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"D-dimer and prothrombin time as a biomarker to predict the outcomes in covid-19 patients: A hospital based study."

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ABSTRACT:
BACKGROUND: Covid-19 disease, which is a global pandemic because of rapid human-to-human transmission, can cause mild to fatal respiratory, cardiovascular, and neurological diseases. The outbreak of the coronavirus disease can be managed efficiently by determining the early and effective predictors of clinical outcomes. **AIM & OBJECTIVE :** We aim to observe the dynamic relation of D-dimer and prothrombin time levels with clinical features and covid-19 disease outcomes. **MATERIAL & METHODS :**This is a retrospective study where in patients that were admitted over a period of 6 months,i.e., from 1st January to 30th June2021 ,were included in the study. The D-dimer and Prothrombin time levels of the patients from isolation ward and ICU which were received in the clinical pathology section of department of Pathology were included in the study. **RESULT & CONCLUSION:**Covid-19 disease is known to cause a hypercoaguable state and in the recent outbreak of novel coronavirus infection, the risk of thrombosis and bleeding has attracted much attention. D-dimer and prothrombin time levels are the lab parameters to determine the hypercoaguable state.We conducted this study to find out the direct relationship between D-dimer and prothrombin time levels with the various outcomes of the disease so that timely intervention, like aggressive anticoagulant therapy can be made available to these patients at the earliest.
KEYWORDS: COVID-19, D-Dimer, Prothrombin time.

I. INTRODUCTION:

COVID-19 disease, which is a global pandemic, is predominantly caused by SARS-CoV-2 virus. This virus belongs to beta-coronavirus 2b lineage, a new strain of RNA viruses which has not been identified in humans previously[1].

COVID-19 disease predominantly affects the respiratory system, other organs can also be involved. Some of the patients have favourable outcome, but few of the patients progress to critical stages with severe respiratory distress syndrome, coagulation dysfunction and multiple organ failure[2,3].The case-fatality rate for COVID-19 is 2.3% but for patients aged 70-79 years is 8.0% and 80 years and above is 14.8%[4].

It is accompanied by various biochemical and cellular changes including leukocytosis,leukopenia,neutrophilia,hypoalbuminemia,hyperglycemia,etc[5,6]. The critically ill patients particularly those requiring ICU admission have high probability of developing hypercoagulability. According to one of the study reports the higher neutrophil-to-lymphocyte ratio is associated with greater incidence of venous thromboembolism[7] .

All of the coagulation profile including activated partial thromboplastin time,prothrombin time,fibrinogen,fibrin,etc can be derranged by covid-19 and there can be contrasting variations in the laboratory results of various patients with the severity of the disease[8].

The outbreak of the coronavirus disease can be managed efficiently by determining the early and effective predictors of clinical outcomes.This study seeks to determine the utility of D-dimer and prothrombin time as a biomarker to predict the outcomes in covid-19 patients by determining the disease severity and prognosis .

II. MATERIAL AND METHODS:

This was a retrospective study done in the Government Medical College, Kathua .In this study only those patients were included who were admitted in the hospital premises.In GMC Kathua, for COVID-19 diseased patients, Isolation wards were made where in moderately ill patients were admitted and 24 hr ICU was kept for severely ill patients with severe disease complications.

This study included the RT-PCR positive patients from isolation ward and ICU who were

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moderately to severely sick and those were admitted over a period of 6 months,i.e., from 1st January to 30th June2021. The RT-PCR negative cases and asymptomatic and mild symptomatic diseased patients were not included in the study.
The COVID-19 patient has been classified into 3 types by the AIIMS, New Delhi guidelines:

1. Mild disease: Upper Respiratory tract symptoms (&/or fever) WITHOUT shortness of breath or hypoxia.
2. Moderate disease: Any one of: (1) Respiratory rate>/=24/min

(2) Spo2 </=93% on room air

3. Severe disease : Any one of: (1) Respiratory rate>30/min

(2)Spo2<90% on room air

According to these guidelines; patients with Mild disease require only home isolation along with the prescribed medications. The patients with moderate disease have to be admitted in wards and discharged only after improvement whereas the patients with severe disease needs to be admitted in ICU. These patients can be discharged after clinical improvement as per the discharge criteria.

So, the D-dimer and prothrombin levels of these admitted patients who were moderately to severely ill were sent in the clinical pathology section of Department of Pathology and were studied.

D-DIMER: The d-dimer kit used in our hospital is Erba DDimer R which is an immunoturbidimetric assay used for the quantitative determination of the fibrin degradation products that contain D-dimer in human plasma.

CLINICAL SIGNIFICANCE:

D-dimer containing moieties are formed by plasmin degradation of factor XIlla cross-linked fibrin. Elevated levels of D-dimer are found in clinical conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC)[9,10,11], Laboratory measurements of fibrin degradation products, including D-dimer, have significance in the assessment of these conditions.

PRINCIPLE

Erba D-Dimer R is a turbidimetric assay that utilises antibody coated latex particles. In the presence of D-dimer, the particles aggregate and turbidity increases.

The increase in scattered light is proportional to the amount of D-dimer in the sample. The latex particles are coated with a monoclonal antibody that reacts with fibrin D-dimer or fragment D of fibrin. The antibody has no cross reactivity with fibrinogen[12]. This allows for the determination of D-dimer in human plasma.

COMPOSITION

Important: The reagents are lot-specific. Lots are not interchangeable.
R1-D-Dimer Buffer: containing buffer and preservatives
R2-D-Dimer Latex: latex particle coated with anti-D-Dimer monoclonal antibody.
WORKING REAGENT :Reagents are ready to use. Avoid reagents contamination.
The Latex (R2) may sediment during storage. Mix thoroughly before use.
STABILITY AND STORAGE

The unopened reagents are stable till the expiry date stated on the bottle and kit
label when stored at 2-8°C

R1-Buffer opened vials are stable:
4 weeks at 2-8°C
2 weeks at 20°C

PROCEDURE:

- Add 75ul of R1 in a cuvette.
- After about 60 seconds, add 15ul of plasma of patient and mix properly with formation of froth.
- Add 60ul of R2 but no mixing at this step should be done
- The machine takes about 150-200seconds to give the reading.
- The reading is multiplied by 2.5 to get value o D-dimer in ng/ml.

PROTHROMBIN TIME: The prothrombin time kit used in our hospital is Erba Protime LS.
PRINCIPLE: The one-stage PT measures the clotting time of plasma after adding a source of tissue factor(thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated factor Xa. Factor Xa in turn activates prothrombin to thrombin,which converts fibrinogen to an insoluble fibrin clot.[13,14]

The time of this clotting process is measurable manually or with optical or mechanical coagulation analysers.
COMPOSITION: Erba Protime LS is a tissue thromboplastin from rabbit brain,which converts calcium ions and sodium azide(<0.01%)as preservative.

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PROCEDURE:

Plastic or siliconised glass should be used throughout.Blood (9parts)should be collected into 3.2% or 3.8% sodium citrate anticoagulant(1part).Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at 18-24°C. Testing should be completed within 4 hours of sample collection,or plasma can be stored frozen at -20°c for 2 weeks or -70°c for 6 months. Thaw quickly at 37°C prior to testing. Donot keep at 37°C for more than 5 minutes.[15]

MANUAL METHOD:

- Mix sufficient Erba Protime LS reagent to complete the anticipated testing for the day and incubate reagent at 37°C no more than 4 hours.
- Add 50ul of patient plasma or control plasma into a reaction tube and incubate at 37°C for 2 minutes.
- Add 100ul of freshly mixed reagent and start simultaneously a timer.
- Note the time for clot formation nearest 0.1 seconds.

AUTOMATED METHOD:

In this method machine automatically gives reading within 15-20 seconds and no need of a timer.

III. RESULT AND DISCUSSION:

1. Demographic characteristics: Out of 115 COVID-19 patients studied, about 65 cases (56.5%) were males and 50 cases (43.4%) were females. Moreover, majority of the patients were between the age group of 61-80 years that is 44 cases out of 115 (38.2%).Table 1 and Table 2 shows the complete data and according to this study, majority of the patients were males and more patients were above 60 years that is consistent with the previous literature report.

TABLE 1: Sex Distribution of COVID-19 patients

GENDER	TOTAL	%AGE
MALES	65	56.5%
FEMALES	50	43.4%

TABLE 2: Age Distribution of COVID-19 patients

AGE GROUP	TOTAL	%AGE	MALE TOTAL	MALE %AGE	FEMALE TOTAL	FEMALE %AGE
0-20yrs	22	19.3%	20	90.9%	2	9.09%
21-40yrs	22	19.3%	16	72.7%	6	27.2%
41-60yrs	20	17.3%	10	50%	10	50%
61-80yrs	44	38.2%	25	56.8%	19	43.1%
>80yrs	07	06.08%	04	57.14%	03	42.8%

2. Classification of COVID-19 patients: The COVID-19 patients were clinically classified according to symptoms, sPO_2 and respiratory rate into mild, moderate and severe cases. The admitted cases in the hospital were either moderate or severe cases only. The mild cases were given treatment with home isolation.

TABLE 3: Classification of COVID-19 cases:

GENDER	MODERATE CASES (TOTAL)	MODERATE CASES (%AGE)	SEVERE CASES (TOTAL)	SEVERE CASES (%AGE)
MALE (TOTAL 65 cases)	15	60%	50	55.5%
FEMALE(TOTAL 50 cases)	10	40%	40	44.4%
TOTAL CASES	25	21.7%	90	78.2%

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According to our study,about 90 cases out of 115 cases (78.2%) were classified as severe cases and 25 cases out of 115 cases(21.7%) were classified as moderate cases as shown in table 3. Both moderate and severe cases showed male predominance which is consistent with the previous literature report.

3.Effect of dynamic changes of D-Dimer and Prothrombin Time on outcome of COVID-19 patients: D-dimer and Prothrombin Time values of moderate and severe cases were observed and about 30 of the 90 severe cases showed raised D-Dimer levels(33.3%) and 28 of the 90 severe cases showed raised raised Prothrombin time levels(31.1%). However, 5 of the 25 moderate cases showed raised D-Dimer levels(20%) and 3 of the 25 moderate cases showed raised Prothrombin levels(12%) as described in detail in table 4.

The outcome was followed in these patients that showed total 35 death cases(30.4%)out of 115 cases. Of these 30 deaths were of severe cases(33.3%) and 5 death cases were of moderate cases(20%) . The rest 80 cases(69.5%) were discharged with or without morbidity as described in detail in table 5.

TABLE 4: D-Dimer and Prothrombin Time values of COVID-19 patients:

TEST	MODERATE CASES				SEVERE CASES			
	NORMAL VALUES		RAISED VALUES		NORMAL VALUES		RAISED VALUES	
	TOTAL	%AGE	TOTAL	%AGE	TOTAL	%AGE	TOTAL	%AGE
D-DIMER	20	80%	05	20%	60	66.6%	30	33.3%
PROTHROMBIN TIME	22	88%	03	12%	62	68.8%	28	33.1%

TABLE 5:OUTCOME OF COVID-19 CASES:

CASES	DISCHARGED CASES		DEATH CASES	
	TOTAL	%AGE	TOTAL	%AGE
MODERATE (25 CASES)	20	80%	05	20%
SEVERE (90 CASES)	60	66.6%	30	33.3%
TOTAL (115 CASES)	80	69.5%	35	30.4%

IV. CONCLUSION:

The results of this study showed that COVID-19 patients have a hypercoaguable state at an early stage that is directly related to the outcome of the disease and its progression to various complications.The total 35 deaths that occurred in covid patients were the patients with raised levels of both D-Dimer and Prothrombin time. Rest of the patients were discharged either with or without morbidity. Therefore, the coagulation indicators like D-DIMER and PROTHROMBIN TIME should be assessed at an early stage to detect and avoid the thrombotic complications. This will also help the clinicians to start preventive treatment for thromboembolism and DIC that occur secondary to coagulation disorder, thereby reducing the morbidity and mortality of the COVID-19 patients.

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REFERENCES:

[1]. Tan W, Zhao X, Ma X, Wang W, Niu P, Xu W, Gao GF, Wu G (2020) A novel coronavirus genome identified in a cluster of pneumonia cases—Wuhan, China 2019–2020. China CDC Wkly 2(4):61–62.

[2]. W. Han, B. Quan, Y. Guo et al., "The course of clinical diagnosis and treatment of a case infected with coronavirus disease 2019," Journal of Medical Virology, vol. 92, no. 5, pp. 461– 463, 2020.

[3]. N. Tang, D. Li, X. Wang, and Z. Sun, "Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia," Journal of Thrombosis and Haemostasis, vol. 18, no. 4, pp. 844–847, 2020.

[4]. Wu Z, McGoogan JM (2020) Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention.

[5]. 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;395:507–13.

[6]. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.

[7]. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Müller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost 2020;18:1995–2002.

[8]. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18:844–7.

[9]. Declerck P et al. (1987) Fibrinolytic response and fibrin fragment D-dimer levels in patients with deep vein thrombosis. Thrombosis and Haemostasis, 58: 1024-1029.

[10]. Lindahl T et al. (1994) Clinical evaluation of a diagnostic strategy for deep venous thrombosis with exclusion by low plasma levels of fibrin degradation product D-dimer, Scand. J. Clin. Lab. Invest, 58:307-316.

[11]. Hansson PO et al. (1994) Can laboratory testing improve screening strategies for deep vein thrombosis at an emergency unit, J. Intem. Med,235: 143-151.

[12]. Holvoet P et al. (1989) Binding properties of monoclonal antibodies against human fragment D-dimer of cross-linked fibrin to human plasma clots in an in vivo model in rabbits, Thrombosis and Haemostasis, 61: 307-313.

[13]. Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice, Am. J. Med. Sci, 190: 501.

[14]. Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition,Blackwell Scientific Publications, London.

[15]. Clinical and Laboratory Standards Institute(2008) Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline,5th edn. CLSI: H21-A5.