Original Article

Changes in the Level of Antiphospholipid Antibodies (Anticardiolipin and Anti-β2-Glycoprotein-I) and Thromboembolic Indices in COVID-19 Patients during 3 Weeks

Somayeh Haghighipour, Mansour Salesi[4], Fereshteh Keikhaei[1], Mohammad J. Tarrahi[2], Zahra Khani[3], Hassan Salehi[1]

Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [1]Department of Infectious Diseases, Medical School, Isfahan, Iran, [2]Department of Epidemiology and Biostatistics, School of Health,Isfahan, Iran, [3]PHD of Internal Medicine, Medical School, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Immune Deficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Introduction: COVID-19 is a respiratory disease caused by infection with severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Thrombotic complications appear to be of particular importance in patients with COVID-19. This study aimed to investigate Changes in the level of Antiphospholipid antibodies (Anticardiolipin and Anti-β2-glycoprotein-I) and thromboembolic indices in COVID-19 patients during 3 weeks. **Methods:** This cross-sectional study was performed on adults with Covid-19 hospitalized at Al-Zahra Hospital in Isfahan. The case group includes the patients admitted to the internal ward or ICU who despite receiving prophylactic or anticoagulant doses suffer from thrombotic complications and the control group includes COVID-19 patients without thromboembolic events. The sample size of 120 people was considered. Anticardiolipin and anti-β2-glycoprotein-I antibodies, coagulation profiles including Fibrinogen, PTT, PT Troponin, ESR, CRP, and D-dimer were examined. After collection, the data were entered into spss24 software and analyzed. **Results:** The results showed that there was no statistically significant difference in the changes of anticardiolipin and anti-beta-2 glycoprotein in IgM and IgG as well as in the changes of ESR, CRP, PTT, PT, and fibrinogen in the two groups ($P > 0.05$). **Conclusions:** Our study showed that there was no statistically significant relationship between anti-phospholipid antibodies (anticardiolipin and anti-beta-2 glycoprotein) and thromboembolic events. Therefore anticardiolipin and anti-beta-2 glycoprotein is probably the puzzles causing thrombosis in COVID-19 patients, and other inflammatory responses should be examined among the cases.

Keywords: *Anticardiolipin, anti-β2-glycoprotein-I, COVID-19, thrombosis*

Introduction

COVID-19 is a respiratory disease caused by infection with severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). This disease first appeared in China in late 2019 and led to an unprecedented crisis and the death of more than one million people.[1,2] Typical symptoms include fever, cough, myalgia, and fatigue.[3,4] The severity of COVID-19 disease varies in patients ranging from mild respiratory disease to acute respiratory distress syndrome, shock, and limb failure.[5]

Thrombotic complications appear to be of particular importance in patients with COVID-19. Early reports indicate coagulopathy among the patients, manifesting thrombocytopenia, increased D-dimer levels, increased prothrombin time, and intravascular coagulation.[6-8] COVID-19-associated coagulopathy comes with a poor prognosis, including hospitalization in the Intensive Care Unit (ICU), the need to use a ventilator, and increased mortality.[8] Since the onset of the disease, several studies have reported the risk of thromboembolism in hospitalized patients. A Chinese study reported recurrent episodes of venous thromboembolism in the case of severe infection with COVID-19.[9] In another report, venous thromboembolism occurred in 27% of Dutch patients admitted to the ICU.[10] Reports from Italy, France, and Switzerland confirm recurrent venous thromboembolism complications. According to the studies, the risk of thromboembolic events in patients admitted to the ICU, obese patients, and patients with frequent clots at the catheter site, dialysis filter, and ECMO oxygenator appear to be significantly higher. Pulmonary embolism has recently been identified as the most common thrombotic event occurring despite

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Address for correspondence:
Dr. Hassan Salehi, Department of Infectious Diseases, Medical school, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: h-salehi@med.mui.ac.ir

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thromboprophylaxis.[11,12] Therefore, a better understanding of COVID-19-related thromboembolic events and the mechanisms involved in optimizing diagnostic strategies, designing, and conducting clinical trials will help prevent these events. Although the pathological impacts of SARS-CoV2 infection on the coagulation system are unknown, the release of various proinflammatory cytokines, damage to vascular endothelial cells, and platelet activation may affect the clotting process.

Increased levels of antiphospholipid antibodies in patients with COVID-19 are likely to cause thrombotic events. Antiphospholipid antibodies such as anticardiolipin and anti-beta 2-glycoprotein cause thrombocytopenia and increase the partial thromboplastin time.[13] According to recent studies, levels of these antibodies have increased in patients with this viral infection. For example, Zhang *et al*. have observed the development of antiphospholipid antibodies in three COVID-19 patients with cerebral infarction who were admitted to the ICU.[14] Harzallah *et al*. studied 56 patients with COVID-19 and observed the presence of antiphospholipid antibodies in more than 50% of them. The researchers suggested that the presence of these antibodies might be used as an indicator for the early initiation of anticoagulant therapy.[15] However, no studies have so far compared the levels of antiphospholipid antibodies among COVID-19 patients with/without thromboembolic events. Due to the magnitude of the Covid 19 epidemic worldwide and the rapid changes in information about the disease, it requires a high degree of awareness about the disease, the results of this study can be helpful in preventing thromboembolic events in these patients. As a result, the present study is to evaluate the changes in the level of Antiphospholipid antibodies (Anticardiolipin and Anti-β2-glycoprotein-I) and thromboembolic indices in COVID-19 patients with thromboembolic events during 3 weeks. In addition, the study is to find out whether the increase in antiphospholipid antibodies turns into antiphospholipid syndrome or not.

Materials and Methods

This is a cross-sectional study on adults (over 18 years old) with COVID-19 (definitive cases based on PCR tests or clinical and radiological evidence) admitted to Al-Zahra Hospital in Isfahan. The case group includes the patients admitted to the internal ward or ICU who despite receiving prophylactic and therapeutic anticoagulation doses suffer from thrombotic complications and arterial ischemia, Deep Vein Thrombosis (DVT), Pulmonary Thromboembolism (PTE), Cerebral Venous Sinus Thrombosis (CVST), Myocardial Infarction (MI) and arterial thrombosis of the limbs (compression ultrasonography for suspected DVT; helical computed tomography [CT] scan for suspected PE). The control group includes COVID-19 patients without thromboembolic events. The cases with a history of previously known thromboembolic diseases, previous ischemia, ischemic and hemorrhagic stroke, rheumatic diseases (antiphospholipid syndrome (presence of anticardiolipin and anti-beta-2 glycoproteins is higher than 99% at the beginning of the study), lupus), atrial fibrillation arrhythmia and those with a history of surgery during the last month and immobility for more than three days were excluded from the study. Antiphospholipid results were also excluded from the study three weeks after being infected with COVID-19. According to a similar study, the sample size was considered to be 60 for each group (total of 120 subjects).[15] The case and control groups were matched by blood oxygen saturation of less than 90%, and lung involvement of more than 50% (according to CT-scan). All patients benefited from the current standard of care for COVID-19 and received thromboprophylaxis according to current guidelines (Heparin 5000 units/subcutaneously/every eight hours). Remdesivir and Methylprednisolone were used to treat Covid 19 in both case and control groups.

Patients were tested for the presence of aPL antibodies, i.e. ACL, aβ2GPI. The ELISA QUANTALite™ (Inova Diagnostics, San Diego, CA) and the Elia β-2 Glycoprotein-1 (Phadia, Uppsala, Sweden) detection kits were used to detect IgG/IgM ACL and IgG/IgM aβ2GPI antibodies, respectively, with a limit of positivity fixed at 15 units/mL (99th percentile of a control population)[16] at the beginning of the study and three weeks later (If the patient died between the beginning of the study and the third week, the patient was excluded from the study).

By considering the ethical issues in this study, necessary permits were obtained from the Research Council of the University and Al-Zahra Hospital. The personal information remained confidential and the data were analyzed as a whole.

The collected data was entered into spss24. It was described by descriptive statistics (for quantitative characteristics; e.g. mean, standard deviation, or median (quadrature amplitude), and percentage for numeric characteristics). Then, it was analyzed by analytical statistics (Mann-Whitney test). The statistical significance level was considered to be 0.05.

Results

A total of 120 COVID-19 patients with and without thromboembolic events admitted to Al-Zahra Hospital in Isfahan were enrolled in July and August 2021. Among them, 60 patients had thromboembolic events but 60 did not. The mean age of participants was 58.64 ± 17.8 years (at least 18 years and at most 98 years). Moreover, 66.7% of the (80 subjects) were male. Among the 60 patients with thromboembolic events, 2 CVA cases (3.3%), 5 DVT cases (8.3%), 15 MI cases (25%), and 38 PTE cases (63.4%) were observed. Data were analyzed

to compare the mean levels of IgM/IgG anticardiolipin anti–β2-glycoprotein antibodies among those with/without thromboembolic events [Table 1]. The frequency of antiphospholipid antibodies markers at the beginning of the study and three weeks later displays in Table 2.

The results of the study showed that the mean level of anticardiolipin IgM in the two groups of patients with thrombotic events in the third week compared to the beginning of the study (3.02 ± 2.5 vs 2.4 ± 1.5) and in patients without thrombotic events (2.8 ± 2.1 vs 2.06 ± 1.7) increased. The mean level of anticardiolipin IgG in two groups of patients with thrombotic events in the third week compared to the beginning of the study (3.86 ± 2.5 vs 2.4 ± 1.7) and in patients without thrombotic events (3.8 ± 2.9 vs 2.1 ± 1.8) increased. While the mean of other markers such as Antibeta-2 glycoprotein IgM, Anti–β2-glycoprotein IgG, ESR, CRP, D-dimer, Troponin, PTT, PT, SWR, and Fibrinogen decreased in both groups.

The mean level of IgM/IgG anticardiolipin and anti–β2-glycoprotein was not higher than 12 for none of the cases. The mean changes at the beginning of the study and three weeks later were used to examine the mean levels of these markers. The results showed that there was no statistically significant difference in the changes of anticardiolipin and anti–β2-glycoprotein in IgM and IgG as well as in the changes of ESR, CRP, PTT, PT, and fibrinogen in the two groups ($P > 0.05$). However, changes in D-dimer and troponin levels in the two groups were significantly different ($P < 0.05$) [Table 3].

Discussion

Results of the current study on COVID-19 patients revealed that there was no statistically significant relationship between anticardiolipin and anti–β2-glycoprotein of IgM/IgG and thromboembolic events.

In line with our results, lupus anticoagulant was positive in 21 patients (35%), while IgM/IgG anti–β2-glycoprotein was positive only in one patient and anticardiolipin was positive in no patient in the study by Najim *et al*. They indicated that the presence of antiphospholipid antibodies was not significantly associated with the development of thromboembolic events in severe COVID-19 patients.[17] Borghi *et al*. also confirmed that there was no positive association between positive antiphospholipid antibodies and thromboembolic events in COVID-19 patients admitted to the ICU.[18]

Frapard *et al*. observed no significant association between thrombosis and positive antiphospholipid antibodies in patients with severe COVID-19.[19] In a cohort study, Galeano *et al*. noticed a low prevalence of antiphospholipid antibodies among COVID-19 patients with venous thromboembolism. Hence, antiphospholipid antibodies

Table 1: Frequency distribution of demographic variables of participants in the study

Varible	Patients with thromboembolic events	Patients without thromboembolic events	Total
Age	61.07±18.2	56.22±17.3	58.64±17.8
Sex			
Male	42 (70%)	38 (63.3%)	80 (66.7%)
female	18 (30%)	22 (36.7%)	40 (33.3%)
Underlying disease			
Blood pressure	1 (1.7%)	0	1 (0.8%)
Diabetes	4 (6.7%)	0	4 (3.3%)
Myocardial infarction	0	1 (1.7%)	1 (0.8%)

Table 2: Evaluation of antiphospholipid antibodies in Covid 19 patients with and without thromboembolic events

Variable	Patients with thromboembolic events		*P*	Patients without thromboembolic events		*P*
	Primary	3 weeks later		Primary	3 weeks later	
Anticardiolipin IgM	2.4±1.5	3.02±2.5	0.43	2.06±1.7	2.8±2.1	0.49
Anticardiolipin IgG	2.4±1.7	3.86±2.5	0.46	2.1±1.8	3.8±2.9	0.302
Antibeta-2 glycoprotein IgM	2.37±1.8	2.4±1.5	0.34	2.6±1.8	2.4±1.9	0.046
Antibeta-2 glycoprotein IgG	2.56±1.9	3.2±1.9	0.68	2.4±2.2	3.03±2.1	0.73
ESR	56.15±26.9	5.9±10.5	0.006	49.13±22.6	4.1±4.8	0.57
CRP	94.83±24.9	6.6±8.4	0.612	87.52±31.3	4.3±4.4	0.38
D-dimer	3079.03±2233.7	23.9±38.3	0.91	1256.47±1005.6	28.1±35.1	0.67
Troponin	235.94±396.4	5.8±10.9	0.654	10.29±21.5	4.29±8.1	0.723
PTT	36.35±13.2	28.67±2.7	0.68	34.33±9.4	29.75±3.6	0.74
PT	13.36±6.8	11.67±1.5	0.35	11.77±1.6	11.5±0.7	0.18
SWR	1.61±1.6	1.16±0.2	0.93	1.3±0.1	1.13±0.1	0.88
Fibrinogen	236.7±129.8	52.5±24.5	0.69	232.3±108.6	51.6±29.7	0.1

Table 3: Evaluation of median changes in antiphospholipid antibody levels in Covid 19 patients with and without thromboembolic events

Variable	Patients with thromboembolic events	Patients without thromboembolic events	P
Anticardiolipin IgM	0.15	0	0.214
Anticardiolipin IgG	1.15	1.15	0.809
Antibeta-2 glycoprotein IgM	0.15	0	0.241
Antibeta-2 glycoprotein IgG	0.45	0.35	0/906
ESR	-51.5	-45.5	0.337
CRP	-93.5	-89	0.6
D-dimer	-2966	-852	*P*<0.001
Troponin	-12.5	-3.3	*P*<0.001
PTT	-3	-2	0.402
PT	0	0	0.878
Fibrinogen	-173.5	-171	0.996

were probably not involved in the pathogenicity of venous thromboembolism in patients with COVID-19 pneumonia.[20]

Contrary to the results of the present study, Alexandre Le Joncour *et al*. showed a meaningful association of anticardiolipin and anti–β2-glycoprotein with the occurrence of thrombotic events.[21]

The results of a review study on the possible role of antiphospholipid antibodies in hypercoagulability and hypofibrinolytic conditions among severe COVID-19 patients showed that they are at risk of arterial and intravenous thromboembolism despite heparin treatment. The role of antiphospholipid antibodies in thrombosis of COVID-19 19 patients is not yet clear. Due to the poor condition of COVID-19 patients admitted to the ICU, it is likely that a large number of patients are not screened by CUS or CTPA, and many thromboembolic indices may be underestimated, making microvascular thrombosis difficult to assess. It is often impossible to distinguish microvascular thrombosis from other causes of organ dysfunction without an autopsy. The results of this study indicated that the data on antiphospholipid antibodies for COVID-19 patients and their association with thromboembolic events are limited and inconsistent. Further studies with longer follow-ups are needed to determine whether antiphospholipid antibodies are a simple and transient secondary cause of COVID-19 or a cause of thrombosis.[22]

According to the results of this study, a statistically significant difference in the changes in D-dimer and troponin levels was observed in the two groups (with and without thromboembolic events). Leonard-Lorant *et al*. found out that COVID-19 patients with pulmonary embolism experience higher D-dimer rates than patients without pulmonary embolism. High D-dimer may occur because of high blood coagulation activity in CODID-19 patients due to secondary systemic inflammatory response syndrome or as a positive consequence of coronavirus.[23]

To examine the second question of this study (whether the increase in antiphospholipid antibodies turns into antiphospholipid syndrome in COVID-19 patients with thromboembolic events compared to patients without it or not), it should be noted that since infections can transiently make the titer of antiphospholipid antibodies positive or high, infection with COVID-19 caused a slight increase in antiphospholipid antibodies in the third week of the study.

The difference between the results of the present study and other studies may be due to the presence of some confounders; e.g. the length of stay in ICU which reflects the severity of the illness, and the history of taking antidepressants at the beginning of the illness. The difference in the results might also originate from defining a positive test, determining the effective cut-off point, using different thresholds in determining the positive results for the two antibodies, and various measurement techniques in different laboratories. As observed in the results, considering the cut-off point 12, there were no positive cases of anticardiolipin and anti–β2-glycoprotein in the patients of the present study. Homogenization of the treatment and control groups in terms of oxygen saturation and lung involvement is one of the strengths of this study.

One of the limitations of this study is the small sample size, it is suggested that future research be done on more sample sizes in a multi-center and cohort study with a longer follow-up period.

Conclusions

Our cross-sectional study showed that there was no statistically significant relationship between anti-phospholipid antibodies (anticardiolipin and anti–β2-glycoprotein) and thromboembolic events. Therefore anticardiolipin and anti–β2-glycoprotein are probably the puzzles causing thrombosis in COVID-19 patients, and other inflammatory responses should be examined among the cases. Future research is suggested to be conducted on a larger sample size and in a multi-center manner as cohort studies with a longer

follow-up period. In this way, patients and the factors affecting them will be closely followed from admission to the end of the study.

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Conflicts of interest

There are no conflicts of interest.

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