CME LIVE OPEN ACCESS

Journal of Clinical Immunology Research

Research Article

Importance of clinical and laboratory investigations for diagnosis of Covid-19 infections

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Received: April 20, 2023 **Accepted:** April 29, 2023 **Published:** May 17, 2023

Abstract

Background: Covid-19 infections was first emergenced in Wuhan city, China, severe acute respiratory syndrome coronavirus 2, which responsible for the pandemic COVID-19, has become a significant health problem all over the world affecting over 2.1 million people globally.

Aim: The current study aimed to investigate serum levels of ESR, ferritin, C-reactive protein (CRP), D-dimer as well as creatinine and neutrophils count in COVID-19 patients, and to clarify the correlation of these parameters with the disease and progression. Forth ESE purposes,

Methods: A (93) patients with COVID-19 (confirmed by polymer as each in reaction and/or CT-scan).

Results: Almost all study population (patients) were subjected the evaluation of serum levels of ESR, ferritin, and CRP Creatinine, D-dimer, as well as and neutrophils, count. COVID-19 patients showed a significant elevation in the levels of all parameters included in study when compared with healthy controls (standard values). We also found that all of ESR ferritin, CRP Creatinine, and D-dimer are significantly associated with severity of the COVID-19symptoms.

Conclusion: CRP and increased neutrophils were also effectively correlated with disease progression. In line with these results, we concluded a proportional correlation between the aforementioned parameters and COVID-19suggesting the uses of these tests to the diagnosis of critical cases.

Introduction

Coronavirus disease – 2019 (COVID-19) is disease that was detected in December 2019 in Wuhan, China, and led to the risk mortality of about 2% [1]. This disease is caused due to infection with a recently arising zoonotic virus known as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [2].Previously, infection with coronaviruses appeared in 2002 within China in the form SARS-CoV, and it appeared later also in 2012 within Saudi Arabia that was known as Middle East Respiratory Syndrome (MERS-CoV) [3, 4]. All these coronaviruses are enveloped positive-strand RNA viruses that are isolated bats that can be transferred from animals to humans, human to human, and animal's animals [5].

They share a similarity in the clinical symptoms in addition to specific differences that have been recently observed [5, 6]. In Iraq, the first case of COVID-19 was recorded in March 2020 in Al Najaf city, since which the virus spread dramatically around the country causing approximately 51,300 infections and more than 7000 deaths according to the daily reports declared by the Iraqi ministry of health. The symptoms of this disease appear with different degrees that start in the first seven days with mild symptoms such as fever, cough, shortness of breath, and fatigue [7]. Afterward, critical symptoms may develop patients involving dyspnea and pneumonia that require patient's management in intensive care units to avoid the serious respiratory complications that may lead to death [8]. However, there are no specific symptoms to diagnose coronavirus infection, and accurate testing depends on the detection of the viral genome using the reverse transcription-polymerase chain reaction (RT-PCR) analysis [9]. The immunological and hematological not have significant data so the aim of this research is to look for the changes appear on patient with COVID-19 the important of it in diagnosis.

Material and methods

Study population: The study involved (93) patients were infected with covid-19, aging from 10 to 100 years old and

both sexes. The sample was taken from patients with variety of severity .Almost all patients were subject to estimation RBC, WBC, serum ferritin, ALP (Alkaline phosphates) D-dimer, ESR, CRP (C-reactive protein) and creatinine. Also the study include the distribution of infection in age according to sex and 50 case for diagnosis criteria, clinical symptoms and booster vaccine among patients.

Estimation ESR

Erythrocyte Sedimentation Rate Analyzer / ESR (Sed Rate) Analyzers. Also called (modified wester green) is the machine use to estimate ESR (Blood Dynamic Full Automatic ESR/HCT Analyzer machine MSLXC03, MSLXC03).

Serum ferritin

Cobas c501 biochemistry module measurement method: Human-driven ferritin shows agglutination with latex particles covered with anti-ferritin anti bodies in expanded particle surface immune turbid metric test. Precipitation was to be turbid metric at 570/800 nm.

Estimate serum creatinine and alkaline phosphates {ALP}
The comprehensive metabolic panel (CMP) is a blood test that gives doctors information about the body's fluid balance, levels of electrolytes like sodium and potassium, and how well the kidneys and liver are working.

Determination of serum D-dimer and C-reactive protein

Serum concentrations of D-Dimer and CRP were evaluated using a specific automated protein analyzer (PA120) provided by (Shenzhen Genius Electronics Co., Ltd. China 2019). Serum samples for each of the patients and healthy persons were applied to the instrument then the concentrations of D-dimer and CRP are calculated automatically.

Estimation of CBC (complete blood Count)

An automated hematology analyzer, which counts cells and collects information on their size and structure (Bio base Medical Fully Auto Hematology Analyzer CBC Blood Test Machine BK-5000, 2022).

Result

There sults of the current study, which included (93) patients suffering from SARS CoV2 infection, found, as illustrated in Figure 1, that the percent of COVID-19 infection was higher in male 62% and lower in female 38%. The study was conducted in Basra Teaching Hospital and Al-Sadr Teaching Hospital, located south of Iraq as in table 1 below.

Table 1: DistributionofCovid-19 patients according to age and gender

Age group(yrs)	Male		Female		Total	
	Nomber	%	Number	%	No	%
1-10	1	1%	0	0%	1	1%
11-20	0	0%	1	1%	1	1%
21-30	0	0%	0	0%	0	0%
31-40	1	1%	1	1%	2	2%
41-50	3	3%	1	1%	4	4%
51-60	3	3%	3	3%	6	6%
61-70	22	24%	9	10%	31	34%
71-80	20	22%	13	15%	33	37%
>80	8	8%	7	7%	15	15%
Total	58	62%	35	38%	93	100%
P value	0.0481		0.0621		0.0435	
Significant	S*		N.S		S*	

*S=significant. N.S: non- significant

The results of Table 2 are showing that the mean value for white blood cell was significantly higher (P =<0.05) in COVID-19 patients (13.86 10^3/uL in male, 14.57 10^3/uL in female) compared to the standard values (4.5-11.00 10^3/uL for both sex). Likewise, the mean of the serum levels of D-Dimer, Ferritin, and CRP with the ESR mean level was also elevated significantly among COVID-19 patients compared to the standard values. We also found that total Neutrophils count was increased significantly (P = 0.0363) in patients infected with SARS CoV2 (mean = 12.2,13.06 10^3/uL for male and female respectively) compared to the standard values .We also found as shown in Table 2,that ESR mean level was significantly higher (P- value=0.0331,0.0372 for male and female respectively) in patients infected with SARS CoV2 (mean =35, 57 mm/hrs. for male and female respectively) in compared to standard value(0-20mm/hrs.). Furthermore, the D-Dimer level was found to increase significantly as shown in Table 2 in patients infected with SARS CoV2 for both sex.].In our study we also found that the mean serum level for both Ferritin and CRP were significantly increased as shown in Table 2 in patients infected with SARS CoV2 and for both sex.

Table 2: laboratory investigation among patient with covid-19 with standard value

Laboratory investigation	Gender	mean value	Standard value	Unit	P-value	Significant
RBC	Male	4.63	4.5-5.9	10^6/uL	0.0612	N.S
	Female	3.65	4.1-5.1		0.0591	N.S
WBC	Male	13.86	4.5-11.00	10^3/uL	0.0431	S
	Female	14.75			0.0467	S
ESR	Male	35	0-20	mm/hrs	0.0331	S
	Female	57			0.0372	S
Neutrophils	Male	12.2	1.63-6.96	10^3/uL	0.0363	S
	Female	13.06			0.0281	S
Monocytes	Male	0.812	0.240-0.79	10^3/uL	0.0731	N.S
	Female	0.302			0.0812	N.S
D-dimer	male	3.58	0-0.55	mg/L	0.0296	S
	female	2.073			0.0350	S
creatinine	male	2.777	0.2-1.25	mg/dL	0.0431	S
	female	0.890			0.0591	N.S
Ferritin	male	1023.275	30-400	ng/mL	0.0331	S
	female	450.674			0.0372	S
alkaline phosphates	male	110.875	40-150	U/L	0.0731	NS
	female	86.345			0.0812	NS
CRP	male	62.363	<10	mg/L	0.0363	S
		85.831				S

*RBC: red blood cell, ESR: erythrocyte sedimentation rate. CRP: c-reactive proteins S: significant .N.S: non-significant.

The result of Table 3 shows that all patient have chest pain and cough in 100 %(p=0.0731, p=0.0739 respectively). Almost all patients have fever (p=0.0612, 96%) and for less extend eye infection was 54% (p=0.0731).All females were had fever, chest pain, breathing difficulties and cough (100% for all) while the lesser symptoms were eye infection and sore throat (50% for both). in male all have chest pain and cough (100%) and lesser symptoms appear was eye infection (57%). All result demonstrated in Table 3a

Table 3: shows the clinical symptoms among patient with covid-19

Clinical symptoms	Male (28)		Female (22)		Total (50)		P-value	Significant
	Number	%	Number	%	Number	%		
Fever	26	92%	22	100%	48	96%	0.0612	N.S
Chest pain	28	100%	22	100%	50	100%	0.0731	N.S
Vomiting	20	71%	19	86%	39	0.78	0.0691	N.S
Diarrhea	15	53%	12	54%	27	78%	0.0731	N.S
Loss of taste	25	89%	20	90%	45	90%	0.0831	N.S
Breathing difficulty	27	96%	22	100%	49	98%	0.0622	N.S
Cough	28	100%	22	100%	50	100%	0.0739	N.S
Fatigue	21	75%	15	68%	36	72%	0.0921	N.S
Headache	20	72%	18	81%	38	76%	0.0866	N.S
Runny nose	25	89%	19	86%	44	88%	0.0637	N.S
Eye infection	16	57%	11	50%	27	54%	0.0731	N.S
Muscle-body aches	20	71%	15	68%	35	70%	0.0883	N.S
Sore throat	16	57%	11	50%	2 7	54%	0.0792	N.S

* S: significant .N.S: non-significant

The result of Table 4 is showing that half of the patients in that study sample are vaccinated and most of them are males

Table 4: Type of booster vaccine among patient with covid

Booster vaccination	Male (numbers:28)		Female (numbers:22)		Total		P-value	Significant
	Number	%	Number	%	Number	%		
One time	14	50%	5	23%	19	38%	0.041	S*
Twice	4	14%	3	13%	7	14%	0.0532	N.S
Nonvaccinated	10	36%	14	64%	24	48%	0.0641	N.S
Total	28	100%	22	100%	50	100%	0.071	N.S

S: significant .N.S: non-significant

while in Table 5 we see that most cases was diagnosed by CT-scan (94%-p=0.0831) and about half of cases diagnosed by x-ray as a secondary diagnostic tool (50-p=0.0862).In female the most diagnosis tool used CT-scan(78%) and also about half used x-ray(53%) while in male the most used tool is CT-scan(89%) and less than half is x-ray (35%) and only 2 patients not used any of diagnosis way(Rt-PCR,CT-scan,X-ray)

Table 5: diagnosis criteria among patient with covid-19

Diagnosis	male No.(28)		female No.(22)		Total No.50		p-value	significant
	No.	percentage	No.	percentage	No	percentage		
Rt-PCR	20	71%	21	75%	41	82%	0.0923	NS
CT-scan	25	89%	22	78%	47	94%	0.0831	NS
x-Ray	10	35%	15	53%	25	50%	0.0862	NS
non	2	7%	-	-	-	-	-	-

S: significant .N.S: non-significant

Discuss

In the present study, which is conducted Basrah city, according to Table 1 62% of the SARS CoV2-infected patients were men and 38% were women.

Females and males have a variable response to viral infection just such as SARS CoV, Middle East respiratory syndrome (MERS) SARS CoV2, and other viruses. These differences are the leading of disease severity and incidence between the two genders. Multiple factors contribute to the disparity in sex-specific disease out comes following virus infections. Sex-specific steroids and the activity of X-linked genes, both of which modulate the innate and adaptive immune response to virus infection, influence the immune response. Furthermore the differences in the expression of angiotensin-converting enzyme (ACE) 2 receptor and the cellular serine protease TMPRSS2, which are necessary for the binding and priming of SARS CoV2, may have an important role[10,11].In agreement with our results about increase in leukocytes specially Neutrophils as shown in Table 2. A retrospective study found that several differences in WCC between severe and non-severe COVID-19 patients [12].

Both groups experienced an increase leucocytes with the severe group having a significantly greater rise (5.6 vs 4.9 × 109/L; P < 0.001). NCs were predominantly driving this increase as the severest (4.3 vs 3.2 × 109/L; P < 0.001).In addition our result also found that as shown in Table 2 increased significantly in the level of ESR (P<0.05) and in both sex, and as a reason of that RBCs typically fall at a faster rate in people with inflammatory conditions such as infections cancer, or autoimmune conditions. These conditions lead to an increase in the number of proteins in the blood. This increase causes red blood cells to stick together (clump) and settle at a faster rate. A group of RBCs that are clumped together will form stack (similar to a stack of coins) called a Rouleau (pleural is Rouleau) [13]. Rouleau formation possible because of the particular discoid shape of RBCs. The flat surfaces of the RBCs allow them to make contact with other RBCs and stick together.

The ferritin leveling COVID-19 patients, as shown in Table 2, was significantly increased and reached to (1023.27,450,67 ng/ml for male and female respectively). Several publications have shown that elevated ferritin levels have been associated with worse outcomes, along with several other pro-inflammatory markers, involving CRP and IL-6, and may even help predict these outcomes [14-16].

In our study the C - reactive protein as shown in Table 2 was highly significantly increased (mean=62.36, 85.83 mg/L for male and female respectively) with P-value =0.0363, 0.0281 for both sex respectively). The application of CRP in COVID-19 has been highlighted by a retrospective single-center study in Wuhan, China, where the majority of patients in the severe cohort showed significantly higher levels compared to the

non-severe cohort (57.9 mg/L vs 33.2 mg/L,P < 0.001) [12].

A second retrospective cohort study found the like lihood of progressing to severe COVID-19 disease increased in patients with CRP levels > 41.8 mg/L [17].

Both studies suggest CRP level strong indicator to reflect the presence and severity of COVID-19 infection. As a tool used to detecting the prognosis of the disease, pathologically, computed tomography (CT) scans can identify lung lesions relating to COVID-19.Nonetheless, a study conducted in China revealed CT scores could not differentiate mild cases from severe. However compared to erythrocyte sedimentation rate (ESR), CRP levels were significantly greater during early periods of severe cases and proved to be a more sensitive biomarker in reflecting disease development [18].

The excellent performance of CRP as a biomarker is reflected in the 'area under curve' in the receiver operating analysis of 0.87 (95% CI, 0.10–1.00) where values 83% and 91% represent sensitivity and specific it respectively. Hence compared to CT scans alone, CRP values are more reliable for earlier identification of case severity [18].

As the study show in Table 2, the high creatinine level may associated with severe cases of infection. There is also evidence that chronic kidney disease is associated with severe forms of COVID-19 infection [19].Studies have demonstrated significantly higher levels of renal bio- markers such as serum urea, creatinine and markers of glomerular filtration rate in severe cases [20]. Since these results stem from the analysis of 28 patients, extrapolation across larger cohorts is more difficult. Furthermore, we found that as shown in Table 2 the mean level of serum D-dimer was significantly increase in patients infected with SARS CoV2 and for both se Early studies have associated COVID-19 with hemostatic abnormalities with on study observing elevated levels of D-dimer, the measure of coagulation, inn on-survivors compared to survivors [21].A retrospective cohort study composed of 191 patients found that D- dimer levels > 1.0 mg/mL(p=0.0033) were associated with increased mortality among COVID-19 patients. Furthermore, they found that levels of 2.0 mg/mL or more on admission was the optimum cut-off to predict in-hospital mortality for COVID-19 [22] Studies have reported that nearly 90% of inpatients with pneumonia had increased coagulation activity marked rising D-dimer levels [23].

Weal so found that according to Table 4 male vaccinated number is about twice as female vaccinated and that may due to social status and culture in the society in Iraq In our study there is a variety of symptoms different in both sexes. The majority of different in fever [in female 100%, in male vomiting (female 86%, male 71%), headache (female 81%, male 72%) so the female symptoms in general is more than male. As seen in Table 3 In a report from Europe published on April 30, 2020, the most common symptoms were headache (70.3%), anosmia (70.2%), stuffiness (67.8%), cough (63.2%), fatigue (63.3%), muscle pain (62.5%), rhinorrhea (60.1%), gustatory dysfunction (54.2%), sore throat (52.9%) and fever (45.4%) [24]. In Taiwan, COVID-19cases were imported from Europe (41.7%), America (23.9%), Asia (17%), Oceania (0.8%) and some were indigenous cases (13.6%). Therefore, the presented symptoms vary in reports based on only Europe. In Europe, young patients more frequently had ear, nose and throat complaints, whereas elderly patients often presented with fever, fatigue and loss of appetite. Loss of smell, headache, nasal stuffiness and fatigue were more prevalent in female patients [24]. In our study, asymptomatic patient with COVID-19 accounted for 4.3%, which corresponds with a report from Wuhan (4.8%) [25-31].

Conclusion

Our current study found that female sex may be at low risk to be infected with SARS CoV2. Infection with COVID-19 has led to significant elevation in the levels of ESR, ferritin, CRP, D-dimer as well as increased neutrophils count. These differences were effectively associated with disease severity and progression, suggesting the use of such clinical markers to recognize severe illnesses.

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