

Assessment of Serum Ferritin, Folate, Vitamin B12, C-reactive Protein, D-dimer and Homocysteine in Patients with Severe and Critical Covid-19 Infection

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Abstract

Background: Corona virus disease 2019 (COVID-19) pandemic need urgent measurement and correlation of laboratory parameters in severe and non-severe cases. Serum ferritin, folic acid, vitamin B12, C-reactive protein, D-dimer and homocysteine was measured in patients who were infected with COVID-19.

Aim of the Study : To evaluate the usefulness of serum ferritin, folate, B12, C-reactive protein, D-dimer and homocysteine level as biomarkers for prediction of patients with severe and critical COVID-19 infection.

Subject, Material, Method: A cross sectional study was conducted at AL-Kindy hospital, Baghdad, Iraq from October 2020 to December 2020. Serum ferritin, folate, Vitamin B12, C-reactive protein, homocysteine and blood D-dimer were measured in 88 patients who suffered from COVID-19 infection, 50 cases with severe infection and 38 with non-severe infection. The age range of patients between 20-59 years old. Samples were collected from AL-Kindy hospital, Baghdad.

Result: Serum folic acid was significantly low in severe group than in non-severe group, while serum ferritin, CRP and blood D-dimer were high in severe group than in non-severe group.

Conclusion: In this study, measurement of serum ferritin, CRP and blood D-dimer were important in follow up of patients who infected with the novel virus as the level of them were higher in severe than in non-severe cases. Folic acid has role in determining the severity of COVID-19 infection because level was low in patients with severe than non-severe infection.

Footnote: COVID-19, folic acid, vitamin B12, C-reactive protein, D-dimer, ferritin and homocysteine.

Introduction

Corona viruses infect humans and animals, in general human corona viruses (HCoVs) cause 15-30% of common colds. Any animal reservoir corona viruses can however be transferred to humans, causing outbreaks in the human population, these zoonotic viruses infect both humans and animals, unlike HCoVs, and cause serious respiratory diseases such as acute respiratory distress syndrome (ARDS) and pneumonia, resulting in death⁽¹⁾.

90 % of patients with COVID-19 have irregular chest radiographs, In serious and critically ill patients,

lymphopenia is common, and in patients with mild symptoms, it is uncommon, COVID-19's chest computed tomography features include bilateral opacity of ground glass, consolidation, and local or bilateral patchy shadowing, In COVID-19 patients, GI symptoms are common, and a meta-analysis has shown that these symptoms occurred in 17.6% of infected patients and are more common in serious patients⁽²⁾.

Respiratory tracts are the primary focus of SARS-CoV-2, contributing to clinical symptoms such as fever, dry cough, fatigue and dyspnea, in 10-20% of patients,

the disease usually progress into a serious type, requiring hospital admission or even intensive care unit⁽³⁾.

Ferritin levels were high in patients who died from COVID-19 upon admission to hospital and during the hospital stay⁽⁴⁾. In COVID-19 infection, pregnant women who classified as severe and critical were less than non-pregnant women, The factor protecting these patients against SARS-CoV-2 infection may be folic acid supplementation during pregnancy⁽⁵⁾.

A significant modifiable risk factor for COVID-19 morbidity and mortality in the elderly and those with diabetes mellitus could be vitamin B12-folate-homocysteine dependent biochemical reactions that are crucial for wide-ranging aspects of DNA synthesis, cellular regulation and body repair⁽⁶⁾.

In most patients with COVID-19, elevated serum C-reactive protein (CRP) levels were observed⁽⁷⁾.

Elevated D-dimer and the increased rate of poor prognosis-related conditions, and the initiation of anticoagulant administration identified with D-dimer as a potentially useful method to avoid complications and have a beneficial effect on the course of corona virus disease 2019 (COVID-19)⁽⁸⁾.

Aim of the Study

To evaluate the usefulness of serum ferritin, folate, B12, CRP, D-dimer and homocysteine level as biomarkers for prediction of patients with severe and critical COVID-19 infection.

Subject, material, method:

Across sectional study was conducted at Al-kindy hospital in Baghdad/Iraq, from October 2020 to December 2020, where 88 patients with COVID-19 infection documented by polymerase chain reaction (50 patients with severe symptoms who need hospitalization and 38 patients with non-severe criteria), the age of the patients range from (18-59) years old.

The permission was obtained from Al-kindy hospital, the blood samples were drawn from the patients after their agreement.

Whole blood samples were collected in a clot activator tube, centrifuge the sample after clotting for 5 minutes at 3000 rpm, clear serum was obtained which is divided into five eppendorf tube (1ml each eppendorf tube). Then the serum was frozen at -50°C. Repeated freeze-thaw cycle were avoided.

For measurement of D-dimer, human whole blood was needed and the test must be done within 24 hours.

Serum level of ferritin was measured by Vidas ferritin is an automated quantitative test for measurement of human ferritin in human serum or plasma using Enzyme Linked Fluorescent Assay (ELFA).

Serum level of folic acid, vitamin B12 and homocysteine were measured by Enzyme Linked Immuno-sorbent Assay (ELISA) for quantitative measurement of human serum.

Serum C-reactive protein was measured by Nycocard which was a diagnostic test for quantitative determination of C-reactive protein in human.

D-dimer was quantitatively measured by Fluorescence immune-assay (FIA).

Statistical Analysis

Data translation was done in computerized database, SPSS version program 25 was used to do analysis, p value less than 0.05 was statistically significant results.

Result

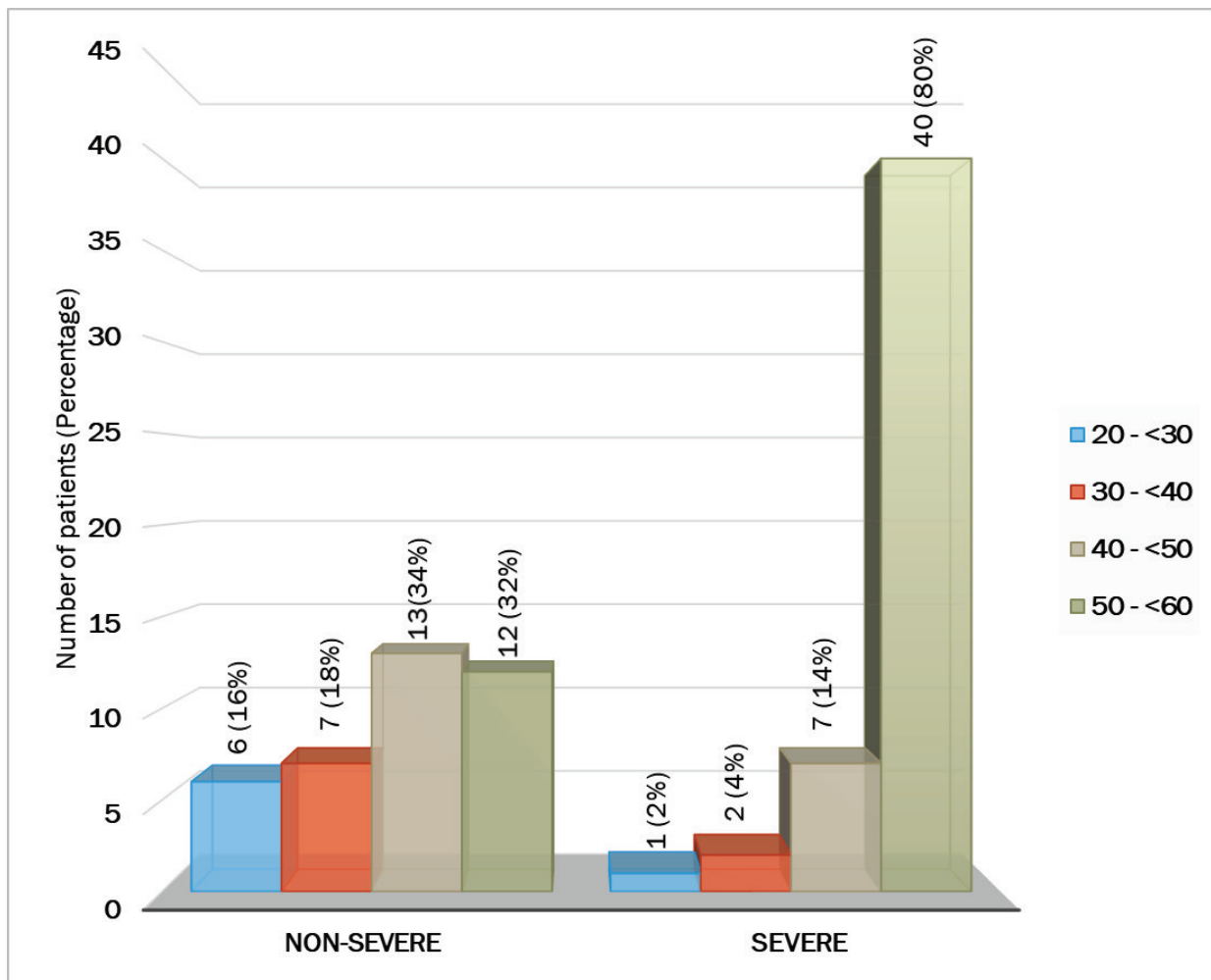
Study conducted on 88 patients who suffer from COVID-19 infection, split into two groups (50 patient with severe infection and 38 patients with non-severe infection).

The most frequently observed category Male ($n = 26, 46.43\%$) and ($n = 30, 53.57\%$) for non-severe and severe groups.

Table 1: Gender distribution of patients according to severity of COVID-19 infection:

Gender	Non-severe n (%)	Severe n (%)	p-value
Female	12 (37.5)	20 (62.5)	0.415
Male	26 (46.43)	30 (53.57)	

In non-severe COVID-19 patients, the most frequently observed category of age was 40 - <50 years ($n = 13, 34\%$). As compared with 50 - <60 years ($n = 40, 80\%$) for Severe cases. Age had an average of 53.24 ± 6.60 , and Median was 55.5 in severe group. For non-severe, the average of age was 42.66 ± 10.14 years, and median was 44 years.



Figure(1): Age distribution in patients with COVID-19 infection.

The most frequently observed category of biochemical parameters in severe and non-severe patients with COVID-19 infection show that the folic acid was significantly lower in severe group than in non-severe group ($p < 0.05$).

There was significant difference in vitamin B12 between the Covid-19 patients' groups, $p < 0.05$. Severe group was significantly lower than non-severe group.

There was significant difference in homocysteine between Covid-19 patients groups $p < 0.05$, Patients with

Severe infection was significantly lower homocysteine level than non-severe group.

There was significant difference in D-dimer level in blood between Covid-19 patients groups $p < 0.05$, Patients with Severe infection was significantly higher level than non-severe group.

There was significant difference in C-reactive protein level between Covid-19 patients groups $p < 0.05$, Patients with Severe infection was significantly higher level than non-severe group.

Table 2 Laboratory findings in patients with COVID-19 according to severity of infection

Laboratory results	Severity of COVID-19 infection				*p-value
	Severe (n= 50)		Non-severe (n= 38)		
	Median + IQR	Mean \pm SD	Median + IQR	Mean \pm SD	
Folic acid (nmol/L)	4.28 (3.22-4.86)	4.22 \pm 1	5.58 (4.69-7.68)	7.61 \pm 5.03	< 0.001
B12 (pmol/L)	463.56 (445.26-480.97)	474.83 \pm 65.74	486.75 (466.66-576.72)	535.69 \pm 105.91	0.0015
Homocysteine (nmol/ml)	2.61 (2.19-3.22)	3.45 \pm 3.83	6.65 (3.03-15.11)	10.86 \pm 10.84	< 0.001
Ferritin (ng/ml)	622.5 (436-702.75)	580.54 \pm 212.41	144.5 (72.50-571.75)	302.26 \pm 303.79	< 0.001
D-dimer (μ g/ml)	1.38 (0.81-3.20)	2.49 \pm 2.51	0.22 (0.12-0.51)	0.36 \pm 0.31	< 0.001
CRP (mg/L)	51 (17.75-90.75)	55.68 \pm 41	5 (5 -12.75)	15.63 \pm 26.66	< 0.001

Discussion

Serum folic acid level was lower in severe group than serum folic acid in non-severe group, the results agree with (Elias J, *etal*, 2020) which was found that Pregnant women seem to have a lower risk of contracting COVID-19 infection, and those who infected were more likely to be asymptomatic. Pregnant women from ethnic groups with low folate RBC concentrations due to genetic factors and/or who do not receive folic acid supplementation, have a higher hospitalization rate⁽⁹⁾.

Serum vitamin B12 levels were normal in patients with severe and non-severe COVID-19 infection. In non-severe COVID-19 infection, mean of serum vitamin B12 level in severe group was higher than non-severe group. These results were disagreeing with (Dalbeni

A, *etal*, 2021) which was found that Excess serum B12 level has been linked to a rise in mortality rates⁽¹⁰⁾.

Serum homocysteine levels were normal in non-severe COVID-19 infection patients, while serum homocysteine levels were low in severe COVID-19 infection. The results were disagree with (Ponti G., *etal*, 2020) which show that plasma homocysteine must be measured on a routine basis as a potential marker for severe disease in SARS-CoV-2 patients⁽¹¹⁾.

Serum ferritin levels were significantly increased in patients with severe COVID-19 infection in comparison with non-severe COVID-19 infection. These results were agree with (Henry B, *etal*, 2020) and (Cheng L., *etal*, 2020). Who show that severe group had substantially higher serum ferritin levels than non-severe also they

recommend that serum ferritin must be used to monitor COVID-19 patients' prognosis⁽¹²⁾⁽¹³⁾.

Serum C-reactive protein showed significantly high levels in patients with severe COVID-19 infection. These results were agree with (Liu F., *etal*, 2020). Their research looked into the connection between CRP and COVID-19 and discovered that patients with CRP levels greater than (41.8 mg/L) were more likely to develop serious disease⁽¹⁴⁾.

D-dimer levels showed significantly high levels in patients with severe COVID-19 infection while the in patients with non-severe COVID-19 infection was within normal range. The results were agree with (Li Y., *etal*, 2020) which show that patients with severe COVID-19 infection had a higher D-dimer than non-severe infection in the patients without cardiovascular disease⁽¹⁵⁾.

Conclusion

1- High Serum ferritin, C reactive protein and whole blood D-dimer levels were important to confirm the severity of the patients with COVID-19 infection.

2- Low serum folic acid level was associated with severe COVID-19 infection.

3- Serum vitamin B12 level was not affected by the severity of the COVID-19 infection.

4- Serum homocysteine level had no role in detection of the severity of COVID-19 infection because it was affected by drugs which were received as a protocol for COVID-19 treatment.

Recommendation:

1- A large sample size of patients are needed, follow up of patients until they discharged from the hospital in addition to calculate the mortality rate.

2- Two groups of patients with severe COVID-19 infection, first group receive folic acid tablet and the other group receive placebo then follow up to the patients.

3- Measurement of other parameters in COVID-19

infection such as lactate dehydrogenase, troponin and procalcitonin to detect the severity of infection.

4- Measurement of serum ferritin , C-reactive protein and whole blood D-dimer levels before intake of vaccine.

Conflict of Interest: No

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Ethical Clearance: Not Required

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