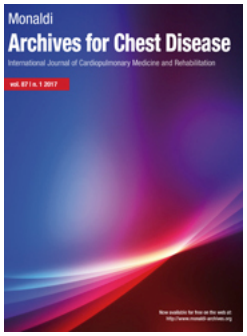

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Serial evaluation of antibody titres in patients recovered from COVID-19 and their correlation with disease severity

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Abstract

On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. According to the findings of various studies conducted around the world, the serological response varies greatly among different populations, with the determinants of variable response still unknown, including the role of disease severity, which is thought to have a definite correlation. The purpose of this study was to assess serial SARS-CoV-2 IgG antibody response in COVID-19 patients and correlate it with disease

severity. It was a longitudinal observational study in which 45 patients (age >18 yrs), were enrolled who had recovered from COVID-19 and were reporting to the post-COVID Care OPD Clinic. Patients who had been on long-term immunosuppressive therapy prior to SARS-CoV-2 infection were not eligible. All patients had not been immunized against SARS-CoV-2 and had no history of contact with recent COVID-19 cases. The patients underwent serial blood tests to determine serum IgG titers specific for SARS-CoV-2 at 30, 60, and 90 days after being diagnosed with COVID-19. Chemiluminescence was used to perform a semi-quantitative evaluation of the SARS-CoV-2 IgG antibody. At 30 days after confirmed SARS-CoV-2 infection, 98.78% had detectable serum IgG levels, and sero-reversion (loss of previously detectable antibodies) occurred in 2.5% at 60 days and 90 days. Serum IgG was found to peak at 30 days out of the three time points of measurement (30, 60, and 90 days from diagnosis). Serum IgG levels at 90 days were significantly lower than those at 30 days ($p<0.0001$) and 60 days ($p=0.002$). The current study's findings shed light on the presence and persistence of serum SARS-CoV-2-specific IgG antibodies following a natural infection. The findings point to a long-lasting immune response with increasing severity of initial COVID-19 disease.

Key words: COVID-19, antibody titres, IgG titres, SARS-CoV-2, seroreversion, immune response.

Introduction

In late December 2019, a pneumonia outbreak of unknown etiology was reported in China (Wuhan City, Hubei Province), and the causative agent was later identified as a novel beta coronavirus, which is closely related to the severe acute respiratory syndrome (SARS) coronavirus (CoV) family, and the disease was named Coronavirus Disease-2019 (COVID-19) [1]. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. SARS-CoV2 was isolated from the lower respiratory tracts of pneumonia patients in Wuhan and named 2019-nCoV [2]. The International Committee on Virus Taxonomy (ICTV), on the other hand, designated it as SARS-CoV-2. Most notably, it shares many similarities with the SARS-CoV genomic sequences [3]. According to previous virological and epidemiological research, the disease spreads primarily through close contact with infected people via respiratory droplets or contaminated objects or surfaces. The average incubation period, which is the time between virus exposure and onset of symptoms, is 5-6 days, but it can last up to two weeks [4].

The cellular tropism of the SARS-CoV-2 virus and the methods by which it enters target cells directly influence the pathophysiology of infection. The Spike protein exposed on the viral particle interacts with cellular receptors to control SARS-CoV-2 entry into cellular targets. Spike must bind to the cellular receptor Angiotensin-Converting Enzyme 2 (ACE2) in order for this two-step procedure to work [5]. The receptor binding domain (RBD), a Spike region at its apex that is momentarily exposed during conformational movements, mediates this initial phase [6]. Although spike and nucleocapsid are thought to be the primary targets of humoral response, antibody reaction can be directed against any viral protein RBD antibodies manifest earlier in the course of infection than nucleocapsid antibodies [7]. Furthermore, compared to antinucleocapsid reactions, anti-RBD antibodies may offer a diagnosis with a better sensitivity and specificity. Patients that have RBD seroconversion do so often and quickly, with little SARS-CoV cross-reactivity [8]. The effectiveness of vaccines and protective immunity are thought to be strongly correlated with neutralizing antibodies [9].

The available data on the pattern of antibody generation, rate of rise or fall in antibody titres in COVID-19 is limited and growing and shows a highly variable picture. A longitudinal estimate of antibody levels may model the future of the pandemic including

the possibilities of reinfection until effective vaccines providing lifelong immunity or preventing re-infections are accessible. Hence the present study has been planned to determine the serial IgG response in patients who have recovered from COVID-19 disease as well as to compare the titres with initial severity of disease in an Indian setting.

Materials and Methods

Study setting

It was a long-term observational study in which all recovered COVID-19 patients who presented to the Post-COVID Care OPD clinic were enrolled. The study lasted one and a half years, with patients being enrolled sequentially. The rapid antigen test and real-time reverse transcription-polymerase chain reaction (RT-PCR) were used to diagnose COVID-19. RT-PCR detects SARS-CoV-2 RNA in nasopharyngeal and throat swab (NT) samples (RAT). Patients were said to have recovered from COVID-19 after symptoms resolved and the severity-based recovery criteria specified in the Ministry of Health, Government of India guidelines [10]. The optimal sample size was calculated using the literature's reported 90% rate of antibody titre detection [1]. A minimum sample size of 35 was calculated based on a 95% confidence level and a 10% margin of error; 45 patients were enrolled in this study, which covered a 20% loss to follow-up.

Inclusion criteria:

- Recovered COVID-19 patients at least 1 month after their initial diagnosis.
- Age >18 years.
- Any gender

Exclusion criteria:

- Patients on long term immunosuppressive therapy prior to SARS-CoV-2 infection.
- Patients with inadequate medical records.
- Those who do not give consent for the study.

Methodology

The “Patient Information Sheet” provided all participants with the necessary study information, and informed consent was obtained. A thorough history was taken, including current symptoms and previous hospitalizations for COVID-19. In the event of

hospitalization, information on baseline disease severity, use of oxygen/ventilator, inflammatory marker levels, steroid use, and length of hospital stay was obtained from patient medical records/discharge cards as well as hospital medical records/discharge cards. COVID-19's baseline severity was classified as mild, moderate, or severe using standard criteria [11].

Following that, all patients had serial blood tests to determine IgG titres specific for SARS-CoV-2. The testing was repeated at 30, 60, and 90 days after their initial COVID-19 diagnosis. The patient enrollment is depicted in Figure 1. Chemiluminescence was used to perform a semi-quantitative evaluation of SARS-CoV-2 IgG antibody. Using appropriate statistical tests, the mean titre, highest titre, and time to highest titre were compared with various patient and disease-related factors such as age, gender, initial disease severity, length of hospital stay, and use of oxygen/ventilator support.

Statistical analysis

The presentation of the categorical variables was done in the form of number and percentage (%). On the other hand, the presentation of the continuous variables was done as mean ± SD and median values. The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used nonparametric tests. The following statistical tests were applied for the results:

1. The association of the variables which were quantitative in nature and not normally distributed data were analyzed using Kruskal Wallis test (for more than two groups) and Mann Whitney test (for two groups). Fried man test followed by *post-hoc* comparison was used for comparing serum IgG level at different time periods.
2. Spearman rank correlation coefficient was used to correlate serum IgG level with disease severity, duration of hospitalization for COVID-19(days), duration of steroids(days), duration of oxygen (days) and duration of HFNO/NIV/Mechanical ventilation(days).
3. Univariate and multivariate logistic regression was used to find significant risk factors of severe disease as compared to non-severe (mild/moderate) disease.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM

manufacturer, Chicago, USA, ver. 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

Observations and Results

Out of 45 patients, 30 (66.67%) patients belonged to age group ≤60 years and the mean age in years of study subjects was 51.89±21.1. The majority (57.78%) of patients were males and non-smokers (60%). Among the study population, 15 (33.33%) patients had hypertension followed by diabetes [10 (22.22%)]. Cardiac disease was present in only 3 out of 45 patients (6.67%). In the majority [22 (48.89%)] of patients, disease was severe followed by moderate [15(33.33%)]. Disease was mild in only 8 out of 45 patients (17.78%) (Table 1).

Serum SARS-CoV-2 IgG levels

Median values of serum IgG level at 30 days, at 60 days and at 90 days of study subjects were 100 (87.8-100), 99 (31.425-100) and 16.5 (5.11-100) respectively. Serum IgG level at 90 days was significantly lower as compared to serum IgG level at 30 days ($p<0.0001$) and at 60 days ($p=0.002$). No significant difference was seen in serum IgG level between 30 and 60 days ($p=0.059$). The highest serum IgG level was obtained at 30 days with median as 100. Median value of fall in serum IgG level at 60 days and at 90 days of study subjects was 1 (0-44.35) and 39.22 (0-89.1), respectively. Median of fall in serum IgG level at 60 days in male was 10.1 (0-63.5) which was significantly higher as compared to female (0 (0-11.8) ($p=0.03$). No significant association was seen in serum IgG level at 30 days, at 60 days and at 90 days with age, co-morbidities and disease symptoms. Median of serum IgG level at 30 days, at 60 days in non-smokers was significantly higher as compared to smokers ($p=0.042$ and $p=0.034$, respectively). Median of serum IgG level at 30 days in severe was 100 (100-100) and moderate was 100 (84.8-100) which was significantly higher as compared to mild [25.2 (12.692-100)]. The median fall in serum IgG level at 60 days in moderate was 40.8 (2-73) which was significantly higher as compared to mild [3.25 (0-9.2)] and severe [0 (0-17.5)] ($p=0.024$) (Figure 2). Significant positive correlation was seen between serum IgG levels at 30 days with disease severity with correlation coefficient of 0.374 (Figure 3). No correlation was seen between serum IgG level at 30 days with duration of hospitalization for COVID-19

(days), duration of oxygen (days) with correlation coefficient of -0.025, -0.009 respectively. Non-significant moderate positive correlation was seen between serum IgG level at 30 days with duration of steroids (days) with correlation coefficient of 0.271. Non-significant mild negative correlation was seen between serum IgG level at 30 days with duration of HFNO/NIV/mechanical ventilation (days) with correlation coefficient of -0.132. Significant positive correlation was seen between serum IgG levels at 60 days with disease severity with correlation coefficient of 0.342 (Figure 4). No correlation was seen between serum IgG level at 60 days with duration of hospitalization for COVID-19(days), duration of oxygen (days) with correlation coefficient of 0.006, 0.035 respectively. Non- significant moderate positive correlation was seen between serum IgG level at 60 days with duration of steroids(days), duration of HFNO/NIV/Mechanical ventilation(days) with correlation coefficient of 0.312, 0.266 respectively. Non-significant moderate positive correlation was seen between serum IgG levels at 90 days with disease severity with correlation coefficient of 0.244 (Table 2). On performing univariate regression, serum IgG level at 60 days, male gender, smoking, dyspnea were found to be significant risk factors of severe disease. With the increase in serum IgG levels at 60 days, the risk of severe disease significantly increased with odds ratio of 1.018 (1.001 to 1.036). Males and smokers had significantly low risk of severe disease with odds ratio of 0.113 (0.028 to 0.445), 0.245 (0.068 to 0.884) respectively. Patients with dyspnea had significantly high risk of severe disease with odds ratio of 6.921(1.644 to 29.131) (Table 3). On performing multivariate regression, none of the parameters was found to be independent significant risk factor of severe disease (Table 4).

Discussion

All patients in the present study were unvaccinated against SARS-CoV-2 and had no contact history with recent COVID-19 cases. The precise duration of time that antibodies persist after COVID-19 is still unknown. In the present study, 98.78% had detectable serum IgG levels at 30 days following confirmed SARS-CoV-2 infection, and seroreversion (loss of previously detectable antibodies) was found to occur in 2.5% each at 60 days and 90 days. Incorporating data available, it becomes evident that more than 95% of the patients show detectable serum IgG levels at end of 30 days following SARS-CoV-2 infection, of which 4-5% shows seroreversion at the end of 90 days [12-14].

In the present study, out of the three time points of measurement (30, 60 and 90 days from diagnosis), serum IgG was found to peak at 30 days. Serum IgG level at 90 days was significantly lower as compared to serum IgG level at 30 days ($p<0.0001$) and at 60 days ($p=0.002$) which points towards significant decline in the immune response by the end of 3 months post disease onset. In a study conducted in the Indian population, there was a gradual decline in antibody titre value in 55% of individuals after 16 weeks when compared to week 1 [15]. The results obtained from other studies conducted in different population suggest that the immune response to SARS-CoV-2 infection in the form of serum IgG levels decline over a period of 3 months as in the present study.

Serum IgG level at 30 days and at 60 days in females was significantly higher as compared to males ($p=0.047$ and $p=0.011$, respectively) in the present study. Males showed significant decline in serum IgG levels at 60 days when compared with females ($p=0.03$). In the case of hospitalized COVID-19 patients, a recent study has shown that women mount a different immune response, produce higher antibody titres and have less mortality. In the above study, anti-SARS-CoV-2 IgG antibodies were detected in 34 (68%) women and 16 (32%) men. These analyses also provide a potential basis for taking sex-dependent approaches to prognosis, prevention, care, and therapy for patient with COVID-19 [16].

In the present study, 18 out of 45 patients (40.00%) were smokers. Median serum IgG level at 30 days and at 60 days in smokers was significantly lower as compared to non-smokers ($p=0.034$ and $p=0.042$, respectively). The lower antibody response in smokers in the present study may be attributed to their dysregulated immune responses which warrants the need of further studies in detail.

In majority [22 (48.89%)] of patients, disease was severe followed by moderate [15 (33.33%)]. The disease was mild in only 8 out of 45 patients (17.78%). Significant association was seen in serum IgG level at 30 days with disease severity. Fall in serum IgG level at 60 days in moderate was significantly higher as compared to mild and severe ($p=0.024$). The significant fall in antibody titres at 60 days in patients with moderate severity in our study may be likely due to IgM seroconversion as well as small sample size and unequal distribution among severity subgroups.

Significant positive correlation was seen between serum IgG levels at 30 days and 60 days with disease severity eliciting robust immune response with increasing disease

severity. Serum SARS-CoV-2 IgG titre was found to have a declining trend throughout the present study with slow decline in severe group, pointing towards a persistent and long immune response with increasing severity of disease, although the correlation was found to be statistically non-significant.

Male gender and smoking history were identified as significant risk factors of severe disease by regression analysis in the present study. Similar observations were identified in several other studies including a meta-analysis which elicited the demographic risk factors for COVID-19 severity in detail [4,17].

A non-significant correlation was discovered between antibody titres and symptoms during follow-up visits. A similar finding was observed in one Japanese study, in which raised levels of antibody titres were associated with long COVID symptoms at the first follow-up visit but not at subsequent follow-ups [18].

The present study has depicted a longitudinal evaluation of antibody titres in the Indian setting, within a cohort of unvaccinated patients, thereby ruling out the interference of COVID-19 vaccination in the natural immune response against SARS-CoV-2. Also it can act as a foundation for future researches to uncover the duration and trend of immune response beyond 3 months of disease onset.

The current study has some limitations, such as the lack of measurement of the initial IgM, IgA, and IgG titres at the time of diagnosis and the non-detection of antibodies by virus-neutralization tests, which leaves the neutralizing activities of these antibodies unknown. There was also no quantitative viral load monitoring. The study only includes 3 months of follow-up after the initial infection and focuses on the immunological response to acute COVID-19 infection. Furthermore, without the inclusion of lung function tests, chest imaging and symptomatology were used as the only tools for infection monitoring. As a result, the impact of immunological aspects in post-COVID sequel could not be elicited, necessitating more detailed and long-term research. Aside from these limitations, the current study had a small sample size and excluded asymptomatic and non-hospitalized patients.

Conclusions

The current study's findings shed light on the presence and persistence of SARS-CoV-2-specific IgG antibodies after COVID-19. Furthermore, a positive correlation between

rising serum IgG antibody titre and increasing disease severity suggests that cases with mild or moderate symptoms require vaccination or booster doses as soon as possible. Severe disease in males and smokers, as well as higher antibody titres in females and nonsmokers, calls for additional research to decode the disease's immunopathology in greater detail. It also emphasizes the importance of serological testing in obtaining more accurate estimates of the extent of the immune response against COVID-19, which may guide strategies for effective pandemic containment.

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Table 1. Characteristics of the study population (n=45).

Variable	**n (%)**
Age in years	51.89 ± 21.1
Gender	
Male	26 (57.78)
Female	19 (42.22)
Comorbidities	
Diabetes	10 (22.22)
Hypertension	15 (33.33)
Cardiac disease	3 (6.67)
Smoking history	
Never-smokers	27 (60.00)
Ever-smokers	18 (40.00)
Symptoms at the time of COVID-19 diagnosis	
Fever	44 (97.78)
Dyspnea Chest pain	15 (33.33) 3 (6.67)
Cough	2 (4.44)
Sore throat	4 (8.89)
Loose stools	2 (4.44)
Baseline of severity of COVID-19	
Mild	8 (17.78)
Moderate	15 (33.33)
Severe	22 (48.89)
Inpatient treatment for COVID-19	45 (100)
Treatment received for COVID-19	
Oral steroids	32 (71.11)
Oxygen requirement	37 (82.22)
HFNO/NIV/Mechanical ventilation	19 (42.22)
Imaging (Chest X-ray/CT scan) findings at the time of COVID-19 diagnosis	
Abnormal and consistent with acute COVID-19 infection	38 (84.44)
Abnormal and inconsistent with acute COVID-19 infection	0 (0)
Normal	7 (15.56)

Table 2. Correlation of Serum IgG level with disease severity, duration of hospitalization for COVID-19 (days), duration of steroids (days), duration of oxygen (days) and duration of HFNO/NIV/mechanical ventilation (days).

Variables	Disease severity	Duration of hospitalization for COVID-19 (days)	Duration of steroids (days)	Duration of oxygen (days)	Duration of HFNO/NIV/ mechanical ventilation (days)
Serum IgG level at 30 days					
Correlation coefficient	0.374	-0.025	0.271	-0.009	-0.132
p-value	0.012	0.870	0.133	0.959	0.589
Serum IgG level at 60 days					
Correlation coefficient	0.342	0.006	0.312	0.035	0.266
p-value	0.031	0.972	0.100	0.843	0.286
Serum IgG level at 90 days					
Correlation coefficient	0.244	-0.075	0.089	-0.066	-0.002
p-value	0.129	0.645	0.646	0.711	0.998
Fall in serum IgG level at 60 days					
Correlation coefficient	-0.246	-0.056	-0.329	-0.039	-0.305
p-value	0.125	0.730	0.082	0.828	0.218
Fall in serum IgG level at 90 days					
Correlation coefficient	-0.112	0.050	-0.058	0.094	0.029
p-value	0.490	0.759	0.764	0.597	0.910
Fall in serum IgG level at 90 days from 60 days					
Correlation coefficient	-0.014	0.011	-0.129	0.018	0.133
p-value	0.932	0.944	0.503	0.922	0.597

Spearman rank correlation coefficient

Table 3. Univariate logistic regression to find significant risk factors of severe disease as compared to non-severe (mild/moderate) disease.

Variable	Beta coefficient	Standard error	p-value	Odds ratio	Odds ratio lower bound (95%)	Odds ratio upper bound (95%)
Age	0.018	0.015	0.227	1.018	0.989	1.048
Gender						
Female				1.000		
Male	-2.184	0.702	0.002	0.113	0.028	0.445
Comorbidities						
Diabetes	0.056	0.717	0.938	1.057	0.259	4.312
Hypertension	0.254	0.634	0.689	1.289	0.372	4.462
Cardiac disease	-0.694	1.262	0.582	0.500	0.042	5.931
Smoking						
Ever smoker	-1.405	0.654	0.032	0.245	0.068	0.884
Pack years of smoking	-0.033	0.051	0.517	0.967	0.875	1.069
Serum IgG levels						
Serum IgG level at 30 days	0.019	0.010	0.066	1.019	0.999	1.040
Serum IgG level at 60 days	0.018	0.009	0.038	1.018	1.001	1.036
Serum IgG level at 90 days	0.012	0.008	0.123	1.012	0.997	1.028
Fall in serum IgG level at 60 days	-0.018	0.011	0.108	0.982	0.960	1.004
Fall in serum IgG level at 90 days	-0.007	0.008	0.371	0.993	0.978	1.008
Symptoms at the time of COVID-19 diagnosis						
Fever	3.303	5.336	0.536	27.2032	0.00078	946720
Dyspnea	1.935	0.733	0.008	6.921	1.644	29.131
Chest pain	0.788	1.263	0.533	2.199	0.185	26.150
Cough	3.648	4.096	0.373	38.3987	0.01252	117739
Sore throat	-3.607	2.752	0.190	0.027	0.000	5.973
Loose stools	0.045	1.447	0.975	1.046	0.061	17.829
Symptoms on follow up visits	0.419	0.960	0.662	1.521	0.232	9.979

Table 4. Multivariate logistic regression to find significant risk factors of severe disease as compared to non-severe (mild/moderate) disease.

Variable	Beta coefficient	Standard error	p-value	Odds ratio	Odds ratio lower bound (95%)	Odds ratio upper bound (95%)
Serum IgG level at 60 days	0.011	0.010	0.297	1.011	0.991	1.031
Gender						
Female				1.000		
Male	-1.686	0.865	0.051	0.185	0.034	1.009
Smokers	0.359	0.918	0.696	1.432	0.237	8.659
Dyspnea	1.190	0.855	0.164	3.288	0.616	17.559

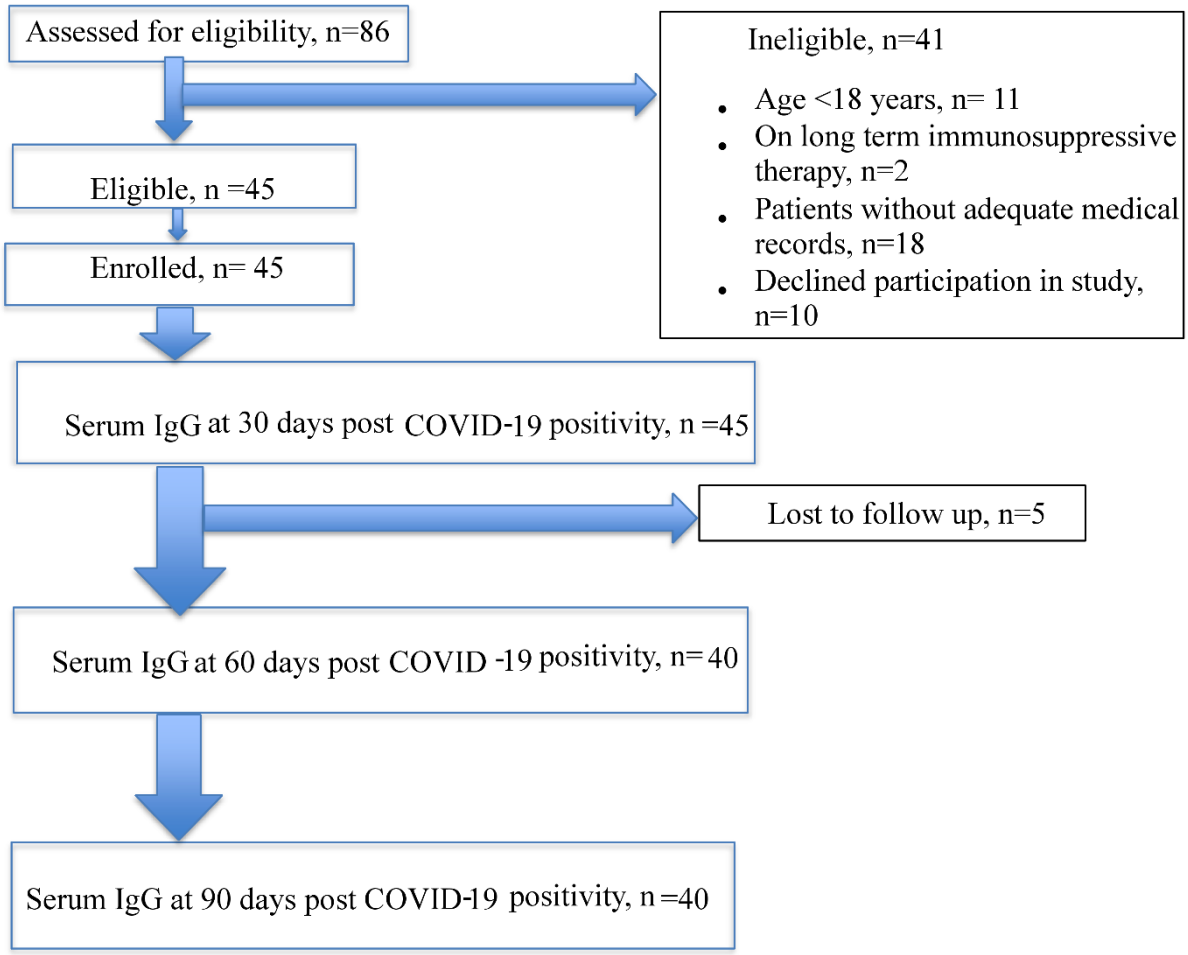

Figure 1. Flow chart of patients' enrolment.

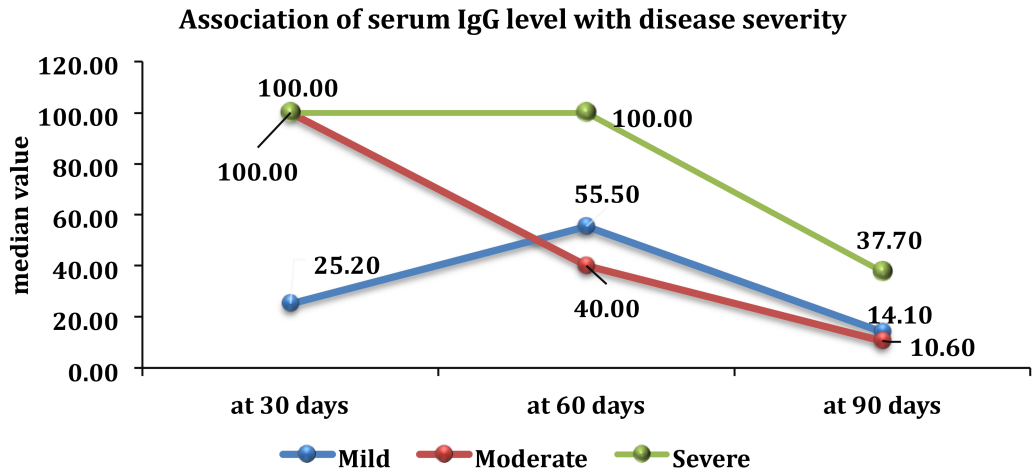

Figure 2. Association of trend of serum IgG level at different time intervals with disease severity.

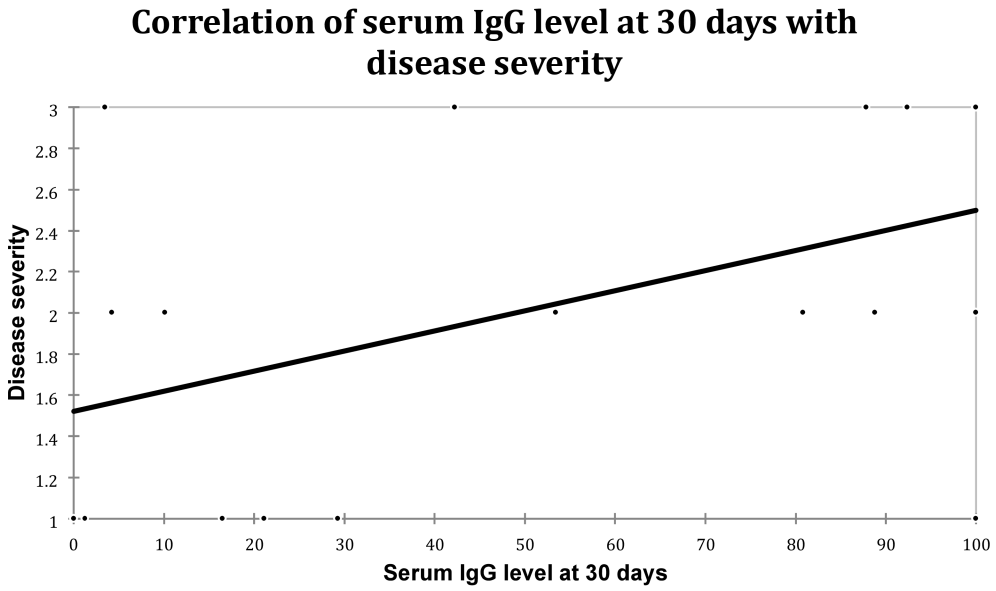

Figure 3. Correlation of serum IgG level at 30 days with disease severity.

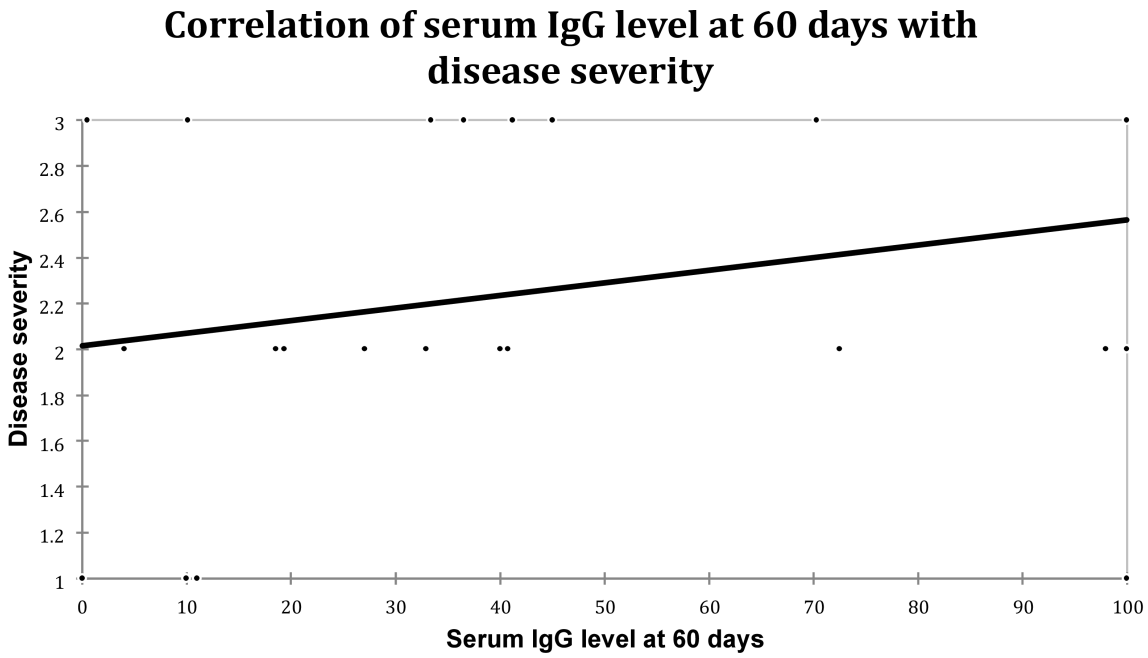

Figure 4. Correlation of serum IgG level at 60 days with disease severity.