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# Evaluating Predictive Role of Inflammatory Biomarkers in Survivors versus Non-Survivors Hospitalized With Covid-19 in a Tertiary Care Hospital

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**ABSTRACT**: In COVID-19, effective employment of inflammatory biomarkers could be helpful in screening, predicting prognoses, deciding clinical management, and preventing serious complications associated with disease. This retrospective single-centre observational study was carried out at tertiary care Hospital, Hyderabad, after obtaining concerned permission from Institutional Ethics Committee. A total of 151 prescriptions of COVID-19 patients were evaluated in the study for a duration of 6 months. The parameters included in the evaluation were:

- Demographic characteristics
- Inflammatory biomarkers in survivors and non-survivor
- Other Biochemical parameters
- Pharmacotherapeutic agents prescribed

In conclusion, the values of inflammatory markers; IL-6, LDH, and CRP gradually decreased in survivors from the time of admission to discharge, whereas Ddimer, Ferritin, and LDH drastically increased in non-survivors prior to their death

**KEYWORDS:**COVID-19, Inflammatory markers, IL-6, D-dimer, Ferritin, Lactate dehydrogenase, Procalcitonin, Cytokine storm.

## I. INTRODUCTION

Coronaviruses are categorized under the Coronaviridae family in the Nidovirales order which is known to produce mild respiratory diseases in humans. Corona represents crown-like spikes on the virus's outer surface of the spike glycoproteins (S); thus, it was named a coronavirus, as shown in fig.1. [1] The viral membrane glycoprotein (M) and envelope glycoprotein (E) are embedded in the lipid bilayer encapsulating the viral RNA. Coronaviruses are minute in size (65–125 nm in diameter) and contain a single-stranded RNA as a nucleic material, sizes varying from 26 to 32kbs in length. In the past two decades, the world has seen coronaviruses emerge and cause outbreaks that have considerably affected global health. The severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) cause acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) leads to pulmonary failure, thus resulting in fatality. These viruses were thought to only infect animals until a severe acute respiratory syndrome (SARS) outbreak caused by SARS-CoV (2002), in Guangdong, China. Then a decade later, another pathogenic coronavirus, known as Middle East respiratory syndrome coronavirus (MERS-CoV) caused an endemic in Middle Eastern countries. In December 2019, China reported an outbreak of pneumonia of unknown origin in the Wuhan city of Hubei Province. The outbreak was considered to have originally started through zoonotic transmission associated with the seafood market in Wuhan, China. Later, the human-to-human transmission was thought to play a major role in the subsequent outbreak. The virus behind the disease was labelled as SARS-CoV-2 and was named Coronavirus disease 19 (COVID-19). A pandemic was declared by the World Health Organization (WHO) on 11 March 2020, [2, 3] and as of current, no effective pharmacotherapeutic measures have been proposed for its treatment.

In May, India recorded the highest one-day tally with over 400,000 confirmed cases and 3523 fatalities of COVID-19, according to the Ministry

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of Health and Family Welfare. It is believed that surge in cases could be due to the Variants first identified in South Africa (known as 20H/501Y or B.1.351), Brazil (P.1), and the UK (B.1.1.7) circulating in India, alongside a newly identified distinct Indian variant (B.1.617) with two mutations (E484K and L452R) first reported in October, however, their extent of involvement remains unknown. [4]

**PATHOGENESIS OF COVID-19**

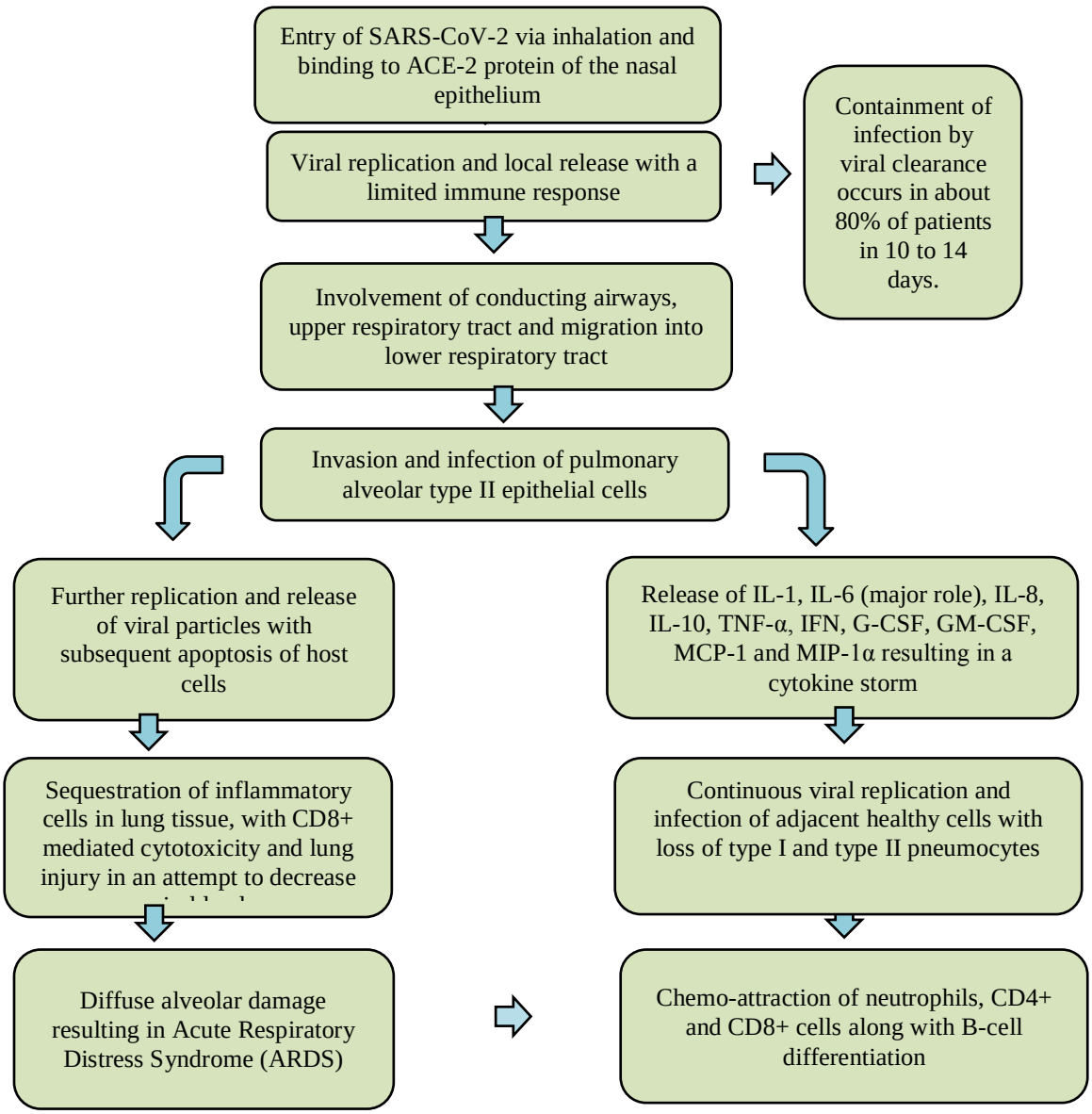


**Fig.1: Potential immunopathogenesis of SARS-CoV-2**

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## ASSOCIATION OF INFLAMMATORY MARKERS AND COVID-19

Several studies have stated that the host's inflammatory response induced by SARS-CoV-2 has a pivotal role in disease severity and mortality. However, the role of inflammatory markers in monitoring and predicting disease progression is still limited. There has been accumulating evidence about the importance of the inflammatory markers, particularly IL-6, D-dimer, ferritin, lactate dehydrogenase (LDH), C-reactive protein (CRP), procalcitonin (PCT), and neutrophil-lymphocyte ratio (NLR).

## IL-6

IL-6 is a pleiotropic cytokine that is produced at the site of tissue inflammation and gets released into the circulation by different cell types such as macrophages, lymphocytes, endothelial cells, and fibroblasts during sepsis and acute organ injury. Elevated levels of IL-6 have helped correlate with disease severity. Plasma and/or broncho-alveolar levels of IL-6 have been employed as early biomarkers of lung injury as well as predictive factors for the requirement of mechanical ventilation, organ dysfunctions, morbidity, and mortality in lung diseases. Multiple studies have implicated that there is an uncontrolled release of cytokines such as IL-1, IL-6, IL-8, IL-12, TNFα, IFN, and other inflammatory mediators which play a major role in reducing alveolar-capillary gas exchange, thereby leaving the pulmonary tissue deprived of oxygen. [5] Thus, evaluation of serum IL-6 levels may be useful as a prognostic tool in assessing disease progression and severity

## D-DIMER

D-dimer is a unique marker of plasmin-mediated fibrin degradation that is generated by sequential actions of the enzymes thrombin, activated factor XIII (factor XIIIa), and plasmin. The process begins when thrombin cleaves fibrinogen into fibrin monomers, which are polymerized to form a template for factor XIIIa and plasmin formation. Then factor XIII bound to fibrin polymers gets activated by thrombin to produce active factor XIII or factor XIIIa. The formation of covalent bonds between the D-domains in the polymerized fibrin is catalyzed by factor XIIIa. It is followed by degradation of cross-linked fibrin by plasmin to release degradation products in the form of D-dimer along with E-fragments. Abnormal coagulation mechanism was associated with elevated D-dimer (>1μg/mL), more commonly in deceased patients with COVID-19, however, this association is not fully understood, leaving scope for further research. [6]

## FERRITIN

Ferritin is a cytosolic protein that is essential for iron homeostasis and plays an important role in the storage of intracellular iron. Ferric iron is stored in the form of ferrihydrate mineral, which is encased in a spherical shell known as apoferritin. Serum ferritin is a well-known acute phase reactant that can be correlated with acute and chronic inflammatory conditions, hematologic, rheumatologic, and malignant disease. Hepatocytes, kupffer cells, proximal tubular renal cells, and macrophages have been shown to secrete ferritin in both in-vitro and in-vivo conditions. The elevated ferritin levels in inflammatory conditions reflect increased iron storage, but paradoxically, these stores are sequestered, making them unavailable for haematopoiesis resulting in anemia of inflammation.This deficiency associated with inflammation and malignancy is presumed to have developed as a defense mechanism to restrict the utilization of iron by pathogens and tumors. [7]

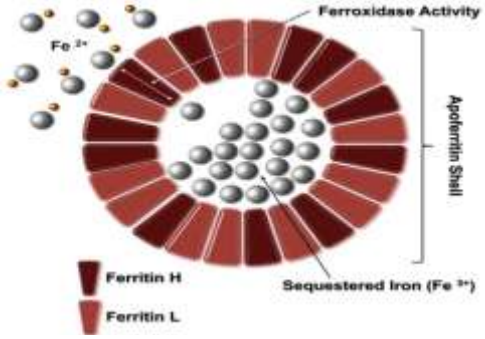


**Fig. 2: Structure of ferritin**

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**LACTATE DEHYDROGENASE (LDH)**

Lactate dehydrogenase (LDH) is an intracellular enzyme, belonging to the class of oxidoreductases. It can be found in almost all tissues but is found in high concentrations in muscle, liver, and kidney. LDH exists in five isomeric forms- LDH1, LDH-2, LDH-3, LDH-4, and LDH-5; assembled in tetramers of either of the two types of subunits, termed as muscle (M) and heart (H). It is implicated in anaerobic glycolysis that catalyzes the oxidation of pyruvate to lactate in the cells of most tissues and was found to be increased following tissue breakdown. Serum LDH has been routinely used clinically for assessing various diseases with poor prognoses, such as severe infections, sepsis, malignancies, and hemolysis. Recent studies have shown that patients with severe COVID-19 have elevated serum LDH levels.[8]

**CRP**

C-reactive protein (CRP) is an acute-phase protein that is a homopentamer in structure. It is primarily synthesized by the hepatocytes of the liver as well as by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. CRP is first produced as monomers (mCRP) and then gets assembled into pentamer (pCRP) in the endoplasmic reticulum (ER) and gets retained in the cell by binding to carboxylesterases (gp60a and gp50b). There has increasing evidence that CRP not only a marker of inflammation but also has a role in the inflammatory process, through activation of complement pathway and subsequent opsonization. Significant data has been accumulating that suggests that mCRP is present within atherosclerotic plaques and is detected within infarcted human myocardium, where it localizes with macrophages and exacerbates tissue injury.[9]

**PROCALCITONIN (PCT)**

Procalcitonin (PCT) is a 116-aminoacid peptide with a molecular weight of 14.5 kDa. It is the precursor of the hormone calcitonin, which is involved in the homeostasis of calcium and phosphorus. The production of PCT occurs in the thyroid cells and is governed by the calcitonin-1 (CALC-1) gene on chromosome 11. Peak levels of PCT are found at 24 to 48 hours after initiation of sepsis. Patients with localized infection have a relatively lower PCT level than those with generalized sepsis, severe sepsis, and septic shock. [10]It is also recognized as a useful tool in guiding antibiotic therapy. Emerging studies have shown that a PCT-guided approach to determine antibiotic therapy and its duration can help avoid unnecessary antibiotic administration. [11]Therefore, PCT has been frequently used as a biomarker to aid in the diagnosis of a bacterial infection or sepsis, to assess the severity of systemic inflammation, and might help in identifying and preventing sepsis-related mortality, if employed at an early stage in COVID-19.

**Neutrophil-Lymphocyte Ratio (NLR)**

Inflammation and immunity play a crucial role in several chronic conditions. The neutrophil-to-lymphocyte ratio (NLR), is estimated as a simple ratio between the neutrophil and lymphocyte counts, measured in peripheral blood. It is a biomarker that is used to contemplate the balance between acute and chronic inflammation. Two features of the immune system: one is indicated by the neutrophil count and the other, adaptive immunity by lymphocyte count. Thus, an elevated NLR indicates a variance of the inflammatory response and thus it can be considered a predictable indicator of disease severity. The acceptability of NLR in detecting viral diseases can be deemed useful, as NLR was reported to be a more sensitive predictor in influenza patients compared to neutrophil or lymphocyte counts individually. In some other studies, NLR was introduced as an independent indicator of clinical outcomes in COVID-19 patients and according to E. V. Moradi et al, higher NLR values were associated with an increased risk of mortality. [12, 13]

## II. METHODOLOGY

The study was conducted with the approval of the Institutional Ethical Committee (IEC), with the designated approval number: AIG/IEC-Post BH&RV 12/02.2021-03 for protocol number: PRIBCOVID 01, dated 27 January 2021. This study was conducted for the duration of six months i.e. from the month of October 2020 to March 2021. The data was collected from the medical records of the COVID-19 confirmed patients who were hospitalized during the months of August 2020 to January 2021.

**SELECTION CRITERIA**

INCLUSION CRITERIA:

1. Age>18 years
2. Laboratory nasopharyngeal swab reverse transcription-polymerase chain reaction (RT-PCR) confirmed COVID-19.

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3. Hospitalized patients at any clinical stage of the disease, i.e., mild, moderate, or severe COVID-19
4. Hospitalized patients with or without co-morbid conditions.
5. Deceased patients with COVID-19.

EXCLUSION CRITERIA:
1. Age < 18 years
2. Pregnant women.
3. Suspected/active autoimmune disorders.
4. Suspected/active bacterial, fungal, viral, or any other infection (besides COVID-19).
5. Concurrent treatment with other agents with actual or possible direct-acting anti-viral/anti-inflammatory activity against SARS-CoV-2 within 24 hours or before hospitalization.
6. Those who do not meet the inclusion criteria

**SAMPLE SIZE:**
The study was carried out with the data collected from 151 patients who were hospitalized with COVID-19 from August 2020 to January 2021.

**STUDY DESIGN:**
This study is a retrospective single-centre observational study.

**STUDY PROCEDURE:**
After obtainingpermission to access the patient's medical records all the eligible participants' data was collected in Case Report Form (CRF). The following data was collected:

- Demographic details such as age, gender, major medical history
- Symptoms on admission
- Final diagnosis
- Laboratory data since admission till discharge/death
- Types of pharmacotherapeutic agents administered
- RT-PCR status

**STATISTICAL ANALYSIS:**
The acquired data was entered into MS EXCEL 2010. Quantitative variables were summarized using descriptive statistics (number of observations, percentages, mean, standard deviation) and chi-square test was used for obtaining study results. The data was then statistically analysed using Statistical Package for the Social Sciences (SPSS) software version 20.

## III. RESULTS

This observational retrospective study was performed among 151 hospitalized COVID-19 RT-PCR positive patients, who fulfilled all the inclusion criteria and exclusion criteria. The data collected includes 96 survivors (discharged patients) and 55 non-survivors (deceased patients).

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**1. AGE DISTRIBUTION**
The survivors and non-survivors were categorized into different age groups is graphically shown below:

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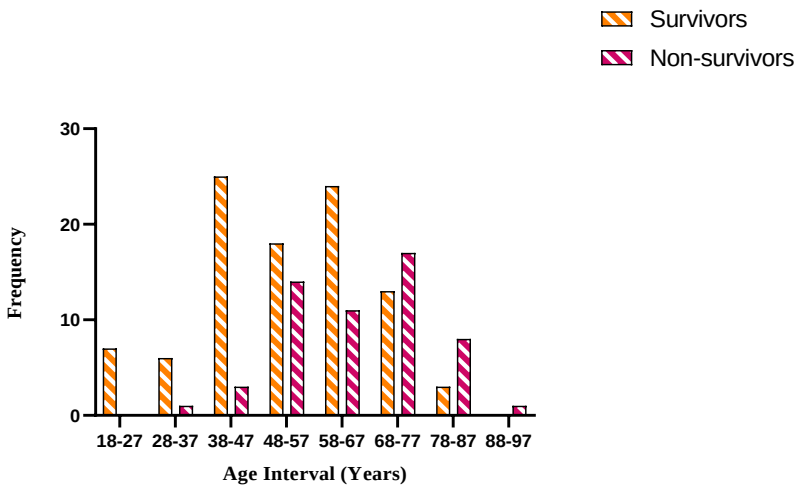


**Fig. 3: Graphical representation of Age-Wise Distribution (in years)**

**2. GENDER DISTRIBUTION**

The gender distribution of the survivors and non-survivors is graphical representation as follows:

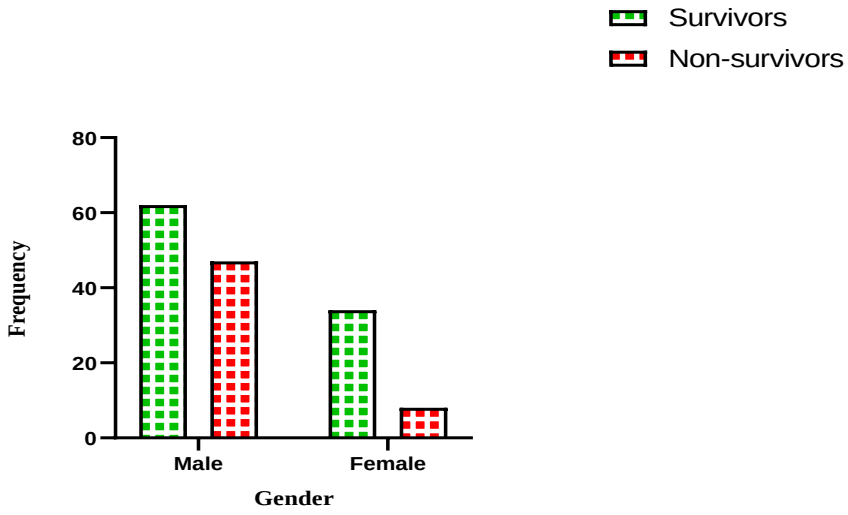


**Fig. 4: Graphical representation of Gender-Wise Distribution**

**9. INFLAMMATORY MARKERS**

The inflammatory markers studied in survivors and non-survivors are IL-6, D-dimer, Ferritin, LDH, CRP, Procalcitonin, and NLR, which are tabulated as shown below:

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| Marker | On Admission | On Discharge | P-Value |
|---|---|---|---|
| IL-6 | | | |
| Survivors | 28.1±21.29 | 16.34±9.72 | 0.0111* |
| Non-survivors | 120.4±110.4 | 164.1±104.1 | 0.1430 |
| D-dimer | | | |
| Survivors | 289.1±1462 | 288.2±166.5 | 0.0879 |
| Non-survivors | 691.6±5189 | 1796±545 | <0.0001* |
| Ferritin | | | |
| Survivors | 392.9±1492 | 355.3±309.4 | 0.3436 |
| Non-survivors | 819.2±457.4 | 1109±688 | 0.0081* |
| LDH | | | |
| Survivors | 519.5±208.2 | 489±177.9 | 0.0465* |
| Non-survivors | 850.6±509.7 | 1218±704.1 | <0.0001* |
| CRP | | | |
| Survivors | 42.23±31.86 | 15.96±11.21 | 0.0001* |
| Non-survivors | 87.74±57.9 | 70.39±58.06 | 0.1631 |
| Procalcitonin | | | |
| Survivors | 1.03±0.18 | 0.88±0.14 | 0.8581 |
| Non-survivors | 3.22±1.19 | 5.35±3.80 | 0.1940 |
| NLR | | | |
| Survivors | 10.89±6.36 | 3.32±1.26 | 0.3567 |
| Non-survivors | 13.1±8.61 | 9.26±4.52 | 0.2113 |

**Table 1: Inflammatory Markers**

### 12. CLINICAL IMPROVEMENT

In survivors and non-survivors, the outcome of hospitalization in the form of clinical improvement in COVID-19 is tabulated and graphically represented below:

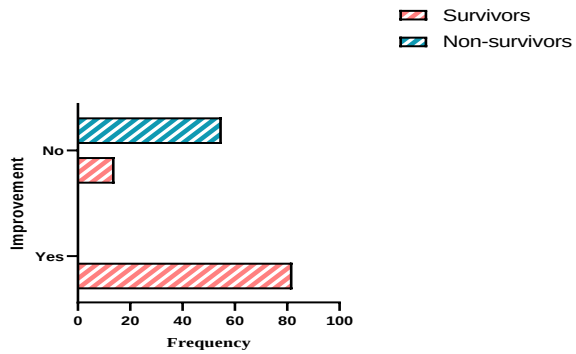


**Fig. 5: Graphical representation of Clinical Improvementin Survivors and non-survivors**.

## V. DISCUSSION

The ongoing pandemic of COVID-19 is a major health crisis that has led to unprecedented changes in the lives of millions globally. Currently, it is still unclear when the pandemic is likely to subside. Therefore, identification of the disease at an early stage is crucial to contain the rapid spreading of the virus

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- It is known that SARS-CoV-2 can infect a population of any age group, but elderly patients are at greater risk of succumbing to the disease. In this study, out of 151 patients, 96 were survivors and 55 were non-survivors of COVID-19. The highest percentage of hospitalized patients, who did not survive COVID-19. i.e. 31% of the patients were between the ages of 68-77 years.
- a statistically significant difference exists in the gender-wise distribution in survivors and non-survivors ($p<0.05$). Among hospitalized COVID-19 patients, the percentage of males admitted for treatment was greater than females, both in survivors (65%) as well as non-survivors (85%).

- IL-6 in survivors during admission showed a mean value of 28.1±21.29 and on discharge 16.34±9.72 ($p=0.0111$), thus a statistically significant difference exists between these IL-6 values, depicting a decrease in IL-6 value from their admission to the discharge. IL-6 non-survivors with mean values of 120.4±110.4 and 164.1±104.1, on admission and discharge respectively, showed no significant difference ($p=0.1430$). In between survivors and non-survivors, a large difference of mean value is seen, as illustrated in table 1.

- The mean values of D-dimer in survivors ($p=0.0879$) during admission and discharge are 289.1±146.2 and 288.2±166.5 respectively. They showed no significant difference while in non-survivors ($p<0.0001$) with the mean of 691.6±518.9 on admission and 1796±545 on discharge shows a statistically significant difference. Hence, there was a large difference in mean values between survivors and non-survivors, as shown in table 1.

- The mean values of Ferritin in survivors ($p=0.3436$) during admission and discharge are 392.9±149.2 and 355.3±309.4 respectively, they showed no significant difference while in non-survivors ($p=0.0081$) with the mean of 819.2±457.4 on admission and 1109±688 on discharge shows a statistically significant difference. Hence, there was a large difference in mean values between survivors and non-survivors.

- As illustrated in table 1, the mean values of LDH in survivors ($p=0.0465$) during admission and discharge are 519.5±208.2 and 489±177.9 respectively, they showed statistically significant difference while in non-survivors ($p<0.0001$) with the mean of 850.6±509.7 on admission and 1218±704.1 on discharge also showed statistically significant difference. Hence, there was a large difference in mean values between survivors and non-survivors.

- The mean values of CRP in survivors ($p=0.0001$) during admission and discharge are 42.23±31.86 and 15.96±11.21 respectively showed a statistical significant difference, while in non-survivors ($p=0.1631$) with the mean of 87.74±57.9 on admission and 70.39±58.06 on discharge showed no significant difference. Hence, there was a large difference in mean values between survivors and non-survivors.

- The mean values of Procalcitonin in survivors ($p=0.8581$) and non-survivors ($p=0.1940$) during admission and discharge are 1.03±0.18, 0.88±0.14 and 3.22±1.19, 5.35±3.80, respectively showed no statistically significant difference, as shown in table 1.

- The mean values of NLR in survivors ($p=0.3567$) and non-survivors ($p=0.2113$) during admission and discharge are 10.89±6.36, 3.32±1.26, and 13.1±8.61, 9.26±4.52 respectively showed no statistically significant difference.

## VI. CONCLUSION

In view of the analyzed data, we observed that majority of survivors are in the age group of 38-47 years, while non-survivors are 68-77 years. The majority of non-survivors are male (85%). IL-6, LDH, CRP values gradually decreased in survivors from admission to discharge. D-dimer, Ferritin, LDH drastically increased in non-survivors before death. Therefore, through close monitoring of D-dimer, Ferritin, and LDH, physicians can make informed decisions and implement adequate management strategies to improve outcomes for patients affected with COVID-19.

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