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Original Research Article

To observe the different hematological parameters including coagulation profile, in patients with SARS -CoV-2 infection

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ABSTRACT:

AIMS**:-** To observe the different hematological parameters including coagulation profile, in patients with SARS -CoV-2 infection.

PRIMARY OBJECTIVE:- To evaluate the various changes in hematological parameters and coagulation profile in SARS- CoV-2 patients during their stay in the hospital.

SECONDARY OBJECTIVE**:-** Whether any of these parameters can be used as a prognostic factor and help us to ascertain the course and final outcome of disease.

MATERIAL AND METHODS:-Hematological parameters and coagulation profile was monitored and tabulated in 160 SARS-CoV-2 patient admitted in a period of one year(April 2020-March 2021) in Apollo Multispeciality Hospital, Kolkata.

CONCLUSION:- There were significant changes in the parameters which even affected the ultimate prognosis (discharge or death).

1. INTRODUCTION

Coronavirus disease 2019 (SARS-CoV-2) is an infectious disease caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). Presently corona virus disease is an ongoing global pandemic.In this study we evaluated the hematological parameters including coagulation profile in SARS-CoV-2 patients for a period of one year (April 2020 to March 2021). The present study correlated the currently available data from various earlier studies from all over the world.

We evaluated 160 RTPCR positive SARS-CoV-2 patients admitted in Apollo Gleneagles Hospital, Kolkata,- a multispeciality tertiary care centre in eastern India.

It has been found that there is significant correlation between hemoglobin, hematocrit, Total Leucocyte Count, ESR, Neutrophil/Lymphocyte ratio and Absolute Lymphocyte Count changes in SARS-CoV-2 patients during their stay in hospital and their final outcome. There were also significant changes in coagulation profile in SARS-CoV-2 patients, observed in our study. Prothrombin Time and Activated Partial Thromboplastin Time values were directly related to poor prognosis. Higher D dimer values were found all throughout and was significantly associated with poor outcome. Lower platelet counts and RDW were also seen in expired patients when compared to those discharged.

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Although RDW,eosinophil and monocyte counts, total platelet counts did not show much variationwhen compared with normal range throughout the disease course.

2. MATERIAL AND METHODS:

STUDY DESIGN **:** Retrospective and Prospective Observational study.
STUDY POPULATION**:** The patients admitted with RTPCR positive Corona virus disease (SARS-CoV- 2) in the hospital from April 2020 to March 2021.

SAMPLE SIZE:
The formula used to calculate the sample size(n) was as follows:
$\mathbf{n = z^2pq / d^2}$
where n= sample size
z = the standard normal deviate, which was 1.96 at 95% confidence interval
p = prevalence in the population of the factor under study
q = 1-p = 0.5
d = Absolute precision

$$\textbf{Thus using the formula } n = \frac{z^2pq}{d^2} = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.0064)}$$

Study was conducted on 160 patients.
Inclusion criteria- RTPCR positive SARS-CoV-2 admitted patients.
Exclusion criteria- SARS-CoV- 2 negative patients.

STUDY METHODOLOGY: A retrospective and prospective observational study of 160 SARS-CoV-2 positive patients was done and their hematological parameters and coagulation profile recorded at:
(1) admission,
(2) one week or midway of hospital stay and
(3) before discharge or just before death.

-Hemoglobin concentration,
-Hematocrit,
-Red cell Distribution Width,
-Erythrocyte Sedimentation Rate,
-Total Leucocyte count,
(percentages of neutrophil, lymphocyte, monocyte, eosinophil and basophils)
-Platelet count
-Neutrophil to Lymphocyte Ratio,
-Absolute Lymphocyte Count,
-Prothrombin Time,
-Activated Plasma Thromboplastin Time,
-Thrombin Time,
-D dimer and
-Fibrinogen levels on three occasions, if done were noted.

Values were tabulated and compared. Prognostic implication was compared between those recovered and those expired.

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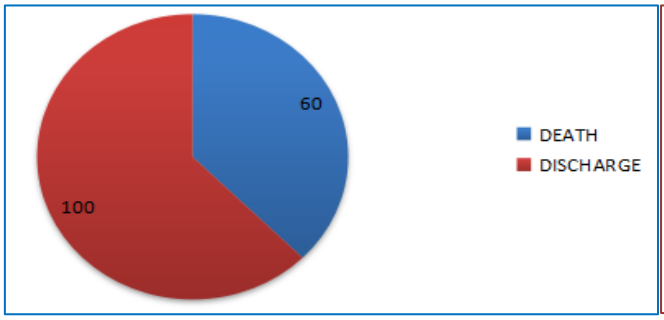
METHOD OF MEASUREMENT OF OUTCOME OF INTEREST: The values were measured by collecting the relevant hematological parameters including coagulation profile in SARS-CoV-2 positive cases from departmental records on admission, after a week or midway of hospital stay and before discharge or death.

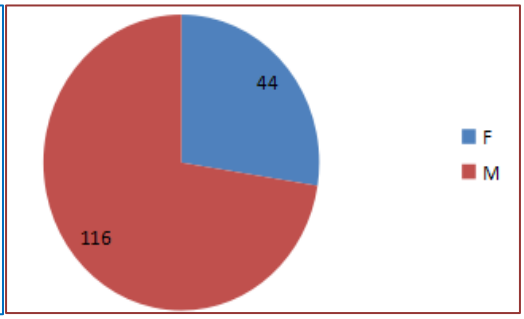
DATA COLLECTION METHODS: The data was collected in a well designed proforma which included relevant patient profile and hematological parameters. Microsoft Excel 2007 was used for data tabulation.

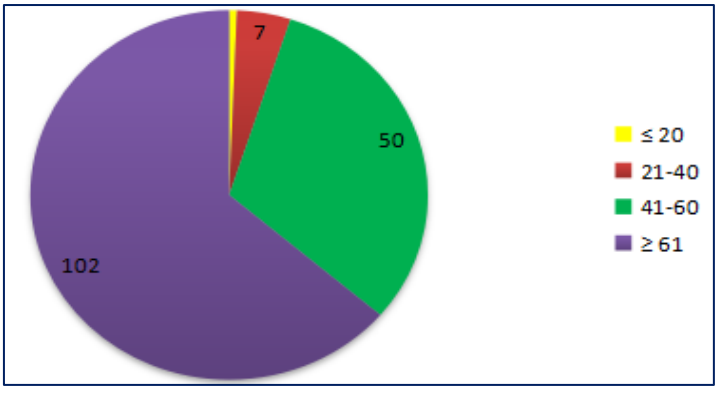
STATISTICAL METHOD:
Categorical variables were expressed as Number of patients and percentage of patients and compared (if required) using Pearson`s Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate.
Continuous variables were expressed as Mean+/- Standard Deviation and compared (if required) using unpaired t test if the data followed normal distribution and Mann- Whitney U test or Kruskal Wallis Test if the data did not follow normal distribution.
The p –value of <0.05 was considered statistically significant.

3. RESULT ANALYSIS

Out of 160 patients in the study, 100(62.5%) were discharged and 60(37.5%) patients expired. Out of 160 patients there were 116 male and 44 females.







Most patients admitted with SARS-CoV-2 were more than 60 years of age (63.8%).
Next group was between 41-60 years (31.3%).

The mean age variability in discharged patients was 62.71+/- 13.98 years. Among those expired the mean age was 67.73+/- 12.15 years. (5 years more than those discharged).

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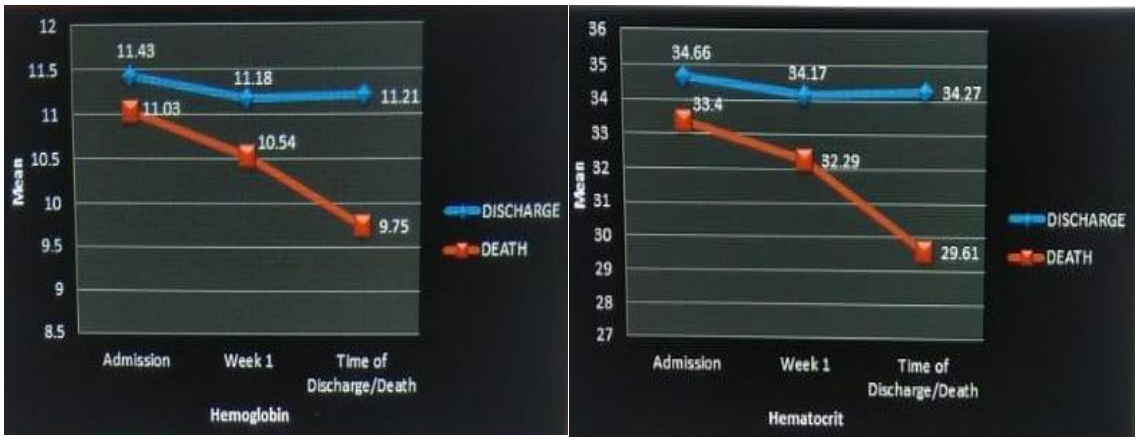
AGE GROUP	FREQUENCY	PERCENT
≤ 20	1	0.6
21 – 40	7	4.4
41 – 60	50	31.3
≥61	102	63.8
Total	160	100.0

PARAMETERS

The mean hemoglobin and hematocrit were significantly lower than normal for age and sex standards. There was a significant($p<0.0001$) decrease in hemoglobin concentration and hematocrit values in those expired.



The mean ESR at admission was higher than normal and remained high all throughout. In our study, those patients who died had significantly($p<0.0001$) much higher values than those who were discharged.

RDW values did not show much significant changes during the course of hospital stay but values were significantly low in expired patients($p =0.0001$)

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Variable	Time		Mean ± SD	Median (Min – Max)	p Value	Significance
ESR (mm/hr)	Admission	DISCHARGE(100)	45.32 ± 22.66	43.00(6.00 – 140.00)	0.001	Significant
		DEATH (60)	58.88 ± 28.60	56.00 (2.00 – 140.00)		
	Week 1	DISCHARGE(28)	55.32 ± 31.11	47.00 (10.00– 131.00)	0.032	Significant
		DEATH (31)	70.03 ± 25.00	69.00 (2.00 - 117.00)		
	Time of Discharge/Death	DISCHARGE(46)	57.93 ± 30.29	58.50 (4.00 – 114.00)	<0.0001	Significant
		DEATH (41)	87.10 ± 26.19	90.00 (4.00 – 140.00)		

Variable	Time		Mean ± SD	Median (Min – Max)	p Value	Significance
RDW (%)	Admission	DISCHARGE(100)	15.30 ± 12.02	14.30(8.50 – 132.00)	0.028	Significant
		DEATH (60)	13.17 ± 3.05	13.60(6.90 – 21.90)		
	Week 1	DISCHARGE(100)	14.20 ± 2.65	14.50(7.90 – 22.40)	0.012	Significant
		DEATH (60)	12.90 ± 3.38	13.00(7.50 – 21.80)		
	Time of Discharge/Death	DISCHARGE(100)	14.10 ± 2.92	14.15(8.60 – 23.00)	0.0001	Significant
		DEATH (60)	12.80 ± 4.64	12.45(5.20 – 35.00)		

The Total Leucocyte Count was on the higher side of normal from the time of admission and the mean values remained high(compared to normal range) during the hospital stay and even during discharge. Those who died had significantly(p=0.07) higher Total Leucocyte Count than those recovered.

Neutrophilia was a constant finding in SARS-CoV-2 infections in our study. Patients expired had significantly higher values than those discharged.

The percentage of lymphocytes remained low all throughout (when compared to normal) and more in deceased patients.

The percentage of eosinophils were low during the course of disease in our study but the values were within normal range.

Monocyte was also within the normal range and not much alterations were found.

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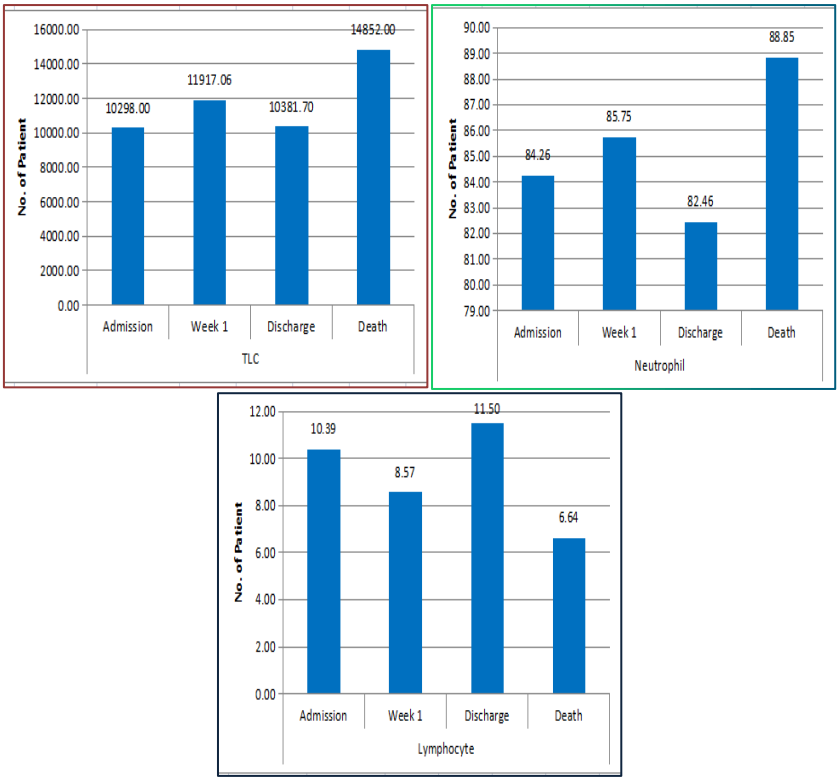
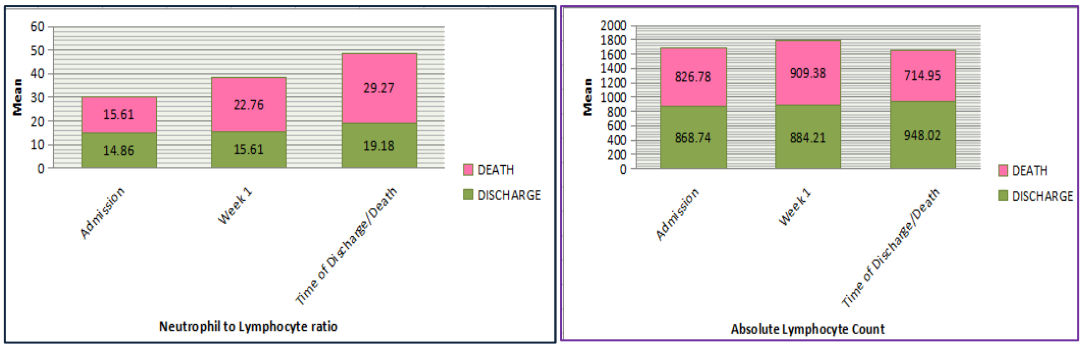


All SARS-CoV-2 admitted patients showed persistently high Neutrophil to Lymphocyte Ratio from admission to discharge. The patients who died had significantly($p<0.0001$) higher values than those recovered.

Absolute lymphocyte count (ALC) was lower than normal. Finally, before discharge or just before death - when values were compared, it was statistically significant($p=0.001$).



The platelet count did not show much variation when compared with the normal range. Expired patients had significantly($p<0.0001$) lower platelet counts.

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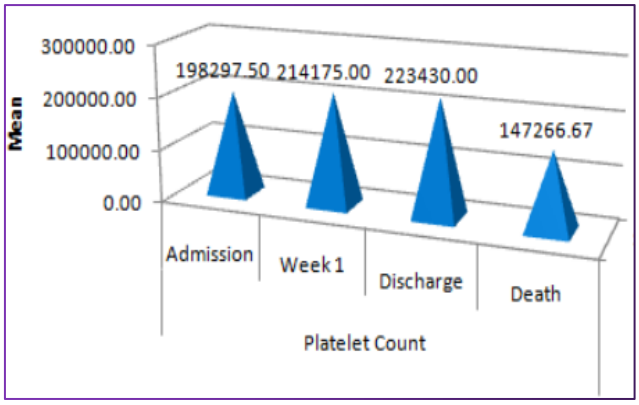
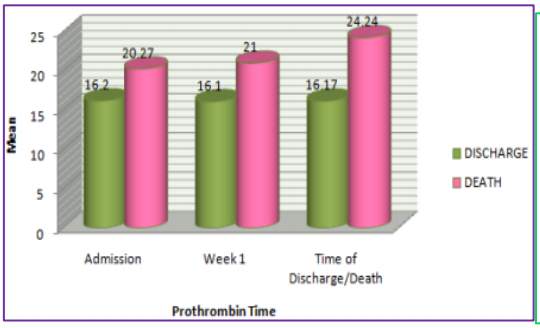
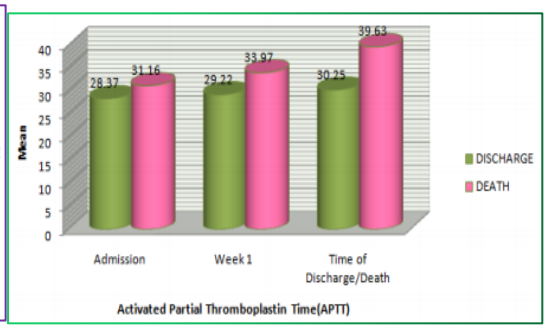


The Prothrombin Time values did not show too much variation. Prothrombin Time was done only in 130 patients out of 160, that is in 81.25% of patients. The patients who succumbed to illness had significantly($p<0.0001$) higher values than those discharged.

Also expired patients had significantly($p<0.0001$) higher Activated Partial Thromboplastin Time values.

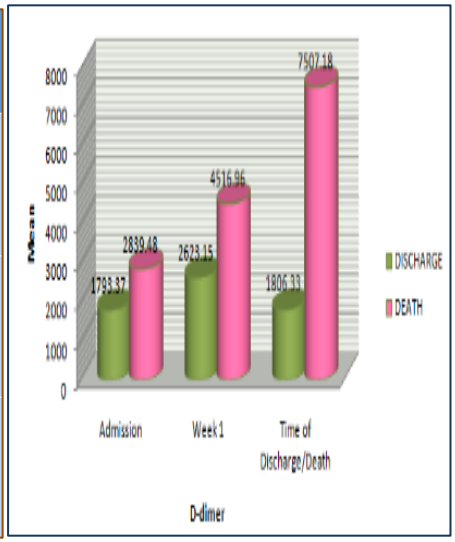
Thrombin Time showed little significance.





131 patients had D dimer done at admission(81.8%). Persistent high D dimer values were noted during the course of disease. Patients who died had significantly($p<0.0001$) very high values than those discharged. There was a sudden spike seen, when recorded just before death.

Variable	Time		Mean ± SD	Median (Min – Max)	p Value	Significance
D-dimer (ng/ml)	Admission	DISCHARGE(81)	1793.37 ± 1961.98	992.50(305.60-1000.00)	0.001	Significant
		DEATH (50)	2839.48 ± 2704.19	1741.50(24.00-1000.00)		
	Week 1	DISCHARGE(28)	2623.15 ± 2949.98	1187.00(463.78-1000.00)	0.007	Significant
		DEATH (16)	4516.96 ± 2904.78	3829.45(578.80-1000.00)		
	Time of Discharge/ Death	DISCHARGE(17)	1806.33 ± 1743.72	1219.40(486.20-7738.20)	<0.0001	Significant
		DEATH (39)	7507.18 ± 2901.55	8753.20(427.80-1000.00)		



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Since Fibrinogen values were done in only 30 patients, no definite conclusion could be made from the results.

Parameters	*Admission*	*Midway or 7 days after Admission*	*At Discharge or death*
Hemoglobin	Not significant	Not significant	Significant
Hematocrit	Not significant	Not significant	Significant
E S R	Significant	Significant	Significant
RDW	Significant	Significant	Significant
T L C	Significant	Significant	Significant
Neutrophil(%)	Significant	Significant	Significant
Lymphocyte(%)	Not significant	Significant	Significant
Eosinophil(%)	Significant	Not significant	Not significant
Monocyte(%)	Not significant	Not significant	Not significant
NLR	Not significant	Not significant	Not significant
ALC	Not Significant	Not significant	Not significant
Platelet count	Significant	Significant	Significant
Prothrombin Time	Not significant	Significant	Significant
APTT	Significant	Significant	Significant
TT	Not significant	Not significant	Significant
D Dimer	Significant	Significant	Significant
Fibrinogen	Significant	Significant	-----

4. DISCUSSION

As we compared our findings with other studies:

Cavezzi et al.[1]in their study demonstrated that as SARS-CoV-2 affected lungs causing hypoxia followed by hemoglobinopathy, and cell iron overload. This finally caused anaemia and correction of anaemia improved prognosis of these patients.

Yuan et al.[2]concluded that severely and critically ill admitted SARS-CoV-2 patients had decreased red blood cell hemoglobin($p<0.01$).

In our study hemoglobin values were definitely lower than normal range for age and sex. Significantly lower hemoglobin concentration was found in those expired in comparison to those discharged($p<0.0001$).

Various studies were done on hemoglobin concentration and hematocrit levels in SARS CoV-2 patients and were compared by **Lippi et al.**[3]. In his study he compared four studies together**(Guan et al.**[4]**, Huang et al** [5]**, Yang et al.**[6]and **Young et al.**[7]) and concluded that in all except one(Yang) of these four investigations, the hemoglobin value was found to be significantly lower in COVID-19 patients with severe disease than in those with milder forms. Similarly, hematocrit values were also low.

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The hematocrit values at admission, one week or midway after admission and at the time of discharge [mean(%)] being 34.19, 33.46, 34.27 respectively, and mean value before death was 29.61%. Hematocrit values were significant with the final outcome of the disease.

Fei et al.[8]demonstrated that SARS-CoV-2 patients were associated with high levels of inflammatory markers ESR, CRP, LDH and serum ferritin.

The ESR values were much higher in patients who expired - from admission till the end when compared to patients who recovered.

Soni et al.[9]in their study concluded that RDW values were significant in COVID-19 patients and was related to mortality and morbidity.

The values of RDW recorded in our study - during admission, one week thereafter and at discharge or before death showed significantly lower values in expired patients than those discharged.

Anurag et al.[10]had already stated that neutrophilia, lymphopenia, eosinopenia were associated with severe COVID 19.

Selim et al.[11]had postulated that neutrophilia might be related to the cytokine storm along with lymphopenia which were common findings.

We found that the Total Leucocyte Count was on the higher side of normal range - during admission, one week after admission and at discharge. Patients who died had significantly raised Total Leucocyte Count done just before death when compared to those discharged($p = 0.007$). Neutrophilia and lymphopenia were significant in those expired when compared to those who were discharged.

Lippi et al.[12]found that low platelet count was associated with increased risk of severe disease and mortality in COVID-19 patients and thus should serve as a clinical indicator of worsening illness during hospitalization.

The SARS-CoV-2 patients had platelet counts in the lower range of normal from admission till discharge. When the platelet counts of patients discharged and those recovered were compared, it showed that there was significant lower levels in expired patients all throughout.

Liu et al.[13]also found that NLR was a predictive factor for early stage prediction of patients infected with COVID-19 who were likely to develop critical illness.

During the course of illness, the Neutrophil to Lymphocyte Ratio was higher from the time of admission till discharge. Those who expired had significantlyvery high Neutrophil to Lymphocyte Ratio in comparison to those discharged($p<0.0001$).

Fan et al.[14]in their study showed that lymphopenia was seen in 36.9% admitted patients and many had reactive lymphocytes of which a subset appeared lymphoplasmocytoid.

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The mean absolute lymphocyte count(cells/µL) were - at admission (853.01), a week after admission (893.65) and at discharge (948.02) and in those expired (714.95). These values were definitely lower than normal values and significantly lower values were seen in expired patients than those who got discharged(p=0.001).

Terpos et al.[15]found that in COVID patients - PT, APTT prolongation with increased FDP

and thrombocytopenia led to death.

Kashinathan et al.[16]reviewed that coagulative features which included prolonged PT,

APTT and decreased antithrombin III were associated with severe disease process and grave

prognosis.

We have found that the mean PT, APTT and TT values were within normal range from admission and all throughout the hospital stay. Those patients who expired had higher values (PT-mean 24.24 seconds, APTT- mean 39.63 seconds, TT- mean 22.56 seconds).

In our study of 160 patients many had co-morbidities and were on anticoagulants for various reasons. These factors could have influenced the coagulation profile indirectly.

So to conclude, the PT, APTT, TT values could reflect different degreesof coagulative dysfunction and their values may be used as predictive factors of severe disease.

Soni et al.[17]found D dimer during hospital stay had the highest C-index to predict in-hospital

mortality in COVID 19 patients.

Shah et al.[18]in their meta analysis demonstrated that patients with COVID-19, who

presented with elevated D dimer levels had an increased risk of severe disease and mortality.

Levi et al.[19]stated that most typical finding in patients with COVID-19 coagulopathy was

increased D dimer concentration.

During admission D dimer was done in 131 out of 160 patients(81.8%). The SARS-CoV-2 patients had a higher mean value at admission(2192.65ng/ml), one week thereafter(mean 3311.80ng/ml), during discharge(mean 1806.33ng/ml) and just before death(mean 7507.18ng/ml). This revealed that D dimer values were always on the higher side of normal. Patients who died had significantly much higher values when compared with those discharged all throughout, and the difference was maximally seen when measured just before death($p<0.0001$). So D dimer values when tested were always significant and manifold higher than normal in most cases of SARS-CoV-2.

This finding typically showed that very high D dimer values were a bad prognostic factor for the severity of disease process and ultimate outcome. D dimer could be attributed to the activation of coagulation cascade - secondary to Systemic Inflammatory Response Syndrome in SARS-CoV-2 patients.

Fibrinogen was done in only 30 patients at admission, 16 patients after one week and only two patients just before discharge or death. Since it was performed in very few patients(compared to 160 patients included in the study) any definiteconclusion could not be made.

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5. CONCLUSION

To conclude decreased hemoglobin, hematocrit, increased Erythrocyte Sedimentation Rate, increased Total Leucocyte Count, neutrophilia, lymhopenia, raised Neutrophil to Lymphocyte Ratio, decreased Absolute Lymphocyte Count and increased Prothrombin Time and Activated Partial Thromboplastin Time (especially done just before death), were noted.

Significantly high D dimer values were seen in admitted patients during the course of illness. Poor prognosis was associated with decreased hemoglobin, decreased hematocrit, increased Erythrocyte Sedimentation Rate, increased Total Leucocyte Count, neutrophilia, lymphopenia, increased Prothromin Time and Activated Partial Thromboplastin Time(especially when measured just before death) and increased D dimer values. Lower platelet counts and RDW were seen only in expired patients when compared to those discharged.

RDW, eosinophil and monocyte counts, total platelet counts did not show much variation when compared with normal range throughout the disease course.

Thus our study findings might help to monitor disease progression, predict final outcome and assist in developing a treatment protocol for these patients at a hospital setup. Larger multi centric studies are required to validate the results.

RECOMMENDATIONS

Monitoring of hematological parameters may help to identify patients with poor outcome. Early prediction may assist clinicians in their management. So these parameters are of immense help to detect disease progression and predict prognosis. So every SARS-CoV-2 admitted patient should have a hemoglobin, Total Leucocyte Count, Differential Leucocyte Count, Erythrocyte Sedimentation Rate, Prothrombin Time and D dimer done during the course of hospital stay to assess the severity and predict outcome.

LIMITATIONS

1. This study was conducted during the first wave of SARS-CoV-2 pandemic in our country. So the results obtained could not be compared with effects in successive waves.
2. This study included a smaller number of patients(160) from a single tertiary centre.
3. The findings may vary from one ethnic group to another and also region wise. In this study it was not taken into consideration.

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