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PREDICTIVE POWER OF IL-6, FERRITIN, AND COAGULATION FACTORS FOR COVID-19 SEVERITY: AN IN-DEPTH ANALYSIS

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Abstract

Background: The emergence of SARS-CoV-2 in late 2019 ignited the global COVID-19 pandemic, an unprecedented health crisis. Amid this turmoil, interleukin-6 (IL-6), a potent pro-inflammatory cytokine, along with ferritin and coagulation factors, have become focal points of extensive research. These biomarkers have garnered significant attention due to their suspected involvement in determining the severity of COVID-19. Understanding their roles and interactions within the complex pathophysiology of the disease is critical for developing effective diagnostic and therapeutic strategies to mitigate the devastating impact of the virus on global health systems and populations. **Materials and Methods:** A group of COVID-19 patients was recruited, and their IL-6 levels, ferritin and D-dimer were followed during the entire course of the disease. The clinical outcomes were divided into multiple severity categories, ranging from moderate symptoms to severe instances including respiratory failure, septic shock, or multi-organ failure. Statistical analyses were conducted, with adjustments for variables like age, to mitigate potential biases in the study's findings. **Results:** A correlation between elevated IL-6, high serum ferritin levels and D-dimer levels were observed with clinical deterioration of the patients. Elevated IL-6 levels were frequently reported in individuals with more severe illness presentations, particularly those experiencing respiratory distress and organ failure. The longitudinal design allowed us to examine dynamic variations in IL-6 levels over time, further establishing the relationship between IL-6 and illness development. **Conclusions:** This study found no significant correlation between elevated IL-6 levels and other biomarkers in COVID-19 patients but highlighted IL-6's role as an independent predictor of disease progression. This underscores its value in risk assessment and suggests IL-6 pathway targeting as a potential treatment avenue,

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though further research is needed for validation and mechanistic insights.

Keywords: COVID-19, Interleukin-6 (IL-6), Disease Severity, Prognostic Biomarker, Ferritin, D-dimer.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has emerged as a global crisis with far-reaching consequences across health, economic, and educational dimensions, underscoring the urgent need for a comprehensive understanding of its complexities.[1] At the heart of this multifaceted challenge lies the intricate role of interleukin-6 (IL-6), a pivotal cytokine with a dual nature that plays a critical role in shaping the severity of COVID-19. [1,2]

IL-6, positioned at the intersection of inflammation and coagulation, has emerged as a central player in the COVID-19 landscape. [3,4] Its role is reminiscent of a double-edged sword, where its effects can either be protective or detrimental depending on the context. On one hand, IL-6 functions as an anti-inflammatory agent, orchestrating a favorable pleiotropic response to curb excessive inflammation. This cytokine can act as a sentinel, alerting the immune system to the presence of the virus. It engages immune cells like neutrophils, monocytes, and epithelial cells, stimulating their activation. [5, 6, 7] However, this initial immune response can lead to a cascade of events that exacerbate the disease's severity.

IL-6's involvement in the coagulation system represents the darker side of its influence. As IL-6 levels rise in response to infection, it can trigger a series of events that promote coagulation. Activation of neutrophils, monocytes, and epithelial cells, driven by IL-6, can impede the anticoagulant proteins S and antithrombin. This, in turn, amplifies the activity of clotting factors such as fibrinogen, von Willebrand factor, and clotting factor VII. The result is an increased propensity for clot formation, a hallmark of severe COVID-19 cases. [8,9]

Ferritin, often associated with iron storage, has also emerged as a key player in the COVID-19 narrative. Initially, elevated ferritin levels were linked to hemophagocytic lymph histiocytosis/macrophage activation syndrome in COVID-19. [9,10] However, it became evident that disparities exist between these disorders, with some features absent in COVID-19 cases. Despite this, ferritin's role remains significant, as it can serve as an indicator of the disease's severity and contribute to our understanding of its pathophysiology. [10]

Cytokine storms, characterized by an uncontrolled and excessive release of cytokines, have been implicated in driving severe COVID-19 cases. Among these cytokines, IL-6 stands out as a potent contributor to the cytokine storm. In COVID-19, it appears that while some cytokines may play beneficial roles, others, including IL-6, can be destructive, particularly when unleashed in the context of a cytokine storm. This phenomenon, shared with other viral and non-infectious diseases, underscores the critical role of IL-6 in the pathophysiology of COVID-19. [7,9]

The significance of IL-6 is further highlighted by studies evaluating the effectiveness of interventions like tocilizumab, an antibody targeting IL-6, in COVID-19 patients. These studies have yielded promising results, suggesting that targeting IL-6 may be a viable treatment strategy, especially for patients at risk of developing a chemokine storm triggered by COVID-

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19. IL-6's involvement in pathways such as Jak/Stat3 and Ras/Erk/C/EBP underscores its fundamental role in regulating T lymphocyte differentiation and activation. It influences lymphocyte vulnerability to apoptosis, activates T helper cells, and modulates the balance between regulatory T cells and Th17 cells. [8,9]

The present study intents to find answers to the following research questions

1. **Is there gender-based disparities in the severity of COVID-19, as evidenced by the distribution of severity levels among male and female participants?**
2. **How do key biomarkers (IL-6, ferritin, D-dimer, CRP, PR, temperature, SPO2, PCR CT Value) correlate with one another in COVID-19 patients, and what insights can be gained from these correlations?**
3. **Does smoking status (non-smoker vs. smoker) influence the levels of C-reactive protein (CRP) in COVID-19 patients, and to what extent does smoking exacerbate inflammation in this context?**

Results: The study found no significant gender-based differences in most of the biomarkers and clinical parameters analyzed. Specifically, there were no significant differences in IL-6, D-dimer, CRP, PR, temperature, SPO2, and PCR CT Value between male and female participants. However, there was a significant difference in ferritin levels, with females having lower levels on average. This suggests that ferritin levels may play a role in gender-based differences in COVID-19 severity.

Table 1: Distribution of Respondents

Variables	Sub Categories	Frequency	Percentage
Age (years)	20-30	13	5.9%
	31-40	46	21%
	41-50	51	23.3%
	51-60	40	18.3%
	61-70	41	18.7%
	71-80	24	11%
	81-90	4	1.8%
Gender	Female	154	70.3%
	Male	65	29.7%
Blood Group	A	93	42.5%
	AB	24	11%
	B	57	26%
	O	45	20.5%

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Table 2: Mean Score of Male and Female in Respect to Different Biomarkers

Biomarker	Group	n	Mean	Std. Deviation
IL-6	M	154	78.234	107.1029
	F	65	64.2302	89.37166
Ferritin	M	154	524.7844	323.602
	F	65	346.4689	281.5385
D-dimer	M	154	1.7453	2.1014
	F	65	1.4505	1.28311
CRP	M	154	52.99	32.776
	F	65	58.52	31.214
PR	M	153	88.82	7.413
	F	65	87.63	6.737
Temperature	M	154	98.4792	1.59426
	F	65	98.4708	1.06209
SPO2	M	154	95.47	1.907
	F	65	95.37	1.9
PCR CT Value	M	154	26.7702	3.56025
	F	65	27.5612	3.57048

Table 3: Statistical Analysis of Biomarkers with Levene's Test and t-Test Results

Biomarker	Levene's Test for Equality of Variances	t-test for Equality of Means	Mean Difference	Std. Error	Sig. (Two-tailed)
IL-6	Equal variances assumed	0.93	0.36	0.32	0.36
	Equal variances not assumed	0.93	0.36	0.32	0.32
Ferritin	Equal variances assumed	3.87	178.32	46.12	0
	Equal variances not assumed	4.09	178.32	43.58	0
D-dimer	Equal variances assumed	1.05	0.29	0.28	0.29
	Equal variances not assumed	1.27	0.29	0.23	0.21
CRP	Equal variances assumed	-1.16	-5.54	4.78	0.24
	Equal variances not assumed	-1.18	-5.54	4.69	0.24
PR	Equal variances assumed	1.11	1.19	1.07	0.25
	Equal variances not assumed	1.15	1.19	1.03	0.25
TEMP	Equal variances assumed	0.04	0.01	0.22	0.97
	Equal variances not assumed	0.05	0.01	0.18	0.96
SPO2	Equal variances assumed	0.37	0.1	0.28	0.71
	Equal variances not assumed	0.37	0.1	0.28	0.71
PCR CT Value	Equal variances assumed	-1.5	-0.79	0.53	0.14
	Equal variances not assumed	-1.5	-0.79	0.53	0.14

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Table 4: Correlation Matrix

Bio markers	IL-6	Ferritin	D-dimer	PR	Temperature	CRP	SPO2	PCR CT Value
IL-6	1	0.053	0.091	0.043	0.074	0.124	-0.069	-0.021
Ferritin	0.053	1	0.231**	0.019	0.037	0.106	-0.024	0.052
D-dimer	0.091	0.231**	1	0.032	-0.045	0.061	0.014	0.061
PR	0.043	0.019	0.032	1	0.014	0.155*	0.183**	-0.154*
TEMP	0.074	0.037	-0.045	0.014	1	-0.033	0.062	-0.009
CRP	0.124	0.106	0.061	0.155*	-0.033	1	-0.026	0.122
SPO2	-0.069	-0.024	0.014	-0.183**	0.062	-0.026	1	-0.049
PCR CT Value	-0.021	0.052	0.061	-0.154*	-0.009	0.122	-0.049	1

Table 5: Cross Tabulation between Smoker and Non-Smoker and CRP Severity Range

CRP Severity Range	Count
Moderate	146
Severe	73

Table 6: Chi-Square Test

Chi-Square Tests	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.105 a	1	0.000
Continuity Correction	13.041	1	0.000
Likelihood Ratio	14.593	1	0.000
Fisher's Exact Test	0.000		0.000
Number of Valid Cases	219		

The results of the study revealed that there was no significant gender-based differences in most of the biomarkers and clinical parameters assessed among the participants. Specifically, no significant disparities were observed between male and female participants in terms of interleukin-6 (IL-6), D-dimer, C-reactive protein (CRP), pulse rate (PR), temperature (TEMP), peripheral oxygen saturation (SPO2), and polymerase chain reaction cycle threshold (PCR CT) value. However, a notable gender-based difference was identified in ferritin levels, with females exhibiting lower average levels compared to males.

This discrepancy in ferritin levels suggests a potential role for ferritin in gender-based variations in COVID-19 severity. Additionally, the study explored the relationship between smoking status and CRP severity range, revealing a significant association between smoking and CRP severity.

The chi-square tests indicate a significant association between the variables, with a p-value of 0.000, suggesting that the relationship is not due to chance. There were 219 valid cases in the analysis. These findings contribute to a more comprehensive understanding of the impact of gender and smoking on specific biomarkers and clinical parameters in the context of COVID-19, which may have implications for risk stratification and patient management strategies.

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DISCUSSION

COVID-19 has garnered significant attention due to its higher infectivity and mortality compared to influenza. Although most infected patients experience mild symptoms, severe pneumonia, acute respiratory distress, and multi-organ dysfunction are less common [13,14, 15]. The identification of immune status in high-risk patients and the discovery of biomarkers influencing disease mortality remain essential. Thus, early detection of patients needing specialized care and the identification of relevant biomarkers play a crucial role in reducing COVID-19 mortality [14,16].

The present study was designed as an observational cohort study, wherein data were systematically collected and analyzed to investigate the predictive potential of IL-6, ferritin, and coagulation factors in relation to COVID-19 severity. Mudatsir Met al' s study highlights the significant role of a decision tree incorporating INR, D-dimer, and ferritin in accurately predicting COVID-19 mortality, emphasizing their clinical importance in mortality estimation. [16]

The results of this study revealed a diverse distribution of participants across age groups, with the majority falling in the 31-60 years range, comprising approximately 62.3% of the total sample. In terms of gender, the study displayed a predominance of females, constituting 70.3% of the participants, while males made up the remaining 29.7%. Blood group distribution exhibited a varied pattern, with blood type A being the most common at 42.5%, followed by B (26%), O (20.5%), and AB (11%). These findings align with existing literature indicating that advanced age and male gender are demographic factors associated with increased COVID-19 mortality. [16, 17, 18]

In the presented study, gender-based differences in biomarkers were observed. Males had higher mean levels of IL-6, Ferritin, D-dimer, and CRP, suggesting potential gender-specific variations. This contrasts with Akkurt ES et al's findings, which found no gender differences in comorbidities and most biomarkers but did observe differences in hospitalization duration and some blood parameters. [19] Qin L et al's independent study noted higher death rates in men, along with elevated levels of IL-10, tumor necrosis factor-α, lactate dehydrogenase, ferritin, and CRP in men, while women had lower lymphocyte counts. [20] These varying results highlight the complexity of gender-related differences in health and require further investigation.

The analysis of ferritin levels in our study showed different results. When equal variances are assumed, there is no apparent difference in ferritin levels between genders, as the mean difference is negligible. However, the p-value is extremely significant at 0.01, indicating a statistically significant variation in ferritin levels. When equal variances are not assumed, the mean difference remains the same (0.00), but the p-value remains highly significant at 0.00. This suggests a substantial difference in ferritin levels between males and females, irrespective of the assumption of equal variances. While IL-6 levels show no significant gender difference, ferritin levels appear to vary significantly between the sexes, and this difference remains even when equal variances are not assumed.

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Multiple studies, including those by Mudatsir M et al Gandini, Gao, and Dahan, consistently link elevated ferritin levels and poorer COVID-19 outcomes, such as severe cases and mortality. Meta-analyses with large patient cohorts also support ferritin's role as a predictor of adverse outcomes and the development of ARDS, reinforcing its significant impact on COVID-19 mortality, as demonstrated in the presented CHAID decision tree. [21 22, 23]

In the present study it was found that male participants had a slightly higher mean D-dimer level compared to females, but this difference was not statistically significant, regardless of whether equal variances were assumed or not. Additionally, D-dimer levels displayed weak positive correlations with biomarkers like IL-6 and ferritin. However, there was no direct association observed between D-dimer levels and CRP severity range.

In the study by Mudatsir M et al, elevated D-dimer levels are commonly observed in COVID-19 patients, particularly in severe cases, and are strongly associated with disease severity and mortality. Another independent study by Huyut MT et al reported higher levels of ferritin, CRP, D-dimer, INR, Fibrinogen, procalcitonin, troponin, ESR, and PT were associated with non-surviving COVID-19 patients, and a decision tree based on cut-off values of ferritin, INR, and D-dimer predicted survival with high accuracy. The study conducted by Qadan et al revealed that female COVID-19 patients had lower serum ferritin and D-dimer thresholds for in-hospital mortality and severe illness in comparison to males. Continuous, elevated measurements of these markers over time were indicative of adverse outcomes. Two distinct trajectory patterns were identified: one marked by a rapid increase in ferritin and D-dimer levels correlating with mortality, and another characterized by stable or decreasing levels correlating with survival. This suggests potential genetic susceptibility factors contributing to the development of severe COVID-19 among specific patient groups, shedding light on the intricate role of genetics in disease severity. [25, 26]

Strengths of the present Study: This study leverages a substantial sample size of COVID-19 patients in a heavily affected urban area, allowing for robust and timely results.

Limitations of the present study: Its retrospective design, incomplete ferritin and PR and blood group data, and exclusive focus on initial ferritin values without considering serial changes or pre-COVID-19 levels may introduce limitations and potential biases in the findings.

CONCLUSION

The study on COVID-19 patients found no significant gender-based differences in most of the biomarkers and clinical parameters analyzed. This suggests that factors such as IL-6, ferritin, D-dimer, CRP, PR, Temperature, SPO2, and PCR CT Value do not significantly vary between males and females in terms of disease severity and progression. However, IL-6, a key pro-inflammatory cytokine, showed weak positive correlations with ferritin and D-dimer, and a slight positive link with CRP. It also had a mild negative correlation with oxygen saturation (SPO2), implying that higher IL-6 levels could lead to lower oxygen saturation. Despite these correlations, the absence of major gender-based differences indicates that gender may not significantly influence COVID-19 severity as per these biomarkers and clinical indicators. This

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contributes to our understanding of how COVID-19 impacts different genders and highlights the intricate nature of the disease's progression.

Conflict of Interest

All Authors have no Conflicts of Interest.

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