

Original Article

Changes of Proinflammatory Biomarkers of COVID-19 Patients and Its Relationship with HRCT Percentage- A Multi-Center Study in Tertiary Hospitals of Sylhet.

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Abstract

Background and objectives- COVID-19 is a coronavirus disease declared pandemic by WHO. The commonly checked proinflammatory biomarkers are Neutrophil-Lymphocyte ratio (NLR), C - reactive protein (CRP), D-Dimer and Ferritin. High-Resolution Computed Tomography (HRCT) of chest is an important tool to evaluate the diagnosis and prognosis of COVID-19. The objective of this study was to evaluate the relationship between the biomarkers and HRCT findings.

Materials and method- This was a multi-center cross-sectional study done in Sylhet Women's Medical College Hospital (SWMCH), Sylhet Shahid Shamsuddin Ahmed Hospital (SSSAH), Northeast Medical College Hospital (NMCH) and Mount Adora Hospital, Sylhet. Data were collected from electronic medical record (EMR). Relationship test were done by independent t-test and one-way-ANOVA test.

Results- Among the total 481 participant, 312 (65%) were male and rest 169 (35%) were female. The average age of the COVID-19 positive group participants was 60.72 year and it was 60.79 year in the COVID-19 negative group. The mean difference of D-Dimer between COVID-19 positive and negative patients was significant ($p=0.0063$). The mean differences of NLR ($p=0.0012$) and CRP ($p=0.005$) were significant between died and survived patients.

Conclusion- All four biomarkers were raised in the higher HRCT percentage involved groups. NLR and D-Dimer were significantly ($p<0.001$) higher in the higher HRCT percentage groups.

Key words: Proinflammatory biomarkers, HRCT of chest, COVID-19.

DOI: <https://doi.org/10.47648/jswmc2021v11i02-01>

JSWMC 2021[11(02)] P: 13-18

Introduction:

The world Health Organization (WHO) has declared COVID-19 a pandemic.

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A novel coronavirus (nCoV) is a new strain of virus that never had been identified in humans in past. The clinical features of the COVID-19 affected patients are fever (98%), cough (76%), dyspnea (55%), myalgia or weakness (44%), sore throat (29%), headache (8%), hemoptysis (5%), and loose motion (3%). Lots of patients had reported with different neurological complaints like stroke, loss of taste, loss of smell, altered conscious level and convulsion.¹

There are few biochemical markers which are presumed to be associated with COVID-19 infection. Ferritin is an acute phase reactant, plays a defensive role in body by limiting iron supply. Ferritin is produced by inflammatory conditions associated with hematologic, malignancy, infectious and rheumatology. Apart from restricting iron availability ferritin also controls the cytokine synthesis and releases in cytokine storm.² C-reactive protein popularly known as CRP, synthesized by the liver is a strong acute phase reactant biomarker. Unlike other acute phase biomarkers, it does not vary in concentration a lot, rather remains nearly constant during acute inflammation. This is considered an accurate marker for sepsis but also involves in the proinflammatory cycle by activating cytokine in the body.³ The cytokine storm is so far known the most important immunopathological feature of COVID-19 infection. The virus achieves quick replication inside epithelium or endothelium cells after invasion the body resulting production of proinflammatory chemokines and cytokines. The severity of this disease is significantly related to the huge quantity of proinflammatory cytokines production which eventually may lead to acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF).⁴ D-dimer assesses the response of host immune system by measuring the coagulation cascade. It plays important role in determining the severity of sepsis in the process of clinical management. It has been documented that increased level of D-dimer is strongly associated with high risk of septic shock and sepsis.⁵

RT-PCR is the commonest available diagnostic tool in most of the countries in order to confirm the diagnosis of COVID-19. Specimen containing SARS-COV-2 virus genetic material, collected from nose or other part of respiratory tract has been presented with variable sensitivity has kept the validity of the diagnosis.⁶ Acute respiratory distress is the commonest form lungs injury in COVID-19 patients usually presents in 30% of cases.⁷ High resolution computed tomography (HRCT) of chest plays the key role to evaluate the lung condition in this infection, which can have positive findings in case even when the patient is asymptomatic. Different studies showed the sensitivity of HRCT in

diagnosing COVID-19 varies from 75% to 97% though the specificity was only 25%.⁸ It has been evident that HRCT has a good prognostic value and mortality predicting role in case of COVID-19 patients. Common HRCT findings are ground glass opacity and consolidation of lung and in most of the cases the lesions are peripherally located.⁹

Methodology:

This was a multicenter cross-sectional study to evaluate the respiratory support pattern of COVID-19 patients admitted in isolation unit of four tertiary hospitals of Sylhet. Study places were Sylhet Women's Medical College Hospital (SWMCH), Sylhet Shahid Shamsuddin Ahmed Hospital (SSSAH), Northeast Medical College Hospital (NMCH) and Mount Adora Hospital, Sylhet. The total Study period was 5 months. Sample size (N) was 481. The objective of the study was to find out the changes of proinflammatory biomarkers of COVID-19 patients and to find out its relationship with HRCT percentage. All the patients aged 18 and above, not suffering from any other infection (except for COVID-19) and willing to participate were included in this study. Ethical approval was granted from the Institutional Review Board (IRB) of the respective institutions. Informed consent was taken from the participants or nearest keen. Electronic data collection sheet (DCS) was made. Each center was assigned one co-primary investigator (co-PI) and at least one clinical research assistant (CRA). Auditing and monitoring was performed by the primary investigator (PI). After admission of patient in COVID-19 isolation unit demographic information, proinflammatory biomarker values (Neutrophil Lymphocyte ratio, D-Dimer, Ferritin and CRP) were documented. HRCT reports were subdivided into four groups ranging from 0 to 25%, 26 to 50%, 51 to 75% and 76 to 100% involvement of lungs. All participants were tested for COVID-19 by RT-PCR. There were two outcome measures, died and survived. The unit of CRP was milligram / liter. The unit of D-Dimer was microgram / milliliter and the unit of Ferritin was microgram / liter. After collection of all data the final DCS of each center were sent to PI by electronic

form. All DCS were added, compiled and master DCS was prepared. After completion of data collection descriptive calculation of mean and percentage were calculated. Independent t-test

and one-way ANOVA were used to find out the significance between the means. Finally, data were tabulated and prepared for final results.

Results:

This was a multi-center cross-sectional study on COVID-19 isolation unit patients to find out the relationship between biochemical markers and

HRCT percentage. Total number of participants were 481. Overall mortality was 11% (total 53 cases).

Table 1: Distribution according to sex.

	COV positive n=272		COV negative n=209		Died n=53		Survived n=428		Grand total N=481	
	No.	%	No.	%	No.	%	No.	%	No.	%
Male	188	69%	124	59%	36	68%	276	64%	312	65%
Female	84	31%	85	41%	17	32%	152	36%	169	35%

Table -1 shows the distribution according to gender. More than two-third (69%) of the COVID-19 positive group participants was male. Male was more than half (59%) in the COVID-19 negative participant group. Male was

64% and 68% in the survived and died group respectively. The total number of participants was 481, of them 312 (65%) were male and the rest 169 (35%) were female.

Table 2: Distribution according to age frequency.

	COV positive N=272		COV negative N=209		Chi-square	p-value
	No.	%	No.	%		
40 year and below	21	7.7%	17	8%	0.027	0.867
41- 50 year	46	17%	28	13.4%	1.121	0.289
51-60 year	67	24.6%	53	25.4%	0.033	0.855
61-70 year	82	30%	65	31%	0.050	0.821
More than 70 year	56	20.7%	46	22.2%	0.142	0.705
Mean	60.72		60.79		t-value -0.05523	0.955
Median	61		62			
Mode	65		65			
Range	75		84			
Minimum	18		16			
Maximum	95		100			

Table-2 illustrates the distribution of the enrolled participants according to their age frequency. COVID-19 positive participants were 272 in number (56.5%) and 209 participants (43.5%) were COVID-19 negative. In the COVID-19 positive most (30%) participants were in the 61-to-70-year age group and the next was the 51-to-60-year age group (24.6%). Oldest

age group (more than 70-year group) had 20.7% and 41-to-50-year age group had 17% participants. Least percentage was (7.7%) in the below 40 year age group. In the COVID-19 negative participants, most participants were in the 61-to-70-year group (31%), one-quarter (25.4%) were in the 51-to-60-year group. Among the COVID-19 participants, lowest

number of participants (8%) were in the below 40-year age group. No significant statistical difference was revealed between the age group frequency of COVID-19 positive and negative groups (chi-square test done). The average age

of the COVID-19 positive participant group was 60.72 year and the same was 60.79 year in the COVID-19 negative group. There was a significant difference between the mean ages of two groups (independent t-test done).

Table 3: Distribution according to mean of biochemical markers.

	COV positive N=272	COV negative N=209	t- value	p-value	Died N=53	Survived N=428	t-value	p-value
NLR	6.9	6.27	-0.939	.1740	10.08	6.10	3.700	0.0012
D-Dimer	570.7	332.5	-2.502	.0063	417.19	429	0.077	.469
Ferritin	574.8	632.5	-0.388	.348	686	602	0.767	.221
CRP	87.9	77.16	1.312	.0950	112	78.2	2.573	.005

Table-3 illustrates distribution of participants according to biochemical markers. The mean NLR, D-Dimer, Ferritin and CRP of COVID-19 positive participants were respectively 6.9, 570.7, 574.8 and 87.9. These means of COVID-19 patients were 6.27, 332.5, 632.5 and 77.16 respectively. The mean difference was

significant ($p=0.0063$) for D-Dimer. The mean NLR, D-Dimer, Ferritin and CRP of died patients were respectively 10.08, 417.19, 686 and 112. These mean readings were 6.10, 429, 602 and 78.2 for the survived cases. The differences were significant in case of NLR ($p=0.0012$) and CRP ($p=0.005$).

Table 4: Distribution according to mean biochemical markers in relation to HRCT percentage.

	HRCT% 0-25%	HRCT% 26-50%	HRCT% 51-75%	HRCT% 76-100%	f-value	P-value
NLR	4	7.12	8.21	10.8	10.71	<0.0001
D-dimer	199	422	523	1516	21.98	<0.0001
Ferritin	499	603.5	687	809	2.0177	.110
CRP	87	74.6	87.2	99.79	0.927	.427

- One-way ANOVA test done.

Table-4 describes the relationship between biochemical markers and HRCT percentages of all participants. There were groups according to the HRCT percentage. They are HRCT 0-25%, HRCT 26-50%, HRCT 51-75% and HRCT 76-100%. All four biomarker's means were

distributed according to the four HRCT percentage groups. The differences of means were estimated by One-way ANOVA test. The means of NLR and D-dimer were significantly ($p<0.0001$) higher in the higher HRCT percentage groups.

Table 5: Distribution according to mean HRCT percentage in different groups.

	COV positive N=272	COV negative N=209	t- value	p- value	Died N=53	Survived N=428	t- value	p- value
HRCT %	41%	38%	-1.939	0.265	43.17%	38.65%	1.56	0.059

Table-5 shows the distributions according to HRCT percentage of different groups. The mean HRCT percentages of COVID-19 positive and negatives cases were 41% and 38% respectively. The difference was not significant ($p=0.265$). On the other hand, the mean HRCT percentages of died and survived groups were 43.17% and 38.65% and the difference was significant ($p=0.059$).

Discussion:

Total 481 participants were involved in this study. Relationship between biomarkers and HRCT findings was evaluated. The result of this study was compared with result of different international studies.

Muhammad Kermalia et al published their study on the role biomarkers to diagnose COVID-19 patients.¹⁰ It was a systematic review on 34 articles. They found C-reactive protein (CRP), Neutrophil to Lymphocyte ratio (NLR), D-dimer, interleukin-6 and lactate dehydrogenase were high in the severely affected groups in comparison to the non-severe group. On the other hand, platelet and lymphocyte counts were low the severe groups. In our study severely lung affected patient's NLR and D-dimer showed significant change in comparison to the less severe groups.

Muhammad Sohaib et al published their study on 364 COVID-19 patients.¹¹ Two-third patients were in isolation ward and rest one-third were admitted in intensive care unit (ICU). Mortality was 27.3%. Mean age of their study population was 52.6 years. Commonest age group was 50 to 75-year age group. There was significant difference in total leukocyte count, neutrophil count, lymphocyte count, CRP level, LDH and D-dimer between survivor and death cases. It was concluded that inflammatory markers like LDH, PCT, D-dimer, CRP, and ferritin were useful guide for predicting mortality. In this study the mean age of the enrolled patients was 60.72 year in COVID-19 group and 60.79 year in the COVID-19 negative group. The commonest age group was 61-to-70-year age group in our study.

Abderrahim et al performed a retrospective longitudinal cohort study on 162 COVID-19 patient's biochemical markers.¹² Changes of biochemical markers in multiorgan involvement was observed. It was detected that CRP more than 90 mg/L and urea nitrogen more than 36g/L were independently strongly associated with stage 3 acute kidney injury related death of COVID-19 patients. At the end they did not find any relationship between inflammatory markers and COVID-19 related death. In our study CRP and NLR were found significantly raised in the died group in comparison to the survived group. Wei Chen et al performed their study to find out the relationship between plasma CRP and severity of COVID-19.¹³ All the patients tested positive for SARS-CoV-2 were enrolled. Plasma CRP was assessed for all the patients. HRCT was performed for all the patients to categorize the patients into mild, moderate and severe group. Then linear regression models were applied to find out the correlation between CRP and severity of COVID-19. After correlation it was found that plasma CRP was positively related to the severity of COVID-19 pneumonia and hospital staying. In our study the CRP was mean was 87 in less severe group (<25% HRCT involved) in comparison to 99.79 in the severely affected (76-100% HRCT involved) group.

Correlation between HRCT severity and clinical parameters was evaluated by Ghufuran et al.¹⁴ COVID-19 infection of 902 patients was confirmed by RT-PCR. The HRCT severity was assessed by 25-point severity score. In the result HRCT severity score was positively related to lymphopenia, increased CRP, D-dimer and ferritin level at a highly significant level. Severity score was also related with oxygen requirement and increased hospital staying. Similar result was concluded in our study also. CRP, D-dimer, ferritin and NLR were high in the severely affected group.

Conclusion: COVID-19 is a highly infectious disease predominantly presents with pneumonia. Biomarkers have shown a significant relationship with the percentage of lung involvement in HRCT. The proinflammatory markers like CRP, D-dimer, ferritin and NLR

could be used to assess the severity of lung involvement.

Conflict of interest: None of the coauthors declared any conflict of interest related to this study. This was a self-funded research.

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