Original Research Article

Evaluation of antibodies against SARS-CoV-2 and their patterns of response in health care workers and general population at a tertiary care Centre

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ABSTRACT

Background: SARS-CoV-2 (previously called 2019-nCoV), and was named in February 2020 as COVID-19 by the WHO.
Objectives: Estimate the seroprevalence of antibodies against SARS-CoV-2 in health care workers (HCW) and general population in the first and second wave and assess the pattern of antibody response in HCW with COVID-19 infected and non-infected over pre and post-vaccination.
Materials and Methods: This was a cohort observational retrospective study done to analyse the seroprevalence in HCW from July-September 2020, in the general population in the first wave (December 2020–February 2021) and second wave (March–September 2021). SARS-CoV-2 testing by RT-PCR (QIAGEN Company). Testing for quantitative IgG and IgM (Abbott) antibodies, Total Antibodies (Roche), Anti-SARS-CoV-2 IgG RBD(Roche), and Anti-SARS-CoV-2 IgG S1/S2 (Diasorin XL), to assess the pattern of antibody responses categorized as baseline (before the first dose), 14 days after 1st dose, before 2nd dose (45 days post first dose), 14 days post-second dose.
Results: Among 1340 HCW, 1268 underwent RT-PCR testing, 540 serology testing and 431 underwent both testing. We identified 164 of 1268 positive RT-PCR and using serology testing 229 of 540 were seropositive. High seropositivity was observed in age group 26-45 years (44.9%) HCW, in males (65.9%), nurses (47.3%), and ward staff (48.6%). High seroprevalence in general population-76.07% in the 2nd wave compared to 1st wave (44.67%).
Conclusion: SARS-CoV-2 antibodies showed gender associated seroprevalence and higher immune response was observed in COVID-19 infected than in non-infected HCW pre- and post-vaccination.

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1. Introduction

SARS-CoV-2, previously called 2019-nCoV was a highly transmissible and pathogenic coronavirus emerged lately in December 2019 which was firstly reported by Wuhan, China as per increasing occurrence of cases of pneumonia. It was classified under Beta corona virus. World Health Organization declared it as a public Health Emergency of International concern on 30th January and a pandemic on 11th March 2020.[1] The cases have been steadily increasing since then.[2] Suitable methods for the diagnosis of SARS-CoV-2 infections are, detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) primarily in sample material from the upper (nasopharyngeal or oropharyngeal smear) or lower respiratory tract (Broncho alveolar lavage fluid, tracheal secretion, sputum, etc.)

Health care workers (HCW) are the frontline workforce who are at a higher risk of SARS-CoV-2 infection and can be a source of nosocomial infection in transmitting disease. Monitoring the prevalence of infection among

HCW (regardless of history of symptoms) was useful for assessing the level of exposure among hospital personnel and identifying high-risk departments.[3]

Many studies have assessed patients of varying disease severity and have found that antibody titers and capacity for neutralization are closely associated with disease severity.[4] In terms of the duration of the antibody response, most studies followed patients for a number of weeks to months.[4] Neutralising antibodies efficiently stop the infection by blocking the interaction between the SARS-CoV-2 virus and the host cells. Most neutralizing antibodies are specific for the receptor binding domain (RBD) of the spike protein, which binds directly to the cell surface receptor ACE2.[5] The nation-wide vaccination drive was started from 16th January 2021 after the approval of two vaccines namely Covishield[TM] (ChAdOx1-nCOV or AZD1222, acquired from Oxford University and AstraZeneca, manufactured by Serum Institute of India, Pune) and Covaxin[TM] (BBV-152, manufactured by Bharat Biotech, Hyderabad in collaboration with Indian Council of Medical Research [ICMR], India).[6]

Seroprevalence studies of antibodies have an important role in identifying the presence of infection. The serological test for the presence of antibodies (IgM or IgG) against SARS-CoV-2 might provide a more accurate estimate of the cumulative prevalence of SARS-CoV-2 infection in a population compared to the viral test, as the antibodies against the virus, in particular IgG, are likely to persist for a longer period of time after the viral infection was cleared.[7] This study aims at estimating the seroprevalence of antibodies against SARS-CoV-2 in HCW's and the general population (patients) visiting the OPD as first objective in following categories:

In study group I: seroprevalence of antibodies were in both who had undergone RT-PCR and serology testing.

In study group II: seroprevalence of antibodies were grouped according to the age group (18-25, 26-45,46-65) years

In study group III: seroprevalence of antibodies were grouped according to the gender.

In study group IV: seroprevalence of antibodies were grouped by occupational status of different health care workers.

In study group V: seroprevalence of antibodies were grouped according to the different departmental sections.

In second objective, to assess the pattern of antibody responses in COVID-19 infected and non-infected health care workers pre and post vaccination.

2. Materials and Methods

2.1. Study population and data collection

This was a cohort observational retrospective study conducted in Department of Clinical Biochemistry, Asian Institute of Gastroenterology Hospitals, Hyderabad, Telangana, India. About 1340 HCW were included in the study for understanding the seroprevalence, about 258 of general population n 1st wave and 1443 in second wave and about 35 health care workers were included to assess the antibody responses of Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2 neutralizing antibodies. All the health care workers were provided Personal Protective Equipment from March 2020. For the post vaccination follow up in COVID-19 recovered individuals and healthy individuals, the samples were collected from the laboratory technicians in the biochemistry department at the baseline (0), 14 days after 1st dose, prior 2nd dose and 14 days' post 2nd dose. The Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2 neutralizing antibodies were assessed accordingly.

2.2. Sample size calculation

Sample size was calculated by using the Cochran's formula and estimated to be about 873 HCW, about 256 of general population in 1st wave and 915 in second wave for estimation of seroprevalence of antibodies and about 35 HCW for the pattern of antibody responses in COVID-19 infected and non-infected HCW pre and post vaccination.

2.2.1. Inclusion criteria
All symptomatic and asymptomatic HCW who were above age of 18 years.

2.2.2. Exclusion criteria
1. HCW who were on chronic steroid use immunosuppressant drugs
2. HCW who had autoimmune disease, HIV/AIDS

2.3. Methods

2.3.1. COVID-19 RT-PCR
SARS-CoV-2 testing by RT-PCR (QIAGEN Company) began June 2020 and was available for any HCW who had COVID-19 like symptoms or suspected exposure. From July 2020 to September 2020 all the HCW were encouraged to be tested and offered free, voluntary antibody testing regardless of symptoms. Nasopharyngeal and throat swabs of the participants were collected. Data was collected from HCW regarding their primary work location, job function, direct patient care, work on a COVID-19 or non COVID-19 ward were taken.

2.3.2. Serological assays
2.3.2.1. SARS-CoV–2 Quantitative IgG and IgM Assay. The SARS-CoV-2 antibody testing Quantitative IgG antibodies (Abbott architect i6000) by Chemiluminescence Micro Particle Immunoassay (CMIA), has a specificity of 99.5% as per kit insert.

2.3.2.2. SARS COV– 2 S. Total Antibodies (IgG and IgM) (Elecsys® Roche) by Electrochemilumniscence Immunoassay (ECLIA) has a clinical specificity of 99.98% and clinical sensitivity of 98.8% according to manufacturer's kit insert. These are the antibodies against Nucleocapsid.[7]

2.3.2.3. Anti-SARS-CoV-2 IgG RBD. The SARS-CoV-2 neutralizing antibody i.e., Anti-SARS-CoV-2 IgG RBD specific (Elecsys® Roche) by ECLIA that quantifies the determination of antibodies to SARS-CoV-2 spike protein Receptor Binding Domain(RBD). SARS-CoV-2 binds to spike protein s1 subunit that was receptor binding domain (RBD). The Elecsys SARS-CoV-2 assay has 99.81% specificity and sensitivity of 100%. The cut off values as per manufacturers kit insert was <0.40 was considered negative and >0.80 was considered positive for Anti-SARS-CoV-2 IgG RBD.

2.3.2.4. Anti-spike IgG S1/S2 neutralizing antibodies. Anti-Spike IgG S1/S2 neutralizing antibodies (Diasorin Liaison XL) by Chemilumniscent Immunoassay (CLIA) that quantifies the determination of IgG anti-S1 and IgG anti-S2 specific antibodies to SARS-CoV-2. Neutralizing antibodies are considered to be protective and Diasorin assay positive agreement with Plaque reduction neutralization test (PRNT90) was 94.4%. Briefly the individual sera were analyzed, about 175 μl sample volume was required for the reaction to take place. The specific recombinant S1 and S2 antigens used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG are linked to a isoluminol derivative. In 2 successive incubations for about 15 minutes each, the antibodies bind to solid phase and reacts with IgG to SARS-CoV-2 already bound to the solid phase. Unbound material was removed with wash cycle in 5 min after each incubation. Starter reagents are added and a flash of chemiluminescence reaction was induced. The reaction time was 35 minutes. It was measured by a photomultiplier as relative light units (RLU) and the analyzer automatically calculates and expresses as Arbitrary units (AU/mL) which was indicative of IgG to SARS-CoV-2 concentration present in samples. The detection limit was ≥3.8 AU/mL; the samples which had >15 AU/mL are considered positive for neutralizing antibodies. The sera which were >400 AU/mL were single fold diluted with 1:10 dilution.

2.4. Ethical statement

The ethical approval was obtained from institutional ethics committee in AIG hospitals (REF NO-AIG/IEC-POST BH&R-EXP-52/09.2021-01).

2.5. Statistical analyses

Statistical analysis was performed on SPSS software. Chi-square test was used to calculate the statistical differences between categorical variables and continuous variables respectively. A p-value of <0.05 considered statistically significant

3. Results

3.1. Clinical characteristics

The first objective, seroprevalence of antibodies in HCW, All the HCW were provided with Personal Protective Equipment (PPE) from March 2020. SARS-COV-2 testing by RT-PCR (QIAGEN Company) began June 2020 and was available for any HCW who had COVID-19 like symptoms or suspected exposure. From July 2020 to September 2020 all the HCW were encouraged to be tested and offered free, voluntary antibody testing regardless of symptoms.

3.2. Seroprevalence of antibodies in HCW

The HCW were grouped based on the age, gender, occupational status and the different departmental sections. The individuals were tested and comparison was done with RTPCR, total antibody status and IgG status. The RT-PCR was been mentioned in Table 1 as per different categories.

In study group 1, the seroprevalence of antibodies in both who had undergone RT-PCR and serology testing -Among 1340 HCW, 1268(94.6%) underwent RT PCR testing, 540 (40%) using serology testing and 431 (32.1%) underwent both testing.

We had identified 164 of 1268 (12.9%) were RT PCR positive and using serology testing 229 of 540 (42.4%) seropositive health care workers. Only 6 (1%) HCW were seronegative though they were PCR confirmed and only 11 (2.03) were IgG negative.

In study group 2: Seroprevalence of antibodies were observed in specific age groups.

The average (SD) age among all employees was 40.3 (20.2).

In age group 18-25 years, antibodies were positive in 64 of 157(i.e., about 40.8%) and about 93 of 157 were seronegative (59.2%).

Similarly, in age group 26-45 years, antibodies were positive in 158 of 352(i.e., 44.9%) and about 194 of 352 (55.1%) were seronegative.

In age group 46-60 years, antibodies were positive in 7 of 131 (i.e., 22.6%) and seronegative was observed in 24 of 131 (77.4%) HCW.

This suggests the seropositive was higher in age group 26-45 years (44.9%) and lower in age group 18-25yrs (40.8%).

In Study Group 3: Seroprevalence of antibodies in accordance to gender in males, seropositive was observed

in 193 of 271 (71.2%) and 78 of 271(28.8%) were seronegative. In females, seropositive was observed in 118 of 269 (43.9%) and 151of 269 were seronegative (56.1%). This suggests the seroprevalence were higher in males compared to females. There was a Significant difference (p-value =<0.00001) in males and females.

In Study Group 4: Seroprevalence of antibodies in occupational status of health care workers like doctors (99), nurses (220), laboratory technicians (48) and others (173). Among all the HCW the seropositive rates were higher in doctors 79 of 99 (79.8%) and nurses 116 of 220 (52.7%) in comparison to other HCW. This indicates high risk of exposure in these occupations.

In Study Group 5: Seroprevalence of antibodies in accordance to HCW working in different departments such as in emergency staff 32 of 98(32.7%) were seropositive and 66 of 98 (67.3%) were seronegative, in endoscopy unit about 21 of 48 (43.1%) were seropositive and 27 of 48 (56.2%)were seronegative, in ICU staff 29 of 70(41.4%)were seropositive and 41 of 70(58.6%) were seronegative, among ward staff 68 of 140 (48.6%) were seropositive and 72 of 140 (51.4%) were seronegative. This suggested that there was a high rate of seroprevalence in Ward staff among other all departments.

3.3. Seroprevalence of antibodies in general population in first wave and second wave

3.3.1. Seroprevalence in first wave
Seroprevalence of antibodies in general population visiting the Out Patient Department (OPD) in first wave and second wave, as per the hospital protocol all were advised routine RT-PCR testing and CT Screening as per the current pandemic situation.

In the first wave, a total of 258 patients were include in the study, all participants underwent RT-PCR testing (239) and serology testing (258). Participants were grouped according to the gender to estimate the seroprevalence. The Mean and SD of age group in males was 55.30±14.40 and in females was 56±24.89. The seroprevalence in the first wave, SARS-CoV-2-S Total Antibody-about 44.57% were positive, of which 55 (42.96%) of 183 males and 60 (80%) of 75 females were seropositive, suggesting no significant difference between males and females (p-value =0.17). Similarly, about 46.51% of which 78 (42.62%)of 183 males and 42 (56%) of 75 females were positive to SARS-CoV-2 IgG antibodies positive, suggesting no significant difference between males and females (p-value =0.68), and SARS-CoV-2 IgM antibodies were developed in about 36 (13.95 %)of 258, of which males about 23 (12.5%) of 183 and females 63(84%) of 75, suggesting a higher rate of infection in females in comparison to males, a significant difference (p-value =<0.0001) was found in males and females. This was statistically significant with p<0.05.

The data has been depicted in Table 3.

3.3.2. Seroprevalence in Second wave
In the second wave, a total of 1443 patients were included in the study, of which about 1411 had undergone RT-PCR testing and 1418 had done serology testing which included SARS-CoV-2-S. Total Antibody (1418), SARS-CoV-2 IgG and IgM (1328). The remaining didn't turn up due to the pandemic situation and partial lockdowns in the state. Similarly, as in first wave the patients have been grouped and compared according to gender in order to observe the seroprevalence. The Mean and SD of age group in males was 54.27±16.25 and females 54.79 ±14.40. About 1411 who had undergone RTPCR testing, about 427 (30.26%) of 1411 were RTPCR positive, of which females (29.33%) and males (30.67%) were RT-PCR positive. The seroprevalence in second wave, SARS COV-2 S total antibody – about 1079(76.07%)of which 700 (87.5%) of 800 males and 379 (61.32%)of 618 females were seropositive, similarly igg about 502 (69.81%) of 719 males and 301 (45.39%)of females and IgM about 742(53.69%) of 1382 of which 428(59.52%) of 719 males and 314 (47.36%) of 663 females were seropositive This overall suggests a high seroprevalence in males compared to females. The p values of Total antibody, IgG and IgM ($\chi 2$ =129.85, p=<0.00001; $\chi 2$ =83.49, p=<0.00001; $\chi 2$=20.04, p=<0.00001) showed a statistically significant differences in positivity between males and females, suggesting and higher infectivity in the second wave and high seroprevalence in comparison to the first wave. The overall seroprevalence was highest in second wave (76.07%) compared to first wave (44.57%). The data was being depicted underTable 4.

3.4. Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2 in COVID-19 infected and non-infected HCW

The Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2 neutralizing antibodies were assessed and analyzed as the second objective as mentioned to assess the antibody responses in COVID-19 infected and non-infected HCW with pre and post vaccination. Among 35 HCW, about 19 (54.28%)were covid-19 positive by RT-PCR, 16 (45.71%) were normal and healthy, we have a observed a low antibody titers (S1/S2) post covid-19 in healthy as well in covid-19 positive HCW, only 1 HCW showed a minimal positive immune response. During the period of testing (July to September 2020) Anti-SARS-CoV-2-IgG RBD wasn't available in the laboratory and has been introduced in January 2021. The data was being represented under table 3 indicating the values of Anti Spike IgG S1/S2 and Anti-SARS-CoV-2 IgG RBD in pre and post-vaccination. Baseline (that was pre-vaccination) values didn't show any immune response with s1/s2 in most HCW but about 24 (68.57%)of 35 showed a positive immune response in accordance to Anti-SARS-CoV-2 IgG RBD, prior to vaccination the k sample analysis for Anti-SARS-CoV-2 IgG RBD (z=4.16, p=<0.0001) and Anti-Spike IgG S1/S2

Table 1: SARS-CoV-2 RT PCR among HCW

Variable	Total RT-PCR (%) n=1268	PCR Negative (%) n=1104	RT-PCR Positive(%) n =164	P-Value
Sex				
Women	678	606(89.3)	72(10.7)	0.0084
Male	590	497(84.4)	93(15.76)	
Age				
18-25	399	370 (92.7)	29 (7.3)	
26-45	756	635 (84.0)	121 (16.0)	0.00014
46-60	113	99 (87.6)	14 (12.4)	
Occupation				
Doctor	179	159 (88.8)	20 (11.2)	
Nurses	729	619 (84.9)	110 (15.1)	
Technician	80	65 (81.3)	15 (18.7)	0.0015
Others	280	261 (93.2)	19 (6.8)	
Department				
ER	140	118 (84.3)	22(15.7)	
Endoscopy	98	86(87.8)	12(12.2)	
icu	320	278 (86.9)	42 (13.1)	0.61
Wards	190	164(86.3)	26 (13.7)	
Procedural	280	252 (90.0)	28 (10.0)	
Others	240	206 (85.8)	34 (14.2)	

(z=5.14, p=<0.0001). 14 days after the first dose there was a minimal immune response, about 10 out of 35(28.57%) s1/s2 and 3 of 35 RBD didn't show immune response after the first dose, but about 25of 35 (71.42%)) with s1/s2 and 31 of 35(88.57%) showed a response suggesting the vaccination efficacy in initiating an immune response. Post 14 days of vaccination the k sample analysis was Anti-spike IgG S1/S2 (z=3.8, p=<0.0001) Anti SARS-CoV-2 RBD (z=2.9, p=<0.0001). In April and may second jab of vaccination were started and samples were collected, analyzed where the antibody titers remained high for COVID-19 positive patients showing no decline, but in non-infected there was a decline in the both Anti-SARS-CoV-2 IgG RBD and Anti-Spike IgG S1/S2. Prior to second dose the k sample analysis of Anti Spike IgG S1/S2 (z=4.0, p=<0.0001) and Anti-SARS-COV-2 RBD (z=2.24, p=<0.0001). 14 days post 2nd dose, the antibodies were exponentially higher in both covid-19 infected as well as covid-19 non-infected HCW, suggesting higher effectiveness of the vaccine as well lower infectivity. Post 2nd dose the k sample analysis was Anti-spike IgG S1/S2 (z=2.46, p=<0.0001) Anti-SARS-COV-2 IgG RBD (z=2.11, p=<0.0001).

4. Discussion

The detection of specific antibodies against defined infectious pathogens was commonly used as a marker of infection and immunity.[8] In this current pandemic situation, Serology testing was an informative tool in knowing the immune status of the individual to COVID-19 infection and also immune status post vaccination that helps in identifying risk of exposure. Many studies were conducted on seroprevalence of antibodies to SARS-CoV-2 infection. Our study mainly aims at evaluating the immune status of the individual by comparing the pattern of antibody responses as per the IgG status and total antibody status and also the immune response by measuring the circulating antibodies prior and post vaccination in covid-19 infected and non-infected health care workers.

The health care worker's immune status was analyzed grouping them into different categories. We observed there was high total antibody in males compared to females, similarly a low prevalence of IgG positive in females, similar pattern was observed in study conducted in Italy on HCW.[9] The total antibody status and IgG status was observed in according to age, occupation and department as mentioned in table 2. The total antibody and IgG prevalence had a statistically significant correlation in immune status of the individual. The high IgG prevalence was observed in younger adults compared to older adults. High seroprevalence (seropositive) in younger age group compared to older group was mainly due to vaccination as younger age group weren't vaccinated yet but older age group were at least partially vaccinated suggesting high risk of infectivity and transmission in younger age. There was a positive correlation in the total antibody Status and IgG positive in the younger individuals. In a study conducted by Ebinger et al., similar results were observed in the young population with higher Seroprevalence in comparison to older adult.[10] High seroprevalence was observed in doctors and nurses as they are in continuous contact with COVID -19 infected patients and involved in providing endless care and treatment of the patients, similar studies

Table 2: Serology testing of total antibody and IgG in health care workers

Variable	Total Antibody (%) n=540	Antibody Negative (%) n= 311	Antibody Positive (%) n =229	P value	IgG Negative (%)	IgG positive (%)	P-value
		311 (57.6)	229 (42.4)		311	229	
GENDER							
FEMALE	269	151(56.1)	118(43.8)	<0.00001	140(52.04)	129(47.9)	<0.00001
MALE	271	78(28.7)	193(62.1)		82(30.2)	189(69.7)	
AGE							
18-25	157	93 (59.2)	64 (40.8)		98(62.4)	59(37.5)	
26-45	352	194 (55.1)	158 (44.9)	0.0485	182(51.1)	170(48.2)	0.4691
46-60	31	24 (77.4)	7 (22.6)		20(64.5)	11(35.4)	
Occupation							
Doctor	99	20(22.2)	79 (79.8)		35(35.3)	64(64.6)	
Nurses	220	104 (47.3)	116 (52.7)	<0.00001	100(45.4)	120(54.5)	0.0064
Technician	48	18 (37.5)	30 (62.5)		28(58.3)	20(41.6)	
Others	173	86 (49.7)	87 (50.3)		95(54.9)	78(45.0)	
Department							
ER	98	66 (66.3)	32 (32.7)		68(69.3)	30(30.6)	
Endoscopy	48	27 (56.3)	21 (43.7)		30(62.5)	18(37.5)	
ICU	70	41 (58.6)	29 (41.4)	0.2525	33(47.1)	37(52.8)	0.0003
Wards	140	72 (51.4)	68 (48.6)		65(46.4)	75(53.5)	
Procedural	108	64 (59.3)	44 (40.7)		68(62.9)	40(37.03)	
Others	76	41(54.0)	35 (46.0)		32(42.1)	44(57.8)	

Table 3: Seroprevalence in the general population from December 2020 to February 2021

Variable	Total RTPCR n = 239		SARS COV-2S Total Antibody (%) n=258		Total IgG AND IgM n=258			
	RTPCR negative n=194	RTPCR positive n=45	Antibody Negative (%) n= 143 (52.42%)	Antibody Positive (%) n =115 (44.57%)	IgG Negative n=138(53.48%)	IgG positive n=120 (46.51%)	IgM Negative n=222 (86.04%)	IgM positive n=36 (13.95%)
Gender								
Male	137(81.06%)	32 (18.93%)	128 (69.94%)	55(42.96%)	105(57.37%)	78(42.62%)	160 (87.43%)	23 (12.5%)
Female	57 (81.42%)	13 (18.57%)	15 (20%)	60(80%)	33(44%)	42(56%)	63(84%)	12(16%)
P-Value			0.17		0.68		0.0001	

Table 4: Seroprevalence in the general population from March 2021 to September 2021

Variable	Total RTPCR N = 1411		SARS COV-2S Total Antibody (%) n=1418		Total IgG and IgM n=1382			
	RTPCR negative n=984 (69.73%)	RTPCR positive n=427 (30.26%)	Antibody Negative (%) n=339 (23.92%)	Antibody Positive (%) n =1079 (76.07%)	IgG Negative n=579 (41.89%)	IgG positive n=803 (58.11%)	IgM Negative n=640(46.30%)	IgM positive n= 742 (53.69%)
Gender								
Male	678(69.32%)	300 (30.67%)	100 (12.50%)	700 (87.5%)	217 (30.18%)	502 (69.81%)	291(40.47%)	428 (59.52%)
Female	306(70.66%)	127(29.33%)	239 (38.67%)	379 (61.32%)	362 (54.60%)	301 (45.39%)	349(52.63%)	314(47.36%)
P-Value			<0.0001		<0.00001		<0.00001	

Table 5: Pattern of immune responses of Anti spikeIgG S1/S2 and Anti SARS COV 2 IgG RBD in pre- and post-vaccination

Subject no	Age	Gender	July - September post Covid-19 Anti SARS COV 2 IgG S1/S2	Jan-Feb 2021 BASELINE		14 days after first dose		April-May 2021 PRIOR 2nd dose		14 days post 2nd dose	
				Anti SARS COV 2 IgG S1/S2	Anti SARS COV 2 IgG RBD	Anti SARS COV 2 IgG S1/S2	Anti SARS COV 2 IgG RBD	Anti SARS COV 2 IgG S1/S2	Anti SARS COV 2 IgG RBD	Anti SARS COV 2 IgG S1/S2	Anti SARS COV 2 IgG RBD
1	36	M	<3.80	<3.80	150	81	1500	1860	22000	3800	45000
2	35	M	<3.80	<3.80	1800	127	6000	2460	35000	4200	85000
3	27	M	<3.80	44.2	280	17.2	2620	230	17500	545	35000
4	25	M	<3.80	<3.80	45	10.5	1785	1330	12800	3500	25640
5	30	M	<3.80	<3.80	220	3.97	2500	26.2	18050	350	35080
6	35	M	<3.80	<3.80	<0.40	3.77	90	59.6	155	250	455
7	25	M	<3.80	<3.80	35	10.2	280	221	15025	2325	34060
8	29	M	<3.80	<3.80	190	15.1	2100	352	23085	3345	45120
9	22	M	<3.80	<3.80	<0.40	9.5	<0.40	62.3	42.92	458	1583
10	23	M	<3.80	<3.80	25	17.6	250	384	7800	950	15040
11	25	M	<3.80	<3.80	15	7.32	125	130	355	550	2540
12	29	M	<3.80	<3.80	60	6.47	1895	242	13585	684	25070
13	22	M	<3.80	<3.80	<0.40	<3.80	55.4	57.87	195	285	1250
14	33	M	<3.80	<3.80	350	76.4	3540	3840	25000	5000	83000
15	38	F	<3.80	7.09	20	<3.8	150	120.8	550.8	4090	20020
16	40	M	<3.80	<3.80	<0.40	<3.8	20	21.6	30	40	230
17	24	M	<3.80	34	300	28.6	2800	239	18514	595	36125
18	26	M	<3.80	<3.80	225	8.58	2300	1260	7850	2500	15000
19	23	M	<3.80	<3.80	<0.40	7.4	38	52.9	85	235	2500
20	32	M	<3.80	<3.80	<0.40	<3.8	<0.40	35.2	45.98	164	355
21	22	M	<3.80	<3.80	99	32.6	1054	1340	17550	2800	38000
22	24	M	<3.80	<3.80	<0.40	<3.8	57.2	115	108.2	495	2653
23	32	F	<3.80	<3.80	22	<3.8	150	120.8	1060	4060	82060
24	34	M	<3.80	<3.80	<0.40	<3.8	<0.40	42.6	49.62	65.3	500
25	32	M	<3.80	<3.80	120	8.35	1125	129	11565	375	27056
26	36	M	<3.80	<3.80	25	12	750	100.2	1800	3800	55454
27	32	M	5.29	<3.80	10	15.7	290	380	15846	900	33046
28	34	M	<3.80	<3.80	29	30	525	240	12587	675	40520
29	32	M	<3.80	<3.80	<0.40	<3.8	29	110	400	250	15484
30	23	M	<3.80	<3.80	35	20	1100	189	19586	532	42854
31	26	M	<3.80	<3.80	<0.40	<3.8	35	58	125	220	400
32	30	M	<3.80	<3.80	<0.40	<3.8	58	75	230	235	658
33	27	M	<3.80	<3.80	26	18	1500	400	13465	1250	35124
34	25	F	<3.80	<3.80	22.4	22	800	485	10220	1386	29658
35	27	M	<3.80	<3.80	28.9	45	1095	1500	18576	3100	38542

of antibody prevalence was observed.[3] The risk of exposure can be prevented by usage of PPE, repeated sanitization and proper infection control protocol. This also high risk of exposure through nosocomial infections. The CDC found that detection of SARS-CoV-2 antibodies was less common among HCW who reported using PPE.[11]

The neutralizing antibodies help prevent re-infection by viruses, memory B cells allow for rapid production of new antibodies in case of re-infection. Similarly, seroprevalence was conducted also in general population that was patients coming to the outpatient department during the first wave

(December 2020 to February 2021) and second wave (March 2021 to September 2021). We have observed an increase in seropositivity in 2nd wave in both genders in spite of RT-PCR being negative. We observed high antibody titers in males than females. The high seroprevalence was observed in second wave (76.07%) compared to the first wave (44.57%). Similar study conducted in Croatian general population showed a significant increase in SARS-CoV-2 seroprevalence after the second pandemic.[12]

We have done follow up studies in COVID-19 infected and non-infected, that was about 35 HCW working in

biochemistry department where neutralizing antibodies and IgG RBD specific antibodies were analyzed for the pattern of antibody responses. Samples were collected and analyzed in HCW post covid-19 infection in July 2020, only neutralizing S1/S2 antibody testing was available, The Anti-SARS-CoV-2 IgG RBD testing was available since January 2021. The vaccination drive stared after 5 months that was from Jan 16th –baseline (0) samples were collected prior to the first vaccine, then periodically -after 14 days of 1st dose, prior to 2nd dose which was about 45 days' post first dose and 14 days after second dose. The age group was between 20-40 years and 95% were males. About 19 (54.28%) of the 35 HCW were infected with Covid-19 in 2020. All were vaccinated with ChAdOx1-nCOV (CovishieldTM®) from January 2021. The Anti SARS-CoV-2 IgG RBD and Anti Spike IgG S1/S2 tested post 2 months of Covid-19 infection to analyze whether they had any immune response, we have observed there was no significant antibody development in infected. on follow up, we have collected samples prior to first vaccine and had observed that there was no antibody response in 70% of the HCW and minimal response in 30%, 80% were previously infected with COVID-19, both the Anti-SARS-COV-2 IgG RBD and Anti-spike IgG S1/S2 were negative. Further Post 14 days of 1st dose – we observed high antibodies with both Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2 in 24 (68.57%) of 35 and other 11 (31.42%) of 35 didn't develop sufficient antibodies. The high antibody levels were observed in HCW who were previously infected with COVID-19, these are due to the memory cells which get activated and respond to the vaccination which targets the RBD protein-the main target site for the corona virus and circulating neutralizing antibodies help prevent re-infection by viruses. Memory B cells allow for rapid production of new antibodies in case of re-infection. Therefore single dose offered a higher immune protection in COVID-19 infected HCW. As a follow up before the 2nd dose, that was 45 days – we observed a decline in antibody levels in non-infected HC in comparison to infected HCW, this suggests to get a second dose in non-infected, and the infected could wait as they had high antibody titers. Post 2nd dose, that was after 14 days, the partially vaccinated (only 1st dose) showed a continuous higher antibody that was Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2- 3(8.57%) of 35, and the 32 of 35 (91.42%) HCW with second dose showed high titers of Anti-SARS-CoV-2 IgG RBD and Anti-spike IgG S1/S2, proving the effectiveness of vaccine and as well the importance of complete vaccination offering the low infectivity and severity of disease, but doesn't explain the disease elimination.

5. Conclusions

We found a high seroprevalence of SARS-CoV-2 antibodies in HCW, with high seropositivity in- age group 26-45 years

(44.9%), males (71.2%), in doctors (79.8%) and nurses (52.7%), ward staff (48.6%). In general population high seropositive was observed in Second wave (76.07%) than first wave (44.57%). The antibody responses showed a gender associated seroprevalence, with highest in males and lowest seroprevalence in females in general population. We found a higher immune response in COVID-19 infected than the non-infected HCW with pre and post vaccination. As such neutralizing antibodies were detected in both the genders and both infected and non-infected groups. In contrast to seroprevalence high neutralizing antibodies was detected in infected HCW. However, a large study group and also further studies are needed.

7. Conflict of Interest

The authors declare no conflict of interest

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