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Comparative study of Some biochemical and Immunological parameters of patients with COVID-19 disease and non-infected people

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Abstract

The objective of the present study was to evaluate eight biochemical and Immunological parameters and comparison their level in the infected and non-infected people. The methodology include two parts biochemical assays included aspartate transaminase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea breath test (UREA), and creatinine, and Immunological parameters included D-dimer, ferritin, interleukin 6 (IL-6), C-Reactive Protein (CRP). The result detected by Cobas e411, Cobas C111 device. Results obtained showed high levels of all parameters of covid-19 patients in comparing with the control group patients, with significant differences ($p<0.05$). Receiver operating characteristic (ROC) curve showed a high area under the curve for all parameters Area under the ROC Curve (AUC) for all parameters were 1.000, except Patent Cooperation Treaty (PCT) was 0.796) and show high sensitivity for all parameters (sensitivity for all parameters were 100%, except PCT was 75%). The present study was observed significant correlations among the studied of the parameters.

*Keywords***:** *Covid-19; liver; kidney; Patients; D-dimer; ferritin.*

1. INTRODUCTION:

According to WHO there are 234,809,103 confirmed cases of COVID-19, including 4,800,375 deaths, until 2 October 2021, A sequence of pneumonia cases of uncertain origin started to spread in downtown Wuhan, China, in December 2019, the virus is now known as SARS-CoV-2 (4) Coronaviruses are a group of viruses that can cause respiratory and gastrointestinal illnesses in both animals and humans [1-4].

Such viruses mostly affect the lungs, generating mild to severe infections like the common cold or, in more serious cases, pneumonia. Humans' coronaviruses have already been discovered so far, such as the observational viruses of an acute respiratory syndrome (SARS)-coronavirus, Middle East respiratory syndrome (MERS)-coronavirus, and most recent acute respiratory syndrome (SARS Coronaviruses syndrome (SARS-Cove) [2].

Apparently, three pandemic viruses shared over half of their genetic sequences and also have similar sequence similarities (3). While the majority of COVID-19 cases have indeed been moderate, more severe diagnoses had to result in respiratory distress, septic shock, and/or multi-organ dysfunction [3].

Aprovouse studies had demonstrated that SARs 2 have ability to infected many organs along with respiratory tract, this systems and organs, including the heart, liver, and gastrointestinal system, in COVID-19 patinas [4-5].

the main method of corona viruses are real time polymerase chain reaction (RT-PCR) and computerized tomography (CT) scan but these test

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need to support to ensure diagnosis where the sensitivity of RT-PCR was reported to be modest (53–88%), d that CT could have a higher specificity (83–100%) if comparing between them [6].

These studies showed concrete bond between COVID SARS 2 infections [7]. most of biochemical change and inflammation marker showed significant change such as s. CRP, LDH, D-dimer, albumin, ferritin, cardiac troponin T , liver enzyme and kidney function test [8-9]. In this work, levels of eight biochemical parameters in patients with covid-19 in the Iraqi province-Diyala have been studied. Results showed high levels of the studied parameters of covid-19 patients with significant differences ($p<0.05$) comparing with non-infected people (control group).

2. Materials and Methods Experimental samples

A total 98 samples were collected for the present study, the samples were collected from Diyala hospital / Baquba governorate, and the duration times were for three months (from May to July, 2020). The samples were divided into two groups non-infected (control) and infected, each group included 49 samples .Serum and plasma of the blood were used to evaluated these eight parameters, where five CC of whole blood toke from each person by using sodium citrate tubs, Plasms opined by centrifugation for 15 min 3000 rbm the samples keep in cooling system (refrigerator) up to -20 C, the patients already diagnosed with covid 19 in central laboratory of Diyala hospital done by RT-PCR. Immunological assays included CRP, D-dimmer and IL6 have been detected by Cobas e411 devices, while the biochemist assays included AST, ALT, LDH, UREA and CREATININE which were determined by using the CobasC111.

2.1 Statistical analysis

The ROC curve was used to predict the specificity and sensitivity of diagnostic procedures (recognition being the ultimate test for identification). For screening numeric dates, Mean ±SD was used. The T method is used to test two numerical parameters. Kind and degree of the association between variables were explained using Pearson correlation (R). The test was conducted using a significance level of 0.05. Current data was analyzed using SPSS v.22 and Excel 2013.

3. Results

Result of conducted study shows the high average for LDH (412.02±133.93), Ferritin (411.96±151.41), PCT (0.02±0.01) and D-dimer (604.94±155.96) parameters in Covid-19 patients comparing with control group, with high significant difference ($P<0.05$). Table 1 shows the comparative biochemical variables between study groups

Other result related with liver function showed a high average for Urea (54.47±10.31), creatinine (1.63±0.18), AST (41.29±4.16), and ALT (41.29±4.17) parameters in Covid-19 patients, with high significant difference ($P<0.05$) between study groups. Table 2 shows comparative Liver functions variables between study groups.

Comparative immunological parameters of the conducted study showed that the mean was high for CRP (18.53±8.99) and IL-6 (25.49±12.44) parameters in Covid-19 patients with high significant difference ($P<0.05$) between study groups. Table 3 shows, the comparative immunological variables for the studied groups.

Table 1. The comparative biochemical variables between study groups using student t-test

Groups		N	Average	SD	P value
LDH	Patients	49	412.02	133.93	0.001***
	Controls	49	133.35	15.98	
Ferritin	Patients	49	411.96	151.41	0.001***
	Controls	49	79.78	33.97	
PCT	Patients	49	0.02	0.01	0.001***
	Controls	49	0.01	0.00	
D-dimer	Patients	49	604.94	155.96	0.001***
	Controls	49	121.90	52.65	

Regarding the sensitivity and specificity parameters, results obtained showed a highest sensitivity (100%) was for LDH, ferritin, urea, creatinine, AST, ALT, CRP, IL-6 and D-dimer, while it was 75% for PCT. For the specificity, the highest specificity was for PCT, and the lowest was for AST (30%). Table 4 shows the Sensitivity and Specificity of parameters.

It is worth to be mentioned that the results obtained showed significant correlation relationships between urea and creatinine (r=0.337*), AST and

ALT (r=0.528**), CRP and creatinine (r=0.289*), CRP and IL-6 (r=0.808**), and D-dimer and ferritin (r=0.305*). Table 5 shows the correlation relationship among variables using Pearson correlation.

Table 2. Comparative Liver functions variables between study groups using student t- test.

Groups		N	Average	SD	P value
Urea	Patients	49	54.47	10.31	0.001***
	Controls	49	25.06	5.50	
Creatinine	Patients	49	1.63	0.18	0.001***
	Controls	49	0.77	0.19	
AST	Patients	49	41.29	4.16	0.001***
	Controls	49	17.43	4.32	
ALT	Patients	49	41.29	4.17	0.001***
	Controls	49	17.51	4.36	

Table 3. Comparative immunological variables between study groups using student t- test.

Groups		**N**	**Average**	**SD**	**P value**
CRP	Patients	49	18.53	8.99	0.001***
	Controls	49	2.57	1.00	
IL-6	Patients	49	25.49	12.44	0.001***
	Controls	49	9.07	4.42	

Table 4. Receiver operation characteristic curve, Sensitivity and Specificity of parameters.

Variables	**AUC**	**Sensitivity %**	**Specificity %**
LDH	1.000	100	53
Ferritin	1.000	100	42
Urea	1.000	100	36
Creatinine	1.000	100	64
AST	1.000	100	30
ALT	1.000	100	40
CRP	1.000	100	52
PCT	0.796	75	100
IL-6	1.000	100	32
D-dimer	1.000	100	49

Table 5. Correlation relationship among variables using Pearson correlation.

		LDH	**Urea**	**AST**	**CRP**	**PCT**	**D_dimer**
LDH	R	1	.067	-.276	.106	-.002	.005
	P		.647	.055	.470	.987	.975
Ferritin	R	.167	.235	.078	-.074	.032	.305*
	P	.251	.104	.594	.614	.829	.033
Creatinine	R	-.090	.337*	.058	.289*	.162	.234
	P	.537	.018	.693	.044	.266	.105
ALT	R	-.185	-.192	.528**	.050	.010	-.148
	P	.204	.187	.000	.732	.945	.311
IL-6	R	.108	-.026	-.075	.808**	.037	-.066
	P	.460	.858	.610	.000	.802	.650
D-dimer	R	.005	.133	.040	-.010	-.032	1
	P	.975	.363	.785	.946	.826	

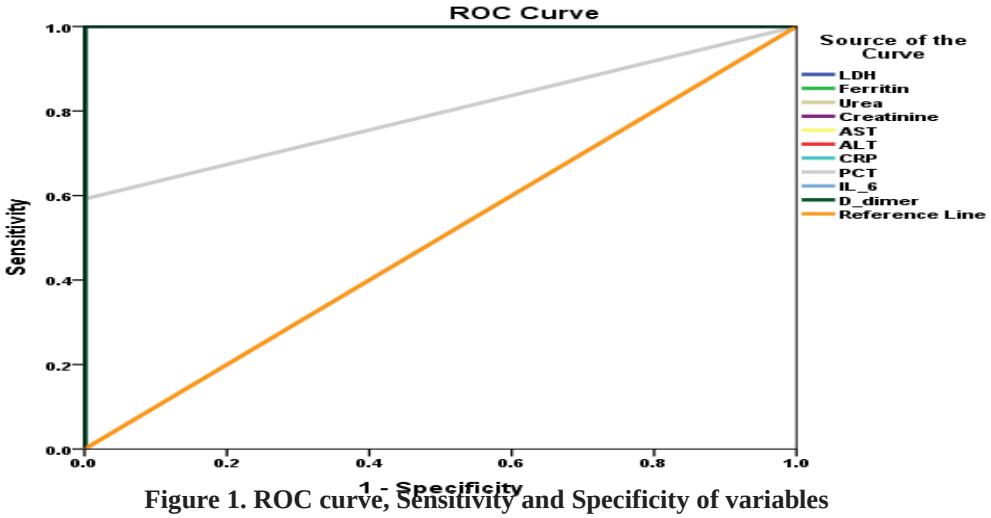

Figure 1. ROC curve, Sensitivity and Specificity of variables

4. Discussion

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) is now confirmed to be a new form of coronavirus that causes COVID-19 infection [10]. Fever is the most prevalent symptom of SARS-CoV-2 infection [11]. Furthermore, in COVID-19 patients, acute respiratory distress syndrome (ARDS) is the most common reason for ICU admission [10]. Lactate dehydrogenase (LDH), a cytosolic enzyme that is found across most tissues and a crucial enzyme inside the glycolytic process, has been related to irritation and cell injury. In the present study, results obtained showed that the levels of LDH, ferritin, pct, and D dimer in Covid-19 patients had significantly increased, in comparison with the control group. In practically all living species, LDH is the main enzyme in anaerobic glycolysis [12].

Serum LDH was found to be increased in serious COVID-19 individuals across several investigations [13-14]. According to our findings, people who are infected with SARS-CoV-2 who had high amounts of LDH are much more prone to developing ARDS. The pathogenic activities of pulmonary organs are complicated by inflammatory and cellular damage [15]. COVID-19 patients had higher LDH values than individuals with negative verified pneumonia [16]. Yuan et al reported that the COVID-19 mRNA elimination ratio was discovered to be strongly linked to LDH levels by [17].

SARS-CoV-2, a positive-sense RNA virus, has been found to trigger inflammasomes, causing intracellular pyroptosis and severe symptoms [18]. Furthermore, COVID-19 patients who had more comorbidities, such as diabetes, stroke problems, and cancer, had a greater ferritin level than those who did not get the same comorbid conditions. Ferritin is an iron-storing protein, which is a very important biomarker. The serum concentration indicates normal iron levels and aids in the diagnosis of anemia due to iron deficiency. Following viral infection, the amount of ferritin in the blood increases, which can be a marker of virus replication [19-20]. Throughout a cytokine and SHLH storm in COVID-19, severe COVID-19 patients [21-22]. had high ferritin levels compared to a cytokine and sHLH storm. Rapidly releasing inflammatory markers such as IL-6, TNF-, IL-1, IL-12, and IFN-, which increase the secretion of ferritin by hepatocytes, Kupffer cells, and macrophages. Multiple organ damage happens as a result of the reaction associated with macrophage activation, hyperproteinemia syndrome, and thrombotic storm. Importantly, ferritin is not just a result of massive inflammation, but it also acts a harmful function inside the inflammation reaction by boosting the expression of pro-inflammatory markers through its connection with the T-cell immunoglobulin and mucin-2 domain (TIM-2) [23].

Furthermore, the H-chain of ferritin has been demonstrated in some experiments to activate macrophages, causing them to produce inflammatory mediators. Thyroid parafollicular C cells generally synthesize and discharge PCT, which would be the 116-amino-acid predecessor of the hormone calcitonin. Following bacterial infection, it can also be synthesized in various extrathyroid tissues, which would be controlled by elevated levels of tumor necrosis factor-alpha (TNF) and interleukin 6 [24].

Many studies have recently found that higher PCT is linked to the intensity of COVID-19

[25-26-27]. According to a meta-analysis, greater PCT values are also linked to a 5-fold increased incidence of severe COVID-19[28-29]. D-dimer levels have been linked to the intensity and treatment outcomes of community-acquired pneumonia across several investigations [30-31].D-dimer, on the other hand, has never been employed as a biomarker for viral pneumonia [32-33].While studies detailing the clinical symptoms of COVID-19 reported a D-dimer elevated. This was not investigated that whether D-dimer level was a predictor of intensity. There is indeed a substantial association between D-dimer values and extent of disease as measured by chest CT, oxygenation index, and clinically staging thus according to interim criteria in just this study.

Furthermore, the current investigation found a higher D-dimer level than previously reported [34-35].This could be owing to the high number of severe and critical patients brought to our facility, indicating that there is a link between D-dimer levels and illness severity. This suggests that checking can be utilized before a chest CT scan as just a sensitivity indicator or even as an addition to medical tomography and radiography.

Individuals with impaired liver results obtained, particularly in hepatocytes or mixture type (i.e. increased ALT / AST, or both ALT / AST) upon admittance or even during hospitalization, had considerably higher odds of advancement than with normal hepatic tests. In extremely severe COVID-19, aggravation of acute pneumonia is a significant clinical outcome that indicates a high death rate and need for ICU or mechanical breathing assistance [36]. In earlier researches, Covoid-19 has been linked to age, sex, and fundamental disorders [37-38].One of those researches was focused on elevated liver tests that are linked to serious disease. SARS-CoV-2 is thought to be not only extremely contagious, but also capable of causing serious organ dysfunction in humans [39-40-41]. and our findings confirm this theory to some extent. When examining the sequencing variations in liver enzyme concentrations and inflammatory indicators, researchers discovered that patients with aberrant concentrations of liver enzymes had increased amounts of ALT and AST, when CRP and ferritin concentrations were at their greatest, suggesting increased levels of inflammatory indicators. Because liver function tests are routinely conducted at the time of enrollment, anomalies in these tests can be utilized as a predictor of symptom severity. The increase in C-reactive protein and ferritin concentrations after a Coronavirus illness implies liver injuries as a result of the acute inflammatory response [42-43].An additional study found that in individuals with severe COVID-19, markers of inflammation such as CRP and LDH, serum ferritin, D-dimer, and IL-6 are considerably higher [44].

Finally, the clinical manifestations of COVID-19 pneumonia in individuals with aberrant liver testing results were evaluated. Patients who had abnormal liver tests were more likely to have a severe illness. The majority of negative impacts on liver injury are connected to medications taken throughout hospitalization, but they should be checked and reviewed on a regular basis.

5. Conclusion

D-dimer, ferritin, IL-6, LDH, liver functions tests (ALT and AST), and kidney functions tests (urea and creatinine) can be used as predictors factors in the diagnosis of COVID-19.

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