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### RESEARCH ARTICLE

#### STUDY OF INFLAMMATORY MARKERS IN HYPOXIC COVID 19 PATIENTS AND CORRELATION WITH DISEASE SEVERITY

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#### Abstract

**Background:** COVID-19 is global pandemic affecting mainly a respiratory system and causing hypoxemia in the majority of those been infected. In our study, we aimed to correlate the inflammatory markers with severity of hypoxemia and predicting the significant markers of disease severity.

**Aims and Objectives:** To correlate the inflammatory markers with degree of hypoxemia and To determine the inflammatory markers useful in predicting the disease severity.

**Materials and Methods:** A cross-sectional observational study was conducted in the month of may 2021 in tertiary care hospital chinnaoutapalli near Vijayawada in Andhra Pradesh. The 296 patients diagnosed as COVID-19 positive by real-time Polymerase chain reaction and admitted in our hospital requiring oxygen (hypoxemic) were included. These patients were divided into supplemental oxygen group (SuO<sub>2</sub> Group) and non invasive ventilation group (NIV group) and inflammatory markers among these groups were compared.

**Results:** Out of 296 hypoxemic patients, 148 were in ward, requiring SuO<sub>2</sub>, 148 were in ICU requiring NIV. There was significant differences in mean D-dimer (p=0.001), and neutrophil-to-lymphocyte ratio (p=0.012), among NIV and SuO<sub>2</sub> group. There was no significant difference in CRP, ferritin.

**Conclusion:** The inflammatory markers are a good guide for predicting the hypoxemia and disease severity. The results concluded Neutrophil-to-Lymphocyte ratio, D-Dimer were effective biomarkers in predicting a severity and prognosis of COVID-19 patients, but could not establish significant association of serum Ferritin, CRP with disease severity.

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### Introduction:-

A mysterious pneumonia like illness causing virus was identified in end of 2019 in Wuhan , Hubei province of China (1) and named as SARS CoV-2 (severe acute respiratory syndrome Coronavirus -2) and the disease is named as COVID-19 (2). It has rapidly evolved into a global pandemic affecting individuals all over the world. Coronavirus family consists of single-stranded enveloped viruses and commonly causes respiratory, neurologic, hepatic, and enteric illnesses. They are divided into 4 subgroups: alpha, beta, gamma, and delta, with human infections caused mainly by the alpha and beta CoVs. Previous epidemics caused by the beta CoVs were due to the SARS-CoV and MERS-CoV (Middle Eastern Respiratory Syndrome Coronavirus) (1). Primarily it was a respiratory tract infection, emerging researches indicate that covid-19 causes an illness which has a wide variety of clinical features, ranging from mild to moderate upper respiratory tract infection to severe systemic disease which involves respiratory as well as other body systems including cardiovascular, gastrointestinal, neurological, immunological and hematopoietic system (3). In spite of worldwide awareness of the infectiousness of COVID-19 and the protective measures, initiatives taken by people, it has already infected more people than SARS. This shows that SARS-CoV-2 is much more contagious (4). Hematological abnormalities in COVID-19 are related with disease progression, severity and mortality. The neutrophil-to-lymphocyte ratio (NLR) is a prominent predictor of disease severity in COVID-19, and levels above 9 determine poor prognosis and high mortality within patients of intensive care units (5). C-reactive protein (CRP) is also an acute-phase reactant whose concentrations significantly raised during sepsis as well as the pro-inflammatory process by the COVID-19 induced inflammatory cytokines (6). Ferritin is an important acute phase reactant which was responsible for the cytokine (pro-inflammatory) storm of COVID-19. These pro-inflammatory cytokines can ultimately lead to Acute Respiratory Distress Syndrome (ARDS) and Multiple Organ Failure (MOF) (7). D-dimer is a measure of the coagulation cascade, and in a way assesses the severity and the risk for the patient to develop sepsis and septic shock (8). Lymphopenia, thrombocytopenia, abnormal coagulation profile and sepsis leading to disseminated intravascular coagulation (DIC) is very well documented in patients of COVID-19. Based on the proinflammatory effects of all the above-mentioned markers and seeing that the main immunopathological mechanism behind severe COVID-19 infection is the cytokine storm. Treatment in intensive care units has become a major challenge, early recognition of severe forms of COVID-19 is absolutely essential for timely triaging of patients, hence we decided to assess their roles as effective prognostic biomarkers for the disease severity and provide a better guide for prompt management of patients which help in decreasing the disease morbidity and mortality.

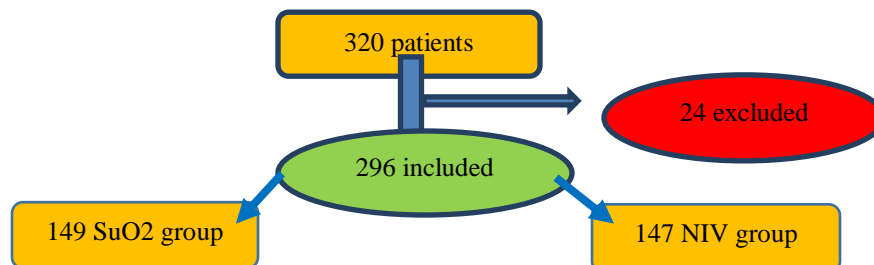
### Aims And Objectives:-

1. To correlate the inflammatory markers with degree of hypoxemia and
2. To determine the inflammatory markers useful in predicting the disease severity.

### Materials And Methods:-

#### Study Population

The study was conducted as a single centered, retrospective observational study in Pinnamaneni Siddhartha hospital, Chinnaoutpalli near Vijayawada, Andhra Pradesh. The data of 320 patients who were diagnosed as COVID-19 positive via either nasopharyngeal or oropharyngeal swab for Real-time Polymerase chain reaction above age of 18 years of both sexes in month of May 2021 were included. Out of 320 patients, 24 were excluded as data of blood investigations was not available. A total of 296 COVID-19 patients requiring O<sub>2</sub> therapy were divided into supplemental O<sub>2</sub> (SuO<sub>2</sub>) and non invasive ventilation (NIV) groups of 149 and 147 patients respectively. SuO<sub>2</sub> group is defined as patients with room air saturation <90% and >80%. NIV group is defined as room air saturation <80%. All of the patients were subjected to the estimation of serum D-dimer, CRP, ferritin, and neutrophil to lymphocyte ratio.



## Enrolment of patients

### Study sampling

Venous blood samples of 5ml is collected from both the study groups and divided into two tubes (plane tubes and Ethylenediaminetetraacetic acid [EDTA] tubes). The first part of the blood samples (EDTA tubes) is applied to the hematology autoanalyzer (Pentra 80. manufactured by the ABX-Horiba group, Minami-Ku Kyoto Japan). The second part of the blood samples was centrifuged to obtain serum which is used for the determination of serum concentration of D-Dimer, CRP, and ferritin.

### Statistics

The results of the current study are analyzed using (SPSS Statistics 20; 1 New Orchard Road Armonk, New York 10504- 1722 USA) to find the variation in the results of the study. Statistical significance was defined as a ( $P < 0.05$ ). Statistical tests such as (independent t-test, percentage) were used for data analysis.

### Results:-

The results of the current study, which included 296 patients of which 149 were SuO2 Group and 147 were NIV group suffering from SARS CoV2 infection were compared. Among 149 patients in SuO2 group 89 (59.7%) were males and 60 (40.3%) were females. Among 147 patients in NIV group 88 (59.8) were males and 59 (40.2%) were females. Though there was no significant gender differences between both the groups, males have higher risk of infection than females. Out of 149 patients in SuO2 group 24 (16.1%) were patients less than 40years of age, 99 (66.4%) were patients between 40-60 years of age and 26(17.5%) were patients aged more than 60years. Out of 147 patients in NIV group 24(16.3%) were patients less than 40years of age, 98(66.6%) were between 40-60 years of age and 25(17.1%) were aged more than 60years. This showed that middle aged patients between 40-60years of age were at highly infected in second wave of covid-19 pandemic. Diabetic patients were 70(47%) in SuO2 group and 74 (50.3%) in NIV group, hypertensives were 40(26.8%) in SuO2 group and 36 (24.5%) in NIV group. Smokers were 34 (22.8%) in SuO2 group and 28(19.1%) in NIV group, 40(26.8%) patients were alcoholics in SuO2 group and 32 (21.8%) were alcoholics in NIV group .This shows that there is no significant difference in comorbidities between both groups. (Table-1)

	• NIV(n=147)	• Su02(n=149)
<b>GENDER</b>	•	•
• Male	• 88(59.8)	• 89(59.7)
• Female	• 59(40.2)	• 60(40.3)
<b>AGE</b>		
• <40yrs	• 24(16.3)	• 24(16.1)
• 40-60yrs	• 98(66.6)	• 99(66.4)
• >60yrs	• 25(17.1)	• 26(17.5)
	•	•
<b>Comorbidities</b>		
• Diabetes	• 74	• 70
• Hypertension	• 36	• 40
• Smoking	• 28	• 34
• Alcohol	• 32	• 40

**Table 1:-** Demographic Characteristics.

Inflammatory markers were studied in both the groups. Mean CRP was 71.3 in NIV and 101.4 in suO2 groups with p value 0.001 which is significant. Mean NLR was 11.19 in NIV and 8.5 in suO2 groups with p value 0.12 which is significant. (Fig-1)

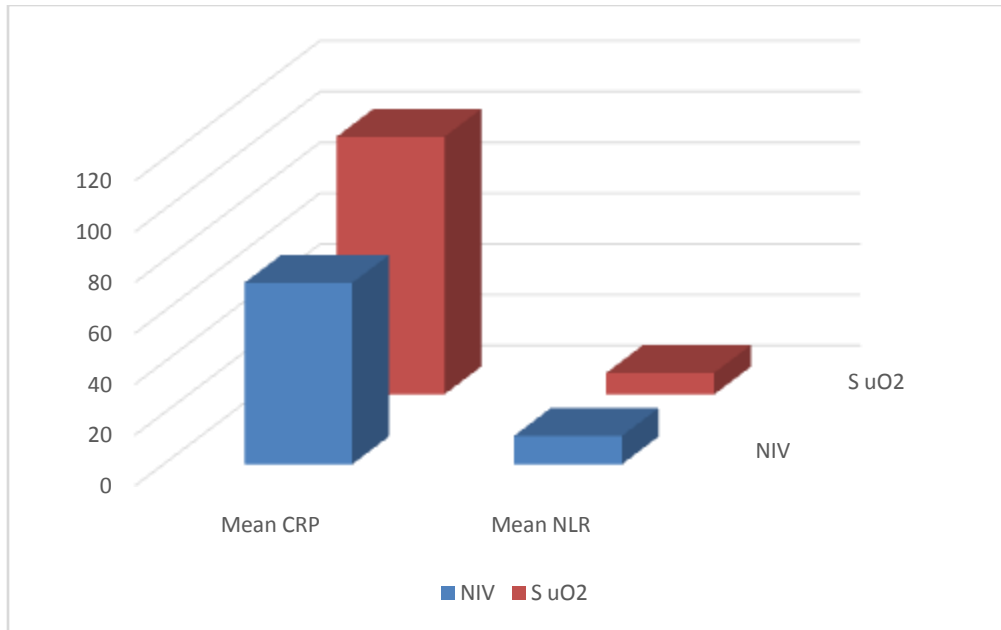


Fig 1:- Comparison of Mean CRP and Mean NLR between both group.

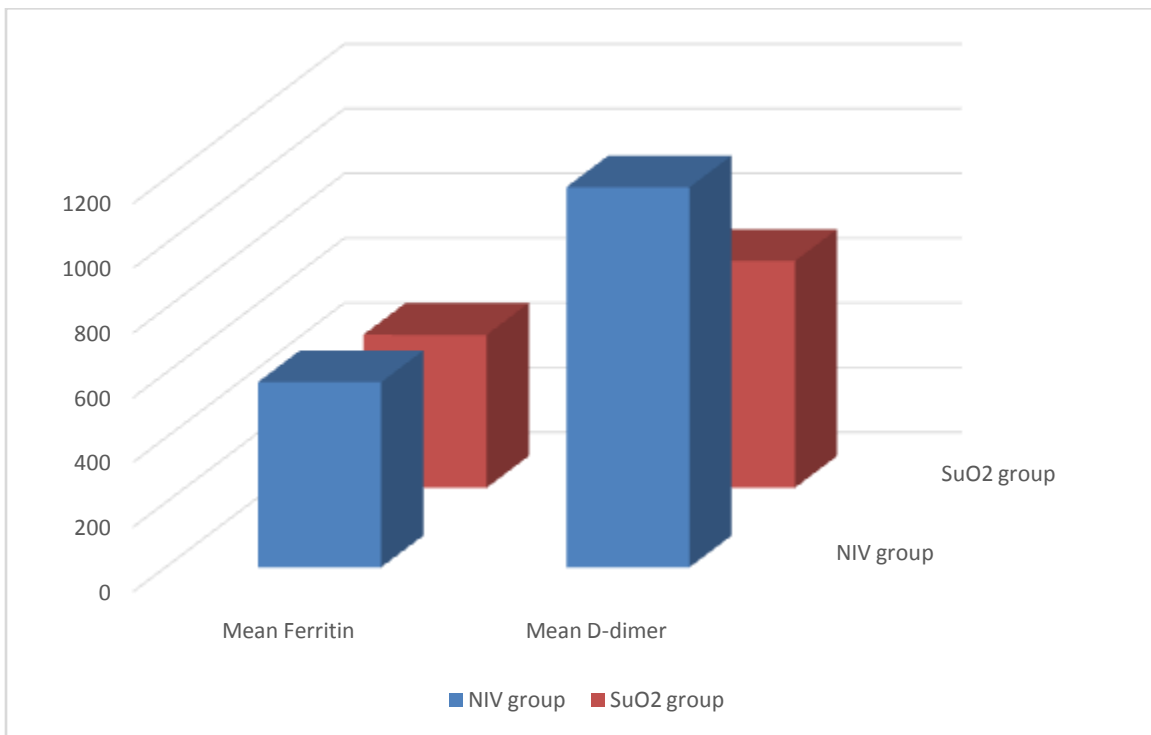
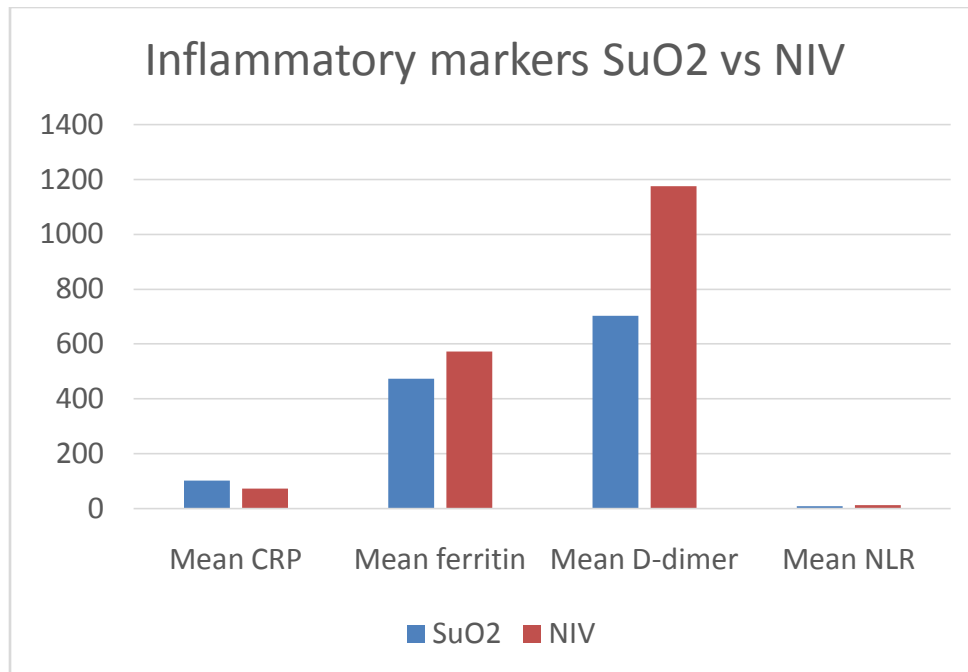


Fig 2:- Comparison of Mean ferritin and D-dimer between both groups.

Mean ferritin was 572.5 in NIV and 473 in suO2groups with p value 1.61 which is not significant. Mean D-dimer was 1175 in NIV and 701in suO2 groups with p value 0.001which is significant. (Fig -2)



**Fig-3:-** Summary of Inflammatory markers in SuO2 group vs NIV group.

### Discussion:-

Based on the data from previous studies it was observed that inflammatory markers and some of the hematological parameters had a role in predicting the course of disease. In our study we have studied the effects of readily available inflammatory markers and hematological parameter on disease severity. In our study the covid -19 infection rate is more in males than females in both groups which is in accordance with S. Taj et al and Jin JM et al who reported that according to the clinical classification of severity, men had more severe disease than the women [3, 9) and contrary to kadhim and Abdullah et al who reported in study conducted in Baghdad city, 60% of the SARS CoV2-infected patients were women and 40% were men(10). Multiple factors contribute to the disparity in sex- specific disease outcomes following virus infections like Sex- specific steroids and the activity of X- linked genes, both of which modulate the innate and adaptive immune response to virus infection, influence the immune response. The differences in the expression of angiotensin-converting enzyme (ACE) 2 receptor and the cellular serine protease TMPRSS2, which are necessary for the binding and priming of SARS CoV2, may have an important role(11).In our study patients aged between 40-60years are at higher infection risk in second wave of covid -19 which is in accordance with Hashem et al study who reported that age of patients admitted to ICU was significantly higher than that of those who did not need ICU admission ( $57.4 \pm 14.0$  vs  $45.1 \pm 17.1$ ,  $p < 0.001$ ) (12). In our study there is no significant difference in comorbidities between both groups as both had moderate to severe infection and mild disease patients were not included. Our results were contrary to Hashem et al (12).Mean CRP was significantly higher in SuO2 group than NIV group which is in contrary to many studies(3,10,12), this can be explained as the mean CRP in both groups were 5-10 times higher than normal range ,but due use of high doses of steroids(10-12mg/kg methyl prednisone) and biologics (tocilizumab etc) in NIV group compared to SuO2 group who are on low dose intravenous steroids or oral steroids and no biologics and other immunosuppressive drugs were used in this group . In COVID-19 patients, CRP levels were increased and it was shown that survivors had a median CRP of approximately 40 mg/L, while nonsurvivors had a median CRP of (125 mg/L), suggesting a good association with the severity and prognosis of the disease(13).In our study there is no significant difference in serum ferritin between both study groups which was in accordance with hashem et al study(12)who showed after adjustment of significant factors in multivariate logistic regression, only anemia, high NLR, high PLR, and high D-dimer levels showed higher risk for ICU admission and our study is contrary to S Taj et al (3) .. The mean serum ferritin in both groups is higher than 1.5 times normal range which indicates that ferritin is higher in both groups but no significant difference between groups because mild cases are not included .Elevated ferritin levels due to secondary hemophagocytic lymphohistiocytosis and cytokine storm syndrome have been reported in severe COVID-19 patients. It has been proposed that COVID-19 could be part of the broader spectrum of hyperinflammatory syndromes such as the secondary hemophagocytic lymphohistiocytosis.(14) Hyperferritinemia is a cardinal

characteristic of these syndromes. COVID-19 severity and worse prognosis mean that mortality could be due to virally induced hyper inflammation which is substantially associated with high ferritin level. However, the circulating levels of ferritin can not only represent a strong phase response but play an important role in inflammation(14). In our study mean D-dimer is very significantly higher in NIV group compared to SuO2 group which is in accordance with many studies (3,10,12). We agree with Zhou et al as D-dimer elevation  $> 1 \mu\text{g/L}$  was the strongest independent predictor of mortality (15) and assess degree of hypoxemia due microemboli at capillaries level which when failed can lead to MODS. D-dimer levels correlate with the severity of the disease and are a dependable prognostic indicator for the hospital mortality in the admitted patients with COVID-19. The elevated D-dimer signify a hyperfibrinolysis state and increased inflammatory burden induced in SARS-COV-2 infection [16]. D-dimer  $> 0.9 \text{ mg/L}$  found to be a sensitive predictor for ICU admission in patients with COVID-19 infection(12). Mean NLR was significantly higher in NIV group compared to SuO2 group which is in accordance with many studies (3,10,12) which implies that this can be used as independent predictor of disease severity. Neutrophil-to-lymphocytosis is a stress and immune parameter. For COVID-19, the increased neutrophils indicate the degree of the inflammatory response, and the decreased lymphocytes indicate the degree of immune imbalance. These associations are amplified by the concept of NLR (17). The normal values of NLR in adults are in the range between 1.0 and 2.3 (18). We believe that NLR and D-dimer are investigations which help in predicting very poor prognosis between severe and critically ill COVID-19 patients though other parameters like CRP and Ferritin values are also elevated than normal range correlation with severity of disease could not be established.

### Conclusion:-

We studied the effect of various inflammatory markers on the hypoxemia and prognosis of COVID-19. Our findings can help in the treatment, effective management, and risk stratifying strategies of COVID-19 patients. The results from our study concluded that the inflammatory markers especially D-dimer and NLR ratio are good predictors of the degree of hypoxemia and disease severity. These markers also predict the adequacy of treatment or require escalation, hence useful in predicting prognosis of moderate to severely ill and critically ill COVID-19 patients. We could not establish significant associations of serum Ferritin and CRP in predicting the severity of COVID-19 disease and hypoxemia.

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