



Estimation of Some Hematological Parameters for Severe Cases of COVID-19 in Babylon City, Iraq

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Abstract

Background: The World Health Organization declared a novel coronavirus disease (COVID-19) as a global pandemic, and the work has begun to combat it. **Objective:** The objective of the study was to investigate whether some biological tests can be used as dependable biomarkers for indicating the severity of COVID-19, with a comparison between patients in urban and rural areas of Babylon province. **Materials and Methods:** One hundred and forty samples of blood (70 patients urban and 70 patients rural) were obtained from patients who had positive results and severe symptoms of COVID-19 infection, accomplished by Marjan Teaching Hospital from December/2021 to February/2022. D-dimer, ferritin, and C-reactive protein (CRP) tests were performed according to the CLIA technique. White blood cell (WBC) counting uses a fully automated hematology analyzer device. **Results:** The results showed a significant difference in D-dimer ($P=0.000$), ferritin ($P=0.001$), and WBC count ($P=0.000$) between patients in both urban and rural areas of Babylon province, while CRP showed an insignificant difference ($P=0.06$). **Conclusions:** It can be inferred that the examination of D-dimer and CRP is considered a biomarker for the severity of COVID-19 patients in urban and rural areas. Whereas the effect of gender is not significant in all parameters. Furthermore, the results of the frequency distribution of patients showed that the concentrations of D-dimer and CRP in blood exceeded the approved normal value in both urban and rural areas. Also, a lot of values of ferritin and WBC in urban patients exceeded the normal value, but not in rural patients.

Keywords: COVID-19, Ferritin, D-dimer, CRP, WBC.

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Introduction

In December 2019, the coronavirus was found for the first time in the respiratory tracts of pneumonia patients in Wuhan, Hubei, China. COVID-19 has resulted in 607,289 deaths in the US [1]. The first study in the USA considered Rural-urban outcome differences associated with COVID-19 hospitalization patients [2]. David [3] expected that lower population density should make the risk of COVID-19 transmission lower in rural areas.

Raheemet *al.* [4] reported that the consideration of the pathophysiology and identifying factors related to a few prognoses are important in improving patient outcomes in COVID-19. Marked elevations in hematologic biomarkers such as D-dimer, ferritin, and CRP are associated with worse outcomes. There is no treatment for the novel infectious disease COVID-19. As a result, biomarkers must be investigated in order to gauge the severity of the disease and the extent of the lung lesions [5]. Early diagnosis of pneumonia is possible using CRP levels [6], and individuals with severe pneumonia have increased CRP levels.

They examined the connection between CRP levels, lung lesions, and disease severity to serve as a guide for clinical care [7]. A pentameric protein with a ring-like structure would be the CRP. It is a component of serum and blood plasma, and in response to inflammation, blood levels of it rise. It is an acute-phase hepatic protein (produced in the liver) that responds to factors like interleukin-6 released by

macrophages and fat cells (adipocytes). CRP, according to Tan *et al.* [8], was the first pattern recognition receptor to be identified. Certain bacteria (or other microorganisms) and dead or dying cells surface-expressed phosphocholine both bind to Crp. As a result, bacteria and necrotic and apoptotic cells are phagocytosed by macrophages (opsonin-mediated phagocytosis) when the complement system is stimulated.

Secondary (also bacterial) infections, CRP has been found in extreme cases. In order to store iron inside cells, ferritin is a necessary component. Most tissues contain the cytosolic protein ferritin. Serum ferritin is utilized as a diagnostic technique for iron-deficiency anemia because it is an indirect measure of the total amount of iron stored in the body [9]. Ferritin is a 24-protein globular protein complex that interacts with various metals to form a nanocage. Ferritin preserves iron in a soluble, non-toxic form [10].

Ferritin that has not been combined with iron is known as Apoferritin. According to data, there may be an increased risk of mortality during the COVID-19 pandemic due to an increase in serum ferritin levels [11]. Hyperferritinemia, which is brought on by infection-induced inflammation and is associated with intensive care unit admission and a high mortality rate, acts as a signal to identify patients at high risk and to direct therapeutic action to control inflammation [12]. After a blood clot is broken down by fibrinolysis, a small protein fragment called the fibrin degradation product D-dimer is discovered in the blood. Its composition, two D-fragments of the

fibrin protein joined by a cross-link, is how it got its name. Its concentration can be determined through a blood test in order to identify thrombosis. D-dimer could be a symptom of severe virus infection, along with thrombosis and pulmonary embolism, and the relationship between it and COVID-19, as well as the changes in amount as infection progresses, was not completely described. Sepsis and coagulation issues brought on by a viral infection are common in the progression of serious illnesses [13].

Additionally, an elevation in D-dimer might be a symptom of an inflammatory response since inflammatory cytokines may lead to an imbalance in the alveoli's coagulation and fibrinolysis, which would activate the fibrinolysis process and raise D-dimer levels [14]. It was discovered that patients with severe COVID-19 had higher D-dimer levels than those with mild COVID-19. Leukocytes, also known as white blood cells, play a well-known role in both the immune system and the response of the host to an infection. White blood cell evaluation has been the focus of numerous studies, and the differential count is an important factor in supporting the COVID-19 diagnosis and predicting disease severity. Leukocyte counts are typically lower in COVID-19 patients than in healthy individuals. However, patients with severe disease have higher leukocyte counts during observation than patients with mild to moderate disease [15]. This study investigates the assessment of some hematological parameters between severe COVID-19 patients and compares the patients of

urban and rural areas in Babylon province.

Materials and Methods

This research was conducted on 140 samples of blood from urban (70 patients) and rural (70 patients) areas of Babylon province, who had positive results and severe symptoms of COVID-19 infection, and were provided by the medical staff of Marjan Teaching Hospital. Blood samples were taken from each patient on the second day of their hospital stay by taking venous blood and putting 3 ml of whole blood in three tubes. Clinical Laboratory Improvement Amendments (CLIA) technique for D-dimer, ferritin, and CRP tests was performed. The first tube contains a non-heparinized tube (gel tube) and the Kit CRP Test Kit by Clia Maglumi 800 (Snibe), an Indian company to test C-reactive protein. It is centrifuged for 10 minutes at 3000 RPM to separate and purify the serum. The second tube includes sodium citrate, an anticoagulant, while the Kit D-dimer test Kit by Clia Maglumi 800 (Snibe), an Indian company. The third tube is used to test ferritin-using plasma, while the kit ferritin is a test kit by the CLIA Maglumi 800 (Snibe) Indian company. Fresh whole blood samples were also utilized for WBC counting using the Mindray BC-3000 plus Bio-Medical Electronics Co., Ltd (Shenzhen, China) as a fully automated haematology analyzer device.

Ethical approval

The study was conducted in accordance with the ethical principles. It was carried out with the patient's verbal and analytical approval before the sample was taken. The study protocol, the

subject information, and the consent form were reviewed and approved by Babylon University College of Science for Women, Department of Biology, a local ethics committee, according to document number 5907 on November 8, 2021, to get this approval.

Statistical analysis

All results were presented as a mean ± SD (standard deviation), *P* value, comparison between patients in rural and urban areas, and between male and female genders was performed by t-test.

Results

A total of 140 patients in urban and rural areas (70 patients for each) of Babylon province with severe COVID-19 disease were enrolled in this study. The results

showed in Table 1, there was no significant difference in CRP levels (*P* =0.06) between the urban and rural patients, and the mean value of CRP of patients in urban and rural areas were (104.113 mg/L) and (99.564 mg/L), respectively. In addition, the statistical analysis showed there was a significant difference in D-dimer (*P* =0.000), ferritin (*P* =0.001), and WBC (*P* =0.000) between the urban and rural patients. The mean values of D-dimer, ferritin, and WBC in patients of urban areas were D-dimer (0.601 mg/dL), ferritin (343.330 ng/mL), and WBC (11.244 x10⁹/L), respectively. While the mean values of D-dimer, ferritin, and WBC in patients of rural areas were D-dimer (0.601 mg/dL), ferritin (343.330 ng/mL), and WBC (11.244 x10⁹/L), respectively.

Table (1): Comparison of studied parameters in patients of COVID-19 urban and rural of Babylon province.

Group Statistics	Variables	N	Mean ± SD	<i>P</i> . Value
CRP	Urban	70	104.113 mg/L ±16.072	0.06
	Rural	70	99.564 mg/L ±12.356	
Ferritin	Urban	70	343.330 ng/mL ±92.781	0.001
	Rural	70	303.666 ng/mL ±20.117	
D-dimer	Urban	70	0.601 mg/dL ±0.231	0.000
	Rural	70	0.740 mg/dL ±0.104	
WBC	Urban	70	11.244 x10 ⁹ /L ±2.144	0.000
	Rural	70	12.876 x10 ⁹ /L ±1.558	

The results showed in Table 2 that there was no significant difference in CRP(*P* =0.916), D-dimer (*P* =0.443), ferritin(*P* =0.139), and WBC (*P* =0.247) between males and females. The means of CRP, D-dimer, ferritin, and WBC for males were CRP (101.740 mg/L), D-dimer (0.680 mg/dL), ferritin (329.999 ng/mL), and WBC (11.800 x10⁹/L), respectively. Whereas the means of CRP, D-dimer, ferritin, and WBC for females were CRP (102.006 mg/L), D-dimer (0.654 mg/dL), ferritin (312.496 ng/mL), and WBC (12.214 x10⁹/L), respectively.

Table (2): Gender distribution for hematological parameters in patients of COVID-19

Hematological Parameters	Gender	N	Mean ± SD	P value
CRP	Male	52	101.740 mg/L ±14.523	0.916
	Female	88	102.006 mg/L ±14.504	
D-dimer	Male	52	0.680 mg/dL ±0.183	0.443
	Female	88	0.654 mg/dL ±0.206	
Ferritin	Male	52	329.999 ng/mL ±72.547	0.139
	Female	88	312.496 ng/mL ±64.024	
WBC	Male	52	11.800 x10 ⁹ /L ±2.166	0.247
	Female	88	12.214 x10 ⁹ /L ±1.957	

The results showed that the range of D-dimer concentration in a blood sample of patients in the study in urban and rural areas was 0.1 -24.1 mg/dL and 0.1 - 30.7 mg/dL, respectively. Figure 1 shows the frequency distribution of D-dimer concentration in blood samples of severe patients within the duration of the study. The results revealed that the

D-dimer was more frequent in rural than in urban patients in the range group 0.1-0.29 mg/dL of D-dimer concentrations. In addition, the results exposed a high frequency of D-dimer concentration distribution at group >0.7 mg/dl in both patients in urban and rural areas.

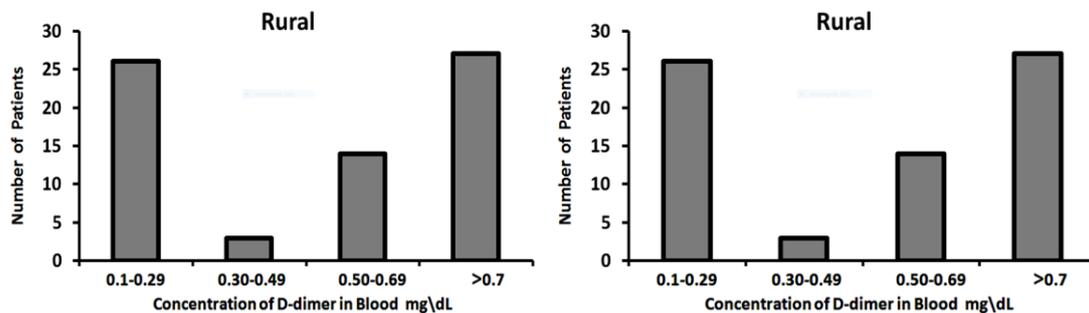


Figure (1): The frequency distribution of patients according to D-dimer concentration in blood sample of patients in urban and rural areas.

The results showed the range of CRP concentrations in blood samples of urban area patients was 39.5-156 mg/dL, while the range of CRP concentrations in blood samples of patients in rural areas was 40-134.5 mg/dL. Figure 2) shows there were no patients who had less than 40 mg/L of CRP concentration in a blood sample of

patients in both rural and urban areas. Also, it shows a high frequency of the distribution in patients of both urban and rural areas at a range group of 80-119.9 mg/L. Additionally, the results showed a high frequency of the distribution at a group of >120 mg/dL in urban area patients compared to rural area patients.

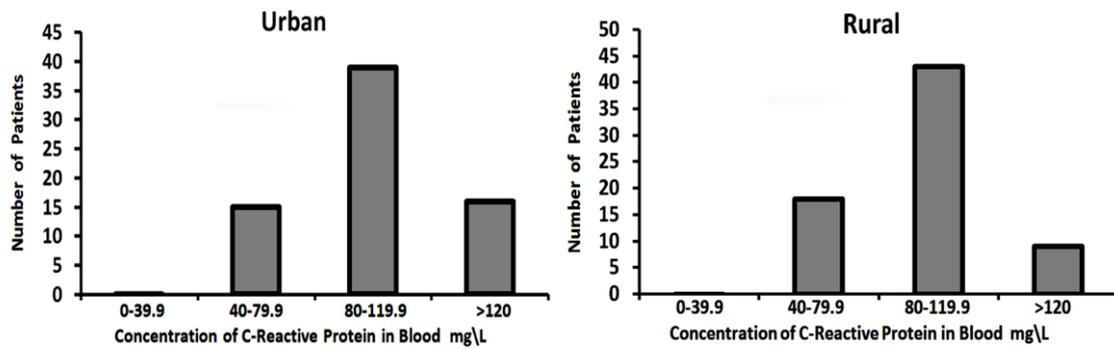


Figure (2): The frequency distribution of patients according to CRP concentration in blood sample of patients in urban and rural areas.

The results showed that the range of ferritin concentration in a blood sample of patients in the study in urban and rural areas was 18.1- 566.5 ng/mL and 14.8- 599 ng/mL, respectively. Figure 3 shows the frequency distribution of ferritin concentration in blood samples of severe patients within the duration of the study. It showed a high frequency of

ferritin concentration distribution in range groups 150-299 ng/mL and 300-449 ng/mL in patients of rural and urban areas. In addition, it exhibited a more frequent distribution in ferritin concentrations in the range group 450-599 ng/mL in patients of urban areas than in rural areas.

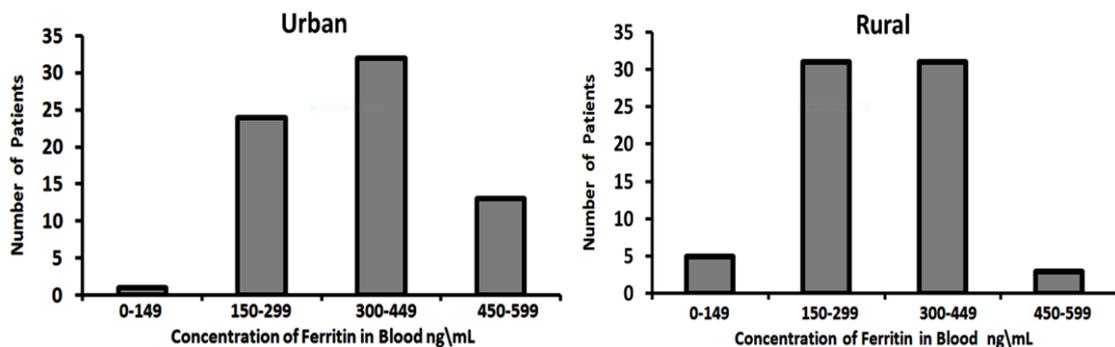


Figure (3): The frequency distribution of patients according to ferritin concentration in blood sample of patients in urban and rural areas.

The results showed the range of WBC counts in blood samples of urban area patients was $3.1 \times 10^9/L$ - $14.8 \times 10^9/L$ while the range of WBC counts in blood samples of patients in rural areas was $4.1 \times 10^9/L$ - $16.5 \times 10^9/L$. Figure (4) shows the frequency distribution of WBC count in blood samples of severe patients within the duration of the

study. The results revealed a high frequency of patient distribution at range groups $(11.0 - 12.9) \times 10^9/L$ and $<11 \times 10^9/L$ of WBC count in blood samples of urban and rural areas, respectively. In addition, it showed that no patients had more than $15 \times 10^9/L$ of WBC count in a blood sample of patients in urban areas.

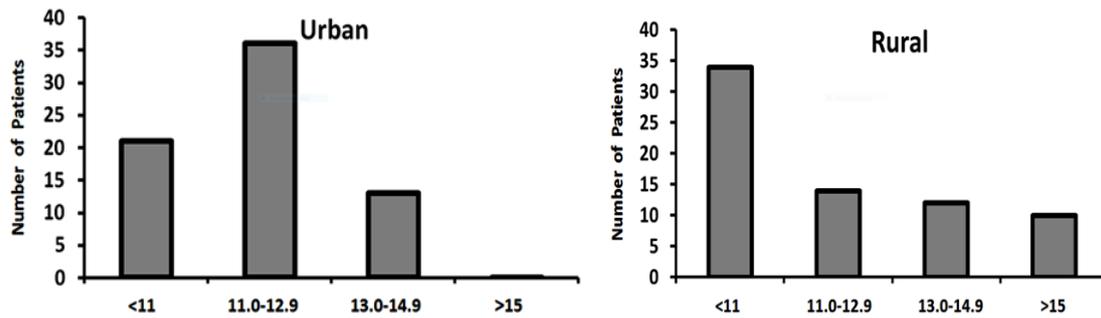


Figure (4): The frequency distribution of patients according to WBC count in blood sample of patients in urban and rural areas.

Discussion

Hematological parameters can play a role in the prediction of disease or indicate inflammation due to being easily measured and widely used for risk diagnosis. D-dimers, which were already involved in fibrin degradation, are now being recognized as useful in a clinical judgment principle for ruling out pulmonary embolism, suggesting their value as a biomarker [16]. It was obvious from the data of the study that the concentration of D-dimer exceeded the approved normal value in both urban and rural patients. According to Yu *et al.* [13], patients with severe COVID-19 have higher D-dimer levels than those with non-severe illness, and D-dimer levels more than 0.5 mg/dL are linked to severe infections in COVID-19 patients. While a normal D-dimer was defined as <0.05 mg/dL [17]. COVID-19 is only diagnosed based on clinical signs. D-dimer assays are crucial for finding infections in the human body, especially in COVID-19 patients [18]. After fibrinolysis dissolves a blood clot, a tiny protein fragment known as a D-dimer is found inside the blood. Additionally, higher D-dimer ranges have been linked to a

lower death rate in pneumonia acquired in the community [19,20]. Moreover, the study revealed that more patients in urban areas had D-dimer levels of 0.7 mg/dL than rural patients, while the lowest distribution frequency was in patients in a group range of 0.3-0.49 mg/dL in urban and rural patients. These results do not agree with Denslow *et al.* [2], who found that patients from rural regions were more likely than patients from metropolitan areas to present to the hospital with test evidence, such as increased D-dimer.

Table (1) shows that the difference in the mean of D-dimer levels between the two areas is highly significant. These findings support, according to Rizal *et al.* [21], considerably high D-dimer values were found in the severe group, which was consistent with the findings of earlier studies that assessed D-dimer levels and showed a relationship between the biomarker (D-dimer) and COVID-19 disease severity. Table (2) shows that the mean of D-dimer level was slightly higher in males compared to females; however, the difference in D-dimer levels between males and females is not significant. These results agree with

the opinion that no sex difference in the D-dimer level between men and women with COVID-19 was detected, according to Saville *et al.*'s study [22]. The variety of D-dimer reporting has an influence on data interpretation and calls for further focus.

The inflammatory biomarker CRP was significantly elevated in severe COVID-19 patients, indicating that overproduction of inflammatory cytokines may be one cause of CRP increases in COVID-19 [11]. CRP levels can stimulate phagocytosis and activate complement, clearing the body of pathogenic microorganisms [20]. The findings demonstrated that in both urban and rural patients, the frequency distribution of patients with a concentration of CRP tests was higher than the permitted normal percentage. These results disagree with Denslow *et al.* [2], who found rural patients had more often shown indicators for hyperinflammation, such as CRP, compared with urban patients.

In the current study, the mean CRP concentrations were 101.740 mg/L and 102.006 mg/L in male and female patients, respectively. It is worth mentioning that the normal CRP value is less than 0.5 mg/dL [23]. Whereas the data of the study showed that the concentration of CRP tests exceeded the approved normal value in both urban and rural patients. Also, the patients in urban areas who had CRP levels above 120 mg/L were more than the rural patients, indicating a high level of inflammation. Patients with more severe symptoms had an average CRP concentration of 39.4 mg/L, according to Gao *et al.* [24], whereas those with milder symptoms had an

average CRP concentration of 18.8 mg/L. Table (1) shows that the difference in mean CRP levels between the two groups is not significant. Abdullah *et al.* [25] results showed that the majority of infected individuals had elevated CRP levels, and *P*-value of less than 0.05 indicated a significant difference in CRP levels between alive and dead patients, also found the median inter quartile range of deceased patients was also found to be significantly higher compared to survivors.

The amount of ferritin that circulates increases during viral infections and can be used as a marker of viral replication [10]. Because of its direct immunosuppressive and pro-inflammatory properties, ferritin is a crucial modulator of immune dysregulation, especially in cases of severe hyperferritinemia [19]. Most COVID-19 patients in both urban and rural areas had ferritin in the normal-moderate ranges of 150-299 ng/mL. 300-449 ng/mL, agree with Sandnes *et al.* [26], who reported ferritin levels were similar in patients who received methylprednisolone compared to those who did not. Other patients of COVID-19 tested in urban Babylon provinces had very high ferritin of 450-599 ng/mL compared to patients in rural Babylon provinces. These results disagree with Denslow *et al.* [2], who found rural patients had more often shown indicators for hyperinflammation, such as ferritin, compared with urban patients. In general, Ferritin levels are (~200–300 ng/ml) considered to be normal [27]. Table (1) shows the *p*-value of ferritin level for the urban and rural Babylon

provinces was 0.001, which indicates that the difference in mean ferritin levels between the two areas is significant.

These results agree with Kaushal *et al.* [28], the biomarker (ferritin) linked to the severity of COVID-19 illness might be utilized to triage patients at the time of admission, identifying those in need of intensive care and enabling the best use of available resources. Table (2) shows that the mean of ferritin level was higher in males compared to females, and the standard errors of the means do not overlap. And the *p*-value was 0.139; therefore, the difference in ferritin levels between males and females is not significant. Falahi and Kenarkoohi [29] reported that this result might show that males and females have the same chance of susceptibility to COVID-19 infection, although there are physiological and hormonal differences between males and females. Also, mention that male cases outnumbered female cases in previous studies, and males appeared to have extra severe COVID-19 or be in a serious state of disease. Recent studies discovered that this sex disparity could be due to several complications, which indirectly raise the risk of death in men.

In a normal healthy adult person, the total number of WBCs in the blood is $4.0 - 11.0 \times 10^9/L$ [30]. On the other hand, the WBC account appeared higher in urban areas than the acceptable normal percentages compared with the patients of rural areas; these results are consistent with the findings of Zhao [16] and may be related to persistent infection. In the current study, the results of WBC

counts in the urban and rural areas were high for some patients due to severe COVID-19 infection. An increase in WBC indicates a promotion in the cellular immune system response to control viral infection and, as a result, an increase in the expression of inflammatory cytokines, such as CRP [31]. In general, the increase of WBC in COVID-19 patients, regardless of age or sex, can be used as a diagnostic marker in the early stages of the disease [32]. According to the findings of Pirsalehi *et al.*'s study [33], severe instances of the illness typically show leukocytosis in addition to a statistically significant increase in the mean number of WBCs. This finding implies that a high WBC count may be a possible sign of a poor prognosis. Taj *et al.* (2021) [34] indicated that the hematological parameters are related to COVID-19 disease, and these markers may be utilized for early disease severity prediction.

Conclusions

The study concluded that the patients of urban and rural areas had a significant difference in D-dimer, ferritin, and WBC count in blood, which could be due to associated factors of the kind of pollution and lifestyle in the city compared with rural areas. In addition, the mean concentrations of ferritin in the blood of urban patients were more than those of rural patients. Notably, the D-dimer and CRP tests can serve as biomarkers for the severity of COVID-19 patients because they were above the normal value in all COVID-19 patients.

Conflict of Interest: Authors declare there is no conflict of interest.

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تقدير بعض معايير فحوصات الدم للحالات الشديدة من كوفيد-19 في مدينة بابل، العراق

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الخلاصة

بعد اعلان منظمة الصحة العالمية عن مرض فيروس كورونا (كوفيد-19) من انها جائحة عالمية بدأ العمل على مكافحة هذا المرض وأجريت لهذا السبب هذه الدراسة. اذ تهدف هذه الدراسة إلى إمكانية استخدام بعض الاختبارات البيولوجية كمؤشرات حيوية التي لها علاقة بشدة مرض كوفيد-19 للمرضى في المناطق الحضرية والريفية بمحافظة بابل. تم الحصول على مائة وأربعين عينة دم من المرضى (70 مريضاً في المناطق الحضرية و70 مريضاً في المناطق الريفية) الذين راجعوا مستشفى مرجان التعليمي للمدة من ديسمبر/2021 إلى فبراير/2022، والذين كانت نتائج فحوصات لكوفيد-19 إيجابية وذوي اعراض شديدة لهذا المرض. أجريت فحوصات الدم الذي دايمر والفيبريتين والبروتين المتفاعل (CRP) على عينات الدم وفق تقنية (CLIA). كما تم عد خلايا الدم البيضاء (WBC) باستخدام جهاز تحليل الدم. أظهرت النتائج وجود فروق معنوية للذي دايمر ($P=0.000$) ، الفيبريتين ($P=0.001$)، وعدد كريات الدم البيضاء ($P=0.000$) لمرضى في المناطق الحضرية والريفية في محافظة بابل، في حين لم يظهر CRP فرقاً معنوياً ($P=0.06$)، فيما استنتج من أن فحص الذي دايمر وCRP يعد من المؤشرات الحيوية على شدة الإصابة بمرضى كوفيد-19 في المناطق الحضرية والريفية، بينما لم يكن لمؤشر الجنس تأثير معنوي في جميع المعايير. علاوة على ذلك، أظهرت نتائج التوزيع التكراري للمرضى أن تراكيز الذي دايمر وCRP في الدم تجاوزت القيم الطبيعية المعتمدة لدى المرضى في المناطق الحضرية والريفية معاً، كما أن العديد من قيم الفيبريتين وWBC تجاوزت المعدل الطبيعي عند مرضى المناطق الحضرية ولم تتجاوزه عند مرضى الريف.

الكلمات المفتاحية: كوفيد-19، الفيبريتين، الذي دايمر، البروتين المتفاعل، كريات الدم البيض