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COMPARATIVE ANALYSIS OF HAEMATOLOGICAL, IMMUNOHAEMATOLOGICAL PARAMETERS AND CLINICAL CHEMISTRY PROFILE AMONG LUPUS ANTICOAGULANT POSITIVE AND LUPUS ANTICOAGULANT NEGATIVE COVID-19 CASES

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) is characterised by a pro-coagulant state that can lead to thromboembolic events. A high prevalence of Lupus Anti-coagulant has been shown in several studies, that may atleast partially explain the procoagulant profile of COVID-19. Few people infected by Coronavirus have become seriously ill while others showed little or no signs or symptoms related to COVID-19. This study was undertaken to analyze Haematological, Immunohaematological parameters and clinical Chemistry profile among LA positive and LA negative COVID -19 cases.

Objectives:

1. To assess clinical, hematological and coagulation profile in COVID-19 lupus anticoagulant positive cases and lupus anticoagulant negative cases ..
2. To correlate findings amongst lupus anticoagulant positive and negative cases.
3. To assess inflammatory status amongst Covid-19 lupus anticoagulant positive and negative cases with markers like Crp-q, LDH, D-Dimer and Ferritin.

Materials and Methods: The study was conducted as a prospective observational study with search data forms from the case records of patients hospitalized as ICU/ non- ICU COVID-19 cases in MGM Hospital and Medical College, Aurangabad during the pandemic for a total duration of 2 years from December 2020 to December 2022. A total of 90 patients with confirmed diagnosis of COVID-19 by RTPCR/RAT were studied. To assess Lupus Anti-Coagulant positivity- Dilute Russel Viper venom time (DRVVT) technique is used for established COVID-19 cases

Conclusion: In conclusion, the current study confirmed no statistical correlation for parameters such as age, gender, comorbidities (hypertension, diabetes mellitus, COPD, IHD) in LA positive and LA negative cases. There was no statistical correlation found for ANA, RA factor, Inflammatory markers and Coagulation profile among LA positive and LA negative cases. Neutrophil : Lymphocyte ratio and Lymphocyte : CRP ratio did not statistically differ in LA positive and LA negative cases. Complications (stroke, IC bleed, DVT, PTE) were observed in 16.27% LA negative patients while none of the above complications were seen in LA positive patients. Further large scale studies are required to comment on differences in Haematological, Immunohaematological and Clinical Chemistry profile between LA positive and LA negative cases.

KEYWORDS : COVID-19, Lupus Anticoagulant (LA), Anti-nuclear antibody (ANA), Dilute Russel's viper venom time(DRVVT).

INTRODUCTION:

Patients with coronavirus disease 2019 (Covid-19) have a profound hypercoagulable state, and complicating venous thrombotic events are common.1-3 Abnormalities in coagulation screening measures, including a prolonged activated partial-thromboplastin time (aPTT), have been reported in patients with Covid-19.4 .A prolonged aPTT may indicate a clotting-factor deficiency or the presence of an inhibitor of coagulation that is either specific (e.g., antibody to factor VIII) or nonspecific (e.g., lupus anticoagulant). Lupus anticoagulant can affect in vitro tests of blood coagulation but typically is not associated with bleeding. Antiphospholipid antibodies, mainly lupus anticoagulant (LAC), contribute to an acquired prothrombotic state. They are associated with significantly increased risk of arterial, venous, and microvascular thrombosis.

Many infections have been shown to be accompanied by an increase in antiphospholipid antibodies, which is often transient, but can persist and trigger thromboembolic events.

Individuals with signs and symptoms of Covid-19 mostly have abnormal results in routine hematology, immunohematology tests . The abnormal results on such test findings are believed to have a supportive and / or suggestive accomplishment for Covid-19 infection. Abnormalities in clinical chemistry are described by many authors in liver enzymes, creatinine values, troponin, D-dimer, ferritin levels and C- reactive proteins.

The elevated aPTT values in COVID- 19 are seen often with raised CRP. Besides , alteration noted in other immunohaematological parameters, C reactive protein levels vary significantly in LA positive cases with or without thrombosis therefore this study was undertaken to record thrombotic events either venous or arterial with raised aPTT and co relationship with CRP levels. The disturbed haematology and immunohaematological profile in covid19 cases result in hyperinflammatory conditions.Such individuals may have increased neutrophils to lymphocytes ratio (N/L) and low lymphocytes to CRP ratio.

MATERIALS AND METHODS:

The study was conducted as a prospective observational study with search data from the case records of patients hospitalized as ICU/ non-ICU COVID-19 cases in MGM Hospital and Medical College, Aurangabad during the pandemic for a total duration of 2 years from December 2020 to December 2022. A total of 90 patients with confirmed diagnosis of COVID-19 by RTPCR/RAT were studied. To assess Lupus Anti-Coagulant positivity- Dilute Russel Viper venom time (DRVVT) technique is used for established COVID-19 cases.

INCLUSION CRITERIA:

1. Positive test from RTPCR in hospitalized ICU and non ICU Wings
2. Patients having positive Rapid antigen test.
3. Patients having Rapid antigen test negative but RTPCR test positive.

EXCLUSION CRITERIA:

1. RTPCR and Rapid antigen test both negative tests excluded.

OBSERVATION AND RESULTS:

Table 1: Distribution of Cases according to antinuclear antibody (ANA) & RA factor positivity.

Sr. No.	Test	Group A LA positive 4 (%)	Group B LANegative 86 (%)	Total 90 (100 %)	OR (95 % CI)	P Value
1	ANA	03(3.33%)	33(36.67%)	36 (40%)	5.18	0.1616
2	RA Factor	00 (0%)	06(6.66%)	06 (6.66%)	1.444	0.811

Table 1 shows distribution of cases according to antinuclear antibody (ANA) & RA factor. ANA positive cases were 36 and RA factor positive cases were 6 out of total 90 cases. 2 LAC negative cases had both ANA and RA factor positivity. Both ANA & RA factor was not found statistically significant indicator for LA positivity amongst COVID 19 cases (p-value > 0.05)

Table 2: Distribution of Cases according to inflammatory markers and coagulation profile

Sr. No.	Biomarkers	Group A (LA +ve)		Group B (LA-ve)		Total Raised n (%)	t value	P value
		Raised n (%)	Mean ± SD	Raised n (%)	Mean ± SD			
1	APTT (seconds)	0	-	3	53.93 ± 10.40	3	-	-
2	PTINR	3	1.12 ± 0.106	72	1.19 ± 0.23	75	0.522	0.603
3	CRP (mg/L)	2	21 ± 12.72	62	76.49 ± 62.48	64	1.246	0.217
4	D-dimer (mg/L)	4	0.74 ± 0.21	67	2.33 ± 2.58	71	1.224	0.225
5	Ferritin (µg/l)	2	425.5	56	502.85 ± 293.73	58	0.368	0.714
6	LDH (U/L)	0	-	63	482.11 ± 254.08	63		

Table 2 shows distribution of cases according to inflammatory marker and coagulation profile. APTT was found raised in 3 cases, PTINR in 75 cases, CRP in 64 cases, D-dimer in 71 cases, Ferritin in 58 cases and LH in 63 cases. No statistically significant difference found for values between LA positive and LA negative patients (p-value > 0.05)

Table 3: Distribution of Cases according to blood investigation

Sr. No.	blood Investigation	Group A (LA+ve) n(%)	GroupB (LA-ve) n(%)	Total n (%)
1	N/L Ratio a. ≤ 3 b. >3	1 3	23 63	24 66
2	L/CRP Ratio a. ≤ 2 b. >2	2 2	61 25	63 27

Table 3 shows distribution of cases according to blood investigation N/L ratio >3 was found in total 66 cases and L/CRP ratio <2 was present in 63 cases. LAC positive and LAC negative patients did not differ significantly with respect to above markers.

Table 4: Distribution of Cases according to following complications

Sr. No.	Complications	Group A (LA+ve) n(%)	Group B (LA-ve) n(%)	Total n (%)	P Value
1	Present a. Stroke b. IC bleed c. DVT d. PTE	00 00 00 00	06 02 02 04	06 02 02 04	1.000
	Total	00	14	14	
2	Absent	04	72	76	
Total	04 (4.44 %)	86 (95.56 %)	90 (100 %)	-	

Table 4 shows distribution of cases according to following complications. H/o Stroke was found in 6, IC bleed in 2, DVT in 2 and PTE in 4 cases out of total 90 cases. No complication observed in LA positive cases amongst these. No statistically significant correlation found in presence of complications in LAC positive and LAC negative patients (p-value = 1.000).

DISCUSSION:

In the present study we have taken 90 cases which are RTPCR/RAT positive COVID-19 patients . We studied the relationship between haematological parameters , immunohematology and clinical chemistry profile among lupus anticoagulant positive and negative cases.

Majority of patients i.e 67 were of >/= 40 years and 23 cases of <40 years age . The correlation between age in LAC positive and LAC negative patients was found statistically insignificant (p-value=0.979) .The pandemic affected older and younger age group equally. Carmine Gazzaruso et al (2020)[5] in Italy conducted a study on 192 COVID 19 patients and observed LAC positivity more in older age group(>65 years).

According to our study , out of total 90 cases ,72 were males whereas 18 were females . Male to female ratio was 4:1 . 3 males and 1 female was Lupus positive , total 4 (4.44%) . No statistical correlation was found between gender in LAC positive and LAC negative patients(p-value = 0.798). Nicolas Gendron et al (2020)[6] in France studied parameters such as Age, Sex between LAC positive and LAC negative COVID - 19 patients. No statistical significant difference found between both groups.(p- value >0.05)

Co-morbidities in study comprised presence of diabetes mellitus, hypertension , chronic obstructive pulmonary disease and ischemic heart disease and were studied , in relation to COVID-19 Lupus anticoagulant positive and LAC negative cases .Diabetes mellitus was present in 28 cases, HTN was present in 20 cases , COPD was present in 5 cases . IHD was present in 7 cases. 43 cases were without any co morbidity . No co morbidity was found statistically significant risk factor for causing LA positivity amongst Covid-19 cases (p-value>0.05). A study conducted by Carmine Gazzarusso et al(2020)[5] in Italy in 192 patients of COVID -19, out of which 95 were LAC positive and 97 were LAC negative. Co-morbidities such as diabetes mellitus, hypertension, cardiovascular disease and lung disease studied among LAC positive and LAC negative patients. There was no statistical significant difference found as a risk factor for causing LAC positivity amongst COVID - 19 cases(p -value>0.05)

We further studied cases according to ANA and non specific immunologic markers . Table no. 1 shows distribution of cases according to antinuclear antibodies (ANA) and RA factor . ANA positive cases were 36 and RA factor positive cases were 6 out of total 90 cases . Both ANA and RA factor was not found statistically significant indicator for LAC positivity amongst COVID-19 cases (p-value>0.05). Both ANA and RA factor were positive in 2 cases . Out of 4 LAC positive cases , one case is ANA negative. This can be attributed to the fact that ANA is a diagnostic hallmark for SLE , having a frequency of 95% or greater in SLE patients. Sero-negativity in lupus patients may be due to technical failure or entrapment of ANA in circulating immune complexes.[7,8] A study carried out by Carmine Gazzarusso, Nicoletta Carlo Stella et al (2020)[5] in Italy in 45 patients of COVID-19 pneumonia, percentage of ANA was 35.6% and LAC positivity was 11.1% which was very high. The high prevalence together with other autoimmune markers, suggest an involvement of autoimmune mechanism in SARS 2 COV Disease.

LAC positivity noted in our study is 4 (4.44%) that mean 95.56 % cases were negative . The positivity of LA varies in literature from 5% to 91 % . The reason for dissimilarity in observations can be attributed to genetic variability , geographical variation , technical methods . However most of researchers have taken Dilute Russel Viper Venom Time (DRVVT) which has cut of value 0.85-1.2 . The present study also taken with DRVVT technique with cut off value of 0.85-1.2. Yan Zhang et al (2020)[9] in China carried a study in COVID-19 critically ill patients. Total 19 patients were included out of which only 1 patient had LAC positivity.(5.26%). Similar results for LAC positivity were found in our study.

The inflammatory markers and ratios of Neutrophils to lymphocyte (N/L ratio) , Lymphocyte to C-reactive protein ratio (L/CRP ratio) are regarded significant parameters of study in either group . The details of inflammatory markers and coagulation profile are shown in table no. 2 . APTT was found raised in 3 cases, PT-INR elevated in 75 cases , CRP in 64 cases , D-Dimer in 71 cases , Ferritin in 58 cases and LDH in 63 cases . No statistically significant difference found for values between LAC positive and LAC negative patients (p-value>0.05). A study carried out by Lousie Bowles et al (May 2020)[10] in Royal London Hospital, Cohort of 540 specimens received for LAC testing, 43(8%) had an aPTT elevated. Carmine Gazzarusso, Giuseppe Mariani et al(2020)[5] in Italy carried a study in 192 COVID-19 patients, out of which 95 were LAC positive and 97 were LAC negative. PT-INR and aPTT both were raised in patients with COVID-19. LAC positive patients had significantly deranged PT-INR and aPTT as compared

with LAC negative patients.(p- value< 0.0001) . Ariella Tvito, Eli Ben Chetrit et al (2020)[11] in Israel carried a study and found CRP and D-dimer which are inflammatory markers raised in COVID-19 patients. But there was no statistical significant difference was found for values, D-dimer(p-value= 0.2), CRP (p-value= 0.7) between LAC positive and LAC negative patients.

In our study , out of 4 LAC positive patients 2 cases have mild score , 1 case has moderate score and 1 has normal HRCT chest.Out of 86 LAC negative patients 17 cases have mild score , 36 have moderate score ,13 have severe score and 20 patients have normal HRCT chest. There was no statistical correlation between CT findings in LAC positive and LAC negative cases (p-value=0.915).

In our study , out of 90 patients , 4 patients were LAC positive . These patients have high Neutrophil to Lymphocyte ratio (N/L Ratio) and significant low ratio of lymphocyte to CRP . Table No.3 shows comparative study of blood investigations such as haemoglobin level , WBC count and platelets count between LA positive and LA negative cases . Hb<10 gm/dl was found in total 13 cases , WBCs >11000 was present in 13 cases . Platelets < 1 lac/ml was found in 5 cases. In our study significant high Neutrophil to Lymphocyte ratio >3 was found in 66 cases and significant low lymphocyte to CRP ratio < 2 was present among 63 cases. A systematic review study carried out by Mistire Wolde in Ethiop. J. Health Dev. (2020)[12] in COVID-19 patients showed presence of high Neutrophil count and high CRP values with low Lymphocytes count will create increased Neutrophil/Lymphocyte ratio and decreased Lymphocyte/CRP ratio. COVID-19 patients have leucocytosis with thrombocytopenia.

We have found complications such as Arterial Thrombosis , IC bleed , Deep venous thrombosis and Pulmonary thromboembolism . Table no.4 shows distribution of cases according to above mentioned complications . H/O arterial thrombosis(stroke) was found in 6 cases , IC bleed in 2 cases , DVT in 2 cases and PTE found in 4 patients out of total 90 cases . No statistical significant correlation found in presence of complications in LAC positive and LAC negative patients(p-value =1.00). Another Study carried out by Ariella Tvito, Eli Ben-Chetrit et al (2020)[11] in Israel on 43 COVID-19 patients,out of which 16 were LAC positive (37%) and 27 were LAC negative (63%). 1 patient with LAC positivity and 2 patients with LAC negativity had thrombotic event. The occurrence of vascular events was not higher in LAC positive patients compared with LAC negative patients.

All four patients from our study with LAC positivity survived . Out of 90 patients , 67 survived and 23 did not survive . The results were statistically insignificant for correlating outcome in LAC positive and LAC negative cases (p-value =0.568) . In our study LAC positive patients did not have any of the complications like IC bleed , Stroke , DVT , PTE probably because of early initiation of anticoagulation as per protocol .Out of 86 patients which were LAC negative , 23 died because of complications such as stroke , PTE , DVT , and IC bleed . Their status of ARDS was severe . Hence more mortality noted. Carmine Gazzarusso, Giuseppe Mariani et al (2020)[5] in Italy carried a study on 192 patients of COVID-19 among which 95 were LAC positive and 97 were LAC negative. The non survivor percentage was 34.7% and 29.9% respectively. No statistical significant difference found betLAC positivity and mortality outcome. (p-value=0.4745).

Lupus anticoagulant positivity may be a transient event in patients which may explain the lack of association of poor prognosis in the LAC positive patients. In our study we did not do a follow up of the patients to know the status of LAC positivity , which is a limitation of our study.

CONCLUSION:

In conclusion, the current study confirmed no statistical correlation for Haematological, Immunohaematological parameters and Clinical chemistry profile among LA positive and LA negative COVID-19 cases.

Larger sample size studies may be better equipped to comment on differences in hematological, immunohematological and clinical chemistry profile between LAC positive and LAC negative cases.

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