Original Article

Evaluation of the Association between Antiphospholipid Antibodies and ICU Admission Outcome in Critically Ill COVID-19 Patients in Iranian Population

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Abstract

Background: The role of anti-phospholipid antibodies (aPLs) in the prognosis of COVID-19 patients is controversial. In order to prove the role of this factor, the necessary measures such as early initiation of anticoagulants should be started even in the early stages of the disease and in outpatients or the use of other drugs in addition to anticoagulants. We decided to investigate the role of these antibodies in ICU admission outcomes in critically ill COVID-19 patients. **Methods:** The case-control study was carried out in Isfahan, Iran, from March to September 2021. One hundred nine patients in the case group were selected, including patients admitted to the ICU with a COVID-19 diagnosis. The 140 patients in the control group were selected from hospitalized and outpatients with COVID-19 with PCR + and pulmonary involvement, similar to the case group without the need for ICU hospitalization. The anti B2GP1 (IgM, IgG) and anti-cardiolipin (IgM, IgG)) were compared in two groups. **Results:** The frequency percentage of patients in the abnormal group of anti-phospholipid antibodies was about 10% in total. No statistically significant difference in these aPLs in continued measures was observed between the two groups of patients admitted to the ICU and those outside the ICU. Also, in the logistics regression analysis, no significant association was observed. **Conclusions:** Therefore, the cause of coagulation in patients admitted to the ICU is not related to these aPLs. This means that aPLs could not be a good predictor of patient admission to the ICU.

Keywords: *Antiphospholipid antibodies, COVID-19, ICU admission*

Introduction

COVID-19 is an infectious illness that can develop as a result of an infection with the new coronavirus causing severe acute respiratory syndrome (SARS-CoV-2). Some individuals experience a far more severe and systemic disease that results in severe lung damage, organ failure, and coagulopathy.[1] In the clinical observations of patients with COVID-19, some patients have a sudden course of respiratory failure and the need for ICU and intubation, while in patients with the same condition and similar lung involvement on CT Scan, the course of the disease is normal, and the patient can be cured without the need for an ICU by conventional methods.[2]

Coagulopathy is a complication of COVID-19 disease that causes organ failure due to micro-embolism in the vascular bed of the patient's organs, including the lungs, heart, liver, brain, and kidneys. Despite anticoagulant treatment, the desired result may not be achieved.[3] Among the causative agents of coagulopathy are anti-phospholipid antibodies against which COVID-19 can act as a trigger. Virus infection may be a probable trigger for APS by increasing the creation of APL antibodies, i.e., through molecular mimicry.[4]

COVID-19-associated coagulopathy (CAC) is of particular interest because it may represent a novel kind of coagulopathy. Patients in intensive care units (ICUs) have a high prevalence of thrombotic complications, which appear mostly as pulmonary embolisms.[5] Only the formation of a general thrombotic stage is suspected, as clinical evidence shows that the existence of APL antibodies alone seldom leads to thrombotic problems.[6]

The COVID-19 coagulopathy was initially described as disseminated intravascular coagulation. Abnormal coagulation

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outcomes, particularly substantially raised D-dimer and FDP, were shown to be prevalent in fatalities caused by novel coronavirus pneumonia.[7]

Most patients with COVID-19 have normal levels of coagulated factors, fibrinogen, and platelets. There are more and more reports of venous thromboembolism in patients with this condition.[8]

The development of coagulopathy, a disease linked with poor outcomes, is one of the poor prognostic markers in critically ill COVID-19 patients.[9] A study showed on day four, 71.4 percent of the patients who died and 0.6 percent of those who survived had disseminated intravascular coagulation (DIC).[10]

The exact mechanism of COVID-19-induced coagulopathy is still unknown. In a case series in China during the early stages of the pandemic, positive aPLs were found, including anti-cardiolipin IgA and anti-2-glycoprotein IgA and IgG. Nonetheless, the incidence of PLs in the literature varies greatly.[11]

Due to the controversy surrounding the role of APL antibodies, we decided to measure the role of these antibodies in two comparative groups (patients with severe thromboembolic complications and respiratory failure requiring ICU and similar patients without ICU and intubation). In this study, we investigate the role of anti-phospholipid antibodies in the prognosis of COVID-19 patients. In order to prove the role of this factor, the necessary measures such as early initiation of anticoagulants should be started even in the early stages of the disease and in outpatients, or the use of other drugs in addition to anticoagulants, including immunomodulators, etc., should be recommended in hospitalized patients before the patients' general condition worsens.

Methods

The case-control research was carried out at the Al-Zahra Hospital and private clinics of supervisors in Isfahan, Iran, from March to September 2021. A case group was selected, including patients admitted to the ICU with a COVID-19 diagnosis (PCR +, HRCT severe, and moderate lung involvement), and no significant underlying disease or use of anti-coagulopathy drugs were chosen and tested for anti-phospholipid antibodies. Two hundred forty-nine samples were included in the study, of which 109 were included in the case group, and 140 were included in the control group.

The control group was selected from hospitalized and outpatients with COVID-19 with PCR + and pulmonary involvement similar to the case group without the need for ICU hospitalization and without the underlying disease affecting coagulopathy with matching conditions, similar pulmonary involvement rate in HRCT returns.

Patients who have other diseases that affect the development of anti-phospholipid syndromes, such as autoimmune diseases (SLE, RA, etc.), or take drugs that affect it, such as Cotri Moxazole, are excluded from the study.

Blood serum samples were taken from patients in both groups to measure anti-phospholipid antibodies (anti B2GP1 (IgM, IgG) and anti-cardiolipin (IgM, IgG)) and were compared in groups that did not require ICU and intubation (as a control group) and samples from the ICU and required intubation (as a case group). Anti-phospholipid screening was carried out according to the International Society on Thrombosis and Haemostasis's defined standards. For all patients in the present condition, di-Dimer, CRP, is also checked for comparison with anti-phospholipid antibodies.

The ISTH criteria for anti-cardiolipin IgG/IgM and anti-2-glycoprotein IgG/IgM were followed. The fluoro enzyme immunoassay (FEIA) on the Phadia 250 platform was used for the tests. Based on the manufacturer's instructions, anti-cardiolipin IgG/IgM was judged positive at a threshold value of >12 GPL or MPL, whilst anti-2-glycoprotein IgG/IgM was deemed positive at a cutoff value of >12 U/mL.[12,13]

Statistical analysis

The statistical analysis will be divided into two sections: univariate and multivariable. The mean and standard deviation, or median and interquartile range, were presented for quantitative variables according to the potential distribution of the measured variables. For qualitative data, frequency and frequency percentage are also supplied. In univariate analysis proportional to the probability distribution of variables, an independent t-test is used to compare the mean of variables in the case and control groups if the normality hypothesis is established, and if the normality hypothesis is not established, the non-parametric Mann-Whitney test is used. The Chi-square test is used to look into the association between a categorical variable and an ICU admission outcome. A logistic regression model was employed in the multivariable analysis section to evaluate the association between included variables and predictive power with the occurrence of ICU admission. Tests were deemed statistically significant at 0.05. All statistical analyses were conducted using the SPSS version 25.0 software program.

Results

In our study, COVID-19 resulted in the hospitalization of 249 critically ill patients. With the youngest being 18 and the oldest being 98, their average age was 59.11 ± 16.52 years. Patients admitted to the ICU (n = 109) had an average age of 56.15 ± 15.07 years, whereas those not admitted to the ICU (n = 140) had an average age of 69.18 ± 17.44 years. The difference between these groups was statistically significant (P = 0.007, independent t-test). In addition, whereas 45 percent of non-hospitalized ICU patients were female and 55 percent were male, ICU hospitalized

patients were 34.9 percent female and 65.1 percent male. The difference between these groups was statistically significant (P = 0.096 Chi-square test). Among those with comorbidities, the highest number of hospitalized patients had diabetes, followed by hypertension. There was a statistically significant difference between comorbidities and hospitalization in the ICU, and more people with hypertension were admitted to the ICU (P = 0.001, Chi-square test). The frequency percentage of patients in the abnormal group of anti-phospholipid antibodies was about 10% in total. The frequencies according to the type of antibodies were as follows: IgM aCL 4.8% and IgG aCL 2% and IgM anti-β2GPI 2.4% and IgG anti-β2GPI was 0.8%.

The anti-cardiolipin IgG/IgM, anti-2-glycoprotein IgG/IgM, CRP, and Di-dimer of patients who were admitted to ICU and those who were not admitted to ICU were analyzed using the non-parametric Mann-Whitney test because the One-Sample Kolmogorov-Smirnov test results showed that the distributions of these parameters were not normal [Table 1]. Also, we used the Mann-Whitney test for comparison of Di-dimer and CRP between normal and abnormal groups of aPLs. Only Di-dimer in the abnormal group of anti-β2 GPI (IgM) was significantly higher than in the normal group (P-value = 0.028).

Then, using multivariable analysis and logistic regression with consideration of anti-phospholipid antibodies (in binary mode using clinical cutoffs) and adjusting for gender and age and CRP and Di-dimer, the results showed only Di-Dimer had a predictive role in the hospitalization of Covid-19 patients in the ICU (P-value <0.001, OR = 17.41) but aPLs were not significant.

Discussion

Although the entire scope of COVID-19 is still being investigated, growing data shows that coagulopathy affects the majority of critically ill individuals. Anti-phospholipid antibodies are thought to be one of the processes that contribute to a proinflammatory and hypercoagulable condition.[14]

According to our findings, aPLs were found in 10% of the research participants. In most studies, in severe COVID-19 patients, the prevalence of lupus anticoagulant was high, and the prevalence of aPLs was low, as in the meta-analysis study, they estimated pooled prevalence of aCL (IgM or IgG) 13.9% and anti-β2 GPI (IgM or IgG) 6.7%.[15]

In our study, no statistically significant difference in aPLs in continued measures was observed between the two groups of patients admitted to the ICU and those outside the ICU. In the logistics regression analysis with aPLs, considering the effects of CRP and Di-dimer, only Di-dimer was significant. This means that aPLs could not be a good predictor of patient admission to the ICU.

Few studies have been performed on the association of aPLs with the outcome of ICU admission. The results of our study are in line with other studies. In Wahono *et al.*'s[16] study, anti-phospholipid antibodies were found in 5 of 50 patients (10.0%), although there was no association between the presence of anti-phospholipid antibodies and ICU admission.

Also, in the meta-analysis conducted by Taha and Samavati, they discovered that critically ill COVID-19 patients showed considerably greater aCL (IgM or IgG) and anti-β2 GPI (IgM or IgG) prevalence than non-critically ill patients. There was no association between the presence of aPL and clinical outcomes like thrombosis, invasive ventilation, or death. This meta-analysis was done with cross-sectional and retrospective studies.[15]

Xiao and colleagues studied 66 critically ill patients admitted to the ICU and 13 patients who were not critically ill. Findings suggest that aPLs might be involved in the hypercoagulable condition in COVID-19. Anti-phospholipid antibodies appear 35–39 days following the onset of the disease. Patients who had multiple aPLs had a significantly higher risk of cerebral infarction than those who did not have any. One of the reasons for the difference in results with our study could be the difference in the days of disease onset. Also, the hospital admission time was recorded as an average of eight days. The sample size in our study was

Table 1: Comparison of anti-phospholipid antibodies parameters at the ICU hospitalization outcome in Covid-19 patients

anti-phospholipid antibodies		ICU hospitalization outcome		*P*
		No	Yes	
Anticardiolipin IgM	Mean±SD	4.10±5.52	2.94±2.48	0.827
	Median (Interquartile Range)	2.24 (3.00)	2.10 (1.90)	
Anticardiolipin IgG	Mean±SD	4.35±8.10	3.02±2.36	0.471
	Median (Interquartile Range)	2.50 (3.10)	2.10 (2.20)	
anti-beta2-glycoprotein IgM	Mean±SD	3.65±4.66	3.26±6.46	0.924
	Median (Interquartile Range)	2.28 (2.80)	2.30 (2.60)	
anti-2-glycoprotein IgG	Mean±SD	3.27±3.64	2.69±2.03	0.815
	Median (Interquartile Range)	2.20 (3.33)	1.80 (2.00)	

greater than in this study. Since we did not consider the simultaneous existence of several antibodies, in the case of a single antibody, the results were consistent with our study, and no significant correlation was observed.[17]

The advantage of our study was that the sample size was larger than other research performed. This can make the results of statistical tests more reliable.

One of the limitations of this study, and most other studies conducted in this field, is the type of study. In this case-control study, we have moved from the outcome to the exposure; that is, we have measured between the control group and the case group, and therefore, the percentage of people with aPLs abnormal in each subgroup is very low. Perhaps if there were more, we could see the statistical significance in relation to other subgroups. It was better to use a longitudinal prospective study to measure these antibodies in patients and then follow up with the desired clinical consequences, including hospitalization in the ICU. As in Vollmer *et al.*'s[18] cohort study, they found a high correlation was detected between thrombosis and positivity of anti-cardiolipin IgM (41 percent), which was also confirmed at a follow-up of 3–6 months. In another cohort study done by Hamadé *et al.*,[19] they reported aPL Antibodies were shown to be substantially associated with ICU admission. Although the number of samples in this study was only 41.

Therefore, the cause of coagulation in patients admitted to the ICU is not related to aPLs. Only the di-dimer factor was significant in this study. When compared to non-admitted patients, SARS-CoV-2 patients have significantly abnormal coagulation function; monitoring D-dimer may be useful in detecting more likely patients who require hospitalization in the ICU early.

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Conflicts of interest

There are no conflicts of interest.

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