Correlation between covid-19 infection and inflammatory biomarkers in hospitalized patients

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Abstract:

Background: Pulmonary inflammation during COVID-19 that cause tissue destruction along with coagulopathy and heamatological dysregulation were noticed among many patients which are overlapping and did not elucidate a specific pattern among those patients, therefore;

Objective: The study aimed to study the haematological markers along with biomarkers in patients infected with COVID-19 in comparison with healthy control to obtain a specific profile aid in infection identification and progress monitoring.

Setting: The study was case control study conducted on 150 inpatients in Fallujah Teaching Hospital and 50 healthy controls from January to September 2021.

Materials & Methods: our study was case control study conducted on 150 inpatients in Fallujah Teaching Hospital and 50 healthy control from January to September 2021, blood samples collected from the patients were classified to whole blood, serum and plasma samples to obtain eligible samples for each type of tests, the tests that accomplished on those samples were measuring the haematological markers including haemoglobin, leukocyte with differential count and platelets, along with inflammatory biomarkers consisting of C-reactive protein, Erythrocyte sedimentation rate, Lactate dehydrogenase, ferritin and D-dimer.

Results: Most of haematological, inflammatory and coagulation markers were high in patient group compared with control healthy group significantly. Elderly are more prone to highly elevated ESR compared with younger ages significantly. Despite the high count of WBC, neutrophils and lymphocytes represent lower values in patients compared with control which exacerbated with increasing of age especially in lymphocytes.

Conclusions: The study concluded poor outcome in hospitalized patients with high destruction and inflammatory response. Ferritin elevation noticed in patients compared with control caused by highly inflammatory response. Elderly would be more prone to secondary bacterial and viral infections due to decreased immunological response represented with lower counts of lymphocyte compared with healthy group.

Key words: SARS-Cov2, LDH, ESR, CRP, Lymphocytopenia, Ferritin.

1. Introduction:

SARS CoV-2 virus infection known as COVID-19 represented a significant emergency during the last two years due to rapid transmission and adverse effects with high mortality (1). symptoms varies according to disease stage ranging from viral pneumonia, and patients may be hospitalized for an extended period of time or placed on a ventilator along with pulmonary inflammation and coagulopathy during the second stage. Therefore; acute respiratory distress syndrome were detected with poor clinical course, with elevated inflammatory biomarkers, such as C - reactive protein (CRP), ferritin, Interleukin 6 (IL-6), Interleukin 1 (IL-1), and d-dimer (2). Reliable laboratory diagnosis is one of the main concerns for facilitating public health actions. Reverse transcriptase (RT-PCR) is used routinely to detect causal viruses from respiratory secretions in acute respiratory infections (3). While the incubation period is approximately 6 days, and viral load significantly increases during this period and early testing may be falsely negative due to disease kinetics (4). Accurate laboratory testing is crucial in the diagnosis and management of the disease (5). A series of changes start immediately after disease initiation and tissue injury representing inflammatory response including many proteins and ferritin (6).

The study aimed to study the impact of SARS-Cov2 infection on haematological parameters with assessing inflammatory markers to establish a pathway to detect and assess disease progression. Using some haematological and inflammatory variables.

2. Materials and methods:

The study was conducted on 150 inpatients with diagnosed COVID-19 infection in the isolating theatre during the period from January to September in 2021 in Fallujah Teaching hospital in comparison with 50 control group without any diagnosed infection. Blood sample were collected aseptically to EDTA and gel clot activator tubes.

2.1. Haematological parameters measuring:

All haematological parameters conducted automatically using CBC device (SYSMEX, Germany) on EDTA-anticoagulated blood sample.

2.2. D dimer detection:

D dimer was conducted on sodium citated sample separated to obtain plasma, the test performed using quantitative test of ichroma ™ device (Boditech Med Inc., South Korea) according to the instructions of the supplemented kit.

2.3. Inflammatory parameters

2.3.1. Ferritin test

Ferritin measured on serum sample using quantitative test of ichroma ™ device (Boditech Med Inc., South Korea) according to the instructions of the supplemented kit.

2.3.2. CRP test

This test done using latex agglutination kit provided by (LINEAR, Spain) by mixing 50 µl of Serum, Positive control or Negative control on separate reaction circle on glass slide with same amount of CRP latex reagent to each of the circles, the results appear after 2 minutes for visible agglutination.

2.3.3. ESR test

The test conducted using kit supplemented by (Dispette, Switzerland) by adding 1 ml of EDTA anticoagulated blood to the container with mixing, then filling Wintrobe ESR tube, results obtained after standing for 1 hour.

2.3.4. Lactate dehydrogenase (LDH) test

The test conducted using kit provided by using Mindray BS-200 Analyzer (MINDRAY, China) according the instructions of the kit.

2.4. Statistical analysis: the data were analysed using T test, chi square test, one way ANOVA test using SPSS program (Version 24) at 0.01 and 0.05 probability.

3. Results:

In the study many parameters in COVID-19 patient were measured in comparison with control group. one of those parameters was CRP, as an acute phase protein elevated after infection nonspecifically, males showed high percentage of positive CRP results (58.1%) than females (68.6%) non significantly indicating absence of relationship between gender and CRP results as presented in Table (1).

Table (1): relationship between CRP result and gender in COVID-19 infection.

Gender	Positive		Negative		P-value¥
	N	%	N	%	
Male	75	58.1%	35	68.6	0.193[N.S]
Female	54	41.9	16	31.4	
Total	129	100%	51	100%	

Significant increasing obtained by LDH in the patient (297.00 $\mp$ 104.17) compared with control healthy group (144.50 $\mp$ 10.16) significantly (p value = 0.00) Moreover; ESR was significantly (p value = 0.00) higher in patients (50.21 $\mp$ 20.73) than control (12.29 $\mp$ 5.07), and D-dimer showed highly significant difference between patients (571.13 $\mp$ 289.86) and control (254.56 $\mp$ 23.89) were patients showed elevated values than normal values. Ferritin was higher than normal values in the patients (341.14 $\mp$ 202.38) compared with control (175.45 $\mp$ 51.08) that chowed normal values of ferritin as shown in Table (2).

Table (2): shows the effect of COVID-19 infection on LDH, ESR, D dimer, Ferritin, compared with the control.

Variabale	patient(N=150)	Control(N=34)	P-value
	Mean $\mp$ SD	Mean $\mp$ SD	
LDH	297.00 $\mp$ 104.17	144.50 $\mp$ 10.16	0.000**
ESR	50.21 $\mp$ 20.73	12.29 $\mp$ 5.07	0.000**
D-dimer	571.13 $\mp$ 289.86	254.56 $\mp$ 23.89	0.000**
Ferritin*	341.14 $\mp$ 202.38	175.45 $\mp$ 51.08	0.000**

* LDH (Lactate dehydrogenase), ESR (Erythrocyte sedimentation rate).

Non significant difference noticed in LDH, ESR, D-dimer and ferritin between males (298.18 $\mp$ 118.51) and females (295.35 $\mp$ 80.34), where LDH in males is higher than females, in addition to ESR in males and females (52.76 $\mp$ 22.75 and 46.6 $\mp$ 17.00) respectively, and ferritin in males and females (323.56 $\mp$ 132.73 and 300.76 $\mp$ 223.07) respectively, in contrast with D dimer where females is higher than males (614.24 $\mp$ 379.43 and 540.76 $\mp$ 201.72) respectively. ctor that defect Ferritin causing elevation to high levels with an extent reflex the severity of disease. According to study of (13). With increasing reflect the increased severity especially in ICU patients or those on ventilator that may be associated with liver or renal injury.

Table (3): shows the effect of COVID-19 infection on LDH, ESR, D dimer, Ferritin by gender.

Variable	Male(N=88)	Female(N=62)	P-value
	Mean $\mp$ SD	Mean $\mp$ SD	
LDH	298.18 $\mp$ 118.51	295.35 $\mp$ 80.34	0.871N.S
ESR	52.76 $\mp$ 22.75	46.6 $\mp$ 17.00	0.73N.S
D-dimer	540.76 $\mp$ 201.72	614.24 $\mp$ 379.43	0.127N.S
Ferritin*	323.56 $\mp$ 132.73	300.76 $\mp$ 223.07	0.435N.S

* LDH (Lactate dehydrogenase), ESR (Erythrocyte sedimentation rate).

Non significant relationship between inflammatory parameters and age of the study groups except for ES, where elderly (>50 years) showed elevation in ESR value compared with the other groups, while the age group of 30-49year represent the highest average among all of those parameters. The lowest percentage was in the 50-year-old group and the lowest was <30 years with non significant differences observed in other parameters including LDH, D-dimer and ferritin as indicated in Table (4).

Table (4): shows the effect of COVID-19 infection on LDH, ESR, D dimer, Ferritin by age groups.

Variable	<30year(n=32)	30-49year(n=66	>50 year(n=52)	P-value
	Mean ∓ SD	Mean ∓ SD	Mean ∓ SD	
LDH	299.3 ∓ 95.99	310.85 ∓ 120.17	278.03 ∓ 84.14	0.235N.S
ESR	44.47 ∓ 20.599	48.25 ∓ 17.997	56.24 ∓ 22.859	0.023*
D-dimer	535.42 ∓ 234.642	586.61 ∓ 281.504	573.47 ∓ 331.497	0.715N.S
Ferritin*	290.79 ∓ 172.61	334.49 ∓ 184.45	302.67 ∓ 165.68	0.435N.S

* LDH (Lactate dehydrogenase), ESR (Erythrocyte sedimentation rate).

The total count of WBC in the patients are higher than healthy group significantly (P-value =0.001), Neutrophils are influenced none significantly with slight elevation. In contrast, lymphocytes decreased significantly in the infected patients (P-value =0.001) the values range from (4870 – 44700 /µl) with mean 24.57∓19.7 / µl (Table 5).

Table (5): shows the effect of the Covid-19 infected on the number of the differential of white blood cells, platelets count and haemoglobin compared with control.

Variable	Patient(N=102)	Control(N=34)	P-value*
	Mean ∓ SD	Mean ∓ SD	
WBC	47.82 ∓ 18.5	34.24 ∓ 10.17	0.001**
Neut.%	57.6 ∓ 19.8	60.24 ∓ 17.10	0.456N.S
Lymph.%	24.57 ∓ 19.7	40.03 ∓ 15.16	0.001**
Mon.%	3.83 ∓ 4.1	5.07 ∓ 1.44	0.001**
Eso.%	0.63 ∓ 0.7	7.95 ∓ 3.51	0.001**
Bso.%	0.06 ∓ 0.1	0.71 ∓ 0.43	0.001**
plate count	308.4 ∓ 127.6	271.1 ∓ 87.9	0.596N.S
Hb*	12.9 ∓ 2.1	15.3 ∓ 2.2	0.000**

* Neut. (Neutrophil), Lymph. (Lymphocyte), Mon. (Monocyte), Eso.(Eosinophile), Bso.(Basophile), Hb(Haemoglobin)

Infection caused decreasing in haemoglobin value, where the average was recorded in patients (12.9 ∓ 2.1) compared to the recorded average in healthy group was (15.3 ∓ 2.2), as appear in Table (6). In elderly, higher than 50 years of old; lymphocytes significantly decrease (P-value = 0.001).While monocytes illustrated the highest value significantly then followed by the mean value of 2.19 in the age group > 30, with no effect of age on other leukocytes values.

8

Table (6): shows the effect of COVID-19 infection on the differential count of white blood cells, platelets and hemoglobin for age groups.

Variable	<30year		30-49year		>50 year		P-value
	N	Mean ∓ SD	N	Mean ∓ SD	N	Mean ∓ SD	
Neut.%	32	54.9 ∓ 21.59	50	60.68 ∓ 17.09	53	58.50 ∓ 19.31	0.314
Lymph.%	32	48.79 ∓ 21.10	50	34.50 ∓ 19.86	53	19.47 ∓ 18.42	0.001**
Mon.%	32	2.19 ∓ 2.65	50	4.2 ∓ 3.49	53	5.40 ∓ 3.92	0.001**
Eso.%	32	2.66 ∓ 3.36	50	3.00 ∓ 3.99	53	1.83 ∓ 3.53	0.253
Bso.%	32	0.30 ∓ 0.45	50	0.23 ∓ 0.33	53	0.17 0.33	0.261
plate count	35	305.31 ∓ 93.4	52	291.53 ∓ 94.43	45	295.88 161.1	0.853
HB	35	13.61 ∓ 2.21	52	13.65 ∓ 2.32	46	13.53 2.37	0.811

* Neut. (Neutrophil), Lymph. (Lymphocyte), Mon. (Monocyte), Eso. (Eosinophile), Bso. (Basophile), Hb (Haemoglobin)

No significant gender-based differences of types of leukocytes noticed between males and females in the study group for WBC, neutrophils, lymphocytes, and platelets, moreover; Platelets are normal in both infected patients and control. Females Hb is lower than males Hb values (12.4 ∓ 2.2 and 14.1 ∓ 2.1) respectively (P-value =0.000). Along with monocytes (3.17 ∓ 3.88 and 6.4 ∓ 3.51) sequentially, without differences in other haematological parameters including other types of WBCs and platelet as presented in Table (7).

Table (7): shows the effect of COVID-19 infection on the differential count of white blood cells, platelets and hemoglobin for sex.

Variable	Male		Female		P-value*
	N	Mean ∓ SD	N	Mean SD	
Neut.%	89	58.12 ∓ 17.89	46	58.53 ∓ 21.51	0.912
Lymph.%	89	31.99 ∓ 21.76	46	31.99 ∓ 24.39	1
Mon.%	90	6.4 ∓ 3.51	46	3.17 ∓ 3.88	0.034*
Eso.%	90	2.77 ∓ 3.86	46	1.86 ∓ 3.25	0.153
Bso.%	90	0.25 ∓ 0.37	46	0.17 ∓ 0.33	0.237
plate count	90	294.8 ∓ 111.5	45	307.4 ∓ 135.5	0.596
HB	90	14.1 ∓ 2.1	45	12.4 ∓ 2.2	0.000**

* Neut. (Neutrophil), Lymph. (Lymphocyte), Mon. (Monocyte), Eso. (Eosinophile), Bso. (Basophile), Hb (Haemoglobin)

4. Discussion:

Many physiological parameters were related with the infection of COVID-19 and it's used as diagnostic parameters when the immunological or molecular tests fail to produce reliable results because of disease kinetic that delay identification of the virus in the nasopharyngeal and oropharyngeal swab along with immune response that detected by IgM and IgG antibodies. While significant increasing obtained by LDH in the patient compared with control healthy group which was agreed with (7).The reason for the elevate   9

in people with covid 19 is that LDH is present within the lung tissue (isozyme3) and during severe covid 19 infection caused by cytokine-mediated tissue damage, Thus, a large amount of LDH is expected to be released. (8); (9).

Same finding also applicable on ESR where significant difference noticed between patients and control that agrees with the results of (10). This value confirmed the existence of a large discrepancy between the averages for infected people and healthy people. A high ESR is not a definitive indication that a person has COVID-19, it is elevated in several diseases such as rheumatoid arthritis, but since ESR can be affected by the shape, size, concentration of red blood cells and plasma characteristics (11), it is possible that COVID-19 may Changes the RBC shape and plasma properties and thus leads to an increase in ESR. The elevation of D dimer in patients which is consistent with (10). The inflammatory marker (ferritin) was high in accompany with other inflammatory markers, which indicates that the relationship is highly significant which agrees with (12).

The results illustrated convergence of LDH, ESR, D dimer and ferritin averages for males and females, where males are higher than females none significantly which does not correspond to the results of (1) who indicated significant relationship showed in table (3). COVID-19 represent a confounding factor that defect Ferritin causing elevation to high levels with an extent reflex the severity of disease. According to study of (13). With increasing reflect the increased severity especially in ICU patients or those on ventilator that may be associated with liver or renal injury. No effect of age on inflammatory parameters but elderly showed elevation in ESR value which almost corresponds to (14) as the ESR rises in the age groups above 50 more than other age groups.

The elevation of total count of WBC in the patients compared with healthy group, which indicates that total white blood cells rise in those with COVID 19 and this is consistent with (15) while it important to detect which type of leukocytes being influenced by the infection. Neutrophils are influenced non significantly with slight elevation. In contrast, the main effect appears on lymphocytes leading to lymphocytopenia which confirms that the lymphocyte is reduced in those with Covid 19 and this is consistent with (16). In our study we revealed normal total WBC count with decreased lymphocyte count, Several studies demonstrated that neutrophilia might be related to the cytokine storm (absolute neutrophil count above the normal range; $3-7.5 \times 10^9$/L) and/or lymphocytopenia (lymphocyte count $< 1.5 \times 10^9$/L) were present in severe cases of COVID-19 and were associated with poor prognosis.(17) and (18) concluded that hemocytometric changes, especially the presence of lymphopenia and an elevated neutrophil-lymphocyte-ratio, in patients infected with SARS-CoV-2 virus may assist clinicians in diagnosing and predicting disease progression of COVID-19. Virus may directly inhibit the proliferation of nucleated cells in bone marrow, resulting in significantly low LYM, granulocyte and PLT counts in peripheral blood (19).

Lymphocyte may return to increase following treatment with anti-viral and anti-inflammatory medications (18). Neutrophil to lymphocyte ratio (NLR) has also been found to predict disease severity in the early stages of SARS CoV-2 infection (17). The reason of lymphocytopenia is due to lymphocytes express the ACE2 receptor on their surface (20); thus SARS-CoV-2 may directly infect those cells and ultimately lead to their lysis. Add to it, the cytokine storm is characterized by markedly increased levels of interleukins (mostly IL-6, IL-2, IL-7, granulocyte colony stimulating factor, interferon-γ inducible protein, MCP-1, MIP1-a) and tumor necrosis factor (TNF)-alpha, which may promote lymphocyte apoptosis (21). All other leukocytes are lower in infected group than the healthy control significantly for monocytes, eosinophils and basophils as presented by (22). Researchers have suggested a possible causal role for the reduced number of basophils in the increased risk of severe COVID-19 disease, possibly due to an inadequate innate immune response to SARS-CoV-2 (23). It also showed a p-value = 0.596 platelets representing not significant increase of platelets in the infected patients limited to the normal value as shown by (24).

No significant gender-based differences of types of leukocytes noticed between males and females in the study group for WBC, neutrophils, lymphocytes, and platelets that represent convergence with the results of (19) moreover; in the same study, Platelets are normal or decreased in non-severe patients and significantly decreased in severe patients. Thrombocytopenia noticed in many severe infections other than COVID infections, which may be a result of antibodies damaging thrombocytes or infected hematopoietic stem cells leading to hematopoietic inhibition (19).

A slight decrease of haemoglobin values in patients and this is consistent with (25). And (19), they presented decreased values of MCV as well as RBC, hemoglobin; hematocrit and mean corpuscular hemoglobin (MCH) were generally low in COVID-19 patients at onset. In elderly, higher than 50 ye

10

old; lymphocytes significantly decrease, same finding obtained by (26). Suggesting that older patients (>60 years) who have more systemic symptoms, lymphopenia and thrombocytopenia are critical factors associated with disease severity and mortality (18). Monocytes is high in the age group > 30 and this agrees with (27), with no effect of age on other leukocytes values. Patient characteristics such as young or advanced age or pregnancy may also influence hemocytometric parameters (19), thus may indicate partially the reason of slight differences between males and females. But MCV increased after day 8 of admission, indicating RBC recovery. When recovery did not occur, MCV remained low as has been found in COVID-19 patients who died. Moreover; by Inflammation and direct infection of precursor cells of RBC by the virus itself, Hb decrease.

Thrombocytopenia may also be caused by increased consumption of platelets and/or decreased production of platelets in damaged lungs in severe pulmonary conditions. Higher platelet turnover leads to macrothrombocytes together with increased release of young platelets that have higher volumes and, as such, may result in a high MPV as is found in COVID-19 patients (19).

5. Conclusion:

Elderly patients are more prone to many adverse changes especially decreasing in immunity represented by lymphocytopenia, many inflammatory markers elevated during infection suggesting high inflammatory response caused by cytokine storm. As for many infections, ferritin represent biomarkers that could be used for prediction of infection with prognosis of the disease along with CRP and ESR. Slight decreasing in Hb that accompany with little and non significant difference between age groups and males with females.

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العلاقة بين الإصابة بكوفيد -19 وبعض العلامات الالتهابية في المرضى الراقدين في المستشفيات

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الخلاصة:

الخلفية: ان الالتهاب الرئوي المصاحب لـ COVID-19 الذي يسبب تدمير الأنسجة إلى جانب تجلط الدم واضطراب الدم لدى العديد من المرضى والتي من الممكن ان تتداخل ولا ينتجون نمطًا معينًا بين هؤلاء المرضى.

الهدف: هدفت دراستنا إلى دراسة المؤشرات الدموية جنبًا إلى جنب مع المؤشرات الحيوية في المرضى المصابين بـ COVID-19 مقارنة بمجموعة سيطرة من اشخاص اصحاء للحصول على نمط تعريف محدد في تحديد العدوى ورصد التقدم.

الإعداد: كانت دراستنا عبارة عن دراسة حالة مراقبة أجريت على 150 مريضًا داخليًا في مستشفى الفلوجة التعليمي و 50 مريضًا صحيًا من يناير إلى سبتمبر 2021.

المواد والطرق: بعد الحصول على عينات الدم من مجموعتي الدراسة ، تم تصنيف عينات الدم التي تم جمعها من المرضى إلى عينات الدم الكامل والمصل والبلازما للحصول على عينات مؤهلة لكل نوع من الاختبارات ، كانت الاختبارات التي تم إجراؤها على تلك العينات هي قياس مؤشرات الدم بما في ذلك الهيموجلوبين ، الكريات البيض ذات العد التفاضلي والصفائح الدموية ، إلى جانب المؤشرات الحيوية الالتهابية المكونة من بروتين سي التفاعلي ، ومعدل ترسيب كرات الدم الحمراء ، وانزيم اللاكتات النازع للهيدروجين ، وبروتين فيريتين ، وبروتين د- دايمر.

النتائج: كانت معظم علامات الدم والالتهابات والتجلط عالية في مجموعة المرضى مقارنة مع مجموعة السيطرة بشكل ملحوظ. كبار السن هم أكثر عرضة لارتفاع ESR بشكل كبير مقارنة مع الأعمار الأصغر بشكل ملحوظ. على الرغم من ارتفاع عدد خلايا الدم البيضاء ، فإن العدلات والخلايا الليمفاوية تمثل قيمًا أقل في المرضى مقارنة مع مجموعة التحكم والتي تتفاقم مع زيادة العمر خاصة في الخلايا الليمفاوية.

الاستنتاجات: خلصت الدراسة إلى نتائج سيئة في المرضى المقيمين في المستشفى مع تدمير عالي واستجابة التهابية. لوحظ ارتفاع فيريتين في المرضى مقارنة مع السيطرة الناتجة عن الاستجابة الالتهابية الشديدة. سيكون كبار السن أكثر عرضة للعدوى البكتيرية والفيروسية الثانوية بسبب انخفاض الاستجابة المناعية المتمثلة في انخفاض عدد الخلايا الليمفاوية مقارنة بالمجموعة السليمة.

الكلمات المفتاحية: اضطراب الدم، الالتهاب الرئوي، كوفيد 19