



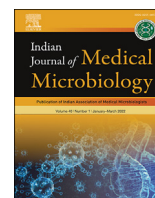
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Original Research Article

Evaluation of SARS-CoV-2 antibody responses in healthcare workers at a tertiary care COVID 19 dedicated hospital in Delhi

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ABSTRACT

Purpose: Amidst the on-going SARS-CoV-2 pandemic, healthcare workers have been at a greater risk of disease exposure as they are working in environments chiefly involved in the COVID-19 patient care since March 2020. SARS-CoV-2 antibody testing can prove to be a valuable tool for better understanding of prevalence of disease exposure in this population. Therefore, we conducted this study to grasp the sero-prevalence of COVID-19 antibodies in our hospital to better comprehend the duration of IgG response.

Methods: This was a longitudinal study involving 305 healthcare workers at Rajiv Gandhi Super Speciality Hospital spanning over a period of four months starting from October 2020 to January 2021. Serum samples were obtained from the study group taken as Day 0 of the study and were screened for the presence of SARS-CoV-2 IgG antibodies using semi-quantitative enzyme linked immunoassay technology from ERBALisa (India). The Antibody Index was determined. Those showing reactive in the screening test were further followed up on a monthly basis till January 2021 for serial antibody testing.

Results: The overall seroprevalence for IgG response among the workers was found to be 21.96%. Seropositivity rate was observed to be significantly higher in those having a history of RT-PCR confirmed COVID-19 infection (45.09%)

Conclusions: Our study demonstrated that healthcare workers have a higher sero-prevalence. Our study also demonstrated that the antibodies developed following COVID-19 infection had a waning effect of protective response following infection.

1. Introduction

The World Health Organisation (WHO) declared coronavirus disease 2019 (COVID 19) caused by a novel coronavirus, Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) to be a pandemic on March 19, 2020 [1]. Since then, the virus has wreaked havoc across continents, leading to increased mortality, morbidity and disruption of normal life. Amidst this, healthcare workers are at a greater risk of disease exposure as they have been working in environments chiefly involved in the SARS-CoV-2 patient care since March 2020 especially in COVID-19 dedicated care centre like ours.

This raises concerns about the safety of frontline workers as well as risk of transmission to other patients and fellow healthcare workers. SARS-CoV-2 antibody testing can prove to be a valuable tool for better understanding of prevalence of disease exposure in this population as it also adds to our existing knowledge of the extent of infection among

people who are not identified through active case finding. Antibody test will be critical to know the immune status of the frontline workers. However, due to limited peer-reviewed literature, it is not yet known whether these antibodies will be protective in nature and how long will they persist in the body.

Therefore, we conducted this longitudinal study to grasp the sero-prevalence of COVID-19 antibodies in our hospital and to better comprehend the duration of IgG response.

2. Materials and methods

This was a longitudinal study done at Rajiv Gandhi Super Speciality Hospital spanning over a period of four months starting from October 2020 to January 2021, on healthcare workers involved in the provision of care for COVID-19 patients.

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A total of 305 healthcare workers were initially enrolled in the study and were divided into the following groups: a) Doctors & Nurses working in different hospital wards/Intensive Care Units & Flu Out-patient department; b) laboratory personnel and technicians in Operation Theatre, cardiology department, radiology department and SARS-CoV-2 diagnostic units; c) patient transporters/Nursing orderly/Multi-task worker; d) cleaning personnel; e) others (guards, physical therapist & administrative workers). Any participant reporting flu-like illness at the time of screening was excluded from the study group. Written informed consent was obtained from all the participants as approved by the Ethics Committee of the Institute (ECR/1436/Ins/DL/2020). After consent, the healthcare workers were asked to fill in a form that detailed their department, history of exposure to COVID patients, history of COVID-like symptoms and history of a positive COVID test if any. Serum samples were obtained from the study group taken as Day 0 of the study and were screened for the presence of SARS-CoV-2 IgG antibodies using semi-quantitative enzyme linked immunoassay technology from ERBALisa (India). The Antibody Index was determined for all patients which had a positive value of 1.1 and negative below 0.9. The results were communicated to the tested healthcare workers with explanation about the implications of a positive or negative result. Those showing reactive in the screening test were further followed up on a monthly basis till January 2021 for serial antibody testing.

3. Result

A total of 305 healthcare workers participated in the study. Initial samples were collected in the month of October 2020. The average age of participants was 33 years (range 19–59 years) with male: female ratio of 1.9:1. Maximum participation was from nursing staff (22.62%; 69/305) followed by nursing orderly (20.32%; 62/305), housekeeping & multi-task workers (16.72%; 51/305), doctors (16.06%; 49/305), security guards (15.73%; 48/305) & technicians from various departments (8.52%; 26/305).

The overall seroprevalence for IgG response among the workers was found to be 21.96% (67/305) with highest reactive rate reported among nursing staff (27.53%; 19/69) followed by technicians included O.T, ECHO, Cath. Lab., phlebotomist & those involved in COVID-19 diagnostics (26.92%; 7/26) and security guards (22.9%; 11/48) as shown in Fig. 1.

While most of the participants were asymptomatic in the last 6 months (83.25%; 254/305), 16.72% (51/305) had past history of RT-PCR (real time-polymerase chain reaction) confirmed COVID-19

infection. Seropositivity rate was observed to be significantly higher in this category (45.09%; 23/51) as shown in Table 1.

Initially 67 individuals tested as positive for IgG SARS-CoV-2 antibodies that were evaluated further on monthly basis to assess the duration of antibody response. Sixty-three participants tested reactive in the 1st follow-up done in the month of November 2020. Of these, 57 returned next month for 2nd follow up testing. All but 20 showed a reactive result for SARS-CoV-2 antibodies. In the 3rd and final follow up testing done in January '21, 6 more became non-reactive for SARS-CoV-2 antibodies as depicted in Fig. 2.

Among the 30 healthcare workers who became non-reactive during the study period, 18 were those who were previously asymptomatic with no past history of COVID-19 infection and 12 had a past history of COVID 19 infection reported before the month of July 2020.

Out of all the positive samples, 23 cases were such in which both history of COVID-19 infection and subsequently presence of antibodies was detected. An overall declining trend was observed in the antibody detection rate in this group as the time to COVID-19 infection advanced. The number of patients with antibodies following COVID infection gradually declined from 1st month to 7th month from the onset of infection as illustrated in Fig. 3.

Of the above 23 patients, it was observed that the average antibody index gradually increased in the first and second month following the infection. This was followed by a fall in antibody index which then again rose till the fifth month. Afterwards antibody levels followed a downward trend till the 9th month when the study was concluded. This is reflected in Fig. 4. The comparison of antibody levels in asymptomatic exposure vs symptomatic infection is also depicted in Fig. 4 which shows a delayed rise in peak antibody levels in asymptomatic individuals.

Table 1
Seroprevalence rate among no previous history of COVID, symptomatic and asymptomatic individuals.

	TOTAL TESTED	TOTAL POSITIVE	SEROPREVALENCE RATE
No history of COVID-19 symptoms	254	44	17.32%
Past history of confirmed COVID-19 infection associated with symptoms	35	20	57.14%
Past history of confirmed COVID-19 infection with no symptoms	16	3	18.75%

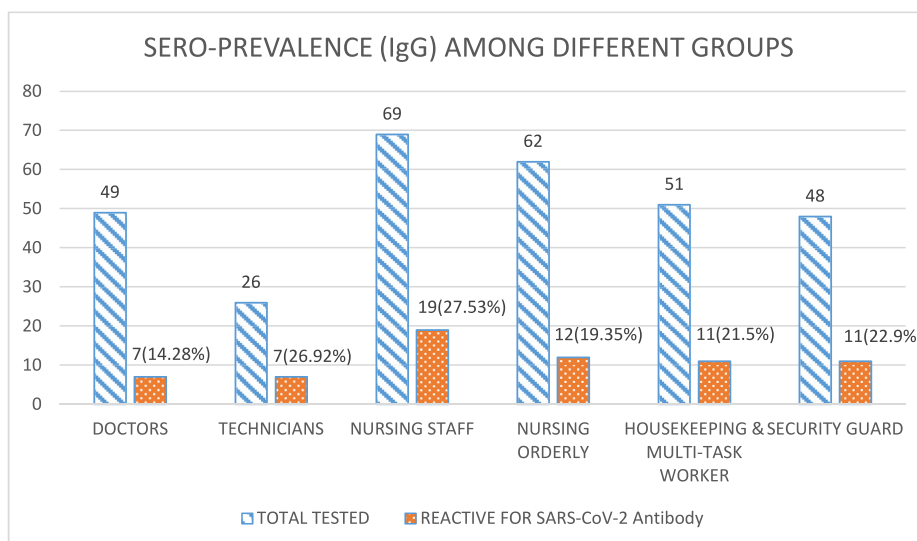


Fig. 1. Seroprevalence (IgG) among different healthcare groups.

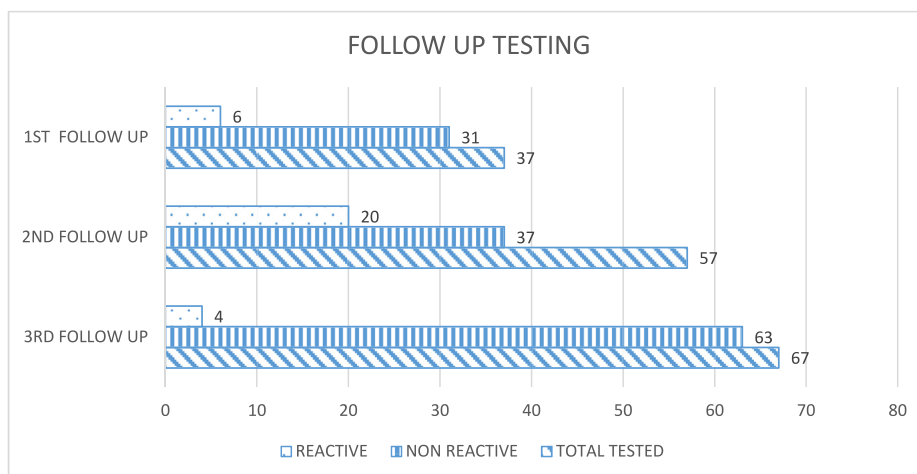


Fig. 2. Follow up testing.

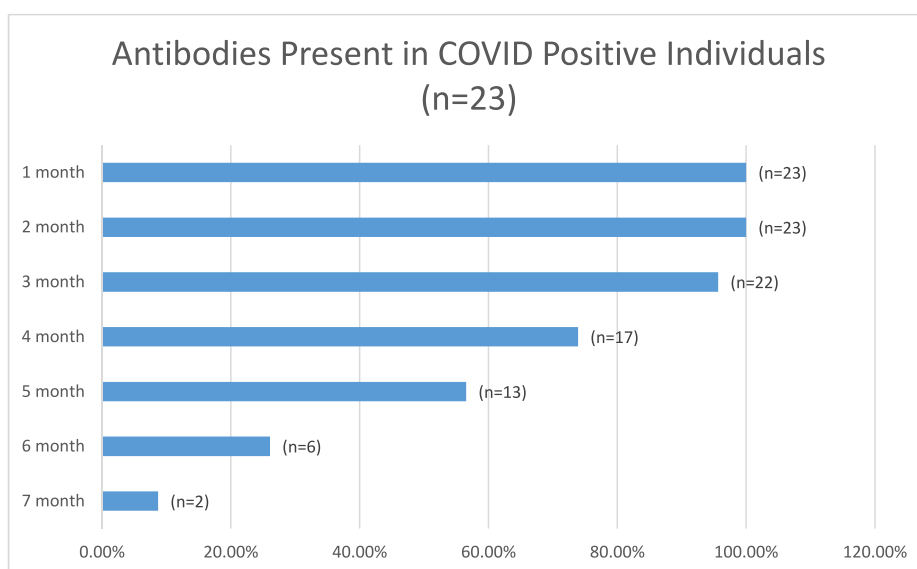


Fig. 3. Antibodies present in COVID positive individuals.

4. Discussion

With increase in reporting of COVID 19 infection, seroprevalence studies among healthcare workers are coming into prominence. In our study, we enrolled a total of 305 healthcare workers (HCWs) and the overall seroprevalence was found to be 21.96%.

A cross-sectional seroprevalence study among HCWs in a New York City hospital also reported 27% positivity for SARS-CoV-2 antibody [2]. Another study from New York City at a tertiary academic hospital noted HCW seroprevalence to be as high as 32.6% [3]. Yet another study done on 2149 healthcare workers in Sweden indicated seroprevalence of 19.1% similar to ours [4]. A study done at a London NHS trust found a seropositivity rate of 31.6% among healthcare workers [5]. Although, there are few studies that have reported seroprevalence of as low as 9-11% [6,7], this data suggests that high rate in healthcare worker can be related to high burden of local transmission, thus increased risk of exposure. This can also imply that a study done at the time of peak infection like ours may report a higher seroprevalence rate. Another explanation suggested for varying sero-prevalence rates is the possibility of the short lived antibody response or the timing of the antibody test post-exposure [8]. A study from India reporting seroprevalence rate of 11.94% amongst healthcare worker mentions that difference in

prevalence may be related to the period of study, prevalence in the local community, hospital policy in terms of triage, social distancing, hand sanitisation and use of personal protective equipment (PPE) [9]. However, we did not carry out a direct assessment of the above factors in our study.

Among the various occupational groups, we found highest positivity rate among nursing staff. Joseph Grant et al. also found a high seropositivity rate among staff working in a clinical environment with direct patient contact [5]. Another study mentions that high seroprevalence among nurses further supports transmission from patients to healthcare worker when considering that these occupations involve the most patient near contact [4].

Notably we also found a high seroprevalence rate in security guard, housekeeping staff and technicians from various departments. Another study from India also reports a high seroprevalence rate among housekeeping staff, food and beverage staff, lab. Assistant and technicians [9]. One study postulates that this may be due to a decreased perception of risk in this group in contrast to other job categories that may take precautions due to their direct contact with COVID 19 patients [10]. We also observed that doctors reported lowest seropositivity rate. Hence, we can also infer from our findings that differential risk of SARS-CoV-2 exposure exist within the hospital environment. The reasons for the same can be

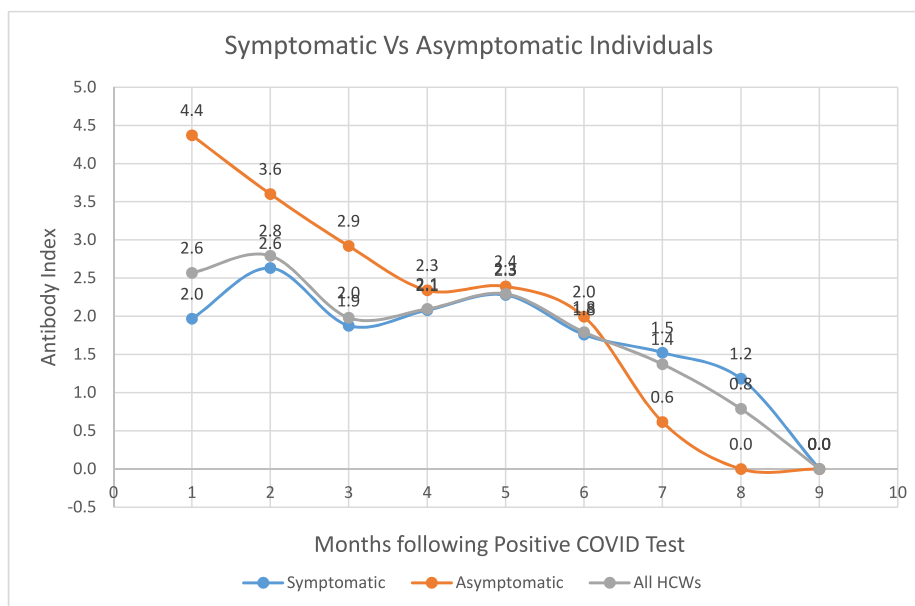


Fig. 4. Comparison of antibody levels in symptomatic Vs asymptomatic individuals.

multifactorial such as better adherence to PPE among doctors than housekeeping staff and security guards and decreased understanding of the disease among other job categories as compared to doctors.

An important finding of our study was seroprevalence rate of 17.32% was observed in subset with no history of COVID-19 infection and 18.75% in those having asymptomatic infection. Similar data was reported by another study wherein 21.9% did not report any COVID-19 symptoms [5]. In a cross sectional study examining SARS-CoV-2 antibodies in a university study population, they reported more than one-third of the positive cases without any prior illness at all [11]. This finding points out the fact that a sizeable proportion of our study population were pauci-symptomatic or asymptomatic. It also indirectly emphasise on the fact that these individuals may have acted as vector for nosocomial transmission as they were not isolating at the time of infection. Ko et al. mentions in their paper that recent evidence showed asymptomatic and subclinical COVID-19 patients producing detectable amounts of anti SARS-CoV-2 antibodies suggesting that subclinical infection could be screened by serologic tests [12].

In extension to the above findings, we also observed a seroconversion rate of 57.14% in those reporting symptoms as compared to 18.75% in asymptomatic group. Another study reported 44% seroconversion in symptomatic cohort compared to 13% in without symptoms similar to our study [2]. Adrian Shield et al. observed that participants who reported prior symptomatic illness had higher seroprevalence (37.5% vs 17.1%) and quantitatively greater antibody responses than those who had remained asymptomatic [13]. These findings imply that developing the disease following the infection is a major influencer of antibody production as it is a well-known fact that the magnitude of the antibody response depends on the severity of infection; therefore, patients with asymptomatic infection may not mount a measurable antibody response. S. Brandstetter also concluded in their study that exposure to COVID 19 positive co-workers in a hospital setting is not leading to development of measurable immune response in a significant proportion of asymptomatic contact person [14].

We also followed the seropositive group for 3 months to assess the duration of antibody response.

Among the 67 individuals who were followed up, we found that 30 became non-reactive during the study period and 10 lost to follow up. Another key finding of our study was that the quantitative antibody index rose initially till 2 months post infection, followed by a slight decline and then again increase in titres was observed. This double peak of average IgG

antibody index levels can be attributed to increase in cases in that particular time period, long half-life of IgG and the fact that it reaches stable titer late that is maintained over time. Moncunill et al. also observed this similar pattern in their study wherein 2 individuals who had seroreverted at month 1 (1 for IgG and 1 for IgA) had detectable antibody levels again at month 3¹⁰. In our study, we observed the IgG antibody index levels started to decline steadily from fifth month post infection and continued to fall over the course of study period. Similar fall of antibody levels were observed by Patel et al. wherein they documented 58% of sero-positive individuals reverting to sero negativity after 60 days [15]. Another study also demonstrated declining neutralizing antibody titres within the follow up period of 95 days' post onset of symptoms [8]. Pyoeng Guyn et al. also reported similar waning antibody responses in asymptomatic and symptomatic SARS CoV-2 infection [16]. In a study by Self et al. levels of antibodies in healthcare personnel declined at follow up at 2 months which is similar to results in our study [17]. Another study by Marot et al. also noticed declining level of antibodies at follow up in healthcare workers [18].

These results indicate that SARS CoV-2 infection elicits an antibody response at least temporarily and negative serology result doesn't exclude the possibility of previous infection. A study conducted by Wang To et al. demonstrated a correlation of *anti-SARS-CoV 2* IgG levels with viral neutralization titres [19]. However further studies are required to equate the antibody decline with loss of protective immunity. Another question that remains unanswered is the need for repeated possible boosters needed to augment this waning effect of antibodies.

5. Conclusion

In conclusion, our study demonstrated that healthcare workers have a higher sero-prevalence than expected. Symptomatic participants showed a greater rate of seropositivity compared to those without symptoms. Our study also demonstrated that the antibodies developed following COVID-19 infection initially rose and fell. Subsequent to this there was another rise which sustained till 5th month post-infection. By the 7th month, levels were almost undetectable. This demonstrated the waning effect of antibody response following infection.

6. Limitations

In our study, total IgG levels were ascertained, however the levels of neutralizing antibodies were not determined.

CRediT authorship contribution statement

Concepts: Dr. Sonali Bhattar. **Design:** Dr. Sonali Bhattar. **Definition of intellectual content:** Dr. B.L. Sherwal. **Literature research:** Dr. Sukriti Sabharwal. **Data acquisition:** Dr. Sukriti Sabharwal, Dr. Sonali Bhattar, Dr. Ajit Jain, Dr. Mona Bhargava, Dr. Vikas Dogra. **Data analysis:** Dr. Sukriti Sabharwal, Dr. Sonali Bhattar, Dr. Shikhar Saxena. **Manuscript preparation:** Dr. Sukriti Sabharwal. **Manuscript editing:** Dr. Sonali Bhattar, Dr. Shikhar Saxena. **Manuscript review:** Dr. B.L. Sherwal.

Author contribution

Dr. Sonali Bhattar: Concepts, design, data acquisition, data analysis, manuscript editing, Dr. B.L. Sherwal: Definition of intellectual content, manuscript review, Dr. Sukriti Sabharwal: Literature research, data acquisition, data analysis, manuscript preparation, Dr. Ajeet Jain: Data acquisition, Dr. Mona Bargoty: Data acquisition, Dr. Vikas Dogra, Data acquisition, Dr. Shikhar Saxena, Data analysis, manuscript editing

Declaration of competing interest

The author declare no conflicts of interest.

References

- [1] WHO. Coronavirus disease (COVID-19) outbreak. 2020. <http://www.who.int/emergencies/diseases/novel-coronavirus-2019>.
- [2] Venugopal U, Jilani N, Rabah S, Shariff AM, Jawed M, Batres MA, et al. SARS-CoV-2 seroprevalence among health care workers in a New York City hospital: a cross-sectional analysis during the COVID-19 pandemic. *Int J Infect Dis* 2021;102:63–9.
- [3] Mansour M, Leven E, Muellers K, Stone K, Mendu RD, Wajnberg A. Prevalence of SARS-CoV-2 antibodies among healthcare workers at a tertiary academic hospital in New York city. *J Gen Intern Med* 2020;35(8):2485–6.
- [4] Rudberg SA, Havervall S, Månberg A, Falk JA, Aguilera K, Ng H, et al. SARS-CoV-2 exposure, symptoms and seroprevalence in healthcare workers in Sweden. *Nat Commun* 2020;11:5064.
- [5] Grant JJ, Wilmore SMS, McCann SN, Donnelly O, Lai LWR, Kinsella JM, et al. Seroprevalence of SARS-CoV-2 antibodies in healthcare workers at a London NHS Trust. *Infect Control Hosp Epidemiol* 2020;1–3.
- [6] Galan I, Velasco M, Casa ML, Goyanes MJ, Rodríguez-Caravaca G, Losa JE, et al. Hospital-wide SARS-CoV-2 seroprevalence in health care workers in a Spanish teaching hospital. *Enferm Infecc Microbiol Clin* 2020;18(S0213-005X(20)):30418–3. Available <https://www.medrxiv.org/content/10.1101/2020.05.29.20116731v1>.
- [7] Paderno A, Fior M, Berretti G, Schreiber A, Grammatica A, Mattavelli D, et al. SARS-CoV-2 infection in healthcare workers: cross-sectional analysis of an otolaryngology unit. *Otolaryngol Head Neck Surg* 2020;163(4):671–2.
- [8] Seow J, Graham C, Merrick B, Acors S, Steel KJA, Hemmings O, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. *Nat Microbiol* 2020;5(12):1598–607. Available, <https://www.medrxiv.org/content/10.1101/2020.07.09.20148429v1>.
- [9] Goenka M, Afzalpurkar S, Goenka U, Das SS, Mukherjee M, Jajodia S. Seroprevalence of COVID-19 amongst health care workers in a tertiary care hospital of a metropolitan city from India. *J Assoc Phys India* 2020;68(11):14–9.
- [10] Moncunill G, Mayor A, Santano R, Jiménez A, Vidal M, Tortajada M, et al. SARS-CoV-2 seroprevalence and antibody kinetics among health care workers in a Spanish hospital after 3 Months of follow-up. *J Infect Dis* 2021;223(1):62–71.
- [11] Tilley K, Ayvazyan V, Martinez L, Nanda N, Kawaguchi SE, O’Gorman M, et al. A cross-sectional study examining the seroprevalence of Severe Acute respiratory Syndrome coronavirus 2 antibodies in a university student population. *J Adolesc Health* 2020;67(6):763–8.
- [12] Ko JH, Joo EJ, Park SJ, Baek JY, Kim WD, Jee J. Neutralizing antibody production in asymptomatic and mild COVID 19 patients in comparison to pneumonic COVID 19 patients. *J Clin Med* 2020;9:2268.
- [13] Shields A, Faustini ES, Perez-Toledo M, Jossi S, Aldera E, Allen DJ, et al. SARS-CoV-2 seroprevalence and asymptomatic viral carriage in healthcare workers: a cross sectional study. *Thorax* 2020;75:1089–94.
- [14] Brandstetter S, Roth S, Harner S, Buntrock-Dopke H, Toncheva A, Borchers N, et al. Symptoms and immunoglobulin development in hospital staff exposed to a SARS-CoV-2 outbreak. *Pediatr Allergy Immunol* 2020;31(7):841–7.
- [15] Patel MM, Thornburg NJ, Stubblefield WB, Talbot HK, Coughlin MM, Feldstein LR. Change in antibodies to SARS-CoV-2 over 60 days among health care personnel in Nashville, Tennessee. *J Am Med Assoc* 2020;324(17):1781–2.
- [16] Choe PG, Kang CK, Suh HJ, Jung J, Song KH, Bang JH, et al. Waning antibody responses in asymptomatic and symptomatic SARS-CoV-2 infection. *Emerg Infect Dis* 2021;27(1):327–9.
- [17] Self HW, Tenforde WM, Stubblefield BW, Feldstein RL, Steingrub SJ, Shapiro IN, et al. Decline in SARS-CoV-2 antibodies after mild infection among frontline health care personnel in a multistate hospital network - 12 states, april-august 2020. *MMWR Morb Mortal Wkly Rep* 2020;69(47):1762–6.
- [18] Marot S, Malet I, Leducq V, Zafilaza K, Sterlin D, Planas D, et al. Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. *Nat Commun* 2021;12:844.
- [19] To KK, Tsang OT, Leung WS, Tom AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;20(5):565–74.