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Total SARSCoV2 Anti-Nucleocapsid Antibodies Levels in Patients Hospitalised with COVID-19 Infection- A Prospective Study

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Abstract **Original Research Article**

Background: There are recent advances in the management of COVID19 infection, but there is still a need to develop methods of predicting the course of the illness which are reliable yet easy to perform. Determining the rate and timing of detection of total SARSCoV2 anti-nucleocapsid antibodies may help to predict the course of illness in COVID19. ***Methodology:*** Patients with COVID19 infection admitted at Ramaiah medical college hospital were categorized based on clinical severity into asymptomatic, mild-moderate and severe/critical illness. Their blood samples were collected at three different time intervals with respect to onset of clinical symptoms to test for the presence of total Anti SARSCoV2 N antigen antibodies (IgG and IgM) and the rate of seroconversion. ***Results:*** The findings of this prospective study on the antibody response to COVID19 infection failed to demonstrate a significant difference with respect to anti-nucleocapsid antibody titers among those with mild and severe categories of infection. However based on the results of the present study, it appears that the virus is able to suppress the generation of antibodies and in the process, escape the clutches of the host immune system. ***Conclusion:*** The progression or recovery from COVID19 disease can be possibly determined by the rate of seroconversion of total COVID19 anti-nucleocapsid antibodies.

Keywords: COVID19, anti-nucleocapsid antibodies (N), SARSCoV2, anti-spike protein antibodies (S), seroprevalence.

INTRODUCTION

COVID19 has now become a near-endemic infection. The rapid spread of the COVID19 infection in the initial stages of the pandemic overwhelmed the healthcare systems in several countries around the world. The course of the illness is difficult to predict clinically, with many patients progressing rapidly from mild to severe disease. The recovery time for severe/critical category of patients is prolonged. There is still a need to devise methods to predict the course of the illness using more reliable methods. A deeper understanding of the underlying immune response to the viral infection could offer deeper insights into the factors responsible for progression or recovery from COVID-19 infection.

Anti-nucleocapsid antibodies have not been studied as well as the anti-spike protein antibodies in COVID19 infection. The pattern of antibody response of anti-nucleocapsid antibodies may provide a potential screening tool to identify the patients who may be at high risk for severe disease. Hence in this study, we sought to determine the association of the total anti-nucleocapsid antibodies to COVID19 infection among hospitalized patients with different categories of illness.

MATERIALS AND METHODS

A prospective study was conducted on patients with COVID19 infection admitted at Ramaiah medical college hospital over a period of three months from October 2020 to December 2020.

Inclusion Criteria

1. Patients with COVID19 infection confirmed by RT-PCR or Rapid Antigen test from a nasopharyngeal and/or oropharyngeal swab as per ICMR guidelines.
2. Age > 18 years and willing to sign an informed consent to participate in the study.

These COVID19 positive patients were subdivided into the following categories:

COVID-19 disease severity		
Asymptomatic	**Absence of clinical features of COVID19 infection**	
Mild disease	**Upper respiratory infection**	Symptomatic* patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia
Moderate disease	**Pneumonia**	Adult with clinical signs of pneumonia (fever, cough, dyspnoea) but no signs of severe pneumonia, including peripheral Oxygen saturation (SpO2) ≥ 90% and respiratory rate < 30/ minute on room air. The diagnosis can be made on clinical grounds; chest imaging (radiograph, CT scan, and ultrasound) may assist in diagnosis and identify or exclude pulmonary complications.
Severe/ Critical disease	**Severe Pneumonia**	Adults with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air

Exclusion Criteria

a) Patient admitted after 5 days of symptom onset
b) Patients who had received COVID19 vaccines
c) Patients on long term immuno-suppressant medications
d) Patients with Liver cirrhosis, Multiple myeloma.

Serum samples were collected from the patients during their hospital stay on a minimum of 3 occasions with respect to the onset of symptoms:

1. Day 0 (day of admission)
2. Day 3
3. Day 7

Total SARSCoV2 anti-nucleocapsid Antibodies testing was done in the Cobas e-immunoassay analyzer using electrochemiluminescence immunoassay (ECLIA).

Results obtained were interpreted as cutoff index (COI) <1.0 as non- reactive and COI>_1 as reactive for anti-SARS-Cov-2 antibodies.

The specificity of the test with 95% CI is 99.80% and the sensitivity of the test is 66.5% at 0-6 days post PCR confirmation, 88.1% at 7-13 days and 100% at >_14 days.

Post this, the rates of seroconversion was analyzed with respect to the severity of the cases.

Statistical Analysis

Data recording was done in MS Excel. Continuous variables are reported as Mean+_Standard Deviation (SD), Median (Interquartile range) (IQR) and Range. Categorical variables are summarized in terms of frequencies and percentages.

Shapiro-Wilk test was used to determine whether data sets differed from a normal distribution

For comparison of continues variables between two Group Unpaired t test and Mann Whitney U test were used based on normality testing and for categorical variable Chi square test were used.

All statistical analysis was performed using "R Studio version 1.4.1103". A two-tailed p value of <0.05 was considered to be statistically significant.

RESULT

Table 1: Baseline profile of COVID19 infected patients in the study

	Frequency	Percentage
Age Group (years)		
21 – 30	14	12.96%
31-40	15	13.89%
41-50	19	17.59%
51-60	25	23.15%
>60	35	32.41%
Gender		
Female	39	36.11%
Male	69	63.89%
Category		
Mild-moderate	57	52.78%
Severe	51	47.22%
Long term comorbidities		
No	43	39.81%
Yes	65	49.07%

It was observed COVID19 affected individuals were in the age group of 50 to 60 years with more common in males. 49.07% of patients had long term comorbidities affecting their quality of life.

Table 2: Correlation of CRP with COVID infected patients on the consecutive days

	N	Mean ± SD	Median (IQR)	Range	p value
CRP Admission					
Mild-Moderate	56	6.2545±22.2801	0.890 (0.370 – 2.475)	0.03 – 154.60	0.000002
Severe	50	6.7710±6.8539	3.835(2.180 – 10.510)	0.03 – 28.76	
CRP at 3rd day					
Mild-Moderate	31	1.9468±3.7534	0.530 (0.243 - 2.368)	0.040 – 19.140	0.003644
Severe	38	4.0734±6.2510	1.660 (0.840 - 4.950)	0.01 – 30.68	
CRP at 7th day					
Mild-Moderate	11	2.1082±2.3749	0.760(0.393 - 3.813)	0.09 – 6.790	0.976525
Severe	30	2.7310±4.4928	0.795 (0.410 - 2.270)	0.170 – 21.65	

The above table shows CRP value was high in the initial days of admission followed by not much significance after one week of admission.

Table 3: The trend of LDH on different days of admission

	N	Mean ± SD	Median (IQR)	Range	p value
LDH Admission					
Mild-Moderate	55	241.109±59.232	240(205.25 – 257.75)	137 - 397	0.001316
Severe	47	299.042±99.781	290(218.50 – 370.50)	161 – 611	
LDH at 3rd day					
Mild-Moderate	29	271.344±128.943	226(182 – 313.50)	156 – 742	0.010767
Severe	38	313.342±97.306	293(242 - 347)	152 – 568	
LDH at 7th day					
Mild-Moderate	9	244.778±71.2597	233(187.75 – 310.50)	152 – 351	0.632685
Severe	28	340.571±269.939	236.5(195 - 372)	27 – 1222	

From the above table, it was observed LDH value was significant up to 3rd day of admission

Table 4: The trend of serum ferritin on the different days of admission

	N	Mean ± SD	Median (IQR)	Range	p value
Ferritin Admission					
Mild-Moderate	55	141.501±130.62	93.70(46.575 – 191.50)	6.76 – 524	0.000439
Severe	46	346.056±383.054	289.50(87.20 - 448)	9.60 - 2296	
Ferritin at 3rd day					
Mild-Moderate	29	198.751±160.382	173(80.725 – 241.75)	8.99 – 647	0.007030
Severe	38	395.336±292.239	383.50(109 - 559)	20 – 1000	
Ferritin at 7th day					
Mild-Moderate	8	132.012±90.351	119(75.70 – 149.50)	38.70 – 329	0.030079
Severe	28	353.15±265.175	355(137.50 - 486)	13.70 – 921	

Serum Ferritin levels were found to increase gradually as the severity of the disease increased.

Table 5: The trend of D Dimer on different days of admission

	N	Mean ± SD	Median (IQR)	Range	p value
D Dimer Admission					
Mild-Moderate	57	0.8482±0.6033	0.630(0.50 – 0.973)	0.37 – 4.010	0.041400
Severe	48	1.1427±1.3241	0.80(0.58 – 1.14)	0.15 – 7.66	
D Dimer at 3rd day					
Mild-Moderate	31	0.8961±0.7548	0.740(0.503 – 0.917)	0.15 – 4.080	0.840511
Severe	36	1.2272±1.4376	0.700(0.460 – 1.170)	0.15 - 6.510	
D Dimer at 7th day					
Mild-Moderate	10	0.9960±0.6987	0.66(0.58 – 1.650)	0.23 – 2.34	0.859553
Severe	29	1.6041±1.9522	0.74(0.498 – 1.663)	0.31 – 7.18	

D-dimer value showed significance only in the initial day of admission, later it remain stable with no effect on the duration of infection.

Table 6: Total N antibodies trend in COVID infected patients

	Category				
	Mild		Severe		
	Frequency	Percentage	Frequency	Percentage	p value
Change in Total N antibodies from admission to 3rd day					
No change	42	75.00%	34	69.39%	0.5231
Positive Change	14	25.00%	15	30.61%	
Change in Total N antibodies from admission to 7th day					
No change	15	53.57%	15	44.12%	0.4622
Positive Change	13	46.43%	19	55.88%	
Change in Total N antibodies from 3rd day to 7th day					
No change	20	71.43%	25	73.53%	0.8548
Positive Change	8	28.57%	9	26.47%	

Table 6 compares the findings of the antibody responses between those with mild vs. severe COVID infections. There was no significant difference with respect to the rise in antibody levels based on the severity of illness on the day of admission, 3rd day or 7th day after admission to hospital.

DISCUSSION

The findings of this prospective study on the antibody response to COVID19 infection failed to demonstrate a significant difference with respect to anti-nucleocapsid antibody titers among those with mild and severe categories of infection. This is counter-intuitive as the expected response of the host immune system to severe infection should be to enhance the antibody synthesis. However based on the results of the present study, it appears that the virus is able to suppress the generation of antibodies and in the process, escape the clutches of the host immune system. Hence these revelations warrant further research into the pathogenesis mechanisms underlying the causation of severe COVID19 infections. These findings were from the pre-COVID19 vaccination era which further highlights the validity of these findings. This also suggests that the anti-nucleocapsid antibodies are not suitable for either predicting the clinical course of the infection since the level of antibodies on repeated measurements failed to demonstrate a significant difference in response among those with mild vs. those with severe infection.

A study by Baoqing Sun *et al*., on 38 COVID patients (11 ICU and 27 non-ICU) on the IgM and IgG responses against SARS-Cov-2 nucleocapsid (N) and spike protein (S) showed that S-IgG was higher in ICU patients in the third week as compared to the non-ICU patients, while N-IgG was higher in non-ICU patients. The increase of S-IgG positively correlated with the fall in CRP levels in non-ICU patients [1].

A serial investigation was conducted by Zhang youchen *et al*., among 21 individuals infected with SARS-Cov-2, non-severe and severe cases underwent seroconversion in the hospitalization or follow up period, and also the severe patients showed immediate antibody responses as compared to the non-severe patients. In contrast, only one asymptomatic case underwent seroconversion [2].

Some researchers speculate that antibodies can enhance infectivity as higher antibody levels and chronological order of IgM and IgG antibody appearance are highly variable supporting detection of both antibodies simultaneously [3, 4].

The increased severity and presence of co-morbidity were associated with an increase in the odds ratio for seroconversion at discharge even after adjustment for duration of symptoms, for both IgG and IgM antibody. With each passing day, the odds of IgG and IgM seroconversion increased by 10% and 5%, respectively [5]. These observations coincides with our study as well.

Patients with more severe disease appear to have higher levels of important neutralizing antibodies as compared to patients who had mild or asymptomatic COVID-19 having low levels of neutralizing antibodies (or even undetectable levels). In these persons it is possible the innate immune response and the T cell response cleared the virus. Recent studies have shown that neutralizing antibodies may disappear after 3 months [6].

A study by Yannick Galipeau *et al*., on Humoral responses and serological assays in SARS-CoV-2 infections, showed that individuals suffering from severe COVID-19 are known to exhibit a dysregulation of pro-inflammatory cytokine release, also known as a cytokine storm. How this large release in cytokines alters the humoral response compared to asymptomatic, mild or moderate COVID-19 cases that do not exhibit this same cytokine storm is not yet clear [7].

Murhekar MV *et al*., in a study of SARS-CoV-2 antibody seroprevalence in India, showed that among 15 084 randomly selected adults (one per household), the weighted and adjusted seroprevalence was 7·1% (6·2-8·2). Seroprevalence was similar across age groups, sexes, and occupations. Seroprevalence was highest in urban slum areas followed by urban non-slum and rural areas [8].

In a study by Vargas *et al*., many individuals with diabetes exhibited elevated serum ferritin levels, and it is known that they face a higher probability to experience serious complications from COVID-19. Similarly, in our study also we found with increase in severity, serum ferritin value also increased [12].

Hannah M Hemek et al in a study of Correlation of D-dimer and Outcomes in COVID-19 Patients observed, ninety-seven patients met criteria for inclusion in the study, Mean age was 63.2 years, median admission D-dimer 2.35ug/mL, and median peak D-dimer 2.74ug/mL. Average time to peak D-dimer was 3.2 days. Patient's requiring intubation had higher admission D-dimers (3.79ug/mL vs. 1.62 ug/mL) [13]. Similarly in our study also, we found that D-dimer value was high during initial 3 days of admission with mean value of .1.1427±1.3241 for 48 patients categorized as severe.

Ray A *et al*., in hospital based seroprevelance study on 212 patients observed. Sero-surveys are thought to be invaluable in diagnosing the proportion of infected individuals irrespective of symptoms. The data from this sero-survey indicate that the IFR of SARS-CoV2, is around 0.084% (4082 deaths were reported in Delhi till 8th Aug 2020; population of Delhi was taken to be 18710922, which is much lower than that previously estimated. It would be worthwhile to remember that during the initial period of the pandemic, Crude Fatality Rate (CFR) as suggested by WHO was around 3.4%. However, subsequently the mortality rate has been revised several times and appears to shrink further in the light of the results of their study [9].

Yadav Ak *et al*., in the study conducted on the hospitalized patients with COVID-19 shows a consistent increase in IgG and IgM antibody response over a period of time. However, as per the initial finding of the study, the majority of the patients (approximately 65%) did not have detectable antibodies at the time of discharge. The comparison among the group of clinical similarities was not carried out as the numbers are very small in severe and moderate cases to do any meaningful comparison as of now [10].

Mar K Young *et al*., in IgG antibodies against SARS-CoV-2 correlate with days from symptom onset, viral load and IL-10 showed COVID-19 positive patients had significantly higher antibodies against all SARS-CoV-2 proteins compared to COVID-19 negative patients Specificity was high for all antigens, specifically S1-IgG and S1-IgA had specificities of

97.6% and S1-IgM that had a specificity of 92%. IgA antibodies against all antigens were elevated in COVID-19 positive ventilated patients compared to not ventilated COVID-19 positive patients. IgG antibodies against S1, S2 and RBD were significantly increased in ventilated patients compared to not ventilated COVID-19 positive patients, and antibodies against N were trending higher in ventilated patients [11].

CONCLUSION

Our results showed that higher antibody levels correlate with more severe form of infection, thus affecting the prognosis and pathogenesis. This may provide further insights regarding the host's immune response against COVID19 infection at different stages of the illness. It may also help to determine if the pattern of appearance of the antibodies indicate the severity of manifestation of the disease.

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