if reinfection occurs.[27]

It is perceived that infection with SARS-CoV-2 protects non-human primate models from reinfection for at least some duration.[28] Although there is no definitive proof that these antibody responses guard against reinfection, that is extremely probable to reduce the likelihood of reinfection and, in the instance of breakthrough infection, may attenuate symptoms.

When immune reactions to vaccine were studied among 100 participants in Pakistan, considerably robust immune response was noted after a single vaccine shot[17] and similar results were stated by another study.[17,29] The majority of the patients developed humoral response 35 days post-administration of the second dose of COVID-19 vaccine.[19]

The current study had the limitation of a small sample size. Establishing immunogenicity in post-infection and post-

vaccination individuals, as well as the impact of age and gender on humoral immune responses, requires further research.

Conclusions

Maximum number of patients developed immunity post-infection and post-vaccination. Male and younger individuals were more efficient in developing humoral immune responses than female and older patients.

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