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Biomarkers Analysis of COVID-19 Patients: Lessons Learnt from the Deadly Pandemic

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ABSTRACT

Background: Coronavirus disease-2019 (COVID-19) manifests as inflammation, leading to a raised level of associated biomarkers, which assists in risk stratification. We investigated the correlation between demography and biomarkers, namely, Interleukin-6 (IL-6), serum C-reactive protein (CRP), D-dimer, procalcitonin (PCT), and serum ferritin with prognosis among COVID-19 patients.
Method: This is a retrospective cohort study conducted on COVID-19 patients amidst the second wave of the pandemic. IL-6, PCT, serum ferritin, CRP, and D-dimer levels were analyzed among patients diagnosed as COVID-positive by real-time polymerase chain reaction.
Result: Out of 1663 patients included in this study, 65% were males, with the median age of the study population being 48 years. The mean levels of IL-6, ferritin, CRP, and PCT were significantly raised in the older age group (47-95 years) than the younger population (4-46 years), whereas D-dimer was found to be raised in all age groups. The mortality rate was 5% (median age- 59 years), with males showing high severity and a mortality rate 67.4%.
Conclusion: Evaluating and tracking the biomarkers at the outset of the disease has been proven to give a substantial edge in assessing disease prognosis and preventing mortality. Henceforth, they become the guiding force for management strategies in this era of precision medicine.

Key-words: Coronavirus, Immunosenescence, Inflammation, Precision medicine, Prognostic markers

INTRODUCTION

The pandemic of the coronavirus disease 2019 (COVID-19) has brought myriad challenges to clinical and diagnostic communities. Even though Severe acute respiratory syndrome-coronavirus (SARS-CoV-2) is transmitted through the respiratory route, it eventually produces multisystem havoc, a cumulative outcome of the coagulative, immunological, and inflammatory cascades. The only constant with COVID-19 is change in the form of mutations, variants, diagnostic approaches, and management strategies.[1,2]

Biomarkers have aided early suspicion, classification based on severity, yardstick for hospitalization, risk categorization, ICU admission criteria, rationalizing pertinent therapies, response to therapies, prognosis, and discharge. [3-5] Augmented inflammatory response of the body reflected in the raised values of various laboratory biomarkers has been relied upon for deciding personalized management protocols.[6-8]

During the pandemic, every wave presented the healthcare system with newer challenges of rising infection rates and varying severity. Despite the vast literature database available, treating clinicians require updated facts from time to time to offer the optimum care at the bedside. This article aims to assess levels of

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some of the biomarkers and how they correlate to demography and mortality in COVID patients for future reference, as we need to be prepared before the next affliction.

MATERIALS AND METHODS

This is a single-center, retrospective, observational study conducted in a multispecialty hospital.

Inclusion criteria- The study population includes patients admitted with signs and symptoms of COVID-19. Later, the clinical suspicion was confirmed through real-time polymerase chain reaction. All consecutively admitted COVID patients from January 2021 to May 2021 were included in this study, and their baseline laboratory data and outcome parameters were analyzed. A total of 1663 patients were included in the final analysis.

Exclusion criteria- COVID-19 patients who left against medical advice were excluded from the study.

Definition- Biomarkers are "characteristics that can be objectively measured and evaluated as an indicator of normal biological and pathological processes, or pharmacological responses to a therapeutic intervention".[7]

Sample collection- Two-millilitre venous blood samples were collected to test CRP, ferritin, PCT, and IL-6 in a red-top plain vial with a clot activator. In contrast, two-millilitre venous whole blood samples for D-Dimer were collected in a blue top vial with anticoagulant sodium citrate. Verbal consent was obtained from all patients before the sample was drawn.

Methodology-For D-Dimer and PCT, Immuno-fluorescence assay rapid test kits were used (Getein Biotech Inc.) and the tests were performed in Getein 1100 immunofluorescence analyser. Ferritin quantitative measurement was done using an immunoturbidimetric-based kit (Chemlex S.A. Barcelona). CRP was done using turbidimetric immunoassay-based reagent (Q-LINE Mfg.) and the test was performed in a fully automated biochemistry analyser based on spectrophotometry (Selectra ProM-Elitech). IL-6 test was performed using the ELISA (Enzyme-based immunosorbent assay) method (DIA source immunoassay SA, Belgium) in an ELISA-based analyzer (Rayto Mfg).

Statistical Analysis- Data was summarized using frequency tables and percentages. A chi-square test/ Fischer's exact test was used to identify a statistically significant difference between COVID-19 severity, demographical characteristics, baseline laboratory biomarkers and mortality. A statistically significant difference was detected for variables with a $p<0.05$.

Ethical Approval- Data was extracted from patients' admission, follow-up, and discharge files, and all data were kept anonymous. Approval has been obtained from the Institutional Ethical Committee for the exemption of consent from the patient and patient information sheet since the isolates were anonymized, coded by randomization, and delinked from any identity of the patients.

RESULTS

A total of 1663 patients with COVID-19 were part of this study, with 13% of patients having severe disease, 39% moderate and 48% mild disease. The median age of the study population was 48 years (Range: 4-95, IQR-26), with no significant difference in the median age of affected males: females. There was a strong male predominance, with 1079 males and 584 females with Male: Female ratio of 1:1.9. The mortality rate was 5% (86/1663) among COVID-positive patients, with males showing high severity with a mortality rate 67.4% (58/86). Furthermore, the median age group in mortality cases was 59 years (Mean-56.7 years, IQR-20) (Table 1).

Table 1: Baseline characteristics of the study Population

Variables	N=1663
Age (Years)	
Mean Range	47.4 ± 16.9 (4-95)
Gender	
Male Female	1079 (65%) 584 (35%)
Mortality Status	
Death Live	86 (5%) 1573 (95%)
Severity Status	
Severe	223 (13%)
Moderate	656 (39%)
Mild	784 (48%)

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The mean level of CRP and IL-6 were higher in males than females, with p<0.001. Although ferritin, D-dimer, and PCT were also raised in males, they were not found to be significantly raised compared to females (Table 2).

Table 2: Comparison of Inflammatory markers based on gender in COVID-19

Gender	Male (n=1079)	Female (n=580)	p-value*
CRP	63.31±37.99	51.56±42.20	<0.0001
IL-6	99.75±24.86	94.76±24.05	0.001
Ferritin	237.51±139.07	230.76±127.71	0.33
D-dimer	3.29±2.49	3.12±2.01	0.15
PCT	9.13±3.11	9.03±3.13	0.53

*Footnotes: CRP- C-reactive proteins, IL-6- Interleukin-6, PCT-Procalcitonin. *p-value is calculated by comparing males and females. p-value <0.05 is considered as statistically significant.*

The age was distributed into two groups: younger (4–46 years) and older (47–95 years). All the inflammatory markers were elevated in the older age group. The mean levels of CRP, IL-6, ferritin, and PCT were significantly raised in the older age group (p<0.0001), whereas D-dimer was found to be raised in all age groups (Table 3).

Table 3: Comparative analysis of Inflammatory markers based on age in COVID-19

Age group/ Biomarkers	Younger (4-46 years) (n=801)	Older (47-95 years) (n=858)	p-value
CRP	43.42±34.53	51.36±34.62	<0.0001*
IL-6	142.26±90.60	348±95.98	<0.0001*
Ferritin	224.32±125.23	339.23±212.08	<0.0001*
D-dimer	3.21±2.44	3.30±2.34	0.44
PCT	3.19±1.16	8.91±2.62	<0.0001*

*Footnotes: CRP- C-reactive proteins, IL-6- Interleukin-6, PCT-Procalcitonin. *p-value is calculated by comparing males and females. p-value <0.05 is considered as statistically significant.*

Our study suggests a statistically significant positive correlation between ferritin, IL-6 levels (r=0.30, p<0.05), and CRP (r=0.40, p<0.05), while no correlation was found between the other inflammatory markers. On the other hand, IL-6 was positively correlated with PCT (r=0.80, p<0.05, and D-dimer (r=0.40, p<0.01). Furthermore, we also observed PCT showed a strong positive correlation with D-dimer (r=0.42, p<0.05) (Table 4). CRP and D-dimer significantly increased, with 93% (80/86) cases and 80% (69/86) respectively. Levels of IL-6 & ferritin were also higher in cases of mortality, with 71% and 67%, respectively.

Table 4: Correlation of inflammatory markers in the Study Population

Parameters	Ferritin (ng/ml)	IL6 (pg/ml)	CRP (mg/l)	PCT (ng/ml)	D-dimer (µg/ml)
Ferritin (ng/ml)	1	r=0.30 p=0.046*	r=0.40 p=0.008*	r=0.30 p=0.154	r=-0.11 p=0.445
IL-6		1	r=0.22	r=0.80	r=0.40

(pg/ml)			p=0.132	p=<0.001*	p=0.005*
CRP (mg/l)			1	r=0.31 p=0.121	r=0.23 p=0.095
PCT (ng/ml)				1	r=0.42 p=0.038*
D-dimer (µg/ml)					1

Footnotes: CRP- C-reactive proteins, IL-6- Interleukin-6, PCT-Procalcitonin. r=Pearson correlation; p-value is calculated by comparing control versus case; p-value<0.05 is considered statistically significant.

DISCUSSION

The present study analysed inflammatory biomarkers with demography and their correlation in a cohort of 1663 COVID patients with varying severity and mortality. The severity profile of COVID-affected patients varies depending on the population's geography, medical amenities and vaccination status. Our data showed 13% of patients with severe disease, 39% moderate and 48% mild. Two multicenter studies have shown a severity profile similar to ours, with severe cases varying from 16 to 26%, except Jurado *et al.* which showed 30.5% critical cases.[6-8]

Various studies have found a lot of heterogeneity regarding the age of COVID-19 patients. The median age of the COVID patients in our study was 48 years, irrespective of gender. The average age in most of the literature ranges from 36 to 58 years old, similar to our study.[6-10] This trend can be explained by the fact that the thirties to fifties is the age of the workforce of any country, which is more outgoing; hence, the chances of them getting infected and manifesting into the disease are higher. However, a few reports have shown an average age of more than 60 years.[8,11]

Furthermore, mortality is higher in older age groups. The older population suffers from co-morbidities associated with ageing, especially noncommunicable chronic diseases combined with treatments for these diseases and age-related deterioration of the immune system, referred to as immunosenescence.[12] Immunosenescence is linked to adaptive immune variations within B and T cells without affecting the number of circulating lymphocytes rather than diminishing their repository and vitality. These phenomena lead to increased susceptibility to infection and a lower vaccination response, thus leading to severe disease with a worse prognosis.[8,12,13] We observed a strong male predominance, with a male-to-female ratio of 1:1.9, which aligns with previous studies reporting a higher susceptibility of males to COVID-19.[6,9,10] Moreover, males suffered more severe disease with worse outcomes, with a mortality rate of 67.4%. This gender association could be correlated with the skewed sex ratio of our country in conjunction with the general demographic gospel of a shorter life expectancy in men when compared to women worldwide.

Summarizing the parameters of the laboratory, our findings had similarities with previous reports, showing an increase of acute phase reactants like CRP, ferritin, D-dimer with severity and prognosis of patients.[6,10,14] Our analysis revealed that IL-6 and CRP had substantially raised levels in males compared to females. Previous studies have reported higher CRP levels in women and higher IL-6 levels in men in various inflammatory conditions. Raised levels of IL-6 and CRP have shown association with worse outcomes and disease severity in COVID-19 patients, suggesting their potential role as biomarkers for disease prognosis. In addition, COVID-19 is strongly associated with immune inflammation and depression, with increased levels of CRP and IL-6 found in both conditions.

Comparing inflammatory markers based on age groups, we observed higher IL-6, CRP, ferritin, and PCT levels in older patients. [14-16] This suggests that advanced age is associated with a more pronounced inflammatory response to SARS-CoV2 infection. These findings are coherent with previous studies indicating that old age is an added risk to severe SARS-CoV-2 infection associated with dysregulated immune responses. [14,17] Considering age-specific management and monitoring strategies in COVID-19 patients becomes crucial due to these age-related differences in inflammatory markers. Correlation analysis revealed significant positive correlations between ferritin and IL-6, as well as CRP. These findings suggest a potential interplay between these markers in

the inflammatory response during SARS-CoV-2 infection. Additionally, IL-6 showed positive correlations with PCT and D-dimer, indicating a possible association between IL-6-mediated inflammation and coagulation abnormalities in COVID-19.[16,17] These correlations provide invaluable cognizance into the complex interactions between inflammatory and coagulation pathways in the SARS-CoV-2 pathogenesis.[18,19] Scoring systems based on laboratory biomarkers and clinical profiles can be developed specifically for COVID-19 to assess the status of patients and modulate the treatment strategies accordingly.[20]

LIMITATIONS

Firstly, it is a single-center study with analysis during the second wave of COVID-19. Secondly, only five biomarkers have been considered. Further evaluation of lymphocytes is needed. Overall, our study contributes to understanding inflammatory markers as potential biomarkers for SARS-CoV2 infection. The observed gender-based and age-related differences in inflammatory responses emphasize the importance of considering these factors in disease prognosis and personalized treatment approaches. Such studies are necessary to explore the clinical relevance of these biomarkers for risk stratification, monitoring disease progression, and steering therapeutic modalities in COVID-19 patients.

CONCLUSIONS

COVID-19 has a multitudinous spectrum, with disease culminations varying according to demography, geography and co-morbidities. Biomarkers play a decisive role in initial suspicion, diagnosis, delineating the probable complications, and optimum management of the patients. Biomarkers need to be pertinently integrated with the clinical evaluation of the patients to reach requisite bedside decision-making. A panel rather than single biomarkers with a serial assessment of their levels provides more authentic laboratory corroboration of patient condition. It is quintessential to have constantly evolving national and regional guidelines tailored according to the epidemiology of the disease to guide the management protocols in various healthcare settings. These protocols can then be adapted for individual patients, incorporating the clinical and laboratory data. All these endeavours are part of precision medicine, which healthcare specialists are advocating to make patient management more personalized and effective.

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