ORIGINAL ARTICLE

Study of Antibody levels Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) among Health Care Workers of The Indian Tertiary Care Hospital

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Abstract:

Background: Health care workers (HCWs) are the front-line workers for suspected and confirmed COVID-19 cases. They are apparently at a higher risk of contracting the disease than the general population and if infected, pose a risk to susceptible patients and colleague of HCWs. The aim of the study was to quantitively measure the antibody levels against SARS-CoV-2 in HCWs of tertiary care hospital. *Material and Methods:* Study was conducted at a tertiary care hospital in India. Blood samples of 410 individuals were analysed by Chemiluminescence Immunoassay analyser using commercially available kits for in-vitro quantitative determination of antibodies (including IgG) against SARS-CoV-2 spike (S) protein's receptor binding domain (RBD). *Results:* In HCWs, greater antibody response to ChAdOx1 nCoV- 19 Corona Virus Vaccine (Recombinant) than Inactivated (killed) vaccine (median antibody titre - 1147 vs 194 respectively; $p \leq 0.001$) was observed. Significant difference ($p \leq 0.001$) in antibody levels of HCWs between those who received vaccination and never acquired the COVID-19 and those HCWs who received vaccination but were previously infected with SARS-CoV-2 after one or two doses of vaccination (278, 100 respectively; median antibodies - 363 vs 1292; p-value = 0.05) was found.

Key Words: Corona Virus, antibody, spike protein, quantitative

Introduction:

In January 2020, the World Health Organization (WHO) declared the coronavirus disease 2019 (COVID-19) outbreak a Public Health Emergency of International Concern, and on 11 March 2020, as a pandemic. [1] According to reports, 80% of COVID-19 patients have mild-to-moderate symptoms, while 20% develop severe manifestations such as severe pneumonia, acute respiratory distress syndrome, sepsis, and death. [1, 2]

The causative agent of COVID-19, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), belongs to the genus Beta coronavirus (b-CoV) of the family Coronaviridae. SARS-CoV-2 has a positive-sense, linear, single-stranded RNA genome that encodes four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N).[1, 3]

Coronaviruses, use unique envelope protein complexes for host cell receptor recognition and binding, followed by viral and host cell membrane fusion, which leads to cell entry.[4] To infect human cells, SARS-CoV-2 uses the S proteins on its envelope. The S protein has two subunits, S1 and S2, which compose a trimeric spike structure on the SARS-CoV-2 envelope. Binding to ACE2, the spike protein's S1 subunit triggers S2 subunits to change conformation from an unstable unfused state to a more stable fused state. The hinge-like movement of the receptor-binding domain (RBD) results in "up" and "down" conformational states. The "up" conformation represents the unstable receptor-bound state, and can attach both the receptor and the antibody, whereas the "down" conformation represents the receptor-unbound state. This advocates that inhibiting the interaction between the S1 subunit and ACE2 will prevent SARS-CoV-2 infection. [5]

As S protein is highly exposed on the viral surface, it is possible to be subject to immune surveillance by T cells and antigen-presenting cells, eliciting neutralizing antibodies against specific epitopes and domains of S.[4, 6] Because the coronavirus S glycoprotein is surface-exposed and mediates entry into host cells, it is the principal target of antibodies (Abs) and the focus of vaccine design.[7]

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) interim guidelines on serological testing of antibodies against SARS-CoV-2 issued in this background.[8]

Health care workers (HCWs) including clinicians, nurses, diagnostic laboratory personnel, hospital cleaners are the front-line workers for suspected and confirmed COVID-19 cases. As a result, they are apparently at a higher risk of contracting the disease than the general population and, if infected, pose a risk to susceptible patients and colleague. Measuring antibody levels to SARS-CoV-2 among HCWs (independent of symptom history) is decisive in evaluating the extent of exposure among hospital personnel and identifying high-risk departments. Knowledge of the past infection among HCWs could again be important in avoiding unnecessary quarantines and organizing healthcare resources.[9] In recognition and appreciation for their dedication to the work, the World Health Organization declared 2021 as the International Year of Health and Care Workers. [10]

Anti-SARS-CoV-2 antibodies measurement is of utmost importance to organize an adequate public health response for HCWs and to predict disease dynamics.[11]

At the time of writing this manuscript, two vaccines were available in India;"Covishield" (Adenovirus vector-based vaccine AZD1222), manufactured by Serum Institute of India, Pune under licence from Oxford-AstraZeneca and "Covaxin" (an inactivated-virus vaccine), manufactured by Bharat Biotech Ltd. in collaboration with the National Institute of Virology of Indian Council of Medical Research (ICMR) [12]

Chemiluminescence Immunoassay (CLIA) methods appear more sensitive than enzyme-linked immunosorbent assays (ELISA) or point of care assays for IgG and IgM/IgG assays. Because of the correlation between disease severity and antibody titre, quantitative rather than qualitative analysis should be considered.[13]

Anti-S1-RBD-SARS-CoV-2 antibody assays are useful for screening potential donors for convalescent-phase plasma therapy, assessing natural or vaccine-induced immunity, stratifying individuals for vaccine receipt, and documenting vaccine response.[14]

Therefore, we undertook this study to quantitatively measure the antibody levels in health care workers (HCWs) against SARS-CoV-2 virus.

MATERIAL AND METHODS:

HCWs of both genders, 18 years of age or older, who agreed to take part in this study were included. The cross-sectional study was conducted at the Clinical Biochemistry Laboratory under the Department of Biochemistry, Dr. V. M. Govt. Medical College & Shri Chhatrapati Shivaji Maharaj General Hospital, Solapur (Maharashtra), a tertiary-care teaching hospital, in India.

Health care workers willing to participate in the study were included in the study, ensuring that they underwent a screening assessment and completed a Case Record Form (CRF) designed to collect information about demographics, the nature of work since the pandemic, vaccination status, clinical data, interval between vaccination date and sample collection date including the previous history of exposure to COVID-19. Pregnant HCWs and those who incompletely filled CRF were excluded from study. The study was approved by the Institutional Ethical Committee.

Four hundred thirty-five voluntary HCWs gave written informed consent and completed the case record form at the start of the study. Blood samples were collected in clinical biochemistry laboratory to determine the antibodies to SARS-CoV-2. Total 435 Heath care workers were recruited in the study and of 435 HCWs, twenty-five participants were excluded after re-checking inclusion and exclusion criteria. Thus, 410 participants were included in the study for analysis.

All participants were asked if they had received any vaccination. The vaccines employed in our institute were "CovishieldTM- ChAdOx1 nCoV- 19 Corona Virus Vaccine (Recombinant)", and "CovaxinTM"- Inactivated (killed) vaccine.[15, 16]

Blood samples (5 ml) were collected at the Clinical Biochemistry Laboratory in Li-heparin sampling tubes for the in-vitro quantitative determination of antibodies to the SARS-CoV-2 spike (S) protein RBD.

Blood samples were analysed within 2 hours of collection for the estimation of antibodies to SARS-CoV-2 spike (S) protein RBD. Analysis was performed on Roche Cobas e411 Chemiluminescence Immunoassay analyser (Electrochemiluminescence immunoassay - ECLIA) by using Elecsys Anti-SARS-CoV-2 S reagent kit.

Results < 0.80 U/mL were reported as negative and ≥ 0.80 U/mL were reported as positive for anti-SARS-CoV-2-S antibody. The measuring range of the assay is 0.40-250 U/mL with a Limit of Quantitation (LoQ) of 0.40 U/mL. Diluent Universal was used to dilute values above the measuring range of 250 U/mL according to the assay's pack insert's recommended dilution range of 1:10 up to 1:100.The assay has an analytical specificity of 100%; Clinical specificity of 99.98 % (95 % CI: 99.91 – 100 %); Sensitivity of 98.8 % (95 % CI: 98.1-99.3 %) and a positive agreement rate of 92.3 % for correlation of assay results to serum neutralization capacity.[17] "Mystat" Statistical software was used for statistical analysis.

As descriptive statistics median, minimum-maximum values

for continuous variables and frequency, percentage values for qualitative variables were calculated. Mann-Whitney U test done for continuous variables. For comparison of two data sets in continuous data, Kruskal-Wallis test was used.

Results :

Table 1. Baseline characteristics of study participants

Sr. No	characteristic	Frequency	Percent
1	Male [a]	199	48.537%
2	Female [a]	211	51.463%
3	Diabetic [a]	34	8.293%
4	Hypertensive [a]	67	16.341%
5	Total Covaxin received [a]	257	62.683%
6	Only a single dose of Covaxin received [b]	17	6.615%
7	Both doses of Covaxin received [b]	240	93.385%
8	Total Covishield received [a]	121	29.512%
9	Only a single dose of Covishield received [c]	65	53.719%
10	Both doses of Covishield received [c]	56	46.281
11	Non-Vaccinated[a]	32	7.805%
12	COVID positive before vaccination [a]	53	12.92%
13	COVID positive after first dose of vaccination [a]	33	8.049%
14	COVID positive after second dose of vaccination [a]	14	3.415%
15	Total COVID Negative [a]	293	71.463%
16	Vaccinated COVID Negative [b]	278	73.54%
17	HCW having direct contact with COVID Positive patients (nursing and physicians etc.) [a]	294	71.7 %
18	Nursing Staff [a]	59	14.39 %
19	Physicians, other health care assistants and emergency medical technicians [a]	235	57.31%
20	HCW not in direct contact with COVID Positive patients (pre and para-clinical Departments) [a]	116	28.29%
21	COVID positive after receiving Covaxin vaccine. [c]	34	13.2%
22	COVID positive after receiving Covishield vaccine. [d]	13	10.7%

The median age of study participants was 36 years. The cohort had a slightly greater representation of females with 51.46% participation and 48.53% male. Thirty-four (8.29%) participants reported having diabetes and 67 (16.34%) had hypertension. (Table1)

a =Percentage is out of Total 410 participants

b =Percentage is out of Total 378 vaccinated participants.

c =Percentage is out of Total 257 Covaxin receiving HCWs

d =Percentage is out of Total 121Covishield receiving HCWs

Table 2. Values of age, BMI, Number of duty days working in COVID ward and antibodies

Parameter	AGE	BMI	No Of Duty Days Worked in COVID Ward	Antibody
Minimum	18	15.62	0	0
Maximum	66	47.61	540	22,858
Median	36	25.65	126	478.5

Of the total study participants, 117 (28.536%) were COVID-19 positive confirmed by one of the two investigations i.e., RT-PCR or rapid antigen tests (RAT) and 293 (58.29%) were COVID-19 negative. Of the 117 HCWs diagnosed with COVID-19, 82 (70%) had mild symptoms, 2 (1.7%) had moderate and 1 (0.85%) experienced severe symptoms. Four (3.4%) were home quarantined. Of the 117 COVID-19 positive, 70 (17%) HCWs were diagnosed with COVID-19 before their first dose of vaccination.

Out of 410 HCWs, 378 HCWs were vaccinated. Two hundred fifty seven (62.683%) received Covaxin, 121 (29.51%) received Covishield while 32 HCWs (7.8%) had received no kind of vaccination.

Of the 410 study participants, 392 (95.6%) had developed antibodies to SARS-CoV-2 and 18 (4.4%) did not develop antibodies to SARS-CoV-2. Of the previously infected HCWs (70) before the vaccination program, 2 (1.7%) HCWs did not develop antibodies to SARS-CoV-2. We observed a wide range of antibody levels in the study group, ranging from 0 to 22,858 U/ml.

No statistically significant difference found between BMI, and COVID ward duty days of both genders in terms of antibody response ($p > 0.05$). (Table 2)

Out of 378 vaccinated (Covaxin/ Covishield) HCWs,73.54% HCWs were protected from acquiring SARS-CoV-2 infection until the study.

To assess the effect of vaccination on antibody development,

we compared antibodies in 278 HCWs, Covishield and Covaxin recipients (93 and 185 respectively) who never acquired SARS-CoV-2 infection. Of the 93 Covishield and 185 Covaxin recipients, 95.7% and 94% respectively, showed seropositivity. However, both seropositivity rate and median rise in anti-spike antibody were significantly higher in Covishield (95.7%, 94% respectively, median antibody titer - 1147 vs 194; p<0.001)

We also note a significant difference in antibody titer after comparison amongst the three groups: Covishield receiving, Covaxin receiving, and non-vaccinated group (93, 185, 15 respectively; median antibodies - 1147 vs 194 vs 40, respectively; p< 0.001). We found no statistical difference in age, BMI, COVID ward duty days amongst these three groups.

An interesting finding in our study was a statistically significant difference (p <0.001) in antibody levels of HCWs between those who received vaccination and never acquired the COVID-19 disease and those HCWs who received vaccination who were previously infected with SARS-CoV-2 or acquired infection after one or two doses of vaccination (278, 100 respectively; median antibodies - 363 vs 1292; p-value < 0.05) (Table no. 3). Both seropositivity rate and median rise in anti-spike antibodies were significantly higher in HCWs having SARS-CoV-2 infection in combination with vaccination.

Table no. 3. Median antibody levels in vaccinated HCWs with and without SARS-Cov-2 infection.

Group of HCWs	Median Antibody level.	P value
HCWs who received vaccination and never acquired the COVID-19 disease.	363	< 0.05
HCWs who received vaccination who were previously infected with SARS-CoV-2 or acquired infection after one or two doses of vaccination	1292	

No significant differences in antibody levels of each type of vaccination after the first and second doses were noted.

The professional category of HCW's, daily close contact with COVID-19 cases in COVID-19 wards, comorbidities (DM, HTN) of both genders and BMI did not show any statistically significant association with the development of antibodies to SARS-CoV-2. We also noticed frequency and percentage of acquiring Covid infection are reduced after vaccination.

33 (8.7%) HCWs became Covid positive after the first dose of vaccination while 14 (3.7%) participants became Covid positive after taking two doses of vaccination. 293 participants never acquired Covid infection.

In this study, 116 (28.29%) HCWs are teaching staff of pre-clinical and para-clinical departments, administrative office staff, and pharmacists; not directly dealing with patients. While 294 (71.71%) HCW were in direct contact with COVID-19 patients including nurses 59 (14.39%), physicians in clinical practice and other health care assistants, such as emergency medical technicians 235 (57.31%).

Discussion:

Antibodies are biomarkers of infection, but if antibodies persist over a period, they can confer long-term immune responses. Binding antibody titters are just a surrogate of protection for most clinically approved vaccines. [18]

Recent reports showed that symptom severity positively correlates with enhanced neutralizing antibodies and levels of anti-Spike and RBD IgG, IgM, and IgA. Similar Observations were noted by some studies.[19, 20]

No significant difference in antibodies of different professional groups of HCW was detected. In contrast to our study in an observational cohort study, the risk of SARS-CoV-2 infection in healthcare workers was found related to exposure to infected patients.[21]

Greater antibody response to Covishield than Covaxin in HCWs (median antibody titer - 1147 vs 194 respectively; p<0.001) was found Similar results were reported in study in which both vaccines elicited good immune response after two doses, although seropositivity rates and GMT of anti-spike antibody titre was significantly higher in Covishield compared to Covaxin recipients.[22] Whether this finding is due to the difference between the type of vaccine; vector-based vs. inactivated whole virion, or linked to differential immunogenic response because of the variation in the loading dose of antigen in each vaccine is not exactly known and needs further studies to verify the exact mechanism of action.

Similar to our observation a Spanish study also reported no significant difference in the different categories of HCW's working in COVID-19 units, daily close contact with a COVID-19 case, sex or comorbidities with the presence of antibodies to SARS-CoV-2.[9] Having COVID 19 infection was the most important factor associated with the development of antibodies. Working in COVID-19 wards was not associated with seropositivity, which may be due to

meticulous use of PPE kits, masks, appropriate hand hygiene thus, a lowering the risk of acquiring the infection.

Our observations are in line with the hypothesis that the first vaccine dose serves as a boost in infected individuals. In our study, we found individuals who naturally contracted SARS-CoV-2 have developed an exaggerated antibody response to vaccine. This phenomenon has also been reported by a recent study demonstrating an increased spike antibody response in seropositive individuals after a single dose of a SARS-CoV-2 mRNA vaccine.[23]

Studies reported high antibody titres after the first dose of vaccinated individuals who already had SARS-CoV-2 infection.[24, 25] One study, reported increased reactogenicity after the first dose in COVID-19 survivors.[26] Exaggerated increase in antibodies to the vaccine, supports the fact that the first vaccine dose acts as a boost for the immune responses acquired after natural infection. [27-30]

No significant difference of antibody levels between two doses was found in contrast to other studies.[10, 31]

Strength of this study include the large screening against previous SARS-CoV-2 infections in HCWs in this institute.

Our study has limitation. The age range of the enrolled individuals ranged from 24 to 66 years, and we cannot rule out that younger or older subjects may react differently.

Conclusion:

Individuals who recovered from COVID-19 and received vaccination experienced relatively robust antibody response to the spike protein showing vaccine could act as a booster in previously infected individuals.

Conflict of Interest - Nil

Sources of Support - Nil

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