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## Serum Levels of Biomarkers in COVID-19 Patients in a Hospital-based Population in Lahore

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**ABSTRACT:** COVID-19 disease has affected more than 1.58 million people in Pakistan. The disease has been associated with a number of inflammatory markers such as serum CRP, d-dimer and ferritin. The present study was undertaken to investigate the role of these 3 biomarkers with the severity of disease in a hospital-based population in Lahore. Ninety-three COVID-19 patients (52 males, 41 females, age 18-70 years) were recruited with informed consent. The blood was analyzed for the serum levels of CRP, d-dimer and ferritin using commercially available kits. Severity of disease was determined on the basis of clinical symptoms. Analysis of the data revealed that although there were elevated serum levels of CRP and d-dimer in most patients (54.8% and 60.2%, respectively), yet no significant differences were observed in mean levels of these biomarkers in males and females. Similarly, mean serum ferritin levels were not significantly different between male and female patients. Since the normal ranges of serum ferritin in females and males were different, the comparison among patients showed more males with normal ferritin levels than females. Thus, there were significantly more females with elevated serum ferritin levels compared to males (56.1% vs. 32.7%;  $p=0.015$ ) indicating an important role of this biomarker in Pakistani female patients. No relationship was found between levels of serum ferritin and severity of disease.

**Keyword:** COVID-19 disease; serum ferritin; serum d-dimer; serum CRP; biomarkers

## **INTRODUCTION**

COVID-19 disease has severely affected people of Pakistan. According to the WHO dashboard by the first week of February, 2023 there have been 1.58 million confirmed cases of this disease in Pakistan, while the mortality has been reported to be 30,639 [<http://covid-19.who.int?region=emro&country=>].

The involvement of serum biomarkers in COVID-19 disease has been examined in a number of studies. Variability in levels has been reported and it is based upon the differences in patients' exposure to the virus and status of the immune system of the infected patients. For example, elevated serum levels of ferritin (a non-specific marker of inflammation) and d-dimer (a marker for thrombotic status) have been reported in those individuals who had a poor survival rate (Tang et al., 2020; Kermali et al., 2020). Another study conducted by Xiang et al. (2020), reported significantly elevated serum markers ferritin and C-reactive protein (CRP; a marker for inflammation) in critically ill COVID-19 patients compared to those who had mild symptoms of this disease. Similarly, a study involving 1500 COVID-19 disease patients showed significant differences in these biomarkers' levels on admission in patients who survived

compared to those who did not survive (Loomba et al., 2020). These studies showed a relationship of these biomarkers with severity of COVID-19 disease.

There are only a few studies that have been carried out in Pakistan to investigate the relationship of these biomarkers with COVID-19 disease. (Saeed et al., 2022; Hassan Shah et al, 2021). The present study was undertaken to investigate the relationship of serum levels of CRP, d-dimer and ferritin with severity of COVID-19 disease in a hospital-based population in Lahore.

## **MATERIALS AND METHODS**

In a cross-sectional study, 93 adult consecutive COVID-19 disease suspected patients (52 males and 41 females with ages in the range from 18-70 years) were recruited with informed consent from the Umer Shoaib Surgical Hospital, Lahore from January 21, 2021 to December 11, 2021. They had the confirmed diagnosis of COVID-19 disease based on clinical examination and lab results. Blood samples (10 ml) were collected for the determination of serum levels of biomarkers CRP, d-dimer and ferritin using commercially available kits and following manufacturers' instructions. CRP kit

was obtained from Pol. Ind. Can Castells. C, Barcelona. The method adopted in this kit had a sensitivity of 74% and a specificity of 75%. The d-dimer kit was purchased from EDAN, Shanghai International Corporation, GmbH, Humburg having a sensitivity of 98.9% and a specificity of 92.5%. Ferritin kit was obtained from Cal biotech Inc. California. It was based on sandwich ELISA with sensitivity and specificity exceeding 98%. In order to ensure quality and accuracy, a negative control and standards with known concentrations of biomarker were run along with patients' samples in each assay of these 3 biomarkers. Severity of the disease was assessed by the clinician based on clinical symptoms and the National Guidelines pertaining to symptoms of COVID-19 for diagnosis of mild, moderate and severe disease as mentioned by Hassan Shah et al (2021). These have been briefly outlined below:

**Mild:** Respiratory rate < 24/min; SpO<sub>2</sub> > 94 % at room temp

**Moderate:** Respiratory rate 24-30/min; SpO<sub>2</sub> 90% -94% at room temp

**Severe:** Respiratory rate > 30/min; SpO<sub>2</sub> < 90% at room temp

The study had been approved by the Bioethical and Safety Committee of the Department of Life sciences, University of Management and Technology.

## STATISTICAL ANALYSIS

The data were analyzed in the Statistical Package for Social Sciences (SPSS) version 21.0 using various statistical tests such as Independent Sample t-test and Chi-Square test. P-value < 0.05 was considered significant.

## RESULTS

Among the 93 recruited COVID-19 patients, 52 were males and 41 were females. Table 1 shows the biochemical characteristics of female and male COVID-19 patients. Mean CRP levels among female and male patients were not found to be statistically significant ( $54 \pm 58.2$  mg/l vs.  $76.6 \pm 104$  mg/l;  $p = 0.088$ ). However, 30 male patients (57.7%) and 21 female patients (51.2%) had CRP levels above the normal levels (< 6 mg/l). The mean serum levels of d-dimer in both female and male patients were not statistically different ( $217 \pm 234$   $\mu$ g/ml vs.  $131 \pm 216$   $\mu$ g/ml;  $p = 0.603$ ), but 31 males (59.6%) and 25 females (60.9%) had d-dimer levels above the normal levels (< 200  $\mu$ g/ml). Mean serum ferritin levels in female patients below the age of 40 years were higher compared to the highest normal value of this biomarker in this age group (137 ng/ml). On the other hand, mean serum ferritin levels in male patients above the age of 40 years were well above the

maximum normal serum ferritin levels of 464 ng/ml in this age group. However, the mean serum ferritin levels in both these age groups were not found to be statistically significant among males and females. The data also showed that among the 93 patients, 17 males and 23 females had more than the maximum normal value of serum ferritin level for that gender and age group (Table 2). When the proportions of male and female patients having serum ferritin levels above the maximum normal value for their

categories were compared using Chi Square test, there was significantly higher percentage of females having elevated levels of serum ferritin compared to male patients ( $p = 0.015$ ), indicating serum ferritin as an important biomarker of inflammation in COVID-19 disease, especially among the female patients. Fifty-two percent of our patients had severe to moderate disease. However, no significant difference was observed between male and female patients in terms of severity of disease (Table 3).

**Table 1: Biochemical characteristics of female and male COVID-19 patients in a population in Lahore, Pakistan**

Variables	Total N	Female		Male		P-value*
		N	Mean ± SD	N	Mean ± SD	
<i>C-Reactive Protein (CRP)</i> mg/l	93	41	54 ± 58.2	52	76.6 ± 104.1	0.088
<i>D-dimer</i> µg/ml	93	41	217 ± 234	52	131 ± 216	0.603
<b>Ferritin with respect to 2 age groups (ng/ml)</b>						
≤40**	43	16	278 ± 410	27	341 ± 374	0.236
> 40***	50	25	188 ± 408	25	486 ± 458	0.598
* P-value was obtained by comparing means of males and females using an Independent sample t-test.						
** Normal range of serum ferritin in age groups less than or equal to 40 years for females is 6.24 to 137ng/ml and for males is 17.9 to 464ng/ml						
*** Normal range of serum ferritin in age group above 40 years for female is 11.1 to 264ng/ml and for male is 17.9 to 464ng/ml.						

**Table 2: Frequency distribution of male and female COVID-19 patients having serum ferritin levels above the maximum normal value for their categories.**

Gender	N	n (%)	Chi-Sq. value	P- value*
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<b>Male</b>	52	17 (32.7)	6.652	0.015
<b>Female</b>	41	23 (56.1)		
* P-value was obtained by comparing the percentages of male and female COVID-19 patients having serum ferritin levels above the maximum normal value for their categories by using the Chi-square test.				
<b>Note:</b> Maximum normal ferritin value in males of any age is 464 ng/ml, while the maximum normal value in females below 40 years is 137 ng/ml and above 40 years is 264 ng/ml.				

**Table 3: Comparison between Males and Females with respect to the severity of the disease**

Disease	Male n(%)	Female n(%)	p-value*
<b>Severe</b>	11 (21.1)	6 (14.6)	0.680
<b>Moderate</b>	18 (34.2)	14 (34.1)	
<b>Mild</b>	23 (44.2)	21 (51.2)	

\*P-value was obtained by comparing the proportions between each of the three rows pertaining to severe, moderate, mild disease using Chi Square test.

## DISCUSSION

The serum levels of biomarkers CRP, ferritin and d-dimer were found to be elevated in most of the study participants, nonetheless, the most significant increase was observed in serum ferritin levels in our female patients. These results are different from the results of another study from Pakistan showing higher serum levels of ferritin in COVID-19 male patients with moderate to severe disease (Hassan Shah et al., 2021). This could be due to the reason that they did not take into account the fact that the normal serum levels of ferritin are lower in females compared to males (6.24 ng/ml -264 ng/ml for females and 17.9 ng/ml – 464

ng/ml for males). However, their results pertaining to serum levels of d-dimer indicating no statistically significant difference between male and female patients with moderate and severe disease are in line with findings of this study. Another study carried out in Pakistan showed significantly higher levels of serum ferritin and serum d-dimer in 48 critically ill patients with COVID-19 disease (Alam et al., 2021). However, their sample size was too small to be grouped into mild disease patients and those suffering from moderate to severe disease.

Ferritin is one of the major mediators of immune system dysregulation and contributes to development of “cytokine storm” in COVID-19 disease patients

(Vargas-Bargas and Cortes-Rojo, 2020). In a meta-analysis, significantly elevated serum levels of ferritin were reported in severe to critically ill COVID-19 disease patients indicating its prognostic value (Kaushal et al., 2022). According to a study carried out in China, nearly 63% of the COVID-19 patients had serum levels of ferritin well above the normal range (Chen et al., 2019). Results of our study are in line with this study and 43% of our patients had levels of ferritin well above the normal range.

The above mentioned studies showed a relationship between levels of this biomarker and the severity of COVID-19 disease. However, in our recruited patients, we could not find any relationship between levels of serum ferritin and severity of the disease. This could be due to the small sample size ( $n = 93$ ) of patients analyzed for this biomarker or the immune system of these patients was robust enough to minimize the inflammatory response to viral attack. This is further supported by the fact that there was only one mortality among the 93 recruited patients.

Another study carried out at the Allama Iqbal Medical College/Jinnah Hospital reported that higher levels of serum ferritin ( $> 200$  ng/ml) and d-dimer ( $>$

400 ng/ml) could be a predictor of mortality among COVID-19 disease patients. However, the recruited patients in that study were critically ill with mortality up to 35%. (Yousaf et al., 2022). We had several patients with levels greater than the cut-off values reported in that study, nonetheless there was only one mortality among our recruited patients. This shows that clinical condition of patients apart from these biomarkers is also important in predicting the outcome of the disease.

The current study though with a relatively small sample size is a useful addition to the existing body of knowledge in the country related to coronavirus infection and the role of serum biomarkers, especially serum ferritin in this disease. However, more prospective studies with an increased number of samples are required to clearly determine the role of biomarkers' levels in predicting the disease, its prognosis, and its final outcome.

## **CONCLUSION**

Serum ferritin levels were elevated above the normal levels in significantly more female patients suffering from COVID-19 disease than male patients. This showed that ferritin was an important biomarker for predicting

COVID-19 disease in Pakistani female patients presenting with common symptoms of this disease.

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### **ETHICAL APPROVAL**

The study have been approved by the Biochemical and Bioethical Safety Committee of the Department of Life Sciences, University of Management and Technology, Lahore.

### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest

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