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Original Research Article

Seroprevalance of IgG Antibodies against SARS CoV-2 in A Tertiary Care Hospital in Chennai

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Abstract:

Background: The SARS-CoV-2 pandemic put the world into despair and caused a huge strain on global healthcare network because of its high morbidity and mortality rates. In order to stop the transmission of the virus a better understanding of its prevalence among the population is needed for implementing safety and treatment management protocols for betterment of the community. Understanding the prevalence in a community through RT-PCR testing likely underestimates true prevalence rate as the asymptomatic cases are mostly unlikely to be tested. In this case serological testing can play a vital role in estimating the prevalence rate of COVID- 19 among the population particularly in the asymptomatic cases.

Objective: The aim of our study was to evaluate the seroprevalence of IgG antibody against SARS-CoV-2 among different subgroups of individuals.

Method: The study participants were divided into four groups. The blood samples were collected from different subgroups of patients who visited the COVID -19 testing facility of a tertiary care hospital in Chennai. The samples were tested for COVID- 19 IgG antibody as per the kit insert provided by the manufacture and the results were documented. The antibody responses among different subgroup of patients were evaluated and appropriate statistical analysis performed.

Result: Out of 160 study participants, 97 individuals turned out to be antibody positive and 63 individuals were antibody negative. Highest number of antibody positivity was observed in late COVID-19 positive group (92.5%) followed by the vaccinated group (87.5%). Highest number of antibody negativity was observed in COVID-19 RT-PCR negative group (72.5%) followed by early COVID-19 positive group (65.0%). Age and gender was not associated with the antibody production.

Conclusion: COVID -19 IgG antibody productions do not depend on factors such as gender and age of the individual. The antibody production is influenced by the phase of infection (early or late phase) in COVID -19 positive individuals, higher amount of antibody positivity was seen in late phase of COVID-19 positive (more than 7 days after symptoms onset). COVID- 19 vaccination also produces significant IgG antibody production (72.5%).

Introduction

The SARS-CoV-2 pandemic collapsed the world and caused a huge strain on global healthcare network because of its high morbidity and mortality rates [1]. WHO declared COVID -19 as a global pandemic on March 11, 2020 after it got disseminated to other parts of the world extensively

from Hubei province of China where many people presented with symptoms of COVID -19 [2]. The virus was identified as a new beta coronavirus by isolating it from bronchoalveolar lavage of patients suffering from severe pneumonia. The evidence of human to human transmission was justified by many cases of family clusters and nosocomial infections. [3] [4]

SARS –CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), is a large enveloped positive stranded RNA virus packed inside the helical capsid. The virus has mainly three structural proteins: membrane protein (M), envelope protein (E) and spike protein (S) which forms protrusions surrounding the viral surface to give a crown appearance [5].

Knowledge about the seroprevalence is essential to know about conditions on which COVID- 19 IgG antibody is produced. Determining the prevalence of COVID- 19 in a community through RT-PCR testing likely underestimate true prevalence rate as the asymptomatic cases are mostly unlikely to be tested. In this case serological testing can play a crucial role in estimating the seroprevalence rate of COVID -19 antibody response as the anti-bodies may be produced in the body due to previous COVID- 19 infection or COVID -19 vaccination. [6]

The virus binds to the respiratory cell of the host with the help of receptor binding domain (RBD) located in the spike protein (S1) of the virus thus facilitating the transmission of virus. The antibodies (IgG, IgA, IgM) thus produced by the immune system of the host in response to the viral particles helps in the inhibition of attachment of the virus particle to the host cell.

The antibodies thus produced can be detected in the serum in the span of 1 to 3 weeks after actual incident of infection. Antibodies IgM and IgG rises proportionately but when compared, antibody IgM deteriorate rapidly than antibody IgG. [6]

Detectable levels of IgG are not produced in some individuals even after the specified period after initial infection and the chances of reinfection are comparatively lower in individuals with detectable level of antibodies. [6]

Antibodies production against the viral antigens is very crucial for neutralizing response and would thereby reduce the virions count that could successfully infect the ACE-2 receptor expressing cells. Thus antibody respines to the SARS-CoV-2 in a community responding to the pandemic is very essential as in case of prophylaxis and treatment [7].

In this study, the antibody response of different groups of individuals is evaluated based on the phase of infection, symptomatic status, vaccination status and COVID- 19 positivity.

Objectives

The aim of our study was to evaluate the seroprevalence of IgG antibody against SARS-CoV-2 among different subgroups of individuals.

Methodology

Study design

A prospective cross sectional study (facility based) was done to evaluate the seroprevalence of IgG antibody among different groups of individuals. Institutional Human Ethics Committee (IHEC) has approved this research project (Protocol No: PMCHRI-IHEC-017) and granted ethical clearance.

Setting

This study was done in a tertiary care hospital situated in Chennai, Tamil Nadu. The study was carried over for a period of six months.

Eligibility criteria

The participants from any one of the 4 groups are eligible to participate in the study

1) Known COVID-19 positive patients - more than 7 days after symptom onset (Late phase)

2) Known COVID-19 positive patients – less than 7 days after symptom onset (Early phase)

3) Known COVID-19 negative patients

4) Vaccinated with 2 doses of vaccine against COVID- 19

Risks

No potential risks are involved in this study to the participants.

Data sources

The data was collected from the patients who visit the tertiary care center after getting proper consent from the patients. All the measures were taken for masking the patient identity at all stages of the research.

Bias

The bias was prevented by blinding the results of the RT -PCR test from the technician who was performing COVID-19 IgG antibody test

Statistical analysis

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results revealed that the variables do not follow normal distribution. Therefore, the data was analyzed by non-parametric methods. The values between the genders were compared using independent samples

Mann Whitney U test. To compare values between Groups Kruskal Wallis test was used followed by Bonferroni adjusted Mann Whitney test for multiple pair wise comparison. Spearman Rank correlations were calculated to assess the linear relationship between variables.

The data was analyzed using SPSS (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019). Significance level was fixed as 5% ($\alpha = 0.05$).

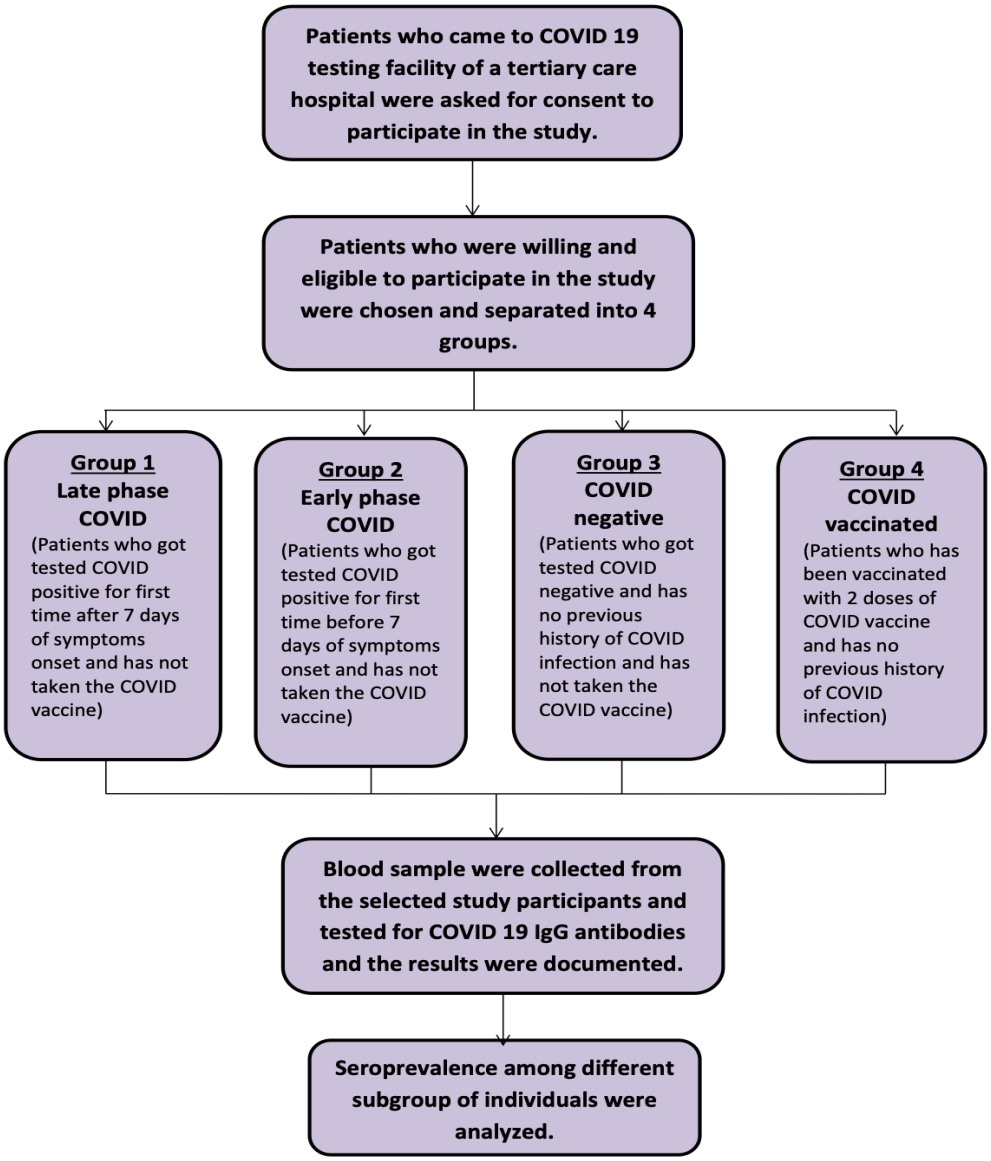
Sample collection

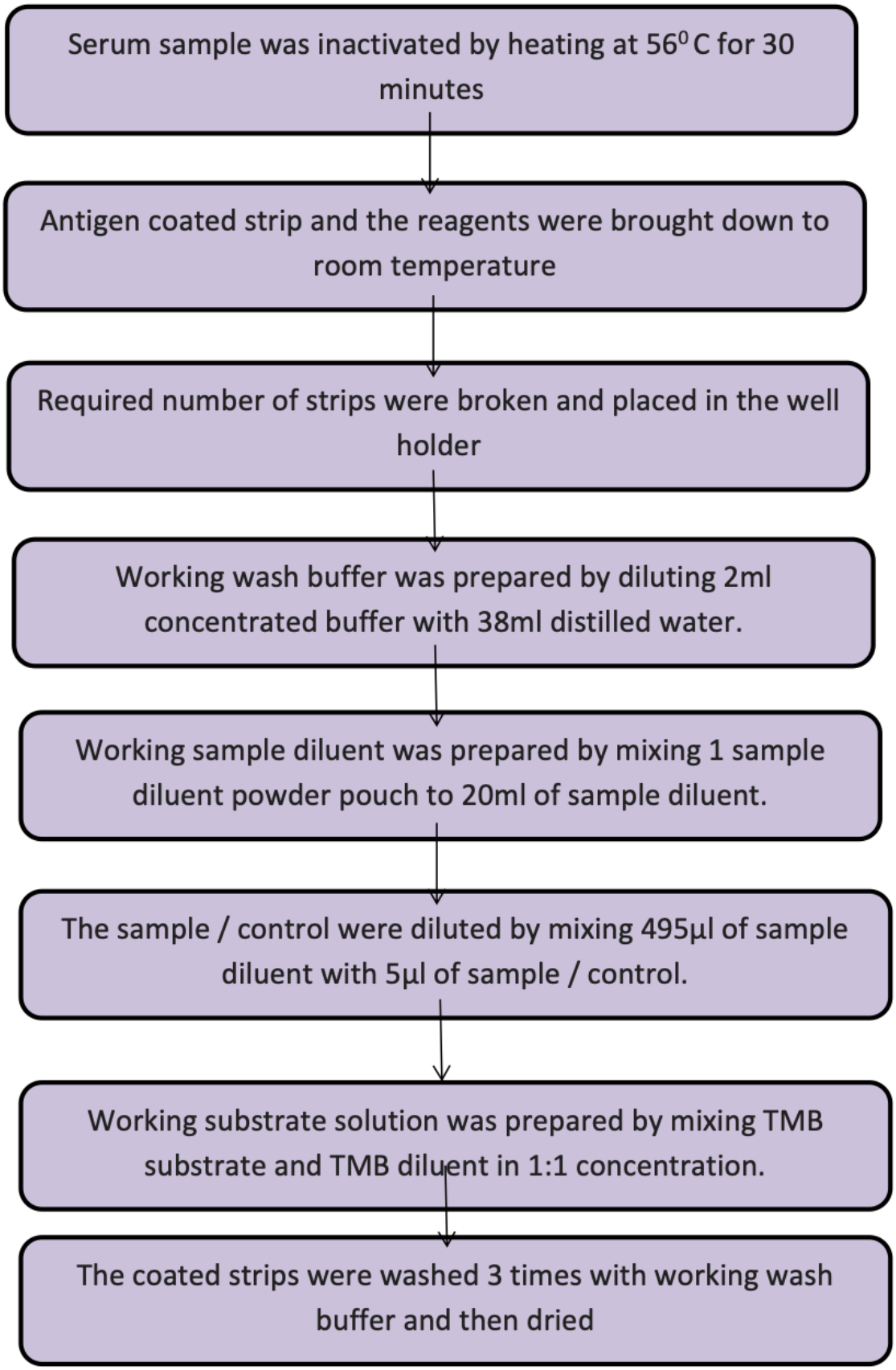
The blood sample for performing the SARS-CoV-2 IgG antibody test was collected by trained phlebotomist after getting proper consent from the patient.

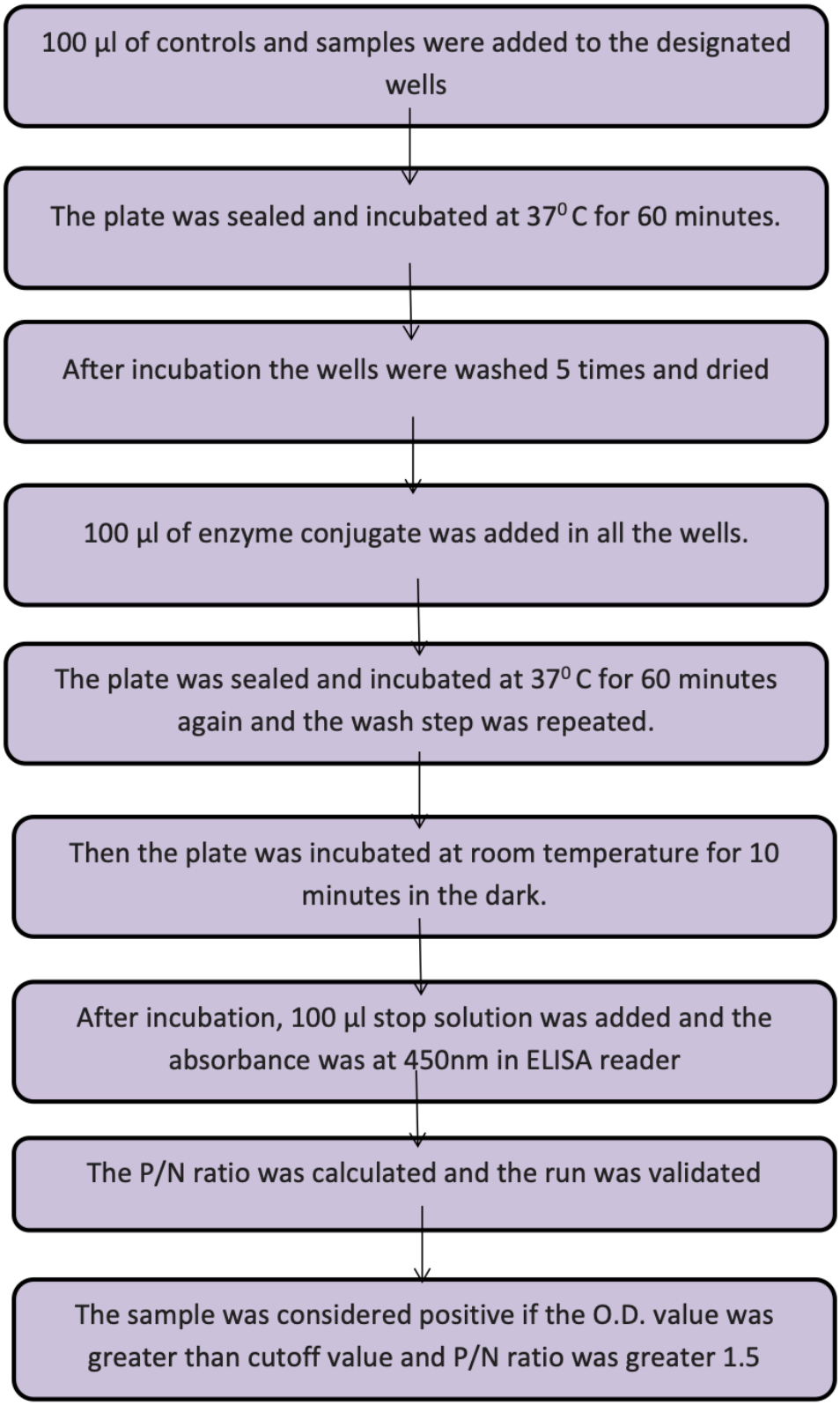
The patient was explained about the blood collection procedure and the preparations needed for withdrawal of blood. Hand hygiene steps were followed and then the vein puncture site was prepared. The needle was inserted into the preferred vein and the blood sample was taken. The serum was separated and was used for testing the SARS-CoV-2 IgG antibody.

SARS- CoV-2 IgG antibody test

The serum sample was collected and inactivated by heating. All the reagents were prepared as per the manufacturers' instruction. The SARS- CoV-2 IgG antibody test is done by ELISA method and all the procedures were done as per the instruction in the kit insert of the manufacturer. [8]




Serum sample was inactivated by heating at 56^0 C for 30 minutes
Antigen coated strip and the reagents were brought down to room temperature
Required number of strips were broken and placed in the well holder
Working wash buffer was prepared by diluting 2ml concentrated buffer with 38ml distilled water.
Working sample diluent was prepared by mixing 1 sample diluent powder pouch to 20ml of sample diluent.
The sample / control were diluted by mixing 495µl of sample diluent with 5µl of sample / control.
Working substrate solution was prepared by mixing TMB substrate and TMB diluent in 1:1 concentration.
The coated strips were washed 3 times with working wash buffer and then dried



Results: The study participants were divided into four groups based on their COVID- 19 report, day of testing after symptom onset and vaccination status.

Group 1 - Late COVID-19 positive - patients tested after 7 days of symptoms onset
Group 2 - Early COVID-19 positive - patients tested before 7 days of symptoms onset
Group 3 – COVID-19 negative patients
Group 4 - Patients who are vaccinated with two doses of COVID vaccine

In this study male participants were found to be in higher number in all the 4 groups than the female participants. Most of the participants of the study belonged to 21 to 40 years age group. The highest frequency of antibody positivity was observed among Late COVID-19 positive individuals (92.5%) followed by the vaccinated individuals (87.5%) (Table 1).

Table 1: Gender & Age distribution

Variables	Group 1		Group 2		Group 3		Group 4	
	N	%	N	%	N	%	N	%
Gender								
Male	27	67.5%	37	92.5%	23	57.5%	28	70.0%
Female	13	32.5%	3	7.5%	17	42.5%	12	30.0%
Age								
<=20 years	0	0%	2	5%	5	12.5%	2	5%
21 - 40 years	29	72.5%	31	77.5%	19	47.5%	27	67.5%
41 - 60 years	11	27.5%	7	17.5%	11	27.5%	11	27.5%
>60 years	0	0%	0	0%	5	12.5%	0	0%
Antibody report								
Positive	37	92.5%	14	35.0%	11	27.5%	35	87.5%
Negative	3	7.5%	26	65.0%	29	72.5%	5	12.5%

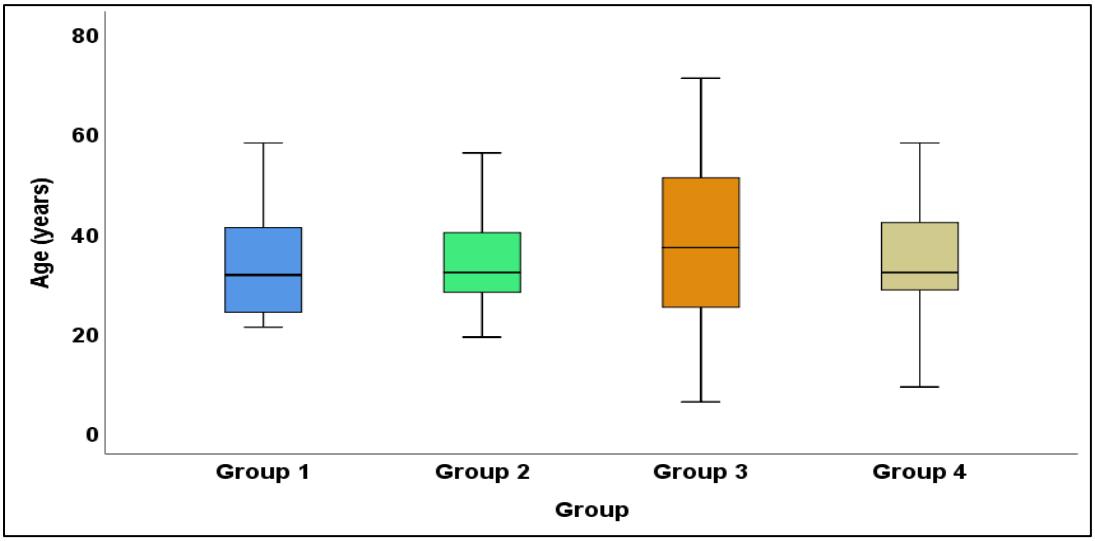


Figure 1: Box plot showing the age distribution in all the groups

The overall mean age of the participants was 35.3 ± 11.97 years. Group 3 (COVID-19 negative) participants had a higher mean age of 38.7 ± 16.63 years when compared to other group of participants (Table 2 & Figure 1).

Table 2: Mean age distribution in the groups

Age (years)	Group				Total
	Group 1	Group 2	Group 3	Group 4	
N	40	40	40	40	160
Mean	33.5	34.2	38.7	34.8	35.3
Std Dev	10.26	8.99	16.63	10.26	11.97
Median	31.5	32.0	37.0	32.0	32.0
Minimum	21.0	19.0	6.0	9.0	6.0
Maximum	58.0	56.0	71.0	58.0	71.0

The antibody OD mean value when including all the 4 groups was calculated to be 0.736 ± 0.856. The highest OD value was observed among the male participants of Group 1 (1.145 ± 0.862) and the lowest OD value was observed in Group 3 female participants (0.241 ± 0.204). No significance was observed between the Antibody OD value and gender in all the group of participants. (Table 3 & Figure 2)

Table 3: Independent-Samples Mann-Whitney U Test to compare Antibody OD Values between Genders

Group	Antibody OD value	Gender		p-value
		Male	Female	
Total	N	115	45	0.469
	Median	0.488	0.501	
	1st Quartile	0.134	0.179	

	3rd Quartile	0.819	0.652	
	Mean	0.736	0.486	
	Std Dev	0.856	0.408	
Group 1	N	27	13	0.475
	Median	0.732	0.775	
	1st Quartile	0.506	0.523	
	3rd Quartile	1.821	0.946	
	Mean	1.145	0.823	
	Std Dev	0.862	0.510	
Group 2	N	37	3	0.401
	Median	0.172	0.079	
	1st Quartile	0.100	0.068	
	3rd Quartile	1.745	0.868	
	Mean	0.863	0.338	
	Std Dev	1.184	0.459	
Group 3	N	23	17	0.978
	Median	0.178	0.197	
	1st Quartile	0.056	0.089	
	3rd Quartile	0.416	0.334	
	Mean	0.282	0.241	
	Std Dev	0.298	0.204	
Group 4	N	28	12	0.873
	Median	0.544	0.519	
	1st Quartile	0.435	0.459	
	3rd Quartile	0.645	0.636	
	Mean	0.545	0.504	
	Std Dev	0.190	0.222	

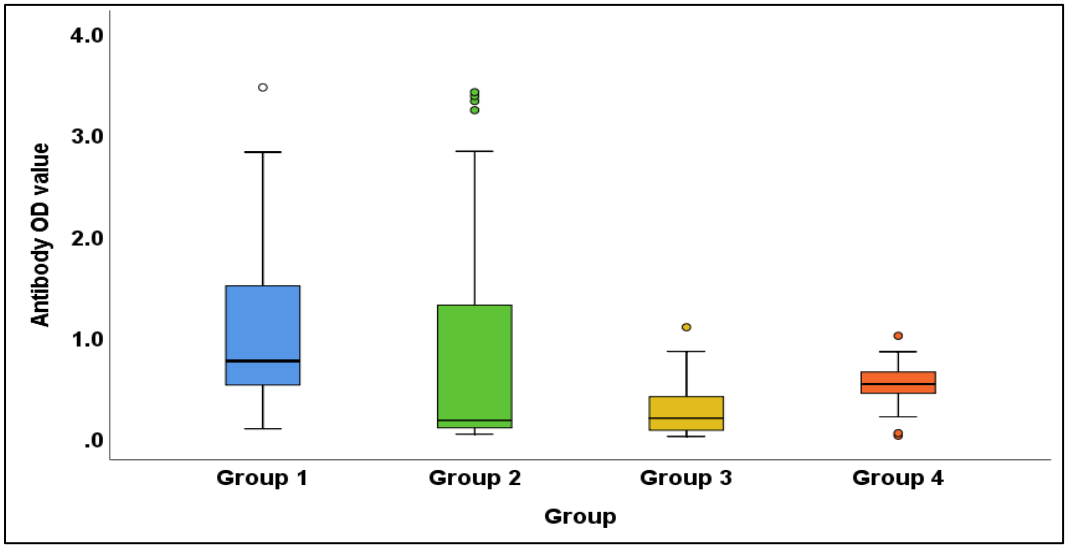


Figure 2: Box plot showing the distribution of Antibody OD value among different groups

The P/N ratio mean was estimated to be 3.973 ± 6.406. The highest mean P/N ratio was observed in Group 1 male individuals (7.330 ± 10.523) and the lowest value was seen in female participants of Group 3 (1.077 ± 0.962). The Mann Whitney test showed that there is no specific significance between the P/N ratio and gender of the individuals. (Table 4 & Figure 3)

Table 4: Independent-Samples Mann-Whitney U Test to compare P/N ratio values between Genders

Group	P/N Ratio	Gender		p-value
		Male	Female	
Total	N	115	45	0.623
	Median	2.080	2.349	
	1st Quartile	0.650	0.730	
	3rd Quartile	3.575	3.075	
	Mean	3.973	2.810	

	Std Dev	6.406	3.978	
Group 1	N	27	13	0.864
	Median	3.152	3.196	
	1st Quartile	1.709	2.560	
	3rd Quartile	7.680	6.620	
	Mean	7.330	5.741	
	Std Dev	10.523	6.391	
Group 2	N	37	3	0.401
	Median	0.840	0.387	
	1st Quartile	0.490	0.330	
	3rd Quartile	8.550	4.250	
	Mean	4.227	1.656	
	Std Dev	5.802	2.247	
Group 3	N	23	17	0.871
	Median	0.830	0.840	
	1st Quartile	0.264	0.419	
	3rd Quartile	1.960	1.240	
	Mean	1.332	1.077	
	Std Dev	1.403	0.962	
Group 4	N	28	12	0.873
	Median	2.566	2.448	
	1st Quartile	2.050	2.165	
	3rd Quartile	3.042	2.998	
	Mean	2.569	2.379	
	Std Dev	.895	1.049	

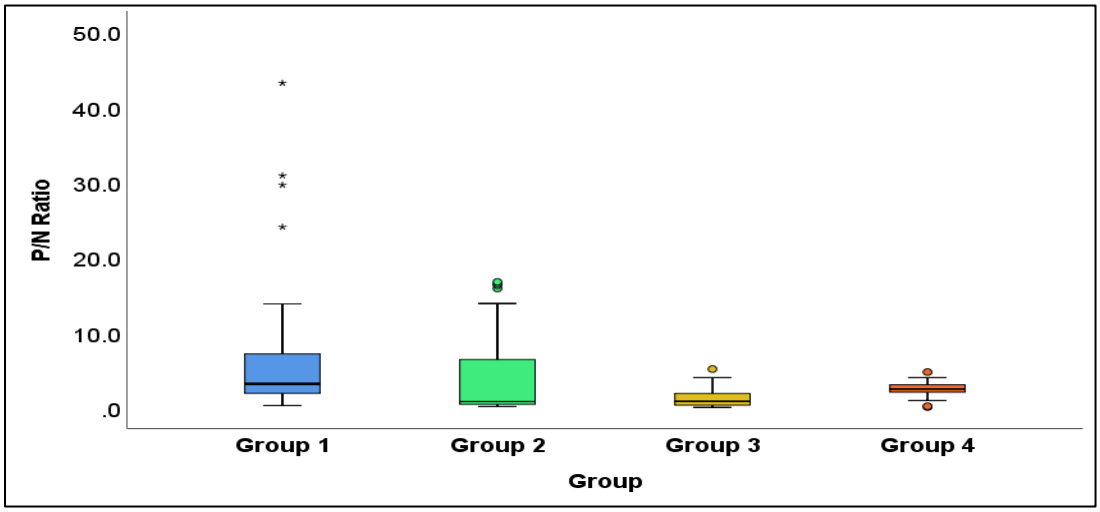


Figure 3: Box plot showing the distribution of P/N ratio value among different groups

Age has no significant relationship with antibody OD value and P/N ratio values in all the 4 groups, which means that age does not affect the COVID - 19 IgG antibody production.

Antibody OD value and P/N ratio value was analyzed to have strong and perfect correlation in mostly all the study groups. In study group 1, age and antibody OD value was found to have a low degree positive correlation & age and P/N ratio had a strong correlation. Age has low degree negative correlation between both the antibody OD value and P/N ratio value in study group 2. Low degree positive correlation was observed between age and both the antibody OD value and P/N ratio value among the COVID-19 negative group individuals (Group 3). Among the vaccinated individuals group (Group 4), strong positive correlation was observed between age and both the antibody OD value and P/N ratio value (Table 5).

When correlation of age was analyzed with the overall participants of the study it was found to have strong negative correlation (Table 6).

Table 5: Nonparametric Spearman's Rank Correlations (Group wise)

Group			Age (years)	Antibody OD value	P/N Ratio
Group 1	**Age (years)**	Correlation value	1.000	0.026	0.090
		p-value	0.0	0.875	0.582
		N	40	40	40

	Antibody OD value	Correlation value	0.026	1.000	0.741
		p-value	0.875	0.0	0.000
		N	40	40	40
	P/N Ratio	Correlation value	0.090	0.741	1.000
		p-value	0.582	0.000	0.0
		N	40	40	40
Group 2	**Age (years)**	Correlation value	1.000	-0.281	-0.281
		p-value	0.0	0.079	0.079
		N	40	40	40
	Antibody OD value	Correlation value	-0.281	1.000	1.000
		p-value	0.079	0.0	0.0
		N	40	40	40
	P/N Ratio	Correlation value	-0.281	1.000	1.000
		p-value	0.079	0.00	0.00
		N	40	40	40
Group 3	**Age (years)**	Correlation value	1.000	0.116	0.076
		p-value	0.0	0.475	0.643
		N	40	40	40
	Antibody OD value	Correlation value	0.116	1.000	0.988
		p-value	0.475	0.00	0.000
		N	40	40	40
	P/N Ratio	Correlation value	0.076	0.988	1.000
		p-value	0.643	0.000	0.00
		N	40	40	40
Group 4	**Age (years)**	Correlation value	1.000	0.091	0.091
		p-value	0.00	0.578	0.578
		N	40	40	40
	Antibody OD value	Correlation value	0.091	1.000	1.000
		p-value	0.578	0.00	0.00
		N	40	40	40
	P/N Ratio	Correlation value	0.091	1.000	1.000
		p-value	0.578	0.00	0.00
		N	40	40	40

Table 6: Nonparametric Spearman's Rank Correlations (Overall)

		Age (years)	**Antibody OD value**	**P/N Ratio**
Age (years)	**Correlation value**	1.000	-0.067	-0.063
	p-value	0.0	0.400	0.429
	N	160	160	160
Antibody OD value	**Correlation value**	-0.067	1.000	0.961
	p-value	0.400	0.0	0.000
	N	160	160	160
P/N Ratio	**Correlation value**	-0.063	0.961	1.000
	p-value	0.429	0.000	0.0
	N	160	160	160

The Independent Sample Kruskal Wallis test was used to compare the variables between the groups revealed that there is no significance when age was compared between the groups. Antibody OD value and P/N ratio value was found to be significant (p=<0.001) when compared between the groups (Table 7). Bonferroni adjusted test for multiple pair wise comparison test found that that Group 1 vs Group 2, Group 1 vs Group 3 and Group 3 vs Group 4 were found to be significant (Table 8).

Table 7: Independent-Samples Kruskal-Wallis Test to compare Age (years), Antibody OD values and P/N ratio between Groups

Variable		**Group**				**p-value**
		Group 1	**Group 2**	**Group 3**	**Group 4**	
Age (years)	N	40.0	40.0	40.0	40.0	0.472
	Median	32.0	32.0	37.0	32.0	
	1st Quartile	24.0	28.0	25.0	29.0	
	3rd Quartile	41.0	40.0	51.0	42.0	

	Mean	33.5	34.2	38.7	34.8	
	Std Dev	10.26	8.99	16.63	10.26	
Antibody OD value	N	40	40	40	40	<0.001
	Median	0.754	0.166	0.188	0.525	
	1st Quartile	0.517	0.092	0.069	0.435	
	3rd Quartile	1.497	1.307	0.400	0.645	
	Mean	1.040	0.824	0.265	0.533	
	Std Dev	0.774	1.151	0.260	0.198	
P/N Ratio	N	40	40	40	40	<0.001
	Median	3.174	0.815	.835	2.476	
	1st Quartile	1.907	00.451	0.321	2.050	
	3rd Quartile	7.150	6.400	1.885	3.042	
	Mean	6.813	4.034	1.223	2.512	
	Std Dev	9.325	5.640	1.228	0.934	

Table 8: Bonferroni adjusted test for multiple pair wise comparison tests

Pair	Antibody OD p-value	P/N Ratio p-value
Group 1 vs Group 2	<0.001	0.002
Group 1 vs Group 3	<0.001	<0.001
Group 1 vs Group 4	0.134	0.858
Group 2 vs Group 3	0.314	0.294
Group 2 vs Group 4	0.405	0.223
Group 3 vs Group 4	0.001	<0.001

Discussion

This study was done to evaluate the COVID- 19 IgG antibody response of different subgroups of patients to Severe Acute Respiratory Syndrome Coronavirus 2. Before the advent of the second wave of the COVID-19 pandemic, studies on seroprevalence were not available. Most of them did not have antibodies against the virus and can be prone to attacks by variants in the future. Available studies show that the immunity acquired after infection can last only upto 1 year.

In this study we primarily evaluated the IgG antibody response in individuals who were tested after 7 days of symptoms onset (Group 1), individuals who were tested before 7 days of symptoms onset (Group 2), individuals who were tested negative for COVID- 19 (Group 3) and individuals who are vaccinated against COVID -19 (Group 4). The antibody responses between the different groups of individuals were compared and their significance was found using statistical analysis. The study population included more male study participants than female participants. The variables such as gender and age of the study participants showed no significance on COVID -19 IgG production as in par with study conducted by Serena Marchi et al [1]. But Manoj V Murhekar et al. study concluded that there is a rising trend of Seroprevalance with age which is inconsistent with our study in which we reported that age does not have any relation with antibody production (p = 0.472) [9] (Table 3 & 7) From the study conducted by Wiegand, Ryan E. et al, the more number of study individuals belonged to 18 to 24 years age group coincides with our study in which we had more number of study participants in the age group 21 to 40 years. [10]

Nwosu et. al study reported female participants to be high in number than male participants but in our study we had more number of male study participants than female participants. (Table 1) [11] Javed W et al. reported that male participants showed higher seropositivity rate than females which compatible with our study in which we also had a higher seropositivity rate among the male individuals. [12] (Table 1)

The study done by Sood N et al, have identified significant higher prevalence rate in the vaccinated group of individuals which is agreeable with our study findings in which we have reported higher rate of seropositivity among the vaccinated group when compared with other unvaccinated individuals. [13] (Table 1) Higher IgG antibody positivity was observed in individuals who were tested after 7 days of symptom onset (Late phase COVID -19) followed by the vaccinated individuals group. (Table 1)

The study analysis revealed that age and gender have a very low correlation with antibody OD value within the group. Statistical analysis revealed that the antibody OD value and P/N ratio had good significance within the study groups. (Table 5 & 6) Bonferroni adjusted test for multiple pair wise comparison test found that that Group 1 vs Group 2, Group 1 vs Group 3 and Group 3 vs Group 4 were found to be significant. (Table 8)

Limitation

The study analyzed only the prevalence of COVID-19 IgG antibodies and not the other antibodies. The titre of IgG gradually wanes in time and the time duration up to which protection is offered remains a subject of concern. So COVID-19 antibody testing is not recommended to evaluate the necessity for vaccination in the case of unvaccinated individual or to decide the quarantine needs of an individual who has been exposed to a positive case.

Conclusion

COVID- 19 IgG antibody production does not depend on factors such as gender and age of the individual. The antibody production is influenced by the phase of infection (early or late phase) in COVID- 19 positive individu-als, higher amount of antibody positivity was seen in late phase of COVID-19 positive (more than 7 days after symptoms onset).

COVID- 19 vaccination also produces significant IgG antibody production. The prevalence rate was higher in late phase COVID-19 positive group (92.5%) followed by the vaccinated group (87.5%). The level IgG antibodies produced during the early phase of COVID- 19 was found to be lower in early phase of COVID- 19 when compared to late phase of COVID -19.

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