ORIGINAL ARTICLE

Immunologic markers, vasculitis-associated autoantibodies, and complement levels in patients with COVID-19

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Background: The cause of coronavirus disease 2019 (COVID-19) is a virus which can lead to severe acute respiratory syndrome-CoV-2 (SARS-COV-2). There are evidences of involvement of immune system in pathogenesis of this disease. We investigated the presence of various vasculitis-associated autoantibodies and complement levels in patients with COVID-19. **Materials and Methods:** Patients with severe or critical type of COVID-19 were evaluated for symptoms, signs, and laboratory tests of vasculitis syndromes including rheumatoid factor (RF), antinuclear antibody (ANA), anti-double-stranded DNA, c and p anti-neutrophilic cytoplasmic antibody (c ANCA and *P* ANCA), and complement levels. **Results:** The study was performed in forty patients with severe or critical illness. The mean age of the participants was 48.5 ± 9.8 years. All patients had pulmonary involvement in lung computed tomography scans. Vasculitis laboratory test results included RF in two patients, ANA in three patients, and ANCA in one patient. Seventeen (42.5%) patients had hypocomplementemia in one or more complement tests. Four patients expired, of whom three had a decrease in complement level. **Conclusion:** Decrease in complement levels may predict a critical state of COVID-19 disease. Therefore, measuring its levels may be of great benefit in making earlier decisions to initiate disease-suppressing treatments including corticosteroids.

Key words: Antinuclear antibody, complement activation, COVID-19, rheumatoid factor, vasculitis

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INTRODUCTION

Coronavirus disease-2019 (COVID-19) is caused by severe acute respiratory syndrome (SARS) coronavirus and the first cases were classified as "pneumonia of unknown etiology." The disease represents a potentially fatal disease that is of great global public health concern. By January 8, 2021, the outbreak of COVID-19 has resulted in 88,615,325 confirmed cases and 1,908,948 deaths globally.[1-3] Clinical presentations and laboratory manifestations of the disease include fever, cough, pulmonary and cardiac complications, headache, lymphopenia, increased lactic dehydrogenase, coagulation disorders, increased liver and muscle enzymes, and electrolyte disturbances.[2,4-8] Severe COVID-19 can result in acute respiratory distress syndrome and multiple organ dysfunction, which are the leading causes of death.[9] The infection is associated with numerous direct and indirect cardiovascular complications including acute myocardial infarction, myocarditis, arrhythmias, and venous thromboembolism.[5] Information about kidney involvement as a pathological diagnosis is relatively incomplete and limited.[10] There are some reports of vasculitis syndromes and COVID-19.[11-13]

Due to the lack of specific antiviral drugs, current treatment of the disease is mainly supportive.[2,4] However, several therapies are used to treat this life-threatening

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disease.[14-16] Growing consensus about the pathophysiology of SARS-CoV-2 infection has led to use some antirheumatic drugs as possible treatment options in COVID-19.[17-19] Among them, glucocorticoids have unique action on immune system including blocking the inflammatory cascade from the origin, global availability, low cost, quick action using the pulsed method, and its half-life compared to the long-lasting biologic agents and monoclonal antibodies.[20]

Studying the relationship between vasculitis syndromes or related laboratory findings with COVID-19 may be interesting in several ways (1) could the similarity of symptoms between both diseases suggest a similar mechanism of injury? (2) Understanding the pathways of damage may be of great help in identifying the treatment, and (3) Can the treatments modify or ignite the pathophysiological processes of the disease? Designing and conducting this study may suggest ways to answer these questions, the safety pathways involved, and possibly the use of appropriate treatments. The current study also aimed at investigating the seropositivity of the tests used for the diagnosis of vasculitis syndromes in patients with severe and critical COVID-19.

PATIENTS AND METHODS

Study population

We conducted a cross-sectional study in April 2020 in hospitalized patients with COVID-19 admitted in a referral hospital in Sari, North of Iran. The patients were consecutively screened by a rheumatologist for possible inclusion. Due to the lack of prior research studies on this topic, the study was performed in forty eligible patients.

Selection criteria

The diagnosis of patients with COVID-19 was based on compatible clinical symptoms with positive PCR and/or lung involvement as inclusion criteria for all patients.[21]

Definition of disease severity

Based on the symptoms of the disease, it was classified into these categories: asymptomatic (positive SARS-COV-2 test and no symptom), mild (fever, cough, or change in taste or smell and no dyspnea), moderate (clinical or radiologic evidence of lower respiratory tract disease and oxygen saturation ≥94%), severe (shortness of breath, respiratory rate ≥30/min, oxygen saturation ≤94% or pulmonary infiltration more than 50% within the first 24–48 h), and critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure.[22]

Inclusion and exclusion criteria

Patients aged 18–60 years old diagnosed with either severe or critical infection of COVID-19 were enrolled. Patients with a history of malignancies, chronic liver or kidney diseases, history of rheumatic inflammatory diseases (including systemic lupus, rheumatoid arthritis, scleroderma, and vasculitis associated with anti-neutrophil cytoplasmic antibodies [ANCA] or anti-nuclear antibodies), and patients using corticosteroids, immunosuppressive, and biological drugs were excluded.

Ethics approval

The current study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (Ethic code: IR.MAZUMS.REC.1399.7427). Patients or their companions were explained about the study.

Data collection

After selecting eligible patients, their consent was obtained, and clinical and demographic information was recorded. Laboratory data including complete blood count, biochemistry, coagulation tests, urine analysis, and reports of the lung computed tomography (CT) scans were recorded. Ten milliliters of blood were drawn from patients and the sera were stored at 2–8 C.

Vasculitis syndrome tests include rheumatoid factor (RF) tests, antinuclear antibodies (ANA), antidouble-stranded DNA (anti--dsDNA), ANCA, and measurement of complement levels (C3, C4, and C50). RF was estimated by Latex method (Bionic, Iran) and ANA, anti-dsDNA antibodies, and ANCAs were performed by enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN, Germany). A value greater than the upper limit of normal was considered positive. The levels of complement were also measured by ELISA (Pars Azmoon, Iran and Diametra, China) and values less than the lower limit of normal were considered low.

Leukopenia was defined as WBC <4400 cells/μL and lymphopenia was considered if absolute count was <1000 cells/μL. Anemia was defined as Hgb < 12 g/dl in women and <13.5 in men. Thrombocytopenia was considered as plt <150,000/μL. High serum creatinine level in men and women was considered as 1.13 and 0.93 mg/dl, respectively. Alanine transferase was regarded elevated if it was >55 IU/L in males and 32 IU/L in females. Hyperbilirubinemia was defined as total bilirubin >1 mg/dl and direct bilirubin >0.4 mg/dl. Creatine phosphokinase and troponin were described high if the levels were >195 U/L in men and 170 U/L in women and >100 ng/dl, respectively.

Statistical analysis

Descriptive statistics such as mean and percentage were used to describe the clinical/laboratory findings IBM SPSS ver 20, Chicago, USA.

RESULTS

Forty patients with severe or critical COVID-19 were entered in this study. All patients had significant changes indicating COVID-19 pneumonia in their CT scans and also clinical manifestations of the disease. The mean age of patients was 48.5 ± 9.8 years old and 27 (67.5%) were males. Demographic and basic clinical data of patients are shown in Table 1. The mean respiratory rate and saturation pressure of O_2 were 23.17 ± 5.8/min and 87.15% ± 7.46%, respectively. All patients received hydroxychloroquine and lopinavir/ritonavir combination therapy. Twenty-seven patients also received interferon beta-1 (n = 27) and 17 patients was treated with additional parenteral glucocorticoid.

The results of vasculitis tests are shown in Table 2. Of the forty patients studied, 17 (42.5%) had hypocomplementemia in one or more components (low C3 in nine patients, low C4 in five, and low CH50 in 14 patients). Six of the 15 patients with severe type of the disease (40%) and 11 of the 25 critically ill patients (44%) had low complement levels (P = 0.804). There were no significant differences between the two groups with low and normal complement levels in the percentage of lung involvement or the number of lobes involved (P > 0.05). Of the patients who had a decrease in complement, 2 were RF positive and 2 were ANA positive, in one patient, both tests were positive. Among the patients expired (n = 4), three had a significant reduction in complement levels.

Table 1: Demographic and basic clinical data of patients with coronavirus disease 2019 involvement

Variables category	*n* (%)
Demographic data	
Age, years (mean±SD)	48.50±9.84
Male (sex)	27 (67.5)
Urban residency	27 (67.5)
Underlying diseases	
Diabetes mellitus	11 (27.5)
Hypertension	5 (12.5)
Ischemic heart disease	6 (15)
Hyperlipidemia	1 (2.5)
Asthma or COPD	3 (7.5)
Pregnancy	1 (2.5)
COVID-19 disease manifestation	
Disease duration, days (mean±SD)	14.07±5.94
Severe/critical illness	15 (37.5)/25 (62.5)
Fever or chills	22 (55)
Myalgia	20 (50)
Headache	5 (12.5)
Loss of consciousness	6 (15)
Dyspnea	28 (70)
Cough	22 (55)
Nausea and/or vomiting	8 (20)
Diarrhea	2 (5)
Abdominal pain	2 (5)
Chest pain	3 (7.5)
Pericardial effusion	1 (2.5)

SD=Standard deviation; COPD=Chronic obstructive pulmonary disease; COVID-19=Coronavirus disease 2019

DISCUSSION

In the present study, we investigated immunologic and vasculitis tests and complement levels in patients with COVID-19 infection. Three patients had a positive ANA result, of whom two had hypocomplementemia and were also treated with interferon. All three patients were fully recovered and discharged from the hospital, despite severe pulmonary involvement initially. One patient was positive both for C and *P* ANCA, with pancytopenia but without any other ANCA-associated vasculitis manifestations. Seventeen patients showed a decrease in one or more components of the complement and of the 4 patients expired, three had a decrease in complement. The vasculitides are defined by the presence of inflammatory leukocytes in the vessel walls with damage to mural structures. They are diagnosed based on patterns of organ injury, the size of the vessels affected, histopathological and imaging features. ANA, ANCA, and serum complement levels are laboratory tests that help the physician in understanding the pathophysiology and making accurate diagnosis.[23] In this study, we did not find any classic vasculitis syndrome which might be due to small sample size, but the variety of symptoms in patients with COVID-19 can be similar and mimic the syndromes of vasculitis.

The complement system is a major component of innate immunity that acts as the effector arm of humoral immune system. The complement system is primarily perceived as a host defense system, but it may be a potentially more harmful side of innate immune pathway as an inflammatory mediator.[24] Severe sepsis is an acute condition that leads to the activation of the complement and coagulation cascades and may play central roles in multiple organ failure and severe complications.[25] Intrapulmonary activation of complement can cause acute lung injury that is complement and PMN dependent, resulting in a cytokine storm.[26] Evidence on the role of complement system as a major host mediator of SARS-CoV-induced disease suggests that complement activation regulates a systemic pro-inflammatory response to SARS-CoV infection. Inhibition of complement signaling in a mouse model is reported to be an effective treatment option following SARS-COV infection.[27] In adult patients, hypocomplementemia is an early diagnostic marker of parvovirus B19 infection.[28] Activation of the complement pathways has been reported in chronic hepatitis C virus (HCV) infection and there is an association between HCV pathogenesis and abnormal complement profiles.[29] Creating an immune complex and activating the complement system are the basic mechanisms in

Table 2: Laboratory and imaging findings in patients with coronavirus disease 2019 infection

Variables category	*n* (%)
Laboratory findings	
CBC	
Lymphopenia	19 (47.5)
Anemia	27 (67.5)
Thrombocytopenia	8 (20)
Acute phase reactants (mean±SD)	
ESR (mm/h)	52.42±22.92
CRP (mg/dL)	53.34±52.19
Blood chemistries	
Elevated ALT	6 (15)
Elevated CPK	15 (37.5)
Elevated troponin	2 (5)
Raised creatinine	8 (20)
Hyperbilirubinemia (total, direct)	19 (47.5), 18 (45)
Coagulation tests study	
Prolonged PT	11 (27.5)
Prolonged PTT	2 (5)
Urine analysis	
Abnormal UA	7 (17.5)
Immunologic and vasculitis lab tests	
Positive RF	2 (5)
Positive ANA	3 (7.5)
Positive anti dsDNA	None
Low complement levels	17 (42.5)
Positive C ANCA	1 (2.5)
Positive *P* ANCA	
Lung CT scan findings	
Number of involved lobes (mean±SD)	4.08±0.87
Percentage of lung involvement (mean±SD)	56.62±14.77
Peripheral opacity	8 (20)
Grand glass opacity	34 (85)
Consolidation	16 (40)

SD=Standard deviation; CBC=Complete blood count; ESR=Erythrocyte sedimentation rate; CRP=C-reactive protein; ALT=Alanine transferase; CPK=Creatine phosphokinase; PT=Prothrombin time; PTT=Partial thromboplastin time; UA=Urine analysis; RF=Rheumatoid factor; ANA=Anti-nuclear antibody; dsDNA=Double stranded DNA; ANCA=Anti-neutrophilic cytoplasmic antibody; CT=Computed tomography

pathophysiology of many autoimmune and vasculitis syndromes. In the present study, 40% of patients with severe disease and 44% of critically ill patients had low complement levels that could have been caused by activating and consuming complement as a result of the virus invading or reacting to it. Comparing these cases with mild cases of the disease may reveal more findings related to hypocomplementemia.

A major concern in the treatment of critically ill patients is drug-induced side effects. There is a broad spectrum of interferon-related complications including glomerulonephritis, systemic lupus erythematosus (SLE)-like syndrome, and thrombotic microangiopathy that may be associated with hypocomplementemia.[30,31] Drug-induced lupus erythematosus is a syndrome with clinical and serological features similar to SLE that is temporally related to continuous drug exposure and resolves after discontinuation of the drug. More than 90 drugs, including interferons, are involved in causing the disease.[31] In this study, 15 of the 27 patients receiving interferon showed a deficiency of complement. Low levels of complement in these patients may be due to disease or interferon use.[32] Therefore, in addition to proper and appropriate use of the drug, care should be taken in selecting patients and monitoring its side effects. Since the onset of the COVID pandemic, several treatment regimens have been evaluated to treat this potentially dangerous disease.[33] Knowing the possible pathways in the pathogenesis of the disease may help to select appropriate treatments.

We encountered limitations in the study. This study was performed as a pilot on patients with COVID-19, and according to our knowledge, evaluation of vasculitis tests and complement levels in these patients have not yet been published. Therefore, there was no any previous study to estimate the number of samples based on them. Furthermore, patients were examined after receiving certain medications such as interferon or steroids, so subsequent studies can be performed before treatment interventions or on different days of treatment.

CONCLUSION

Based on current findings, a decrease in May predict progression to a critical COVID-19. Therefore, measuring its levels may be helpful in making earlier decisions to initiate disease suppressing treatment including corticosteroids.

This was a small pilot study and for a definite conclusion, further studies with larger sample size and inclusion of milder forms of the disease as control group are needed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Tu H, Tu S, Gao S, Shao A, Sheng J. The epidemiological and clinical features of COVID-19 and lessons from this global infectious public health event. J Infect 2020;81.
2. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, evaluation, and treatment of coronavirus (COVID-19). Saint Petersburg, Florida: Stat Pearls Publishing; 2020.
3. Available from: https://www.worldometers.info/coronavirus/. [Last accessed on 2021 Jan 08].
4. Goh KJ, Choong MC, Cheong EH, Kalimuddin S, Duu Wen S, Phua GC, *et al*. Rapid progression to acute respiratory distress syndrome: Review of current understanding of critical illness from COVID-19 infection. Ann Acad Med Singap 2020;49:108-18.
5. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, *et al*. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. J Am Coll Cardiol 2020;75:2352-71.
6. Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, *et al*. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J Med Virol 2020;92:797-806.
7. Zhang X, Cai H, Hu J, Lian J, Gu J, Zhang S, *et al*. Epidemiological, clinical characteristics of cases of SARS-CoV-2 infection with abnormal imaging findings. Int J Infect Dis 2020;94:81-7.
8. Omrani-Nava V, Maleki I, Ahmadi A, Moosazadeh M, Hedayatzadeh-Omran A, Roozbeh F, *et al*. Evaluation of hepatitis enzymes changes and association with prognosis in COVID-19 patients. Hepat Mon 2020;20:e103179.
9. Li HC, Ma J, Zhang H, Cheng Y, Wang X, Hu ZW, *et al*. Thoughts and practice on the treatment of severe and critical new coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2020;43:396-400.
10. Wang HJ, Du SH, Yue X, Chen CX. Review and prospect of pathological features of corona virus disease. Fa Yi Xue Za Zhi 2020;36:16-20.
11. Oda R, Inagaki T, Ishikane M, Hotta M, Shimomura A, Sato M, *et al*. Case of adult large vessel vasculitis after SARS-CoV-2 infection. Ann Rheum Dis 2020. doi: 10.1136/annrheumdis-2020-218440.
12. Vaschetto R, Cena T, Sainaghi PP, Meneghetti G, Bazzano S, Vecchio D, *et al*. Cerebral nervous system vasculitis in a Covid-19 patient with pneumonia. J Clin Neurosci 2020;79:71-3.
13. Hanafi R, Roger PA, Perin B, Kuchcinski G, Deleval N, Dallery F, *et al*. COVID-19 neurologic complication with CNS vasculitis-like pattern. AJNR Am J Neuroradiol 2020;41:1384-7.
14. Bhatnagar T, Murhekar MV, Soneja M, Gupta N, Giri S, Wig N, *et al*. Lopinavir/ritonavir combination therapy amongst symptomatic coronavirus disease 2019 patients in India: Protocol for restricted public health emergency use. Indian J Med Res 2020;151:184-9.
15. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, *et al*. COVID-19, an emerging coronavirus infection: Advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. Hum Vaccin Immunother 2020;16:1232-8.
16. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, *et al*. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020;56:105949.
17. Favalli EG, Ingegnoli F, De Lucia O, Cincinelli G, Cimaz R, Caporali R. COVID-19 infection and rheumatoid arthritis: Faraway, so close! Autoimmun Rev 2020;19:102523.
18. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 2020;14:72-3.
19. Tanaka T. IL-6 blockade therapy for inflammatory diseases: current perspectives and future directions. Nihon Rinsho Meneki Gakkai Kaishi 2015;38:433-42.
20. Owlia S, Owlia MB. Glucocorticoids in COVID19; A friend not foe. J Res Med Sci 2020;25:45.
21. Liu KC, Xu P, Lv WF, Qiu XH, Yao JL, Gu JF, *et al*. CT manifestations of coronavirus disease-2019: A retrospective analysis of 73 cases by disease severity. Eur J Radiol 2020;126:108941.
22. Gandhi RT. The multidimensional challenge of treating COVID-19: Remdesivir is a foot in the door. Clin Infect Dis 2020;ciaa1132. [Online ahead of print].
23. Morita TC, Trés GF, Criado RF, Sotto MN, Criado PR. Update on vasculitis: An overview and dermatological clues for clinical and histopathological diagnosis-part I. An Bras Dermatol 2020;95:355-71.
24. Ricklin D, Reis ES, Lambris JD. Complement in disease: A defence system turning offensive. Nat Rev Nephrol 2016;12:383-401.
25. Holers VM. Complement and its receptors: New insights into human disease. Annu Rev Immunol 2014;32:433-59.
26. Bosmann M, Ward PA. Role of C3, C5 and anaphylatoxin receptors in acute lung injury and in sepsis. Adv Exp Med Biol 2012;946:147-59.
27. Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, *et al*. Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis. mBio 2018;9:e01753-18.
28. Hashizume H, Kageyama R. Hypocomplementemia is a diagnostic clue for parvovirus B19 infection in adults. J Dermatol 2017;44:e27.
29. El-Shamy A, Branch AD, Schiano TD, Gorevic PD. The complement system and c1q in chronic hepatitis c virus infection and mixed cryoglobulinemia. Front Immunol 2018;9:1001.
30. Gianassi I, Allinovi M, Caroti L, Cirami LC. Broad spectrum of interferon-related nephropathies-glomerulonephritis, systemic lupus erythematosus-like syndrome and thrombotic microangiopathy: A case report and review of literature. World J Nephrol 2019;8:109-17.
31. Araújo-Fernández S, Ahijón-Lana M, Isenberg DA. Drug-induced lupus: Including anti-tumour necrosis factor and interferon induced. Lupus 2014;23:545-53.
32. Vedove CD, Del Giglio M, Schena D, Girolomoni G. Drug-induced lupus erythematosus. Arch Dermatol Res 2009;301:99-105.
33. Shirani K, Sheikhbahaei E, Torkpour Z, Ghadiri Nejad M, Kamyab Moghadas B, Ghasemi M, *et al*. A narrative review of COVID-19: The new pandemic disease. Iran J Med Sci 2020;45:233-49.