STUDY OF ANTIBODY RESPONSE IN PATIENTS WITH NOVEL CORONA VIRUS WITH SPECIAL REFERENCE TO INDIVIDUALS WITH AND WITHOUT CO-MORBIDITY

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LIST OF ABBREVATIONS	
COVID -19	Corona Virus disease 2019
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
S - antibody/ protein	Spike
M - antibody/ protein	Membrane
E - antibody/ protein	Envelope
N - antibody/ protein	Nucleocapsid
Ig M	Immunoglobulin M
Ig G	Immunoglobulin G
n Co V	Novel Corona virus
MERS	Middle East Respiratory Syndrome
CVA	Cerebrovascular Accident
HTN	Hypertension
T2DM	Type 2 Diabetes Mellitus
COPD	Chronic Obstructive Pulmonary Disease
RT – PCR	Reverse Transcription – Polymerase Chain Reaction
ELISA	Enzyme Linked Immunosorbent Assay
CKD	Chronic Kidney Disease
IHD	Ischemic Heart Disease

ABSTRACT

AIM: The aim of the study is to assess the specific antibody response in SARS-CoV-2 patients with and without comorbidities.

MATERIALS AND METHODS: This is a prospective observational study conductedto measure SARS-CoV-2–specific antibodies in COVID-19 infected patients. The study included total 60 patients, 30 with co-morbidities and 30 without co-morbidities.

Both inpatient and outpatient patients, with positive COVID-19 RTPCR were included and they were followed up at 21 daysand 4 monthspost infection. The blood samples were collected after obtaining the consent from the study participants. Serum was separated by centrifuging the blood samples and were further subjected to ELISA to detect specific IgG antibodies against N and S proteins of SARSCOV2. Data was entered in MS excel and analysed using SPSS 23.0 version software.

RESULTS: In the study out of 60 patients, most patients i.e., 48.3% (29/60) were in age group of 41-59 years; followed by 36.7% (22/60) were in age group of <40 years and 15% (9/60) were in age group of ≥60 years. Mean age of patients was 36.4 ± 6.8 years. It was found that, anti-N IgG levels at 21 days were positive in 78.3% (47/60) and negative in 21.7% (13/60) with increase in seropositivity to 93.3% (56/60) and fall in negative rate to 6.7% (4/60) at 4months. On the other hand, anti-S IgG levels at 21 days were positive in 83.3% (50/60), negative in 16.7% (10/60) with positive rate of 96.7% (58/60) and negative rate of 3.3% (2/60) at 4months. Both the antibody assays, anti-N IgG, and anti-S IgG in successive time periods, demonstrated the significant increaseinseropositivityandmaintainedlongevityofantibodieswithtime.Inthestudy, 5/5 patients with IHD and 3/3 patients with COPD had 100% serologic response to both N and S proteins of SARS-CoV-2 at 21 days and 4 months. But no significant strong correlation can be established between the seropositivity and longevity of antibodies with various comorbidities due to limited sample size.

CONCLUSION: It was concluded that most of the patients after contracting SARS-CoV-2 infection mount humoral immune response regardless of their age, gender, severity of disease and their association with various comorbidities. The study demonstrated a sustained seropositivity towards the SARS-CoV2 nucleocapsid and spike proteins for at least 120 days post PCR confirmation of COVID-19 patients.

KEYWORDS: SARS-CoV-2, Anti- N IgG, Anti – S IgG

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is an ongoing pandemic across the

worldcausedbytheSARS-CoV-2.[1]ItwasfirstrecognizedinChinainDecember2019, since then, it has spread to various regions of the world.[2 3 4]SARS-COV-2 belongs to enveloped virus in family of corona virus, under the genera beta-corona virus.[1 3]It shares around96% and 79.5% similarity withthetwoother coronaviruses, namely, bat and SARS-COV coronavirus, respectively.[2 3]

The severity of the infection can range from asymptomatic to severe. The most common adverse outcomes are acute respiratory distress syndrome and septic shock; however, cardiac, renal and multiorgan failure have also been documented. In most countries, the fatality rate among confirmed cases is somewhere around 3 percent; nevertheless, this number can rangeanywhere from 9 to 10 percent depending on the country. Even in patients where the disease itself is mild or asymptomatic, recent studies reveal that COVID-19 infection may predispose to long-term symptoms or future repercussions.[9 10]

Newer treatment modalities are continuously being developed. Currently, existing drugs are administered at various stages of illness depending on the pharmacologic mode of action and the predominant pathophysiology of the disease phase. Antiviral medications such as remdesivir, ritonavir-boosted nirmatrelvir, and molnupiravir are only some of the options that are currently available. Examples of monoclonal antibodies that have been used for treatment, postexposure prophylaxis, and preexposure prophylaxis include tocilizumab, sarilumab**,** Bebtelovimab, sotrovimab, casirivimab-imdevimab, bamlanivimab, etesevimab, and tixagevimab-cilgavimab. However, the use of these monoclonal antibodies is contingentondexamethasone, which has also been linked to a considerable reduction in the mortality rates of individuals who require oxygen supplementation. It is recommended to use numerous immunomodulators in conjunction with corticosteroids in patients who require high-flow oxygen therapy or noninvasive ventilation.Remdesivir may or may not be included in this treatment plan.[8]

Studies have shown that the SARS-CoV-2 can trigger both IgM and IgG responses in infected patients. The ability of the immune system to mount humoral response is crucial to clear the cytopathic viruses and in the prevention of reinfection. However, our knowledge of the nature, kinetic changes, and persistence of the humoral immune response during the clinical course of severe acute respiratory syndrome coronavirus 2 is limited. This is because our understanding of the nature of the response is restricted (SARS-CoV-2).

MATERIALSANDMETHODS

1. StudyDesign:Prospectiveobservationalstudy
2. StudyDuration:18months
3. Sampling technique: Purposive sampling

n = Z2pq/d2

n=Desiredsamplesize

Z = Level of confidence according to the standard normal distribution (Z= 1.96 at 95% confidence interval) q = 1-p

p=Prevalence(COVID-19prevalencetakenas4%)

d = Tolerated margin of error (for example we want to know the real proportion within 5%)

Basedontheaboveformulatherequiredsamplesizeis60.

a) Study setting and method of collection of data: The study group included 60 patients, who were further divided into 30 patients each, with and without co-morbiditiesafterthepositiveCOVID-19RT-PCRatthefluclinicOPDorthoseadmitted in JSS hospital. SARS-CoV-2–specificantibodies againstthenucleocapsid (N)protein and the spike protein were tested with serial samples at two successive periods of 21 days and 4months following onset of symptoms. An informed signed consent of all the participants was obtained before they were enrolled into the study.

b) Study Populationand source ofdata: Patients attending flu clinic OPD and those admitted in COVID isolation wards and ICU of JSS hospital, during the study duration of 18 months fulfilling the inclusion and exclusion criteria.

c) InclusionCriteria:

- HospitalizedpatientsdetectedpositivewithCOVIDRT-PCR
- PatientsattendingFluclinicOPD

d) ExclusionCriteria:

- Patientspositivewithrapidantigentest

e) Ethicalclearance:obtainedfromtheInstitutionalEthicalCommittee

4. Testmethod:

A 5 ml of blood sample was collected after obtaining the consent from the study participants. Serum was separated by centrifuging the blood sample at 13,000 rpm for 5-10 minutes. Serum samples were further subjected to ELISA to detect specific IgG antibodies against N and S proteins of SARSCOV2anti-N IgG and anti-S IgG antibody concentrations were estimated by using a commercial assay procured from Epitope Diagnostics as per the manufacturer's instructions. Both assays were based on ELISA, EDITM COVID-19 Nucleocapsid IgG Quantitative ELISA Kitand EDITMCOVID-19Spike protein IgGQuantitativeELISAKit were used to determine IgG against the N-protein and spike protein of SARS-CoV-2 respectively.

Kit controls and calibrators were used to obtain a cut off value. The test samples with cutofforODvalues≥20 U/mLwereconsideredaspositive,andthevalueof<20U/mL was identified as negative for antibodies against N protein. Similarly, the test samples with cut off or OD values≥80 U/mL were considered aspositive, and the value of < 80 U/mL was identified as negative for antibodies against spike protein.

Theindividualsampleswereretestedforantibodiestowards NandS-proteinafter3rdand 16thweek of post Covid infection.

5. Statisticalanalysis:

Data were entered in MS excel and analysed using SPSS 23.0 version software. Descriptive data were presented as mean, standard deviation, frequency, and percentage. *p* values <0.05 was considered as significant.

RESULTS

Outof60 studyparticipant's,48.3%patientswerefrom41to59agegroup,36.7 % frombelow 40agegroupand15were fromabove60agegroup. Thepercentage of maleandfemaleparticipants in the study was 56.7% and 43.3% respectively.

In the study, out of 22 patients in the age group of <40yrs, comorbidity was present in 22.7%

(6/22) of patients. Whereas in 29 patients, under the age group of 41-59 yrs, comorbiditywaspresentin62.1%(18/29)ofpatients.Fromthe9patientsinagegroup of ≥60 yrs, comorbidity was present in 66.7% (6/9) of patients. It was seen that association with comorbidity increased as the age increased and there was significant difference in presence of comorbidity in between different age groups (p=0.026).

TABLE -1:COMPARISONOFCOMORBIDITYINDIFFERENTAGEGROUPSOF COVID POSITIVE PATIENTS

	COMORBIDITY				
AGE GROUP	**PRESENT**		**ABSENT**		
	Number	**%**	**Number**	**%**	**P-Value**
<40yrs	6	22.7	16	77.3	0.026*
41-59yrs	18	62.1	11	37.9	
≥60yrs	6	66.7	3	33.3	
TOTAL	30	50.0	30	50.0	

*P<0.05(Statisticallysignificant).

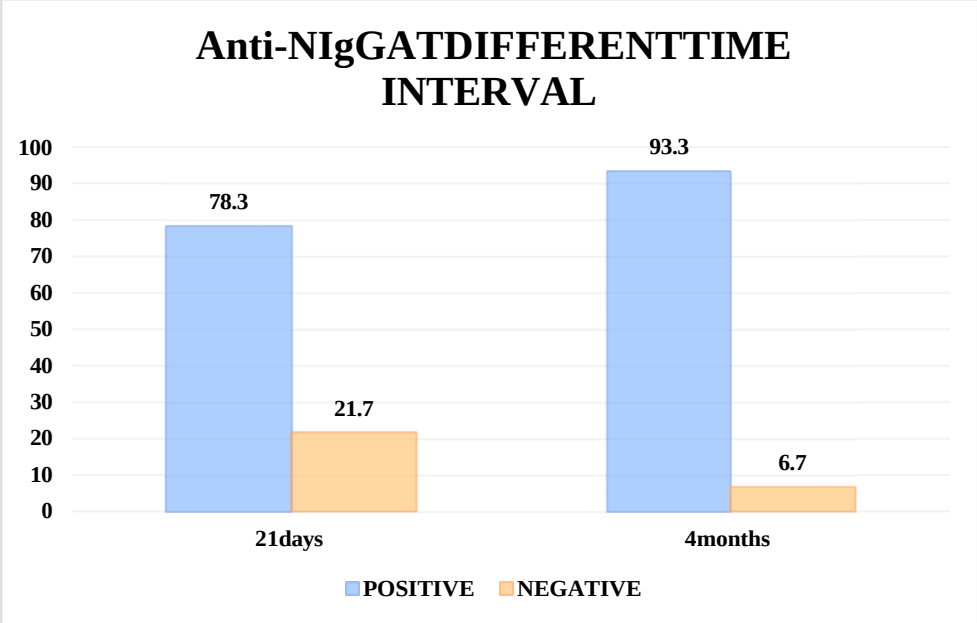
The anti-N IgGlevelsat21dayswerepositivein78.3%(47/60) ofpatientsand negativein21.7%(13/60)ofpatients.Whileat4monthsanti-NIgGlevelswerepositive in 93.3% (56/60) of patients and negative in 6.7% (4/60) of patients. Out of 47 anti-N IgG positive at 21days; 4 turned out to be negative at 4months and from the 13 negative patients at 21days, all turned positive at 4 months. The number of anti-N IgG positive caseswere significantly increased from 21 days to 4 months.

TABLE–2:COMPARISONOFANTI-NIgGATDIFFERENTTIMEINTERVAL IN COVID POSITIVE PATIENTS

	TIME				P-VALUE
Anti-NIgG	**21DAYS**		**4MONTHS**		
	Number	**%**	**Number**	**%**	
POSITIVE	47	78.3	56	93.3	0.036*
NEGATIVE	13	21.7	4	6.7	
Total	60	100.0	60	100.0	

***P<0.05(Statisticallysignificant).**

FIGURE–1:COMPARISONOFANTI-NIgGATDIFFERENTTIMEINTERVAL IN COVID POSITIVE PATIENTS



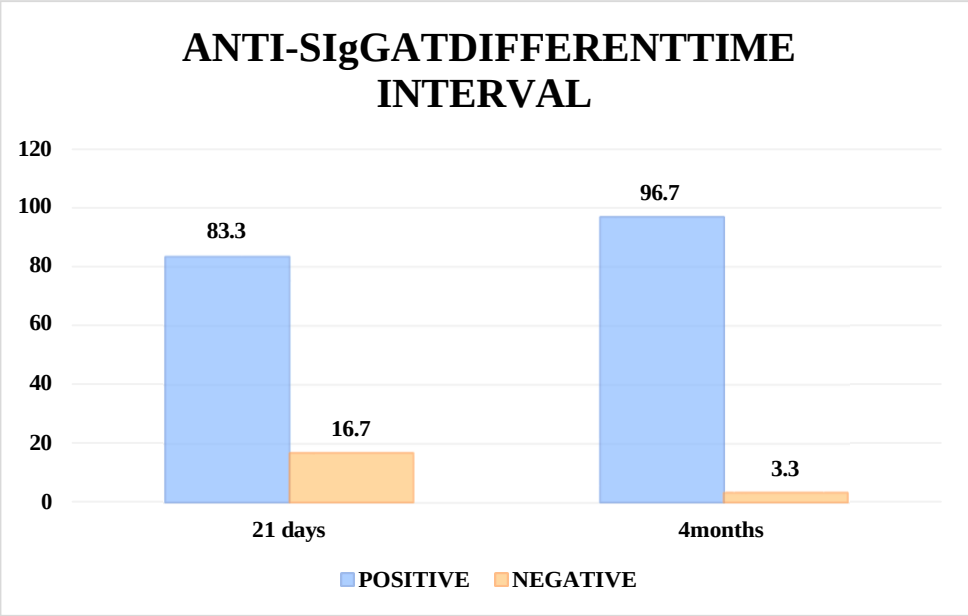
The anti-S IgG levels at 21 days were positive in 83.3% (50/60) of patients and negative in16.7%(10/60)patients. In thestudy anti-S IgG levelsat 4months were positive in 96.7% (58/60) of patients and negative in 3.3% (2/60) of patients. Out of 50 anti-S IgG positive at 21days; 2 turned out to be negative at 4months and from the 10 negative patients at 21days, all turned to be positive at 4 months. The number of positive patients increased from 21 days to 4 months and there was significant difference in number of positive patients from 21 days to 4 months in anti-S IgG.

TABLE-3:COMPARISONOFANTI-SIgGATDIFFERENTTIMEINTERVAL IN COVID POSITIVE PATIENTS

	TIME				P-VALUE
ANTI-S IgG	21DAYS		4MONTHS		
	Number	%	Number	%	
POSITIVE	50	83.3	58	96.7	0.033*
NEGATIVE	10	16.7	2	3.3	
Total	60	100.0	60	100.0	

***P<0.05(Statisticallysignificant).**

FIGURE–2:COMPARISONOFANTI-SIgGATDIFFERENTTIMEINTERVAL IN COVID POSITIVE PATIENTS.



Out of30 patientsassociated with comorbidities, 14 patientshad T2DM, and theiranti-NIgGlevelswerepositivein92.9%(13/14)and85.7%(12/14)at21daysand4months respectively. Accordingly, from the 12 HTN patients, anti-N IgG levels were positive in 83.3%(10/12)and91.7%(11/12)at21daysand4-monthperiods.Inthethreepatients with CKD, positive anti-N IgG levels were exhibited in 66.7% (2/3) and 33.3% (1/3) at successive periods of 21 days and 4 months. Likewise, the levels of anti-N IgG in 3 patients of CVA were positive in 100% (3/3) and 66.7% (2/3) at 21days and 4months respectively.Outof5patientsofhypothyroidism,only40%(2/5)showedpositivelevels at21days with100%seropositivity (5/5)at4months.Also,alltheCOPD(3/3)andIHD (5/5) patients in this study showed 100% seropositivity at 21days and sustained longevity of anti-N IgG antibodies to the end of 4 months.

The anti-N IgG antibodies in association with various co-morbidities in our study displayed that the positivity rate increased in patients associated with HTN and hypothyroidismat 4 months compared to 21 days.

TABLE – 4: ANTI-N IgG AT 21 DAYS & ANTI-N IgG AT 4 MONTHS IN COVID POSITIVE PATIENTS WITH VARIOUS COMORBIDITIES.

	anti-NIgG21Days				anti-NIgG4months			
Comorbidity	POSITIVE		NEGATIVE		POSITIVE		NEGATIVE	
	Number	Percentage %	Number	Percentage %	Number	Percentage %	Number	Percentage %

T2DM	13	92.9	1	7.1	12	85.7	2	14.3
HTN	10	83.3	2	16.7	11	91.7	1	8.3
COPD	3	100.0	0	0.0	3	100.0	0	0.0
CKD	2	66.7	1	33.3	1	33.3	2	66.7
IHD	5	100.0	0	0.0	5	100.0	0	0.0
Hypothyroid	2	40.0	3	60.0	5	100.0	0	0.0
CVA	3	100.0	0	0.0	2	66.7	1	33.3

The study showed the levelsof anti-SIgGin the14 patientsof T2 DM, werepositive in 92.9% (13/14) at 21days with subsequent increase in positive rate to 100% (14/14) at 4 months. From the 12 patients of HTN, anti-S IgG levels werepositive in 91.7% (11/12) and 100% (12/12) at 21days and 4 months respectively. In the 3 patients with CVA, positivelevelsofanti-SIgGweredetectedin100%(3/3)at21daysandweresustained to the end of 4months. Out of 3 patients of CKD; 66.7% (2/3) were positive for anti-S IgG at both the time periods of 21days and 4months. In reference to 5 patients of hypothyroidism with anti-S IgG in our study, only 40% (2/5) patients mounted early antibody response, with positive levels at 21days and the rest 60% (3/5) patients showed delayed seroconversion with 100% seropositivity at 4 months. Also, all the COPD (3/3) and IHD (5/5) patients in this study showed 100% seropositivity at 21days and sustained longevity of anti-S IgG antibodies to the end of 4months.

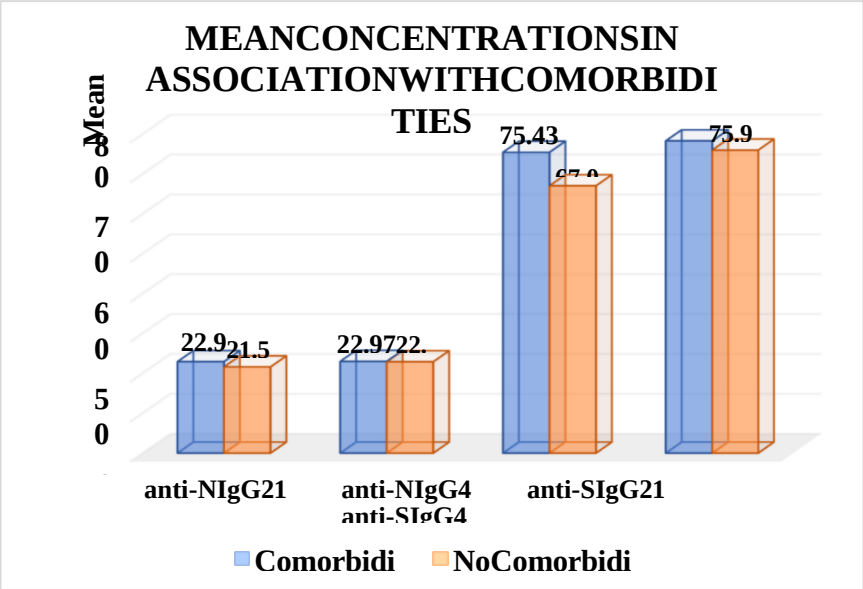
In the study, all the patients with coexisting comorbidities demonstrated proportionate increase in seropositivity of anti-S IgG antibodies at 4 months. In addition, early seroconversion with 100% seropositivity, and sustained longevity of anti-S IgG antibodies to the end of 4months was observed in all the patients associated with COPD, IHD andCVA.

TABLE – 5: ANTI-S IgG AT 21 DAYS & ANTI-N IgG AT 4 MONTHS INCOVID POSITIVE PATIENTS WITH VARIOUS COMORBIDITIES.

	anti-SIgG21Days				**anti-SIgG4months**			
Comorbidity	**POSITIVE**		**NEGATIVE**		**POSITIVE**		**NEGATIVE**	
	Number	**Percentage %**	**Number**	**Percentage %**	**Number**	**Percentage %**	**Number**	**Percentage %**
T2DM	13	92.9	1	7.1	14	100.0	0	0.0
HTN	11	91.7	1	8.3	12	100.0	0	0.0
COPD	3	100.0	0	0.0	3	100.0	0	0.0
CKD	2	66.7	1	33.3	2	66.7	1	33.3
IHD	5	100.0	0	0.0	5	100.0	0	0.0
Hypothyroid	2	40.0	3	60.0	5	100.0	0	0.0
CVA	3	100.0	0	0.0	3	100.0	0	0.0

In the study the mean concentration (Mean ± SD) of anti-N IgG antibodies with comorbidities was 22.9 ± 2.87 and 22.97 ± 1.88 at 21 days and 4 months respectively. The mean concentration (Mean ± SD) of anti-N IgG antibodies without comorbidities 21.58 ±4.4 and 22.87 ±1.78 at 21 days and 4 months respectively. The meanconcentration(Mean±SD)ofanti-SIgGantibodieswithcomorbiditiesis75.43±14.29and78.29±9.03 at21daysand4monthsrespectively.The meanconcentration(Mean±SD)ofanti-SIgGantibodiesinpatientswithoutcomorbiditiesis67.03±12.09and75.98±7.76at21daysand4monthsrespectively.

FIGURE – 3: CONCENTRATION OF ANTI – N IgG AND ANTI – S IgG ANTIBODIES AT 21 DAYS AND 4 MONTHS RESPECTIVELY IN COVID POSITIVE PATIENTS.



However, the estimation of anti-N IgG and anti-S IgG concentration levels at 21days and 4months revealed that antibody concentration levelswere decreased by 55% (33/60) and 25% (15/60) respectively, from the baseline.

DISCUSSION

The prospective observational study was conducted for a period of 18 months in both inpatients and outpatients with positive COVID RTPCR. In the study group of 60 patients, we included 30 patients associated with co-morbiditiesand 30 patients without co-morbidities. These patients were followed up at two successive periods, at 21days and 4 months for detecting the antibody response towards the Nucleocapsid (N) and Spike (S) proteins. From the 30 patients associated with co- morbidities 19 were male and 11 were

female compared to the 30 patients without co- morbidities where 15 were male and 15 were female and there was no significant difference in gender with co-morbidities and without co-morbidities ($p = 0.297$).

In the study there wasno limitation for age group,out of 60 patients22 patients were under 40 years, 29 patients were in between 41 to 59 years age and 9 patients above 60 years of age. In40yearsbelowagegrouponly22.7%(6/22)haveco-morbidities,inbetween 40 to 60 years of age group 62.7 % (18/29) are with co-morbidities and in above 60 years of age group 66.7 % (6/9) are with co-morbidities. There is significant difference in age group ($p = 0.026$)

In the study the number of patients with one comorbidity was 33.3%, with two comorbidities was 11.7%, with 3 comorbidities was 5% and without comorbidities was 50%.

Boththeantibodyassays,anti-NIgG,andanti-SIgGinsuccessivetimeperiods, demonstrates the significant increase in seropositivity and maintained longevity of antibodies with time.

Seropositivity to anti-N IgG was more in patients associated with comorbidities atbothtimepoints21daysand4months.However,therewasnostatisticalsignificance in association with co-morbidity with anti-N IgG positive and negative patients at 21 days ($p = 0.469$) and 4months ($p = 0.604$).

Seropositivity to anti-S IgG was more in patients associated with comorbidities at 21days. However, there was no statistical significance in association with co-morbidity with anti-N IgG positive and negative patients at 21 days ($p = 0.298$) and 4 months ($p = 1.000$).

Insupporttoourstudy,Alfego*etal*[7]conductedresearchonlongevityofSARS- CoV-2 antibodies which showed that the positivity rate against both proteins *S* and *N* was linearataround 90% for 21 days following the infection. However, afterabout 293 days, it showed that the rate of positivity for the antibodies against the N-protein decreased significantly to 68.2% while *S*-antibody seropositivity maintained a rate of 87·8% through 300 days.

In contrast to our study, Lumley *et al* [8], studied the duration and dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody responsesin 452 patients over a period of 6 months. They found that after the first positive RT- PCR test,the levelofthe IgGN antibodiesstartedtodecreaseby1month,witharound 50% of then becoming negative after seven months. In contrast anti-S IgG antibodies remained positive in 94% of patients up to 7 months.

The anti-N IgG antibodies in association with various co-morbidities in our study displayed thatthepositivityrateincreasedinpatientsassociatedwithHTN, hypothyroidism and CVA at 4 months compared to 21 days. Also, all the COPD (3/3) and IHD (5/5) patients in this study showed early seroconversion with 100% seropositivity at 21days and sustained longevity of anti-N IgG antibodies to the end of 4months.

Whereas, for anti-S IgG antibodies all the patients with coexisting comorbidities demonstrated proportionate increase in seropositivity at 4 months. In addition, early seroconversion, and sustained longevity of anti-S IgG antibodies was observed in all the patients associated with COPD, IHD and CVA.

Inreferenceto5patientsofhypothyroidisminourstudy,only40%(2/5)patients mounted early antibody response and the rest 60% (3/5) patients showed delayed seroconversion for both anti-N IgG and anti- S IgG with 100% seropositivity at 4 months.

In our study, 5/5 patients with IHD and 3/3 patients with COPD had 100% serologic responsetoboth NandSproteinsat21daysand wasmaintained totheend of 4 months. However, no significant strong correlation can be established betweenthe seropositivity andlongevity ofanti-N IgG andanti S IgG at successive periods with various comorbidities in our study.

A study carried out by Rimesh pal *et al* [9], showed that the patients with prolonged and uncontrolled T2DM were unableto mount antibody response towardsN protein at 2 weeks after the infection. When compared to non-diabetic patients in the study, this could suggest a possible delayed seroconversion. Our study further strengthens their understanding of the possibility of delayed conversion in diabetics.

Comorbidities increase the risk of developing a severe coronavirus infection in 2019. (COVID-19). Strong evidence points to the fact that after contracting SARS-CoV-2, most individuals generate detectable levels of IgM and IgG antibodies. IgG levels peak around 25 days after the onsetof symptomsandmaybedetectable foratleast 120 days. Data also suggests that higher antibody levels may be related to older age, more severe disease, and the occurrence of symptoms.

Longerfollow-upisrequiredtoevaluateandcommentontheperiodoflongevity of IgG antibody responses in SARS-CoV-2 infection.

CONCLUSION AND RECOMMENDATIONS

It was concluded that most of the patients after contracting SARS-CoV-2 infection mount humoral immune response regardless of their age, gender, severity of disease and theirassociation with various comorbidities. However, the statusof antibodies and the concentration levels dependon the specific type of protein and thenumber of days following the positive PCR. We have demonstrated a sustained seropositivity towards the SARS-CoV2 nucleocapsid and spike proteins for at least 120 days post PCR confirmation of COVID-19 patients. However, estimation of antibody concentration levels revealed decrease in anti-N

IgG and anti-S IgG antibodies with time by 55.5% and 25% respectively from baseline. In addition, all the COPD and IHD patients in our study displayed 100% serologic response to both N and S proteins at two-time intervals. But no significantcorrelation can be established between the seropositivity and longevity of antibodies with various comorbidities due to limited sample size. Althoughthe present study was a pilot one, this could be a gateway for furtherresearch.

Extensive studies with longer follow-up involving serial assessment of antibodies responses can provide valuable insight into the extent and longevity of IgG antibody response to the SARS-CoV-2 infection.Meta analysis of the similar comprehensive studies with profoundresearch can guide the health care system to establish the newcost-effective guidelines for COVID vaccination.Also, help to assess the possibility of cross protection against similar new strains and other virus belonging to thecorona virus family with this seropositivity.

LIMITATIONS OF STUDY

• Limited sample size to comment on the kinetics and longevity of antibodies in association with various comorbidities.

• Shorter duration of follow up to reflect on the dynamics and sustenance of antibodies and their concentration levels following the SARS-CoV-2 infection.

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