

Research article

Is there any relationship between antiphospholipid antibody and COVID-19?Rajlaxmi Sarangi¹, Jyotirmayee Bahinipati¹, Amrut Kumar Mohapatra², Andrew Abel Lamare³, Mona Pathak⁴, Srikrushna Mahapatra¹, Roma Rattan³¹Department of Biochemistry, ²Department of Pulmonary Medicine, ⁴Biostatistics, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, 751 024, Odisha, India³Department of Biochemistry, SCB Medical College, Cuttack, Odisha, India

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Corresponding author: **Jyotirmayee Bahinipati**. Email: jyotirmayee.bahinipati@kims.ac.in**ABSTRACT**

Introduction and Aim: Secondary antiphospholipid syndrome (APLS) and rise in antiphospholipid antibody (APLA) has been linked to the development of Disseminated Intravascular Coagulation in COVID-19. But still controversies exist regarding the increase in APLA in COVID -19. Hence, the present study aimed to estimate the levels of APLA in COVID patients and its relationship with the severity of the disease.

Materials and Methods: 40 RT PCR positive COVID-19 cases and normal control were recruited for the study. Biochemical and hematological findings were compared in both the groups. COVID-19 patients were further subdivided into survivor Vs non-survivor and based upon the CT findings of thorax they were grouped with vs without CT findings.

Results: CRP, PCT, ESR were found to be significantly increased in COVID-19 patients. IgM & IgG APLA antibody (3.02±1.32 U/ml & 3.54±1.85 U/ml) were found to be within normal range in COVID cases. APLA did not show any correlation with serum ferritin, CRP, PCT, N/L ratio and MPV in COVID-19. No statistical difference was seen in the levels of APLA when compared in non-survivors vs survivors. Even APLA was within normal range in the patients who presented with pulmonary embolism (PE), venous thromboembolism (VTE) and succumbed to the disease. Serum ferritin and neutrophil to lymphocyte (N/L) ratio was found to be significantly higher in non-survivors.

Conclusion: Hence, in our study APLA was within normal range and was not related to the severity of the disease.

Keywords: Pulmonary embolism; venous thromboembolism; anti-cardiolipin antibody; β_2 glycoprotein; angiotensin converting enzyme -2; ARDS; DIC; CAD.

INTRODUCTION

Novel Beta (β)corona virus officially classified as Severe Acute Respiratory Syndrome Corona Virus-2 (SARS COV-2) is the etiological agent of this ongoing pandemic. According to Global data reported by WHO there have been 20,162,474 number of confirmed cases of COVID-19 including 737,417 deaths by 12 August 2020 (1). The pathophysiology of COVID-19 is still indeterminate. Emerging evidence shows that severe COVID-19 manifests coagulation abnormalities. This coagulopathy leads to pulmonary microvascular thrombosis, broncho- alveolar fibrin deposition, the hallmark of respiratory distress syndrome (RDS) and thromboembolic complications (2).

In an Italian study in 388 patients 36% showed venous thromboembolism, 7.7% were found with pulmonary embolism, coronary artery disease (CAD) was found in 3.6% and overt Disseminated Intravascular Coagulation (DIC) was found in 2.2%. According to them 50% of these thromboembolic events was seen in 24 hours of hospital admission (3). Tang *et al.*, in their retrospective study found 71.4% of non-survivors and 0.6% of survivors of COVID-19 met the criteria of DIC (4). In another study they showed 69%

of patients have coagulation index abnormalities which were more frequent in severe disease. Significantly higher D-dimer and Fibrin degradation product (FDP) level, longer Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) level were found elevated in severe group compared to the mild group (5).

Anticoagulant treatment given to COVID-19 cases reduces the mortality rate. In a study, it was found that mortality after 28 days of treatment was positively associated to the rise in D-dimer, PT, and age but negatively to platelet count (6). Though coagulation abnormality in COVID-19 mimics other systemic coagulopathy associated with severe infection such as DIC or thrombotic micro-angiopathy but it has distinct features. Combination of profound thrombocytopenia, prolonged PT and moderate increase in D-dimer is suggestive of DIC. COVID-19 usually presents with higher median D-Dimer concentration, mildly prolonged PT, and mild thrombocytopenia. Other laboratory abnormalities suggestive for coagulopathy in COVID-19 are increase LDH, strikingly high ferritin concentration implicative of thrombotic microangiopathy. These evidences together suggest that there is combination of low grade DIC and

localized pulmonary thrombotic microangiopathy leading to coagulopathy the cause behind organ dysfunction in severely affected patients (4).

Anti-Phospholipid antibody syndrome (APS) is an autoimmune disorder characterized by multiple episodes of venous and arterial thrombosis, recurrent fetal losses with moderate thrombocytopenia and positive antiphospholipid antibody directed against cardiolipin, β -2 glycoprotein I (β 2GPI), phosphatidyl serine, inositol, ethanolamine, and phosphatidic acid. Secondary antiphospholipid syndrome has also been reported due to viral infections Hep-C, Herpes Zoster, even in certain bacterial, fungal and parasitic infections. APLA is also found to be positive in 13% patient with stroke, 11% with Myocardial Infarction (MI), 9.5% with Deep Vein Thrombosis (DVT). Even it has been observed that 4.5-5.5% of healthy individuals are positive for antibody against cardiolipin and β 2 GPI (7).

Viral syndromes may lead to endothelial dysfunction triggering inflammatory response, activating coagulation cascade, thereby increasing the risk of thrombus formation. Hence, thromboembolism may not be only restricted to critically ill patients due to sepsis associated coagulopathy conditions like DIC or microvascular thrombosis, but it can also be due to transient rise in APLA related to viral syndrome (8).

Few studies have hypothesized that there is association between APLA and thrombotic events in critically ill patients with COVID-19, which may be attributable to a transient rise in APLA related to viral syndrome. In the study of 3 patients done by Zhang and his colleague they reported coagulopathy, thrombocytopenia, and presence of anti-cardiolipin-IgA, anti- β 2 glycoprotein IgA and IgG antibodies (9).

HarZallah *et al.*, reported the result of APLA in 56 patients and found 25 were positive for lupus anticoagulant, 5 with either IgG or IgM anticardiolipin or β 2 GPI antibody. They even suggested the role of anticoagulation in COVID -19 patients (10). Contradicting studies have also been done implicating that APLA might not be involved in the pathogenesis of venous thromboembolism in patients with severe COVID-19 pneumonia (11). In another study they found only 8.6% of patients were slightly positive for APLA but the values were $< 3X$ of the cut off value (12).

In the present scenario it is still controversial if APLA in COVID-19 is an epiphenomenon or if it is involved in hemostatic abnormalities and thrombus formation leading to COVID severity.

Hence, the aim of our study is to analyze APLA (IgM, IgG Auto antibodies against cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and β 2 GPI in COVID-19 cases and find out any role of APLA in increase thrombus formation.

MATERIALS AND METHODS

After obtaining approval from the Institutional Ethics committee (IEC: KIIT/KIMS/IEC/303/2020) and registering in CTRI bearing number (CTRI/2020/06/026118), a hospital-based case control study was conducted in the Department of Biochemistry in collaboration with COVID hospital of KIMS, Odisha, India over a period of one month (20/06/2020 to 21/07/2020). Written and informed consent were obtained from the participants prior to this study and patient confidentiality was strictly maintained. The study recruited 40 RT PCR positive COVID-19 cases (both symptomatic and asymptomatic) admitted to COVID hospital consecutively .40 age matched non-smoker, non-alcoholic participants who came for routine health checkup without having any history of respiratory illness or infection/inflammation were enrolled as control.

RT PCR positive COVID -19 cases having history of smoking, systemic autoimmune diseases, recent surgery and on estrogen or chronic steroid therapy or using anticoagulant medications or vitamin K antagonists were excluded from the present study. Data regarding age, gender, history of exposure, clinical symptoms at presentation and duration of hospital stay were collected by direct communication with the participants. COVID-19 patients were further divided into two groups survivor and non-survivor (those who succumbed to the disease).

Following overnight fast 10ml of venous blood samples were collected for estimation of different biochemical and hematological parameters. 2 ml of blood was stored at -20°C for APLA estimation. 1 ml of blood was kept in fluoride vial for glucose estimation. 2 ml of sample was kept in a red top vial for estimation of serum urea, creatinine, Liver Function Tests (LFT), sodium (Na^+), potassium (K^+), C- Reactive Protein (CRP), Pro-calcitonin (PCT) and ferritin. Remainder of the sample was collected in EDTA and Citrate vials for estimation of CBC and Erythrocyte Sedimentation Rate (ESR) respectively. Serum was separated by centrifugation at 3000 RPM for 10 mins. Fasting Blood Sugar (FBS), Serum Urea, Creatinine, LFT, Na^+ , K^+ , CRP, PCT, ferritin was measured in an autoanalyzer OCD-5600 using commercially available kit according to the manufacturers protocol. All the hematological parameters were analyzed in Complete Blood Count (CBC) analyzer Transasia-XN 1000. ESR was done by Vesmatic Easy from Transasia. APLA (Both IgM and IgG) were measured in automated ELISA EVOLIS Twin Plus from BIORAD after calibrating and running standard. APLA level IgM < 10 U/ml, IgG <10 U/ml was considered normal. Values IgM ≥ 10 U/ml, IgG ≥ 10 U/ml was elevated.

CT thorax was done in SOMATOM sensation 64 eco by Siemens. In accordance with the CT finding

COVID cases were divided into two groups, those who presented with the findings Vs those having no changes in CT thorax.

Statistical analysis

All the continuous variables were reported with Mean \pm standard deviation or Median (Inter Quartile

range). Normality of the distribution was tested by Sapiro-Wilk test. Non-parametric test, i.e., Wilcoxon Rank Sum test was used to compare various parameters in two groups. p-values are interpreted at 5% level of significance. Stata 15.1, Stata corp, Texas, USA was used for analysis.

Table 1: Comparison of different parameters between control and COVID-19 cases.

Parameters	Cases (Mean \pm SD)	Control (Mean \pm SD)	P value
Age (Yrs)	42.42 \pm 13.82	50.2 \pm 15.35	0.22
Temp ($^{\circ}$ F)	98.41 \pm 0.79	97.9 \pm 0.32	0.09
Pulse Rate(/min)	84.22 \pm 5.36	80.8 \pm 5.76	0.17
RR(/min)	21.97 \pm 2.22	22.6 \pm 1.67	0.58
SBP (mm of Hg)	115.32 \pm 20.65	120 \pm 4.89	0.10
DBP (mm of Hg)	74.6 \pm 6.56	76 \pm 5.47	0.57
IgM (U/mL)	3.02 \pm 1.32	2.70 \pm 0.53	0.81
IgG (U/mL)	3.54 \pm 1.85	3.56 \pm 0.91	0.48
FBS (mg/dl)	138.38 \pm 113.22	88.6 \pm 5.59	0.07
S. Urea (mg/dl)	36.55 \pm 45.10	22.2 \pm 8.43	0.44
S. Creat (mg/dl)	1.61 \pm 5.43	0.64 \pm 0.12	0.42
T.Bil (mg/dl)	0.75 \pm 0.48	0.82 \pm 0.13	0.24
D.Bil (mg/dl)	0.18 \pm 0.17	0.23 \pm 0.08	0.14
SGOT (U/L)	57.42 \pm 71.04	24.8 \pm 1.48	0.02*
SGPT (U/L)	41.87 \pm 30.36	25.4 \pm 6.69	0.42
GGT(U/L)	61.22 \pm 83.33	19.8 \pm 1.92	0.08
ALP(U/L)	93.4 \pm 56.16	86.4 \pm 24.74	0.81
T.Prot (g/dl)	6.97 \pm 0.91	7.74 \pm 0.36	0.03*
Alb (g/dl)	4.04 \pm 0.74	4.66 \pm 0.05	0.02*
S. Na ⁺ (meq/L)	133.8 \pm 6.04	135.4 \pm 4.61	0.63
S. K ⁺ (meq/L)	4.25 \pm 0.71	4.46 \pm 0.28	0.16
CRP (mg/L)	34.47 \pm 74.10	0.22 \pm 0.08	< 0.001*
PCT (ng/ml)	5.19 \pm 18.18	0.018 \pm 0.008	< 0.001*
Ferritin (ng/ml)	259.21 \pm 410.49	33 \pm 9.05	0.006*
WBC (10 ³ u/L)	9.16 \pm 5.28	8.54 \pm 2.47	0.69
N (%)	64.57 \pm 15.17	60.4 \pm 15.19	0.67
L (%)	24.95 \pm 11.44	25 \pm 9.19	0.88
M (%)	7.57 \pm 3.78	5 \pm 1.22	0.05
E (%)	2.82 \pm 3.23	9.6 \pm 12.89	0.12
B (%)	0.07 \pm 0.26	0	0.53
N/L	4.36 \pm 4.71	2.70 \pm 1.18	0.97
RBC (10 ⁶ u/L)	4.53 \pm 0.79	4.90 \pm 1.07	0.18
Hb (g/dl)	12.53 \pm 2.25	13.28 \pm 3.0	0.31
PCV (%)	37.48 \pm 6.56	41.24 \pm 10.15	0.15
MCV (fl/ μ m ³)	83.06 \pm 8.25	83.58 \pm 4.59	0.74
MCH (pg)	27.80 \pm 3.29	27.08 \pm 1.37	0.32
MCHC (g/dl)	33.41 \pm 1.28	32.41 \pm 1.48	0.19
Platelet Count (10 ³ /uL)	216.17 \pm 84.37	244.8 \pm 76.24	0.35
MPV (fl)	9.86 \pm 1.37	11.30 \pm 1.99	0.11
ESR (mm)	33.5 \pm 16.24	19 \pm 2.64	0.003*

* P- value < 0.05 considered as significant.

RR: Respiratory Rate, SBP: Systolic Blood pressure, DBP: Diastolic Blood Pressure, FBS: Fasting Blood Sugar, SGOT: Serum Glutamate Oxaloacetate Transaminase, SGPT: Serum Glutamate Pyruvate Transaminase, GGT: Gamma Glutamyl Transpeptidase, ALP: Alkaline Phosphatase, T.Prot : Total Protein, Alb: Albumin, S. Na⁺ : Serum Sodium, S. K⁺ : Serum Potassium, CRP: C Reactive Protein, PCT: Procalcitonin, WBC : White Blood Cell Count,

N: Neutrophil , L : Lymphocyte , M: Monocyte, E: Eosinophil, B: Basophil, N/L : Neutrophil/ Lymphocyte Ratio RBC: Red Blood Cell Count, Hb : Hemoglobin, PCV : Packed Cell volume, MCV : Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin , MCHC: Mean Corpuscular Hemoglobin Concentration, MPV: Mean Platelet Volume, ESR: Erythrocyte Sedimentation rate.

Table 2: Correlation of IgM and IgG APLA with different parameters in COVID cases

Parameters	IgM		IgG	
	r value	p value	r value	p value
Ferritin(ng/ml)	0.18	0.26	0.10	0.51
CRP (mg/L)	0.19	0.23	0.04*	0.77
PCT (ng/ml)	0.13	0.42	0.09	0.57
N/L	0.22	0.16	0.03	0.83
MPV (fl)	0.10	0.52	0.08	0.61

CRP: C Reactive Protein, PCT: Procalcitonin, N/L: Neutrophil/ Lymphocyte Ratio, MPV: Mean Platelet Volume.

Table 3: Comparison of different parameters between survivor Vs Non-survivor and abnormal CT finding Vs normal.

Parameters	Survived /Expired			CT Findings		
	Survived (n=34) (Mean± SD) Median (IQR)	Expired (n=6) (Mean± SD) Median (IQR)	p- value	COVID Findings (n=23) (Mean± SD) Median (IQR)	Normal (n=17) (Mean± SD) Median (IQR)	p-value
CRP (mg/L)	29.14±71.95 5.2(3.5-8.99)	64.71±85.85 38.2(4-81.48)	0.51	37.33±63.94 5.2(4.0-62.8)	23.70±77.86 4.15(2.0-6.4)	0.52
Ferritin (ng/ml)	191.36±338.67 91.85(65-150)	643.7±590.43 501(100-1284)	0.04*	294.60±419.92 121.6(75.26-258.8)	170.80±361.71 67.5(38.0- 94.5)	0.04*
PCT (ng/ml)	6.04±20.02 0.14(0.04-0.5)	0.40±0.52 0.13(0.04-0.82)	1.00	0.82±2.63 0.1(0.04-0.50)	8.59±24.57 0.04(0.03-0.5)	0.76
N/L	3.02±3.15 2.17(1.54-3.25)	11.98±5.07 12.42(11.12-15.5)	0.001*	4.80±4.91 2.54(1.66-6.83)	3.54±3.99 2.29(1.81-3.6)	0.56
MPV (fl)	9.89±1.42 9.75(8.9-10.8)	9.66±1.15 9.7(8.7-10.6)	0.77	9.7±1.08 9.7(8.9-10.6)	10.35±1.80 10.05(9.1-11.4)	0.52
IgM (U/mL)	2.90±1.28 2.65(2.16-3.4)	3.72±1.44 4.07(3.19-4.45)	0.10	3.28±1.28 3.14(2.5-4.21)	2.68±1.19 2.44(2.20-3.23)	0.10
IgG (U/mL)	3.35±1.13 3.28(2.81-3.94)	4.62±4.08 3.29(3.12-3.94)	0.80	3.81±2.29 3.19(2.81-3.94)	3.26±0.92 3.46(2.44-3.94)	0.77

RESULTS

Comparison of different parameters between COVID positive and control group are summarized in table 1. There is no statistically significant difference in mean age between control and cases. Temperature, pulse rate, respiratory rate and blood pressure were within normal range in both cases and control. Mean FBS of control and COVID-19 cases were 88.6 ± 5.59 and 138.38 ± 113.22 mg/dl respectively. Serum urea and creatinine were normal in both the groups. SGOT was significantly increased in cases, total protein and albumin were significantly decreased in COVID-19 cases, whereas other LFT parameters were within normal range and comparable to the normal group. Serum electrolytes were normal in both the groups. CRP was significantly increased in COVID-19 cases (34.47 ± 74.10 mg/L vs 0.22 ± 0.08 , $p < 0.001$). PCT was higher in COVID-19 cases which was statistically significant (5.19 ± 18.18 ng/ml Vs 0.018 ± 0.008 ng/ml). Similarly, serum ferritin was higher in COVID cases at a significance level of 0.05 (259.21 ± 410.49 ng/ml Vs 33 ± 9.05 ng/ml). There was no significant difference of hematological parameters in between COVID-19 vs control. But ESR was found to be significantly higher in cases. APLA (IgG, IgM) were within normal range in both the groups (IgM: cases 3.02 ± 1.32 U/ml Vs control 2.70 ± 0.53 U/ml, p value

0.81) and (IgG: Cases 3.54 ± 1.85 U/ml Vs control 3.56 ± 0.91 U/ml, p value 0.48)

APLA (IgG, IgM) with serum ferritin, CRP, PCT, N/L and MPV shows no significant correlation in table 2. Table 3 shows comparison of different parameters in COVID-19 survivors Vs non-survivors. CT done in COVID-19 cases were further subdivided into those having abnormal and normal CT findings. Different parameters were compared within these two groups. 6 patients out of 40 expired during our study period. There was no significant difference in CRP, PCT between survivors and non-survivors. Ferritin was higher in expired COVID-19 positive cases in comparison to those who survived (643.7 ± 590.43 ng/ml Vs 191.36 ± 338.67 ng/ml, $p = 0.05$). N/L ratio was significantly increased in non-survivor ($p < 0.001$). MPV also shows no significant difference between both the groups. No statistically significant difference was seen in IgM and IgG APLA antibody. 23 cases out of 40 COVID positive participants shows abnormal CT findings, whereas 17 cases had no changes in their CT findings. While comparing the CT finding none of the parameters shows any significant difference between both the groups except ferritin, which was significantly higher in cases with abnormal CT (294.60 ± 419.92 ng/ml Vs 170.80 ± 361.71 ng/ml, $p < 0.05$).

DISCUSSION

Coagulopathy leading to thrombotic complications have been associated with poor prognosis in COVID-19 pneumonia. Abnormal coagulation parameters were even evident early during hospitalization. APLA was first described in subset of patients with SLE and related connective tissue disorders. APLA targets antibody against multitude of phospholipid, phospholipid complexes and phospholipid binding protein. APLA mediated activation of platelets, monocytes and endothelial cells results in disturbances of anticoagulant and fibrinolytic system. This increases the propensity for thrombus formation and pro-coagulant phenomenon (13). As COVID-19 also appears to give rise to a hypercoagulable state with mechanisms still unclear with some hypothesis linked APLA with it, in the present study we included 40 COVID-19 positive cases and control. 7 cases out of them were symptomatic whereas rest were asymptomatic during admission but 7 out of them develop symptoms in due course of their hospital stay. 6 out of 40 COVID-19 cases succumbed to the disease and rest were discharged after 11 days of their hospital stay. Most of the COVID cases admitted to the general ward except 6 non-survivors who were in the ICU. One of the succumbed cases had developed pulmonary embolism and deep vein thrombosis of all the 4 extremities. 8 patients presented with comorbidities (4 of them had Type-2 DM, 2 had Type2 DM with Chronic Kidney Disease, 1 with Hypertension and CVD and 1 with malignancy). 23 patients had positive CT findings, with areas of ground glass opacity surrounded by consolidation predominantly at postero-basal segment of B/L lower lobe of lungs in sub pleural location.

According to IFCC guidelines of COVID-19, monitoring of creatinine should be done in all patients for early identification of acute kidney injury (14). Though there was no significant difference in mean S Urea and creatinine of our study groups, 7.5% had increased level of urea and creatinine.

Impairment of liver function or liver failure is rarely cause of death in COVID-19. Study have shown abnormal liver function tests in hospitalized COVID-19 patients with 46% having elevated AST and 35 % elevated ALT level (15). In our study AST was significantly higher in COVID cases compared to control. Though GGT was higher in COVID-19 cases but was not statistically significant. Transaminases were specifically higher in those who succumbed to the disease. These findings were in accordance with the findings of Wang and Shi *et al.*, where they reported higher transaminases in severely ill patients requiring ICU comparing to mild and moderate cases (16).

Wang and his colleagues in their retrospective analysis found 56.2% of the patient had abnormal ALT, AST, or Total Bilirubin throughout the course of the disease.

These findings are also supported by some studies where they reported higher values of these parameters. Abnormality in LFT in COVID patient may be due to direct hepatocyte injury by virus, hypoxic ischemic micro circulation disorder or due to underlying liver disorder (17).

In our study Total protein and albumin were significantly lower in COVID-19 cases. Compared to those who recovered, the succumbed/ non-survivor had much lower level of total protein and albumin. Over activated inflammation (acute or chronic) results in hypoalbuminemia besides it may because of increased albuminuria in COVID 19 or may also be reflection of concomitant acute liver failure. Studies have suggested lower level of albumin is the predictor of mortality in COVID -19 cases. Albumin as a negative acute phase reactant and having antioxidant property under normal condition it helps to scavenge reactive oxygen species, whereas in oxidative stress it undergoes irreversible oxidation impairing its antioxidant property. Hence increase mortality in COVID-19 may be linked to reduced albumin level (18). Even albumin has anticoagulant property inhibiting clotting and platelet activation. Hypoalbuminemia in turn results in increased risk of arterial and venous thrombosis in different clinical settings. Serum electrolytes shows no statistically significant difference between cases and control, but those who succumbed to death had lower level of serum sodium and potassium. Studies have also associated COVID-19 severity with lower serum concentration of sodium and potassium. Hypokalemia is seen to exacerbate ARDS and acute cardiac injury, the common cardiac complication in COVID-19 (19). SARS COV-2 binds to ACE receptor and reduces ACE2 expression leading to increased angiotensin -2 causing potassium excretion by kidneys (26). Hyponatremia in COVID-19 may be due to increase release of ADH due to cytokine storm and mostly release of IL-6 from monocytes and macrophages (20).

CRP an acute phase protein helps in clearance of pathogens through the complement system and increase phagocytosis. In our study CRP in COVID-19 patient is significantly higher in comparison to the control group. But no statistically significant increase was seen in non -survived COVID-19 patients compared to those who survived. Similarly, there was no difference between the COVID patient who presented with typical COVID CT findings compared to those who had no changes in their CT findings. Our findings were contradictory to the previous findings where CRP level were positively correlated with the severity of the lung lesions (21). This paradox may be because most of the COVID patients in our study group were asymptomatic.

PCT was though higher in COVID patients compared to normal subjects, but there was no significant

correlation of PCT with severity. Some studies they have suggested that PCT is typically normal on admission but may increase in those admitted to ICU (22). Hu *et al.*, in their study found PCT may be the indicator of disease severity and may help in predicting the prognosis of COVID-19 (23).

Ferritin has two subunits (H and L). H subunit is driven by inflammatory stimuli and works as an immunomodulatory molecule having both proinflammatory and immunosuppressive functions. Several inflammatory stimuli in COVID-19 releasing cytokine storm induces ferritin expression and increases ferritin release. Ferritin in turn induces expression of pro and anti-inflammatory cytokines. Hence, ferritin has a potential role during inflammation following COVID-19 disease (24). Studies have showed that higher serum ferritin levels are associated with ARDS development. Even association between higher serum ferritin level with death by univariate analysis was also seen (25).

Serum ferritin in our study shows significant increase in COVID-19 patients. This increase is even more evident with the severity of the disease (non-survivors/survivors) and presence of CT findings. Hence, in consistence with the other studies in our study also higher serum ferritin levels are significantly associated with the severity of the disease.

There was no difference in hematological parameters between COVID-19 cases and control. But N/L ratio was found to be significantly increased in patients who succumbed to the disease. This may be accounted for as Neutrophilia and Lymphopenia was seen in non-survivors.

The most common hematological finding in most of the studies are thrombocytopenia and neutrophilia. They are even associated with greater severity and fatality in COVID-19 cases (26). Our study also shows lymphocytopenia, and neutrophilia in COVID patients who died during the disease. Hence these hematological parameters can be used to assess the progression and prognosis of COVID-19. Possible reasons behind the significant reduction in lymphocyte count includes lysis of lymphocytes by SARS-COV2 virus as it has ACE2 receptors on the surface, cytokine production leading to lymphocyte apoptosis, atrophy of lymphoid organ impairing lymphocyte turnover and lactic acidosis prevalent in cancer patients inhibiting lymphocyte proliferation (27).

ESR in our study was significantly higher in COVID cases in comparison to control. It is a nonspecific inflammatory marker which changes with protein types. As COVID is associated with inflammation ESR is significantly increase in COVID cases (28).

There was no difference in the levels of APLA between cases and control. When APLA was correlated with inflammatory and hematological

parameters (N/L, ferritin, PCT, CRP), it did not show any significant interrelationship. Even when compared to survivors and non-survivors of COVID-19, there was no significant difference seen in APLA levels. Patients with positive CT finding for COVID-19 even had APLA levels within normal range.

In a case series of 3 patients of COVID -19 with thrombotic complications showed evidence of APLA specifically presence of anticardiolipin Ig A antibody & anti β 2 GPI IgA and IgG antibodies. They suggested APLA as the cause behind cerebral infarct and DIC in these 3 critically ill patients (29). Harzhalla *et al.*, in their study of 56 patients found 45 % were positive for Lupus anticoagulant, 10 % showed anticardiolipin or anti β 2 GPI IgM or IgG, with 6% of the patient positive for lupus, anticardiolipin or β 2 GPI (13). In 6 patient case series study by Routi *et al.*, 5 out of 6 had positive lupus anticoagulant without anti cardiolipin or Anti β 2 GPI antibody. Only 1 had both Lupus and Anticardiolipin / Anti β 2 GPI antibody. It was seen that the patient with double positive antibody had greater propensity towards thrombosis (30).

In our study, the levels of APLA were within normal reference range even for the patient who developed pulmonary embolism and DVT and succumbed to the disease process. Our study is in accordance with the study done by Galleano Valle *et al.*, (14) where they found only 8.3% of the patients were weakly positive for anticardiolipin IgM and Anti β 2 GPI antibody Ig M and negative for IgG Ab. Though 45.8% of their patients (11 out 24) presented with Pulmonary Embolism (PE) alone, 37.5% presented with DVT alone and 16.6% patients presented with both PE & DVT (14). APLA targets the Phospholipid proteins. Presence of these antibodies are the key to diagnosis of APLA syndrome. But these antibodies may also increase in transient illness & various infections. Coagulopathy is known to occur in critically ill COVID 19 cases and have high risk of venous thromboembolism. Various mechanisms like endothelial damage, microvascular thrombosis, occlusion and autoimmune related phenomenon may contribute to increased VTE risk. But in our study autoimmune phenomenon like APLA syndrome was not observed as all the patients were normal for APLA including even the one who presented with the thromboembolic events.

Limitations

This study was done in a small group of COVID-19 patients including both symptomatic and asymptomatic with asymptomatic more prevalent. As it was a cross-sectional study APLA levels were determined in a single sample at the time of admission. Prospective study would have been better to compare the disease course with antibody levels. Larger group study can further clarify the role of APLA and can

justify any change in treatment modality for COVID-19.

CONCLUSION

APLA was found to be within normal limits in all COVID patients (symptomatic and asymptomatic). Patient who succumbed during the disease process by VTE and PE also had normal APLA. Hence, the concept of secondary APLS and rise in APLA causing DIC in COVID cannot be suggested in the present study.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in this study.

REFERENCES

1. Coronavirus Disease (COVID-19) Situation report-205.WHO.12 August. 2020.
2. Levi, M. COVID-19 coagulopathy vs disseminated intravascular coagulation. *Blood Adv.* 2020;4(12):2850.
3. Lodigiani, C., Iapichino, G., Carenzo, L., Cecconi, M., Ferrazzi, P., Sebastian, T., *et al.*, Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. *Thromb Res.* 2020; 191: 9-14.
4. Tang, N., Li, D., Wang, X., Sun, Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Throm Haemost.* 2020; 18(4): 844-847.
5. Zou, Y., Guo, H., Zhang, Y., Zhang, Z., Liu, Y., Wang, J., *et al.*, Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. *Biosci Trends.* 2020; 14(4): 285-289.
6. Tang, N., Bai, H., Chen, X., Gong, J., Li, D., Sun, Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Throm Haem.* 2020; 18(5): 1094-1099.
7. Cavalli, E., Bramanti, A., Ciurleo, R., Tchobanov, A. I., Giordano, A., Fagone, P. Entangling COVID-19 associated thrombosis into a secondary antiphospholipid antibody syndrome: diagnostic and therapeutic perspectives (Review). *Int J Mol Med.* 2020; 46: 903-912.
8. Subramaniam, S., Scharrer, I. Procoagulant activity during viral infections. *Front Biosci (Landmark Ed).* 2018; 23: 1060-1081.
9. Zhang, Y., Xiao, M., Zhang, S., Xia, P., Cao, W., Jiang, W., *et al.*, Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. *NEngl J Med.* 2020; 382(17): e38. doi:10.1056/NEJMc2007575
10. Harzallah, I., Debliquis, A., Drénou, B. Lupus anticoagulant is frequent in patients with Covid-19. *J Thromb Haemost.* 2020; 18(8): 2064-2065.
11. GaleanoValle, F., Oblitas, C. M., FerreiroMazón, M. M., AlonsoMuñoz, J., Torocervera, J., Natale, M., DemeloRodríguez, P. Antiphospholipid antibodies are not elevated in patients with severe COVID-19 pneumonia and venous thromboembolism. *Thromb Res.* 2020; 192: 113-115.
12. Previtali, G., Seghezzi, M., Moiola, V., Sonzogni, A., Cerutti, L., Marozzi, R. The pathogenesis of thromboembolic disease in COVID 19 patients: could be a catastrophic antiphospholipid syndrome. *MedRxiv.* <https://doi.org/10.1101/2020.04.30.20086397>.
13. Chaturvedi, S., McCrae, K, R. Diagnosis and management of the antiphospholipid syndrome. *Blood Rev.* 2017; 31(6): 406-417.
14. IFCC Information Guide on COVID-19. IFCC Information Guide on COVID-19. 2020. www.ifcc.org/ifcc-news/2020-03-26-ifcc-information-guide-on-covid-19/
15. Bertolini, A., Peppel, VDIP., Bodewes, FAJA., Moshage, H., Fantin, A., Farinati, F. Abnormal liver function tests in COVID-19 patients: relevance and potential pathogenesis. *Hepatology.* 2020; 72(5); 1864-1872.
16. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Jhang, J., *et al.*, Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* 2020; 323(11): 1061-1069.
17. Wang, Q., Zhao, H., Liu, L. G., Wang, Y. B., Zhang, T., Ming, H, L., *et al.*, Pattern of liver injury in adult patients with COVID-19: a retrospective analysis of 105 patients. *Mil Med Res.* 2020; 7(1): 28. Published 2020 Jun 7. doi:10.1186/s40779-020-00256-6.
18. Violi, F., Cangemi, R., Romiti, G. F., Ceccarelli, G., Oliva, A., Alessandri, F., *et al.*, Is albumin predictor mortality in COVID-19? Antioxidant and redox signalling. 2020.<http://doi.org/10.1089/ars.2020.8142>.
19. Lippi, G. Electrolyte imbalances in patients with severe coronavirus disease 2019 (COVID-19). *Annals of Clinical Biochemistry* 2020; 57(3): 262-265.
20. Berni, A., Malandrino, D., Parenti, G., Maggi, M., Poggesi, L., Peri, A. Hyponatremia, IL-6, and SARS-CoV-2 (COVID-19) infection: may all fit together?. *J Endocrinol Invest.* 2020; 43(8): 1137-1139.
21. Wang, L. C-reactive protein levels in the early stage of COVID-19. *Med Mal Infect.* 2020; 50(4): 332-334.
22. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., *et al.*, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020; 395: 507-513.
23. Hu, R., Han, C., Pei, S., Yin, M., Chen, X. Procalcitonin levels in COVID-19 patients. *Int J Antimicrob Agents.* 2020; 56(2): 106051.
24. Gómez-Pastora, J., Weigand, M., Kim, J., Wu, X., Strayer, J., Palmer, A., *et al.*, Hyperferritinemia in critically ill COVID-19 patients - Is ferritin the product of inflammation or a pathogenic mediator? [published online ahead of print, 2020 Jun 21]. *Clin Chim Acta.* 2020; 509: 249-251.
25. Zhou, F., Yu, T., Du, R., Fan, G., Lio, Y., Liu, Z., *et al.*, Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020 Mar 28; 395(10229): 1054-1062.
26. Ruan, Q., Yang, K., Wang, W., Jiang, L., Song, J., Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* 2020; 46(5): 846-848.
27. Terpos, E., Stathopoulos, I. N., Elalamy, I., Kastritis, E., Sergentanis, T. N., Politou, M., *et al.*, Hematological findings and complications of COVID-19. *Am. J. Hematol.* 2020; 95(7): 834-837.
28. Zeng, F., Huang, Y., Guo, Y., Chen, X., Xiao, L., Denny, G. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *Int J Infe Dis.* 2020; 96: 467-474.
29. Zhang, Y., Xiao, M., Zhang, S., Xia, P., Cao, W., Jiang, W., *et al.*, Coagulopathy and antiphospholipid antibodies in patients with Covid-19. *New England Journal of Medicine.* 2020 Apr 23; 382(17): e38.
30. Beyrouti, R., Adams, M. E., Benjamin, L., Cohen, H., Farmer, S. F., Goh, Y. Y., *et al.*, Characteristics of ischaemic stroke associated with COVID-19. *Neurol Neurosurg Psychiatry.* 2020; 91(8): 889-891.