ORIGINAL RESEARCH PAPER

Pathology

CORRELATION OF SERUM LACTATE DEHYDROGENASE AND D-DIMER LEVEL WITH SARS-COVID-19 DISEASE: A RETROSPECTIVE AND OBSERVATIONAL STUDY

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ABSTRACT

Objective: The aim of this study was role of serum LDH and D-Dimer in Patients with SARS-COVID-19 Disease. **Materials & Method:** COVID-19 Disease due to Coronavirus 2 has been widely spread in India. COVID-19 has infected more than 4 million people within 4 months. Laboratory investigation values are mainly attributed to the inflammatory biomarkers, tissue injury indicators and coagulation parameters. Hence, we aimed to investigate D-Dimer and Serum LDH in COVID-19 patients. **Results:** Examination of total number of 100 Covid-19 positive patients was done with the help of Semi-auto analyzer Erba Chem 7 and Compact Multiparametric Immuno-analyzer, age group between 14-85 years. Out of 100 patients, there were 74 male patients and 26 female patients, the average age was 56 years, while 59% cases (59cases) were between age group 51-70 years. Out of 59 cases, there were 43 male patients and 16 female patients. In 24 cases there is significant increase in D-Dimer and serum LDH values, while 37 cases had either high LDH level or high D-Dimer level. Also, in 24 cases the association between D-Dimer and Serum LDH and correlation between these two factors with routine investigations is stronger. Out of 100 cases, 24 cases (44-84 years) had severe disease and 37 cases (age between 29- 85 years) had moderate disease and 39 cases (age between 14- 77) had mild disease. **Conclusion:** In summary, this study showed that higher levels of D-Dimer and LDH could be identified as powerful predictive factor for severity of Covid-19 disease or powerful predictive factor for early recognition of tissue damage or organ damage and thrombosis in Covid-19 disease. This study also suggests that, older people with higher levels of LDH and D-Dimer has higher risk to develop severe Covid-19 disease.

INTRODUCTION

Coronaviruses are a group of viruses belonging to the family of Coronaviridae, which infect both animals and humans. Human coronaviruses can cause mild disease similar to a common cold, while others cause more severe disease (such as MERS - Middle East Respiratory Syndrome and SARS – Severe Acute Respiratory Syndrome). A new coronavirus that previously has not been identified in humans emerged in Wuhan, China in December 2019.[1]

Coronaviruses are a group of related RNA viruses that cause diseases in mammals and birds. In humans and birds, they cause respiratory tract infections that can range from mild to lethal. Mild illnesses in humans include some cases of the common cold (which is also caused by other viruses, predominantly rhinoviruses), while more lethal varieties can cause SARS, MERS and COVID-19, which is causing the ongoing pandemic. In cows and pigs, they cause diarrhea, while in mice they cause hepatitis and encephalomyelitis.[2]

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Coronaviruses constitute the subfamily Orthocoronavirinae, in the family Coronaviridae, order Nidovirales and realm Riboviria. They are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry. The genome size of corona viruses ranges from approximately 26 to 32 kilobases, one of the largest among RNA viruses. They have characteristic club-shaped spikes that project from their surface, which in electron micrographs create an image reminiscent of the stellar corona, from which their name derives.[4,5]

Charles D. Hudson and Fred Robert Beaudette cultivated the virus for the first time in 1937. The specimen came to be known as the Beaudette strain. In the late 1940s, two more animal coronaviruses, JHM that causes brain disease (murine encephalitis) and mouse hepatitis virus (MHV) that causes hepatitis in mice were discovered. It was not realized at the time that these three different viruses were related.[6,7,8]

Human coronaviruses were discovered in the 1960s using two different methods in the United Kingdom and the United States. E.C. Kendall, Malcolm Bynoe, and David Tyrrell working at the Common Cold Unit of the British Medical Research Council collected a unique common cold virus designated.[9,10,11]

The surface viral protein spike, membrane, and envelope of corona virus are embedded in host membrane-derived lipid bilayer encapsulating the helical nucleocapsid comprising viral RNA (Finlay et al. 2004). The structure of spike (Yan et al. 2020) and protease of SARS-CoV-2 (Zhang et al. 2020) has been resolved, which provides an opportunity to develop a newer class of drugs for treatment of COVID-19.[13,14,15] The size of coronavirus genome is in the range of 26 to 32 kb and comprise 6–11 open reading frames (ORFs) encoding 9680 amino acid polyproteins (Guo et al. 2020). The first ORF comprises approximately 67% of the genome that encodes 16 nonstructural proteins (nsps), whereas the remaining ORFs encode for accessory and structural proteins.[16,17] M and E proteins are required for virus morphogenesis, assembly, and budding, whereas S glycoprotein is a fusion viral protein comprising two subunits S1 and S2. The S1 subunit, which shares 70% sequence identity with bat SARS-like CoVs and human SARS-CoV, comprises signal peptide, N-terminal domain (NTD), and receptor-binding domain (RBD) (Walls et al. 2020).[18]

In adults, age is the strongest risk factor for severe COVID-19. The risk of severe COVID-19 increases with increasing age over 40 years and with increasing number of certain underlying medical conditions.[19]

The structure of SARS-CoV-2 is found to be similar to SARS-CoV with virion size ranging from 70 to 90 nm. Spike, membrane, and envelope surface viral proteins of corona virus is embedded in host membrane-derived lipid bilayer

encapsulating the helical nucleocapsid comprising viral RNA. The genome comprises of 6–11 open reading frames (ORFs) with 5□ and 3□ flanking untranslated regions (UTRs). [20,21] Asymptomatic or symptomatic infections can transmit SARS-CoV-2, with varying rates and timelines for transmission.[16] Both people who have been vaccinated and those who have not been vaccinated can transmit virus. [22]

MATERIALS & METHOD:

A retrospective and observational study to be done on the patient of SARS-COVID-19 Disease at School of Health Sciences, C.S.J.M. University, Kanpur in collaboration with G.S.V.M Medical College Kanpur. Total number of patients will be 100, age group between 14-85 years both genders. This study will be done in duration 6 Months,These will be selected as per inclusion & exclusion criteria.

Inclusion Criteria- Patient of either sex, confirm Cases of SARS-COVID-19 Disease of all ages and patients who have given informed consent.

Exclusion Criteria- patients whose report is negative for SARS-COVID-19, patients suffering with Chronic Heart Diseases and patients who have not given consent to participate in this study.

Sample Collection: Approximately 5-6 ml Venous blood was collected from antecubital vein without anticoagulant for LDH estimation and. with anticoagulant Sodium Citrate for D-Dimer

Statistical Analysis: Data were compiled and analyzed using SPSS 20.0.

OBSERVATION AND RESULTS:

In the present study, examination of 100 patients' serum and plasma were tested with the help of Erba Chem 7 semi-autoanalyzer and Mini Vidas compact multiparametric immunoanalyzer. Examination of total number of 100 Covid-19 positive patients was done with the help of Semi-auto analyzer Erba Chem 7 and Compact Multiparametric Immunoanalyzer, age group between 14-85 years. Out of 100 patients, there were 74 male patients and 26 female patients, the average age was 56 years, while 59% cases (59cases) were between age group 51-70 years. Out of 59 cases, there were 43 male patients and 16 female patients. In 24 cases there is significant increase in D-Dimer and serum LDH values, while 37 cases had either high LDH level or high D-Dimer level. The average D-Dimer level was 0.8666 and average LDH level was 509.259. Also, in 24 cases the association between D-Dimer and Serum LDH and correlation between these two factors with routine investigations is stronger. Out of 100 cases, 24 cases had severe disease and 37 cases had moderate disease and 39 cases had mild disease.

Table No 1 – On the basis of Age group

AGE groups	No. of cases = 100	Percentage
10-20	02	02%
21-30	02	02%
31-40	09	09%
41-50	15	15%
51-60	37	37%
61-70	22	22%
71-80	10	10%
81-90	03	03%

Table 01: show the age group include in study with number of cases and their percentage. We found that the age group between 51-60 is higher than other group with percentage of 37%.

Table No 2 – On the basis of Male and Female Patients

AGE groups	Male Patients	Female Patients
10-20	01	01
21-30	02	00
31-40	07	02
41-50	12	03
51-60	27	10
61-70	16	06
71-80	07	03
81-90	02	01

Graph 1. On the basis of Age group
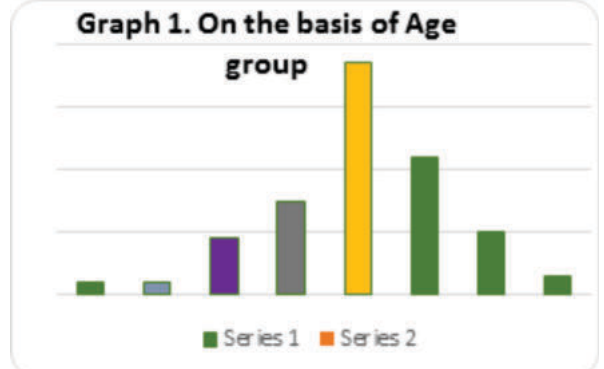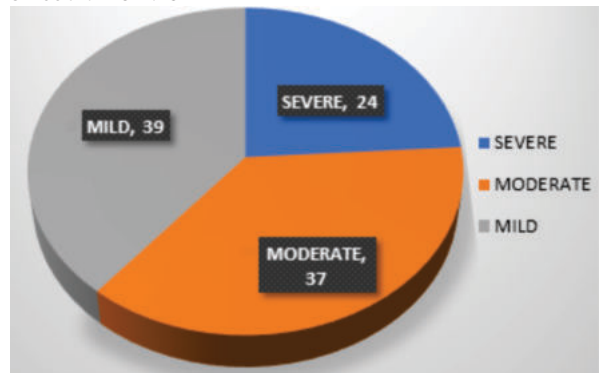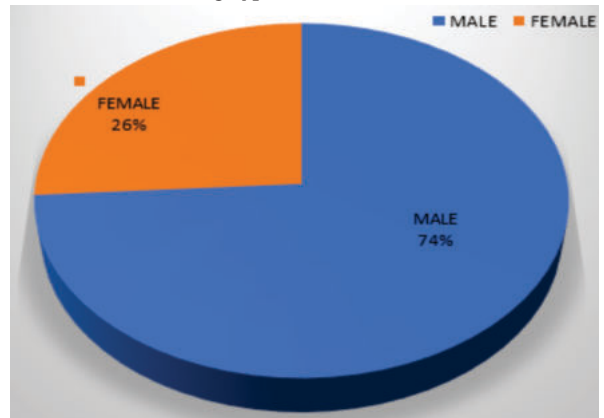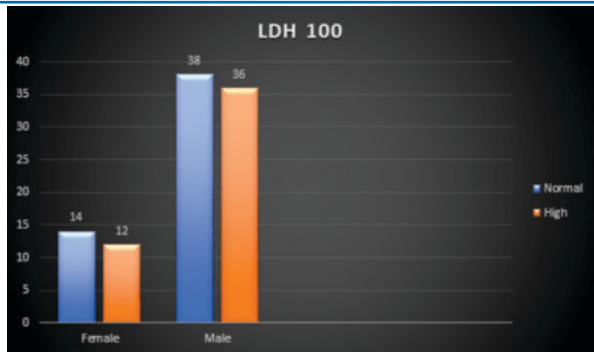

Graph 2. On the basis of Male Female
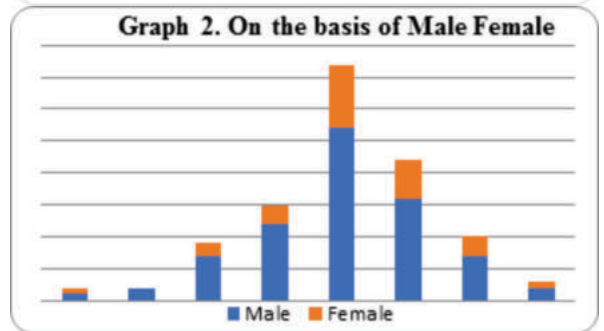
Table 02: show the higher number of male with age group with 51-60 than female.

Graph 3. On The Basis Of LDH And D-dimer Level The Patients Divided Into Following Types- Severe, Moderate And Mild
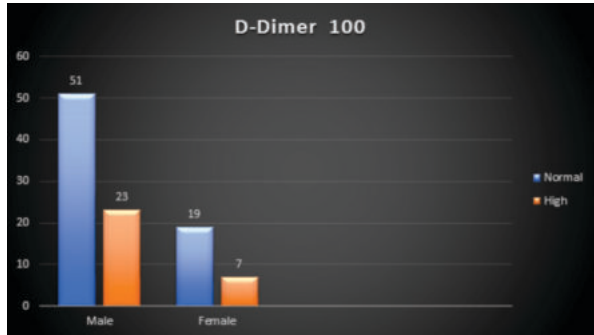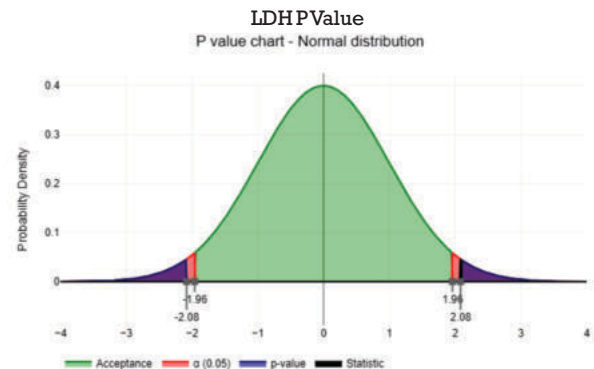

Graph 4. On The Basis Of Gender- In Total Case, We Found Higher Number Of Male With 74% Than Female 26%.
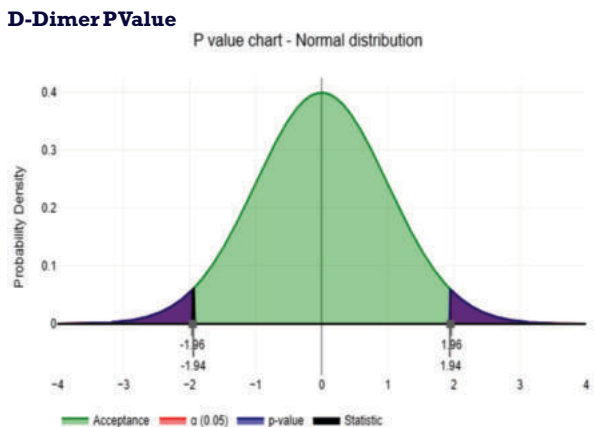
Graph-5 Bar Graph Show The Higher LDH Level In Covid Positive Case, On The Other Hand Some Patients Show Normal LDH Level .
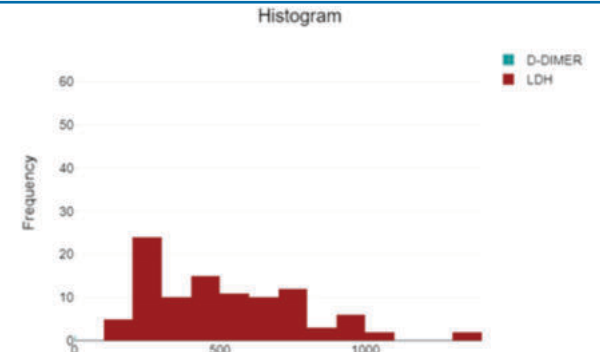
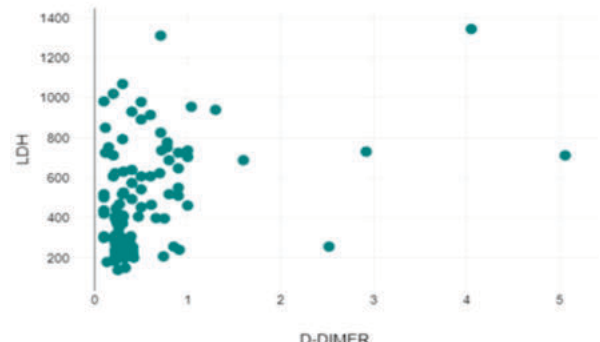**Histogram show the D-Dimer and LDH Mean mode, SD, Maximum and 95% Confidence interval of Mean.**

LDH P Value

Scatter Diagram show the correlation between LDH and D-Dimer.

Graph-6

Box plot show the D-Dimer and LDH level.

D-Dimer P Value

Correlation

	r	p (2-tailed)
D-DIMER and LDH	0.34	.001

Valied Number

	Valid cases
Number	100

Covariance

	D-DIMER	LDH
D-DIMER	0.52	64.28
LDH	64.28	69053.74

Descriptive Analysis of LDH and D-Dimer:

	D-DIMER	LDH
Mean	0.56	509.26
Median	0.32	457.35
Std. Deviation	0.72	262.78
Minimum	0.1	139
Maximum	5.06	1344
95% Confidence interval of Mean	0.42; 0.7	457.75; 560.76
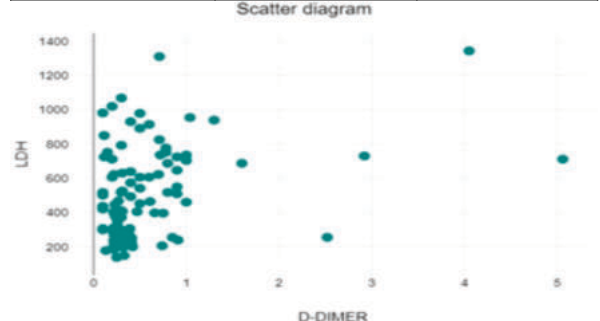
A Pearson correlation was performed to determine if there is a correlation between variables D-DIMER and LDH. There is a medium, positive correlation between variables D-DIMER and LDH with r= 0.34. Thus, there is a medium, positive association between D-DIMER and LDH in this sample.

The result of the Pearson correlation showed that there was a significant correlation between D-DIMER and LDH, r(98) = 0.34, p = .001.

Strength of Correlation

Amount of r	Strength of the correlation
0,0 < 0,1	no correlation
0,1 < 0,3	low correlation
0,3 < 0,5	medium correlation
0,5 < 0,7	high correlation
0,7 < 1	very high correlation

t-Test for paired samples

Descriptive Statistics

	N	Mean	Std. Deviation	Std. Error Mean
D-DIMER	100	0.56	0.72	0.07
LDH	100	509.26	262.78	26.28

t-Test For Paired Samples

	t	df	p (2-tailed)
D-DIMER - LDH	-19.38	99	<.001

95% Confidence Interval Of The Difference

	Mean	Std. Deviation	Std. Error Mean	Lower limit	Upper limit
D-DIMER - LDH	-508.7	262.54	26.25	-560.79	-456.6

The D-DIMER group had lower values (M = 0.56, SD = 0.72) than the LDH group (M = 509.26, SD = 262.78).

A t-test for dependent samples showed that this difference was statistically significant, t(99) = -19.38, p = <.001, 95% Confidence interval [-560.79, -456.6].

This results in a p-value of <.001 , which is below the specified significance level of 0.05. The t-test result is therefore significant for the present data and the null hypothesis is rejected. Therefore, it is assumed that both samples are from different populations

DISCUSSION

The present study includes 100 confirmed cases of Covid-19 (Age group between 14-85 year) who visited the department of Microbiology GSVM Medical College Kanpur, for further investigation. Patients were informed about the procedure to be performed, risks, complications that may occur during or after procedure and informed consent forms were filled. Examination of blood samples were done according to WHO protocol. Venous blood is collected without anticoagulant for LDH estimation and serum is separated. For D- Dimer estimation venous blood is collected with anticoagulant Sodium Citrate and plasma is used for estimation. Examination of total number of 100 Covid-19 positive patients was done with the help of Semi-auto analyzer Erba Chem 7 and Compact 49 Multiparametric Immuno analyzer, age group between 14-85 years. Out of 100 patients, there were 74 male patients and 26 female patients, the average age was 56 years, while 59% cases (59cases) were between age group 51-70 years. Out of 59 cases, there were 43 male patients and 16 female patients. In 24 cases there is significant increase in D-Dimer and serum LDH values, while 37 cases had either high LDH level or high D-Dimer level. Also, in 24 cases the association between D-Dimer and Serum LDH and correlation between these two factors with routine investigations is stronger. Out of 100 cases, 24 cases (44-84 years) had severe disease and 37 cases (age between 29- 85 years) had moderate disease and 39 cases (age between 14- 77) had mild disease.

CONCLUSION

In summary, this study showed that higher levels of D-Dimer and LDH could be identified as powerful predictive factor for severity of Covid-19 disease or powerful predictive factor for early recognition of tissue damage or organ damage and thrombosis in Covid-19 disease. This study also suggests that, older people with higher levels of LDH and D-Dimer have higher risk to develop severe Covid-19 disease. Higher levels of LDH can leads to heart attack or heart failure while higher levels of D-Dimer can lead to formation of clots inside blood vessels.

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