

# THE LEVEL OF INFLAMMATORY MARKERS AMONG THE VACCINATED AND UNVACCINATED GROUPS IN COVID-19 RT-PCR POSITIVE PATIENTS

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**Abstract-** 2019 novel coronavirus (2019-nCoV) or the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)- a public health crisis. COVISHIELD- developed by AstraZeneca/Oxford University and manufactured by the Serum Institute of India (SII). Inflammatory Markers predicts adverse outcomes and severity of disease in patients with COVID-19 infection. **Aim of the study** is to evaluate the level of inflammatory markers among the vaccinated and unvaccinated groups in COVID-19 RT-PCR positive patients. **Results:** The level of inflammatory markers are elevated among the unvaccinated groups when compared to vaccinated. Disease severity, oxygen requirement and number of days hospitalised were lower in Vaccinated group.

**Index Terms-** Inflammatory markers, Vaccination, RT-PCR, Covid-19

## INTRODUCTION:

Cytokines are important factors in the mediation, pathogenesis and progression of novel coronavirus disease (COVID-19) [1]. Cytokine secretion is triggered after internalized virus contacts epithelial cells, macrophages, or dendritic cells [2]. There are cytokines that correlate with the severity of COVID-19, cytokines associated with disease development, and cytokines that can be used as predictors of severe infections. Interleukin-6 (IL-6) is an indicator of activation of the innate immune response. It increases rapidly until it reaches a peak at the onset of symptoms, after which it begins to decline slightly [3]. Overproduction of proinflammatory cytokines causes oversecretion of procalcitonin [4]. Excessive inflammatory responses expressed by cytokine storm, macrophages, and endothelial activation, along with diffuse intravascular coagulation, patient immobilization and hypoxia, can lead to venous thromboembolism. Infected patients are at risk for arterial thrombosis, venous thrombosis, venous thromboembolism, deep vein thrombosis, and pulmonary embolism. D-dimer levels are increased in patients with venous thromboembolism [5]. Ferritin is known to be an acute-phase marker of inflammatory diseases, but it is still unclear whether its role mediates or is a consequence of inflammation. It can also reveal the extent of acute or chronic inflammation in many diseases [6]. Lactate dehydrogenase (LDH) isoenzymes are released following cell injury or apoptosis. They may exhibit vascular permeability in immune-mediated lung injury [7].

According to the World Health Organization (WHO), some people infected with SARS-CoV-2 remain asymptomatic, while others develop symptoms. Most people develop mild or moderate COVID-19, but some develop severe illness that requires oxygen supply, respiratory failure, acute respiratory distress syndrome, and multiple organ failure. , a minority of patients develop serious illness with complications such as thromboembolism [8].

Due to the genetic variation of the virus over time, several worrying SARS-CoV-2 variants have emerged during the pandemic. These emerging variants (alpha, beta, and delta) have several mutations that affect their behavior and may affect the transmissibility and virulence of the virus [9]. In November 2021, a worrying new variant called Omicron was reported, which triggered the fourth wave of COVID-19, which has spread around the world. Omicron variants were associated with reduced severity, affecting younger age groups with higher vaccination coverage [10].

During the pandemic, hospitals were overcrowded and some patients were even denied hospital care. To properly screen COVID-19 patients who develop respiratory failure and may require hospitalization, the following criteria may be helpful: Symptoms, oxygen saturation, respiratory rate, chest imaging, laboratory tests, underlying disease. It is important to note that these criteria are not exhaustive and clinical judgment should always be used when evaluating COVID-19 patients. Classifying patients based on their risk of developing severe or severe COVID-19 at first presentation not only saves lives, but also the economic impact of triaging critically ill patients. Inflammatory markers such as IL-6, C-reactive protein (CRP), D-dimer, LDH, procalcitonin, and ferritin, as well as patient age and gender, can be used as risk indicators. Altering levels of inflammatory markers can distinguish between severe and mild/moderate cases of COVID-19 [11]. Ultimately, the decision to hospitalize a patient should be based on a careful evaluation of the patient's clinical status and laboratory tests. Our aim was to record and analyze the concentrations of the above inflammatory markers according to mild, moderate, severe and severe forms of SARS-CoV-2 infection, vaccination status and viral variants. bottom.

## AIM OF THE STUDY

To evaluate the level of inflammatory markers among the vaccinated and unvaccinated groups in COVID-19 RT-PCR positive patients

**MATERIALS AND METHOD**

Patient selection

60 patients both vaccinated and not vaccinated for covid-19 were taken up for the study.

Inclusion criteria:

Patient aged 18-60 years

Both vaccinated and unvaccinated age group

Exclusion criteria:

Patients who refused to give consent

Sampling technique

Consecutive, convenient sampling

Study design

Prospective cohort study

Study setting

Dhanalakshmi Srinivasan Medical College and Hospital

Study period 4 months (December 2021 to March 2022)

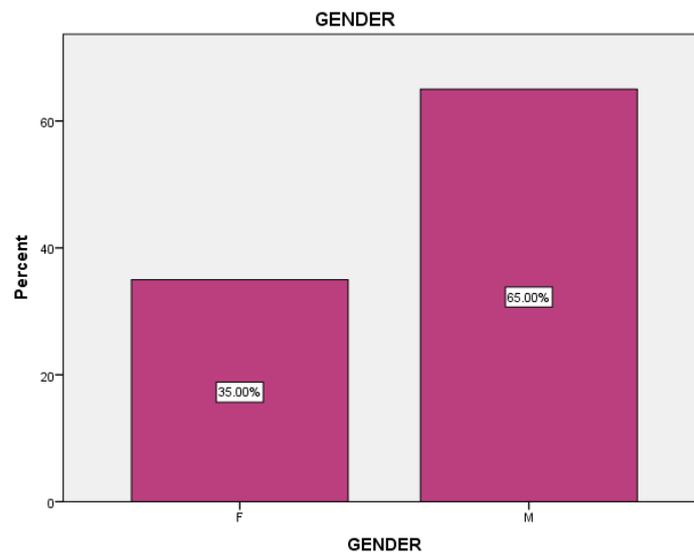
Methodology

Vaccination status of the patients were identified, blood samples were collected and test for RT-PCR status and IL-6, D-Dimer and CRP levels were done.

Statistical analysis

Association between factors using Chi-square and Fishers exact test with Yates correction using Stata Software.

**RESULTS**



**Figure 1: Gender of study participants**

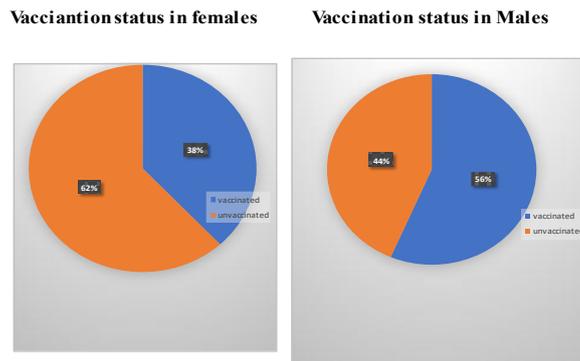
Among the study participants, 35% were female and 65% were males.

**TABLE 1: Vaccination status and Gender of study participants**

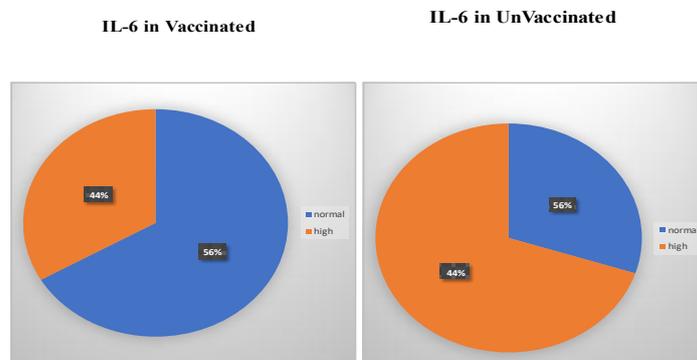
RESULTS

		VACCINATION STATUS		Total
		UNVACCINATED	VACCINATED	
GENDER	F	13	8	21
	M	17	22	39
Total		30	30	60

Among the unvaccinated participants, 13 were female and 17 were males and among the vaccinated group of participants, 8 were female and 22 were males.

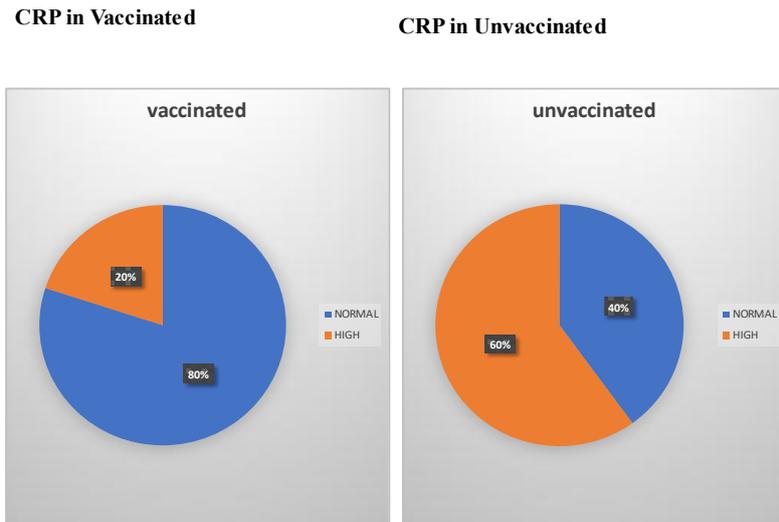


**Figure 2: Vaccination status among male and female participants**



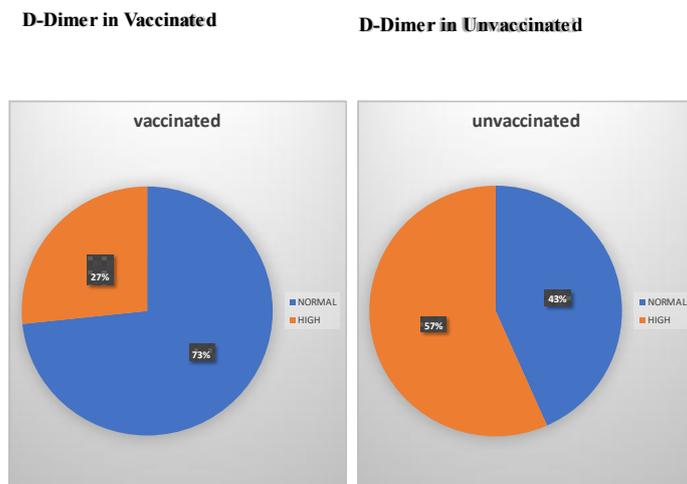
**Figure 3: Level of IL6 among the study participants**

Among the vaccinated group 44% had normal IL-6 levels and remaining 56% had high level of IL-6. Among unvaccinated group 44% had high level of IL6. It was noticed that there was a significant association between IL-6 inflammatory marker and RT-PCR positive status. And it was noted that the IL-6 levels were high among unvaccinated groups with the likelihood ratio of 74.34,  $p < 0.001$ .



**Figure 4: CRP in Vaccinated and unvaccinated group**

Among the vaccinated participants, 20% had high level of CRP while among unvaccinated, 60% had high level of CRP. There is a significant association between CRP inflammatory marker and RT-PCR positive status. And it was noted that the CRP levels were high among unvaccinated groups with the likelihood ratio of 10.210. p value was significant at 0.001. Similarly there was significant association between D- Dimer value and Vaccinations status. Likelihood ratio of 36.15 and p value <0.001. Among the vaccinated group D- dimer was high among 27% and among unvaccinated group 57%.



**Figure5: D-Dimer value among vaccinated and Unvaccinated participants**

**DISCUSSION**

In the current study, we analyzed the inflammatory response of 120 COVID-19 patients, hospitalized in Medical college, during the fourth and the fifth waves of the COVID-19 pandemic. We chose to include patients with any severe form of COVID-19, aiming to compare the parameters according to the disease’s severity and the vaccination status prior to infection. All the vaccinated patients were vaccinated with two doses of a mRNA vaccine. We chose to analyze the IL-6,CRP and D-dimer in the serum collected on the first day.

IL-6, CRP and D-dimer, concentrations increased as the severity of the disease increased ( $p < 0.05$ ). The mean IL-6 concentration that we calculated for each of the four studied subgroups was in the range of values obtained by other author.

Table 2: Comparison of the IL-6 mean value with the results obtained by other authors, depending on COVID-19 severity (pg/mL).

Authorship	Mild Form	Moderate Form	Severe Form	Critical Form	Reference
Sun et al.	5.26 ± 1.25	14.17 ± 11.37	33.22 ± 31.9	34.09 ± 26.47	[13]

Authorship	Mild Form	Moderate Form	Severe Form	Critical Form	Reference
Liu et al.		39.3 ± 71.1	55.6 ± 44.4	81.4 ± 65.6	[14]
Zheng et al.		27.6	64.3		[15]
He et al.		14	14.3		[16]
Liu et al.	7.1		29.1		[17]

The tested inflammatory markers were significantly increased compared to the reference interval for all COVID-19 patient. Our results are in line with Kleymenov et al. (2021), who found that IL-6 showed increased expression associated with disease severity [3].

IL-6, CRP, LDH, and ferritin are higher in men compared to women ( $p < 0.05$ ). These results might be related to the fact that men more frequently show severe forms compared to women. Kleymenov et al. (2021) [3], Scully et al. (2020) [18], and Angioni et al. (2020) [19] suggested that IL-6 is a gender-associated cytokine in COVID-19 because males develop higher concentrations. The findings of Qin et al. (2020) [20] support our results, as they observed that male patients showed greater inflammation, with higher levels of LDH, ferritin, and CRP.

We have performed a comprehensive analysis of scientific papers that aimed to analyze the values of D-dimer depending on the severity of the disease, and we noticed that our mean concentrations are similar to those of other authors (Table 6).

Table: 3 Comparison of the D-dimer mean value with the results obtained by other authors, depending on COVID-19 severity ( $\mu\text{g/mL}$ ).

Authorship	Mild Form	Moderate Form	Severe Form	Critical Form	Reference
Sun et al.	0.16 ± 0.06	0.35 ± 0.25	3.15 ± 3.31	1.95 ± 2.38	[13]
Liu et al.		0.74 ± 2.28	1.17 ± 4.27	4.138 ± 7.5	[14]
Zheng et al.		0.24	1		[15]
He et al.		0.32	0.95		[16]
Liu et al.	0.26		1		[17]
Zou et al.	0.43	-	1.04		[21]
Liu et al.	0.4	-	0.9		[22]
Shang et al.		0.51	0.84		[23]
Chen et al.		0.3	2.6		[24]
Güner et al.	0.33		0.95		[25]

According to the systematic review and meta-analysis of Kaushal et al. (2022) [6], the serum ferritin level was significantly higher in the severe to critical patients compared to the mild to moderate category of patients in 39 of the analyzed studies. Our findings support these outcomes; the ferritin concentrations were similar to those from other studies, according to the severity of COVID-19.

In a meta-analysis, Fialek et al. (2022) outlined the role of elevated LDH levels in assessing the severity of COVID-19. They observed lower LDH levels in mild and moderate groups compared to the severe course of COVID-19, and lower LDH levels in the severe group compared to the critical group. Our conclusions are similar to those described by Fialek et al. (2022). The mean values of LDH in the severe/critical forms of this infection are close to those published in other studies.

Table: 4 Comparison of the LDH mean value with the results described by other authors, depending on COVID-19 severity (U/L).

Authorship	Mild Form	Moderate Form	Severe Form	Critical Form	Reference
Sun et al.	231.75 ± 122.92	217.47 ± 51.12	279.70 ± 84.26	376.89 ± 161.55	[13]
Zheng et al.		194	247		[15]
He et al.		188	276		[16]
Liu Y et al.	221		383		[17]
Liu J et al.	221.5		462.4		[22]
Shang et al.		206	277		[23]
Chen et al.		224	537		[24]
Güner et al.	209		308		[25]
Itelman et al.	298	367.5	538		[26]
Wang et al.	305.6		424.1	542.5	[27]

CRP is a highly sensitive biomarker for inflammation, tissue damage, and infection. This acute-phase protein is a relatively cheap way and within the reach of any laboratory for screening for these pathologies. It was shown in many studies that the CRP value increased in COVID-19 patients; in critical form patients, it reached values 5 to 10 times higher than those in non-severe patients [21]. The same findings were highlighted in our study. The inflammatory reaction in our patients was more important than that described by Sun et al. (2020) [13], Liu et al. (2020) [22], Shang et al. (2020) [23], and Zheng et al. (2020) [15], but it was in line with that described by Trofin et al. (2023) [12].

Table 5: Comparison of the CRP mean value with the results described by other authors, depending on COVID-19 severity (mg/L).

Authorship	Mild Form	Moderate Form	Severe Form	Critical Form	Reference
Sun et al.	5.08 ± 8.58	11.83 ± 17.07	48.92 ± 72.71	30.96 ± 29.46	[13]
Liu et al.		37 ± 43.4	61.5 ± 63.8	75 ± 59.4	[14]
Zheng et al.		6.14	29.9		[15]
Liu et al.	7.6		62.9		[22]
Shang et al.		10.05	43.15		[23]
Chen et al.		22	139.4		[24]
Itelman et al.	68.1	98.5	132.4		[26]
Wang et al.	53.6		91.8	114.9	[27]

As only one-third of the patients included in the study were vaccinated against SARS-CoV-2 before the onset of the disease, we conclude that most hospitalized patients were unvaccinated. As in other studies, significant differences were recorded between the vaccination status and the form of COVID-19 developed by the patients ( $p < 0.05$ ). Even if no significant differences were recorded, the non-vaccinated patients had a more important inflammatory response compared to the vaccinated ones.

#### Strength of the Study

The study considered all the forms of COVID-19, also describing the characteristics of patients with mild and moderate COVID-19. In addition, other variation factors were taken into account, such as the viral variant, the vaccination status, and the gender of the patients.

#### Limitations

The study includes participants who were admitted to a specific infectious disease hospital, which preferentially and mostly hospitalized COVID-19 patients from the Iași metropolitan area, which may not be representative of the broader population. This could limit the generalizability of the study's findings. Confounding factors, such as age, comorbidities, and medications, could impact inflammatory marker levels and COVID-19 severity.

#### CONCLUSION

The level of inflammatory markers are elevated among the unvaccinated groups when compared to vaccinated. Disease severity, oxygen requirement and number of days hospitalised were lower in Vaccinated group

#### RECOMMENDATION

The study should be done among more no. of patients at different settings to identify the trend of disease in various population.

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