Clinical analysis of relationship between clot formation in COVID-19 patients and presence of antiphospholipid- Antibodies by case control study In Holly Karbala City

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Abstract---Objective. One of the hallmarks seen in severely sick individuals with coronavirus illness is coagulopathy (COVID-19). Antiphospholipid antibodies (aPLs) play a role in coagulopathy, although their significance in COVID-19 is unknown. The purpose of this study was to identify the prevalence and features of aPLs in COVID-19 patients. Method :80 patients were admite to the hospital .40 of them were positive inRT- PCR test for COVID-19 infection and the other 40 were healthy control. Both groups were tested for the incidence of Anti-phospholipid-Igm Ab. Result: all patient showed higher levels of antiphospholipids antibodies (0.965±2.07) then in control group (0.023±0.0005) with p-value (0.004). Conclusion. Antiphospholipid antibodies were frequently appeared in COVID-19-infected patients. COVID-19 infection might trigger the development of a condition of autoimmunity resamble the antiphospholipid syndrome (APS), forming what called a "COVID-19–induced APS-like syndrome". tasting reports appears medium to high levels of aPLs that would disappear within a few weeks in infected patients. The identification of aPLs would gave appropriate clew for COVID-19 infection.

Keywords---COVID-19, aPLs, autoimmunity.
Introduction

A new pandemic disease was appeared at present time called COVID-19 or Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that started in Wuhan, Hubei, China. Coronaviruses are enveloped, spherical in shape, positive-sense, single-stranded, RNA viruses within the family Coronaviridae, named because of the presence of spike proteins that gave the ultra-structural “crown-like” (corona) appearance on the virion surface (1). The virus entered to human through lung by using angiotensin-converting enzyme 2 (ACE2) as receptor in order to cause illness (2). An important consequence of COVID-19 infection was causing thrombosis in many cases especially deep venous thrombosis (DVT) and pulmonary embolism (PE) (3).

Antiphospholipid (aPL) antibodies are a heterogeneous family of autoantibodies that are primarily directed against plasma proteins complexes with anionic phospholipids that are found on damaged/activated cellular membranes, including endothelial cells, trophoblasts, platelets, and monocytes. The formation of autoantibodies against many antigenic targets, interfering with many of the biochemical and cellular functions of phospholipids, has led to various pathological clinical manifestations in patients with high levels of these autoantibodies (4). The aim of this research is to use the presence of noticeable levels of aPLs as predictor for severity of infection

Method

40 COVID-19 patients were attended to Al-hayat unit of alhussein medical city – Iraq, and 40 healthy control. The patients were positively diagnosed by RT-PCR. All patients were undergoes multiple investigations including lab tests, physical exam, radiological examination, ultrasound, RT-PCR. The patients where already received antiviral drugs along with anti-fever medication with no previous anticoagulant adminstration. This study was approved by the Ethics Committee of AL-Hussein Medical City – Iraq. The laboratory diagnosis was done by the following: CBC by automated hematology analyzer system of Sysmex Japan, D-dimer and ferritin by MONARCH 300 chemistry analyzer -UK, aPL by Biotek ELISA system

Statistical analysis

Data were showed as means ± standard deviation (SD), T test, Chi secure test. test were used to analyze the differences between the two groups (case and control). P-value < .05 was defined as statistically significant. Statistical analysis was Performed by using SPSS software
**Result**

Both male and female groups were effected by COVID-19 infection with all ages. In terms of biomarkers and biochemical indicators, there was a substantial difference between patients suffered from COVID-19 infection and control group.

Table (1) comparison of anti-phospholipid antibody level according to demographic characteristics of patients and patient with COVID-19 infection and healthy controls

<table>
<thead>
<tr>
<th>variable groups</th>
<th>group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients</td>
<td>Patients</td>
<td>40</td>
<td>0.965</td>
<td>2.07</td>
<td>2.937</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>controls</td>
<td>40</td>
<td>0.023</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age class</td>
<td>&lt;=65 y patients</td>
<td>18</td>
<td>0.616</td>
<td>1.443</td>
<td>-2.958</td>
<td>0.032*</td>
</tr>
<tr>
<td></td>
<td>&gt; 65 y patient</td>
<td>22</td>
<td>1.250</td>
<td>2.478</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gender</td>
<td>Male patients</td>
<td>24</td>
<td>0.568</td>
<td>1.308</td>
<td>-1.992</td>
<td>0.049*</td>
</tr>
<tr>
<td></td>
<td>Female patients</td>
<td>16</td>
<td>1.559</td>
<td>2.823</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>controls</td>
<td>40</td>
<td>0.023</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*α at≥0.05

Figure (1) graphical comparison of aPLs mean level of COVID-19 patients and controls
Table (2) correlation coefficient of antiphospholipid-Ab and parameters among COVID-19 infection

<table>
<thead>
<tr>
<th>parameters</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPL&amp;d-dimer</td>
<td>0.459**</td>
<td>0.003</td>
</tr>
<tr>
<td>aPL &amp;ferritin</td>
<td>0.190</td>
<td>0.241</td>
</tr>
<tr>
<td>aPL &amp;CRP</td>
<td>0.494**</td>
<td>0.001</td>
</tr>
<tr>
<td>aPL &amp;WBC</td>
<td>0.087</td>
<td>0.595</td>
</tr>
<tr>
<td>aPL &amp;platelets</td>
<td>0.008</td>
<td>0.962</td>
</tr>
<tr>
<td>aPL &amp;factor7</td>
<td>0.222</td>
<td>0.169</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
study was showed non-significant correlation between aPL and biomarkers except for d-dimer and CRP

**Discussion**

As shown in table (1) and figure (1) the antiphospholipid Ab level was higher in patients group (0.965±2.07) then in control group (0.023±0.0005). In this side Xiao M et al., (2020) discovered that aPLs were present in a significant fraction of critically sick COVID-19 patients. Although it is unknown whether aPLs contribute to the hypercoagulable state in COVID-19, the data imply that aPLs might be involved in this process. Antiphospholipid antibodies were frequent in COVID-19-infected critically sick individuals. Repeated testing revealed aPL titers ranging from mild to high. Antiphospholipid antibodies were thought to be one of the processes that contribute to a proinflammatory and hypercoagulable condition. (5).

As well as, Zhang, Y et al.,(2020) proved that In COVID-19 individuals with positive aPLs, secondary antiphospholipid syndrome (APS) should be investigated. Dynamic monitoring of aPLs levels may be effective in determining aPLs persistence and confirming the diagnosis of APS. (6). As shown in table (1) and figure (2) the antiphospholipid Ab level was lower in patients >65 y (0.616±1.443) then in <=65 y patients (1.250±2.478). Jean-Charles Piette et al.,(1998) hypothesized that the prevalence of aCL in normal persons would rise with age. aPL, on the other hand, were usually seen in a variety of conditions that frequently occurred in the elderly, including as long-term administration of various medicines, rheumatoid factor, monoclonal gammopathy of unknown etiology, progressive renal or hepatic dysfunction, polymyalgia rheumatica/temporal arteritis, myeloproliferative diseases, lymphomas, and solid cancer. (7)

As shown in table (1) and figure (3) the antiphospholipid Ab level was lower in male patients (0.568±1.308) then in female patients (1.559±2.823). As de Carvalho JF.(2011) showed that Females had a greater prevalence of pulmonary thromboembolism and IgM anticardiolipin positive than males. So, findings indicated that there were gender differences in PAPS (primary antiphospholipid antibody syndrome), suggestion a connection to changes in sex hormones. Especially in estrogen that seems to be a key factor in such gender disparity (8) De Carvalho JF. (2011) also found that females appeared to produce more antibodies in response to main and secondary antigen stimulation. These findings implied that sex hormones had a function in immune system regulation in general (8).

According to table (2) the findings showed a week direct association (r=0.459*) existed between aPL (U/mg) and d-dimer(mg/ml) that was statistically significant at (p-value =0.003). John G. Hanly (2003) studied that of thrombosis risk associated with the presence of antiphospholipid antibodies in blood especially thorough populations with systemic lupus erythematosus, at which the result showed 12%–30% have anticardiolipin antibodies while 15%–34% have lupus anticoagulant (9).
According to table (2) the findings showed a week direct association (r=0.190) existed between aPL (U/mg) and ferritin (mg/ml) that was statistically significant at (p-value = 0.241). Agmon-Levin N et al., (2013) found that hyperferritinemia directly correlated with the presence of APS. Hyperferritinemia was also directly linked with the presence of anti-CMV antibodies in blood of patients with APS. These associations lead to a pathogenic role of ferritin in the pathogenesis of APS. (10). According to table (2) the findings showed a week direct association (r=0.494*) existed between aPL (U/mg) and CRP (mg/ml) that was statistically significant at (p-value=0.001).

De Groot PG et al., (2015) said that Inflammation was not the most noticeable feature of APS. However, clinical and experimental evidence suggested that inflammatory processes were implicated in both the genesis and pathophysiology of the disease. The major antigen of the syndrome had been found was β2-GPI which played an important role in participation of the innate immune response especially the clearance of unwanted objects from blood circulation (11).

According to table (2) the findings showed a week direct association (r=0.008) existed between aPL (U/mg) and platelets (*10^9/ml) that was statistically significant at (p-value=0.962). De Jong A et al., (2000) found that The mechanism of pathogenesis of APS was poorly understood, Nevertheless, as a result of the interplay between antcardiolipin antibodies (aCL), beta-2 glycoprotein-I (beta(2)GP-I) (the aCL cofactor) and blood platelets (12), But Hollerbach A et al., (2019) discovered that Antiphospholipid antibodies (aPL) had been reported to be an activated factor for platelets. This was demonstrated as the most pathogenic properties of aPL, but ability to activate platelets is common to all aPL or depended on antigen specificity (13).

According to table (2) the findings showed a week direct association (r=0.222) existed between aPL (U/mg) and factor VII (pg/ml) that was statistically significant at (p-value=0.169). Boles et al., (2010) proposed that aPL stimulated the expression of TF within the blood vessels and in blood cells, thereby leading to increased thrombosis. Tissue Factor served as the primary initiator of in vivo coagulation. The TF/FVIIa complex in turn served as activator for FIX and FX and leads to thrombin and fibrin generation. Notably FVIIa is a weak serine protease on its own, but upon binding TF, its catalytic activity is increased 2 × 10^7fold (14).

**Conclusion**

This study showed that the serum level of aPL is correlated with d-dimer and CRP. There is a significant difference could be seen in aPL level between patient group and control group between two gender groups and between age groups. This study fairly showed that COVID-10 infection would cause elevated levels of serum aPL which may help in early identification of COVID-related complications especially thrombosis formation and may facilitate supportive medical care for positive patient outcomes.
Acknowledgments

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References


