Original Article

Clinical and Autoimmune Characteristics of Covid-19 Patients in A Tertiary Care Centre: A Cross-Sectional Study

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ABSTRACT

Introduction: Since the end of 2019, the world is witnessing the emergence of the coronavirus disease 2019 (COVID-19) outbreak and pandemic caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This disease presented with a wide array of clinical, inflammatory and possible autoimmune manifestations. Currently, a very few data is available about the involvement of autoimmunity in patients affected by coronavirus disease 2019 (COVID-19).

Aim: To find out the clinical and inflammatory status of COVID-19 patients and whether this disease (SARS-CoV-2) stimulates autoantibody production and contributes to autoimmunity activation.

Methodology: A hospital based retrospective study conducted on 60 COVID-19 patients. All patients were clinically and radiologically evaluated and screened for common inflammatory markers and auto antibodies.

Result: Patients included were 39 men (65%) and 21 women (35%). 33 patients were mild cases, 15 were moderate and 12 were severe cases with a mean age of 44.27. Fever and shortness of breath were the dominant symptoms; most patients had at least one coexisting disorder on admission; the most common characteristic on chest CT was groundglass opacity; the most common findings on laboratory measurements of inflammatory markers were elevated levels of CRP, LDH ,ferritin and altered neutrophil lymphocyte ratio; and prevalence of autoantibodies ,anti SSA/Ro antibody, anti SSB/La antibody, and antinuclear antibody was 20%, 10%, and 15%, respectively and Anti-TPO antibody was positive in 33.3% patients.

Conclusion: We conclude that autoimmune phenomena exist in COVID-19 subjects.

Key words: SARS-CoV-2, Inflammatory markers, Autoantibody, Autoimmunity

INTRODUCTION

Since the end of 2019, we have been witnessing the emergence of the coronavirus disease 2019 (COVID-19) outbreak and pandemic caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of February 2, 2021, total 104,024,884 cases have been confirmed worldwide, including 10,767,206 confirmed cases and 154,522 deaths in India[1]. Since the very beginning of the COVID-19 outbreak, the new disease has demonstrated varying degrees of severity with clinical characteristics. However, there are very few data reported on whether autoimmune phenomena exist in COVID-19 patients. Viral effects and immune-mediated mechanisms are the two pathogeneses of severe acute respiratory syndrome– associated coronavirus (SARS-CoV) infection, and autoimmune responses have been found in SARS-CoV infection[2]. One study suggested that the SARS-CoV antigen can cross-react with autoantibodies in autoimmune diseases[3]. The disease which was declared as a pandemic in early March 2020, is characterized by fever, dry cough, myalgia and or extreme fatigue, may be asymptomatic or with minimal flu-like constitutional symptoms leading to a favorable outcome in many instances. However, some of the patients encounter a severe pneumonia with sepsis leading to an acute respiratory distress syndrome (ARDS) with respiratory failure requiring mechanical ventilation, and at times multiple organ involvement leading to death[4, 5]. Though the exact etiology of autoimmune diseases still remains unknown, there are various factors which are believed to contribute to the development of an autoimmune disease in a host including the genetic predisposition, the environmental factors such as bacterial infections, viral fungal and parasitic infections and the host's immune system dysregulation. All these factors interplay was described by Shoenfeld et al., "The Mosaic of Autoimmunity". The most prominent pathogenic viruses which have been proposed in the triggering and initiation of autoimmune diseases are: Epstein-Barr-virus (EBV), Cytomegalovirus (CMV), Herpes virus-6, HTLV-1, Hepatitis A and C virus, and Rubella virus .These viruses have been implicated in the initiation of chronic inflammatory or autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, primary billiary cholangitis, multiple sclerosis, polymoysitis, uveitis, Henoch Schonlein Puprpura, Systemic Juvenile Idiopathic arthritis, systemic sclerosis, Hashimoto thyroiditis and autoimmune hepatitis [6, 7]. So, just like those viruses SARS-CoV-2 can also initiate autoantibody formation and development of autoimmune diseases. As there is no direct proven correlation between COVID-19 diseases and autoimmune diseases, in this study we present the various clinical and autoimmune profiles of the COVID-19 patients.

METHODOLOGY

It was a hospital based cross sectional retrospective study conducted between 1st December, 2020 and 31st January, 2021. Proper ethical clearance and approval was taken from institutional ethical committee. Inclusion criteria was all the cases of laboratory confirmed COVID-19 infection who are admitted in our hospital in dedicated medicine wards. Exclusion criteria were i)Any case who is pregnant or aged below 12 years or having any surgical emergency and ii) Patients known to have prior autoimmune diseases. Laboratory confirmed cases of COVID-19 infection is defined by suspected patients whose Nasopharyngeal and Oropharyngeal swabs are being tested positive for COVID-19 infection by RT-PCR or CBNAAT .After meeting the inclusion and exclusion criteria our study population turned up to be 60 adult patients (39 men and 21 women), age between 23 and 87 years. All the patients were positive for SARS-CoV-2 as the presence of infection was confirmed by real-time reverse-transcriptase-polymerase chain reaction (PCR) and CBNAAT on nasopharyngeal swab samples. All the samples analyzed in this study were collected after hospital admission and after taking proper consent. Any patient who is pregnant or aged below 12 years or having any surgical emergency and patients known to have prior autoimmune diseases were excluded from the study. After meeting inclusion and exclusion criteria and taking proper consent, detailed history taking and clinical examination was done. Patients were divided into mild (uncomplicated URTI without breathlessness or hypoxia), moderate (Respiratory rate >24/min, breathlessness and/or SpO2< 94% room air) and severe (respiratory rate >30/min, breathlessness and/or SpO2< 90% room air) based on clinical presentation as per ICMR guidelines. The following blood investigations were done: complete haemogram, LDH, CRP, Ferritin, Complement level (C3, C4) . HRCT thorax was done to find out pulmonary changes. We considered the most common autoantibodies associated with inflammation, such as ANA, anti- Ds DNA, Anti JO-1, Anti Scl-70, Anti SSA/Ro, Anti SSB/La, Rheumatoid factor, ANCA and Anti TPO. This study was approved by the ethics committee of the institution.

RESULTS

Demographics, baseline, and clinical characteristics of 60 patients with COVID-19 who were hospitalized are summarized in Table 1 and Table 2. The age of the patients considered in this study was between 23 and 87 years with a mean age of 44.27. Patients included 39 men (65%) and 21 women (35%). 33 patients were mild cases, 15 were moderate and 12 were severe cases. Seven patients (11.6%) died because of SARS-CoV-2 infection. Thirty one patients (51.7%), more specifically all the patients under 60 years of age, presented with fever at hospital admission. The most common clinical symptoms were fever (51.7%) and dyspnea (40%); none of them presented nausea or vomiting and only 8.3% had diarrhea. Only seventeen patients (28.33%) had one coexisting disorder, but none had a previous clinical history of autoimmune disease. The average time of hospitalization was 16.5 days. Whereas 10% needed assisted ventilation and 48 of 60 required oxygen therapy (80%).

COVID-19 highly affects the respiratory system and in particular the lungs. Radiologic investigations were necessary. The specific clinical features observed were: patchy shadowing (15%), interstitial abnormalities (5%) and ground glass opacities(50%). 73.4% of all moderate patients, even 42.4% of all mild patients had ground glass opacities in HRCT thorax.

Like all viral infections, the presence of COVID-19 disease leads to an inflammatory response. Inflammatory status of the 60 patients with COVID-19 was evaluated considering the common diagnostic inflammatory markers: LDH, ferritin, neutrophil lymphocyte ratio, CRP, C3, C4. The laboratory measurements are summarized in Table 4. Table 4 highlights the number of patients having a laboratory value out of the reference range. We observed that ~ 72% of the patients presented an increase in the general inflammation markers (LDH, ferritin, CRP, C3, and C4). It is seen that inflammatory markers are of higher range in moderate and severe disease than mild ones and majority of moderate and severe patients have deranged inflammatory markers.

Table 1: Demographics of study population

Sex	Patients (N=60)	Mean Age (Years)	Severity		
			Mild	Moderate	Severe
Male	39	43.47	19	11	9
Female	21	44.26	14	4	3

Table 2: Symptoms of study population

Symptoms	Total cases with symptom (N=60) (%)	Patient with Symptom		
		Mild cases (n=33) (%)	Moderate cases (n=15) (%)	Severe cases (n=12) (%)
Fever	31 (51.7)	6 (18.1)	13 (86.86)	12 (100)
Dyspnoea	24 (40)	3 (9)	11 (73.33)	10 (83.33)
Dry Cough	26 (43.3)	16 (48.48)	6 (4)	4 (33.33)
Headache	8 (13.3)	5 (15.15)	2 (13.33)	1 (8.33)
Myalgia	10 (16.7)	4 (12.12)	3 (2)	3 (25)
Anosmia	2 (3.3)	2 (6)	0	0
Diarrhea	5 (8.3)	2 (6)	3 (2)	0
Asymptomatic	15 (25)	12 (36.36)	3 (2)	0

Table 3: Radiological features (HRCT Thorax) of COVID-19 patients

Radiological Features	Total cases having radiological feature (n=60) (%)	Patients with Radiological features		
		Mild cases (n=33) (%)	Moderate cases (n=15) (%)	Severe cases (n=12) (%)
Patchy Shadows	9 (15)	0 (0)	2 (13.3)	7 (58.3)
Interstitial abnormalities	3 (5)	1 (3)	2 (13.3)	0 (0)
Ground glass opacities	30 (50)	14 (42.4)	11 (73.4)	5 (41.7)
Normal	18 (30)	18 (54.5)	0 (0)	0 (0)

Table 4: Inflammatory markers in COVID-19 patients

Inflammatory markers	Reference range	Total cases with elevated markers (n=60) (%)	Patients with elevated inflammatory markers		
			Mild cases (n=33) (%)	Moderate cases (n=15) (%)	Severe cases (n=12) (%)
CRP	0-10 mg/dl	52 (86)	28 (84.84)	13 (86.66)	11 (91.66)
C3	75-135 mg/dl	33 (55)	19 (57.6)	6 (40)	8 (66.66)
C4	15-45 mg/dl	31 (51.66)	17 (41.51)	7 (46.66)	7 (58.33)
LDH	230-380U/L	45 (75)	21 (63.63)	14 (93.33)	10 (83.33)
Ferritin	10-250ng/ml	48 (80)	24 (72.72)	13 (86.86)	11 (91.66)
Neutrophil Lymphocyte Ratio	1-3	50(83.33)	26 (78.78)	12 (80)	12 (100)

Table 5: List of autoantibodies detected in study population

Autoantibodies	Total cases with positive autoantibody (n=60) (%)	Cases with positive autoantibody		
		Mild cases (n=33) (%)	Moderate cases (n=15) (%)	Severe cases (n=12) (%)
ANA	9 (15)	3 (9)	4 (26.66)	2 (16.67)
Anti SSA/Ro	12 (20)	4 (12.12)	5 (33.33)	3 (25)
Anti SSB/La	6(10)	2 (6)	3 (20)	1 (8.33)
Anti JO-1	2 (3.3)	1 (3)	0 (0)	1 (8.33)
Anti Centromere	0 (0)	0 (0)	0 (0)	0 (0)
Anti Scl70	0 (0)	0 (0)	0 (0)	0 (0)
ANCA	1 (1.7)	1 (3)	0 (0)	0 (0)
Rheumatoid factor	0 (0)	0 (0)	0 (0)	0 (0)
Anti TPO	20 (33.3)	11 (33.3)	5 (33.3)	4 (33.3)

Autoimmunity is the system of immune responses of an organism against its own healthy cells, tissues and other body normal constituents. The inflammation can either help the organism to fight the virus, or cause a strong harmful autoimmune response. In this study the 60 patients with COVID-19 considered were analyzed for autoimmune auto antibodies to determine the involvement and correlation of autoimmunity with COVID-19 infection. Table 5 summarizes the auto antibodies analyzed in patients with COVID-19. We considered the most common auto antibodies associated with inflammation, such as ANA, anti- Ds DNA, Anti JO-1, Anti Scl-70, Anti SSA/Ro, Anti SSB/La, Rheumatoid factor, ANCA and Anti TPO. Although it is common to detect different type of antibodies also in healthy individuals, the patients who were considered did not have any previous clinical record of antibody presence. Our analysis showed that 15% of our patients were positive for ANA. The prevalence of anti SSA/Ro antibody, anti SSB/La antibody is 20% and 10% respectively. Anti- TPO antibody was positive for 33.3% of patients.

DISCUSSION

In this single-center and observational retrospective study, we have studied on the clinical and laboratory characteristics of 12 severe and 15 moderate and 33 mild cases of SARS-CoV-2 . Our main findings are as follows: fever and shortness of breath were the dominant symptoms; most patients had at least one coexisting disorder on admission; the most common characteristic on chest CT was ground glass opacity; the most common findings on laboratory measurements of inflammatory markers were elevated levels of CRP, LDH ,ferritin and altered neutrophil lymphocyte ratio; and prevalence of autoantibodies anti SSA/Ro antibody, anti SSB/La antibody, and antinuclear antibody was 20%, 10%, and 15%, respectively and Anti-TPO antibody was positive in 33.3% patients.

SARS-CoV-2 infection currently represents the worst global pandemic disease and efforts are being made worldwide to find a possible cure. Different research studies have focused on trying to identify the pathophysiology behind this disease. It has been known that SARS-CoV-2 can initiate a strong harmful immune response in some patients[8]. However, its specific mechanism is not yet completely known.

Different studies have already shown that the SARS-CoV-2 infection determines a higher production of inflammatory cytokines[9, 10]. In particular, it has been seen that this immune response can either be helpful to fight the viruses, or exacerbate the number of inflammatory chemokines leading to a process known as "cytokine storm," that could

worsen the patients' already critical conditions[11, 12]. According to this, the patients with COVID- 19 considered in this study showed increased levels of the classical inflammatory markers. Our data support the concept that high levels of inflammatory markers could be a useful diagnostic value for identifying subjects with a poor prognosis. This hypothesis has been already supported by a study performed by Coomes and Haghbayan[13, 14].

Imbalance in immune response in certain conditions can also lead to the development of autoantibodies. Up to now, no clear data is available to determine whether SARS-CoV-2 infection can activate an autoimmune response or not. One recent study has described the autoimmune characteristics of 21 patients with COVID-19. In this study, it is stated that patients with COVID-19 had a prevalence of anti-52kDa SSA/Ro, anti-60 kDa SSA/Ro, and ANA antibodies (20%, 25%, and 50%, respectively); they have concluded that the autoimmune mechanism is activated in patients with COVID-19[15]. In June 2020, another study on 29 patients was analyzed, defining the presence of autoimmunity after COVID-19 infection[16]. In accordance with these studies, our data sustained that not only inflammation, but also autoimmunity is likely triggered by the COVID-19 infection. The results obtained in this study firmly sustained that COVID-19 is associated with autoimmunity, in particular ANA, Anti SSA, Anti SSB, Anti-TPO antibodies development.

In this study, we found that ANA, Anti-SSA and Anti-SSB are positive for 26.66%, 33.33% and 20% of moderate COVID 19 patients respectively followed by severe ones. Anti TPO antibody is also positive in 33.33% of severe patients. So it is seen that moderate and severe COVID 19 patients shows higher proportion of autoantibody positivity. Although no clinical features of autoimmune disease is seen in those patients during hospital course, these patients have to be followed up in future to identify development of any autoimmune disease. As a significant number of patients shows anti-TPO antibody positive, these patients have to be followed up for development of autoimmune thyroid disorders. Data related to patients with a medium-severe clinical profile highly suggest that there might be a correlation between the response to SARS-CoV-2 and the specific individual autoimmune response. This could be the reason for the wide variability of clinical manifestations related to a single pathogen. Our results sustain the hypothesis that COVID-19 infection correlates with the autoimmunity markers. Our study might help clinicians to: (a) better understand the heterogeneity of this pathophysiolgy and (b) long term follow up of these patients for development of autoimmune diseases.

CONCLUSION

To date, however, the relationship between autoimmune phenomena and SARS-CoV-2 infection has not been reported. Herein we found that prevalence of anti SSA/Ro antibody, anti SSB/La antibody, and antinuclear antibody was 20%, 10%, and 15%, respectively and anti TPO antibody positive for 33.3% of patients. Therefore, we conclude that autoimmune phenomena exist in COVID-19 subjects. So the COVID-19 patients should be followed up long term, to identify development of any autoimmune systemic disease and optimal immunosuppressive therapy in the future.

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