Immunological and Biochemical Markers in Diagnosis COVID-19 disease and their association with clinical severity and mortality in Babylon province

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Abstract

Background: In 2019, China received the first reports of coronavirus illness. The illness has been linked to a number of cases of unusual pneumonia and is mainly spread via touch with the skin and respiratory droplets. The laboratory diagnosis of COVID-19 depends on either molecular detection of virus genes in the respiratory aspirations of patients or serological detection of antiviral antibodies in the blood of patients or biochemical detection of some changes in body physiology and hematology.

Aim of study: evaluate immunological markers including lymphocytes, neutrocytes, interleukin 6) as well as biochemical test including (blood sugar and urea, creatinine, LDH, D-Dimer) in COVID.19 patients.

Material and Methods: All patients who displayed COVID-19 symptoms between November 2 and December 30, 2020, were referred to Medical Merjan City in Babylon Province, Iraq. Real-time PCR ‘’polymerase chain reaction’’ was utilized for confirming SARS-CoV-2 infection from throat-swab samples taken from 238 patients. A serum sample was taken from each group. The XW-100 CBC analyzer from Sysmex was used to test immunological markers such as lymphocytes and neutrophils. Aspira Chemical's ELISA kit was used to test interleukin 6 (IL6). Biochemical tests, including blood urea, creatinine, LDH, and D-Dimer, were measured by their kits, which were supplied by Biogenix© for spectrophotometers.

Results: Statistical analysis revealed significant differences between all biomarkers in severity and mortality groups at levels $P \leqslant 0.05$. The results reveal that neutrophil count, lymphocyte count, and interleukin-6 increase mortality with decreased lymphocyte count. Also, when compared with non-ICU patients, ICU patients had considerably higher LDH and D-dimer levels, and LDH levels were connected with tissue damage and CT scan scores, reflecting the severity of the condition. The levels of kidney biomarkers, including creatinine and urea, increased in mortality compared with severe patients. Finally, the studied biomarker can be used to diagnose COVID-19 infection.

Conclusion: Depending on how severe the illness is, different biomarkers play distinct roles in COVID-19's etiology and assessment of their levels. By doing this, it provides doctors with a tool to classify patients and forecast mortality and prognosis. IL-6, lymphocytes, neutrophils, lactate dehydrogenase (LDH), D-dimers, sugar, and renal indicators are among the biomarkers.

Keywords: *COVID-19, Biochemical markers, Coronavirus, Severity and Mortality*.

1. Introduction

In the final week of December 2019, Wuhan city, Hubei, China, received the first reports of coronavirus illness 2019 , The illness that has been linked to a number of cases of unusual pneumonia and is mainly spread via touch with the skin and respiratory droplets. The laboratory diagnosis of COVID-19

depending on either molecular detection of virus genes in respiratory aspirations of patients or serological detection of antiviral antibody in the blood patients or biochemical detection of some changes in body physiology and hematology. [1].

Blood indicator, coagulation profile and serum biochemistry testing are common exams such as renal and liver function, creatine kinase, lactate dehydrogenase, and electrolytes. The most readily available, effective, and economical test is a complete blood count. The goal in present study was to look backward and examine picture of complete blood of cured and deceased patients through time in order to identify critical markers of illness development and outcome and to offer recommendations for future clinical management. Patients with COVID-19 were shown to have leukocytosis, a drop in lymphocyte count, higher D-dimer levels, neutrophilia, thrombocytopenia, eosinopenia, and basopenia, among other hematological abnormalities [2].And compared to patients with less severe disease, those with severe disease had more obvious laboratory abnormalities , Complete blood counts (CBCs), which measure the quantity of neutrophils, and lymphocytes, are less expensive and simpler to perform than other laboratory tests. When the underlying cause of the sickness is unknown, WBCs can aid in the diagnosis as they are sensitive to several pathological alterations. A frequent laboratory anomaly reported in Coronavirus Severe Acute Respiratory Syndrome patients is lymphopenia [3,8].

Hematological and biochemical characteristics of COVID-19 patients may be taken into account by the practitioners when making future decisions. When making clinical decisions to detect high fatality cases and subpar diagnoses during the initial admission period, these markers may be used [12].

2. Materials and Methods

2.1. Data Collection

All patients who displayed COVID-19 symptoms between November 2 and December 30, 2020, were referred to Medical Mergan City in Babylon Province, Iraq. Real-time PCR ‘’polymerase chain reaction’’ was utilized for confirming SARS-CoV-2 infection from throat-swab samples taken from 238 patients. Patients with COVID 19 had their clinically features and blood biochemical testing analyzed and reported. Age, gender, and etc. Informed consent was obtained from patients.

2.2. Collection of Sample and Data Processing

4.5 ml of venous blood were collected. Into a gel tube were poured blood samples. To obtain the serum, all tubes were centrifuged for 10 minutes at 3500 rpm after standing at room temperature for 30 minutes. immunological markers that measured include lymphocytes and neutrocytes was test by The XW-100 CBC analyzer from Sysmex©. Interleukin 6 (IL6) tested by ELISA Kit from Aspira Chemical©.

Biochemical tests include blood urea, creatinine, LDH and D-Dimer which measured by urea kit, creatinine kit, LDH kit and D-Dimer kit respectively from biogenix©. The turbidity caused by agglutination is detected optically by a chemistry analyzer spectrophotometer. The change in absorbance is proportional to the level of each test indicator in the sample. The actual concentration is obtained by comparing it with a calibration curve with known concentrations.

2.3. Statistical Analysis

Data of current study had been analyzed by using SPSS (Version 20) to find mean, standard

deviation SD and least significant differences by ANOVA. O

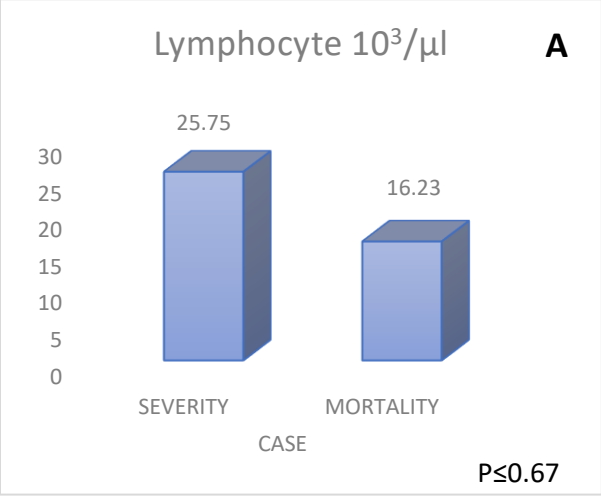
3.Results

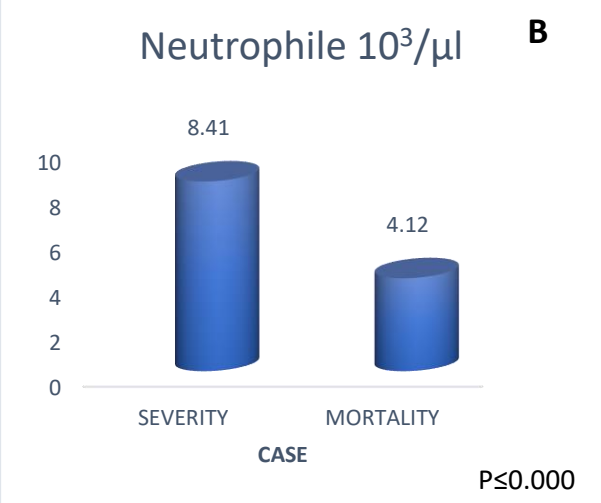
3.1. Immunological Markers

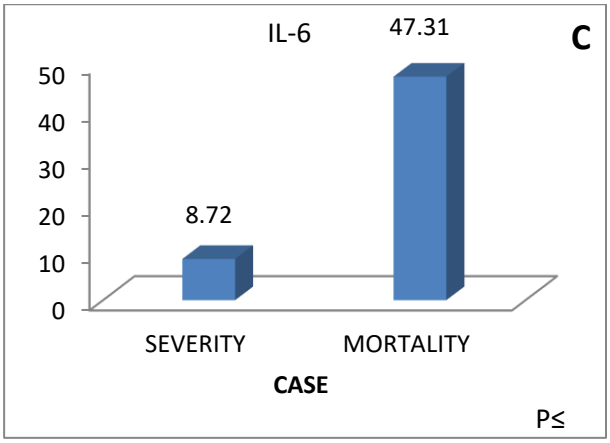
The Figure (2) showed the Mean ± SD of lymphocytes, neutrocytes number and IL-6, the statistical analysis showed significant differences between severity and mortality for neutrocytes numbers and IL-6 markers While lymphocytes numbers which appeared no significant at levels P⩽ 0.05.

Lymphocytes number in severity groups which was reached to 25.75×103/µl ± 5.87 While in mortality groups was decreased to 16.23 ×103/µl ± 4.512 figure (2-A) and neutrocytes number in severity groups was 8.4 ×103/µl 1 ± 2.92 and in mortality groups was decreased 4.12 ×103/µl ± 1.81 figure (2-B) whereas IL-6 concentration in severity groups was reached to 8.72 Pg/ml ± 2.182 and in mortality groups , its concentration was significantly increased and reached to 47.31 Pg/ml ± 6.82 figure (2-C)

Figure (2) Immunological markers in severity and mortality groups of paients with covid-19 (A=Lymphocytes ,B= Neutrophiles and C=Interleukin -6-







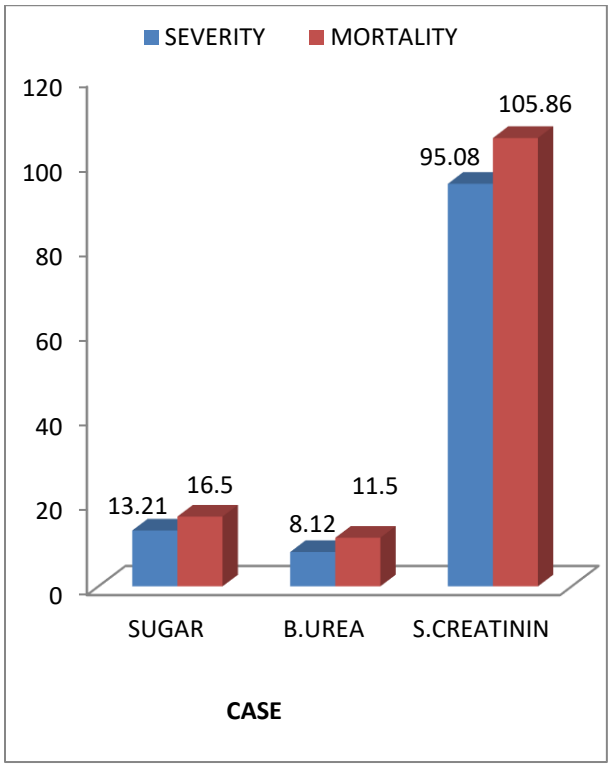
3.2. Biochemical Markers

3-2-1-Blood Sugar and Urea and Serum creatinine Markers

The statistical analysis appeared no significant differences between severity and mortality groups in all markers of blood sugar , blood urea and serum creatinine . Figure (3) showed the Mean ± SD for blood sugar in severity groups 13.21mmol/l ± 3.11 and its concentration in mortality groups, 16.5 mmol/l ± 2.891 , and the kidney function test include (blood Urea and serum Creatinine) the concentration of blood urea was 8.12 mmol/l ± 1.961 in severity groups while its concentration in mortality groups was reached 11.5 mmol/l ± 2.182 , the concentration of serum creatinine was 95.08µmol/l ± 30.18 in severity groups

While in mortality groups was, 105.86 µmol/l ± 25.881,

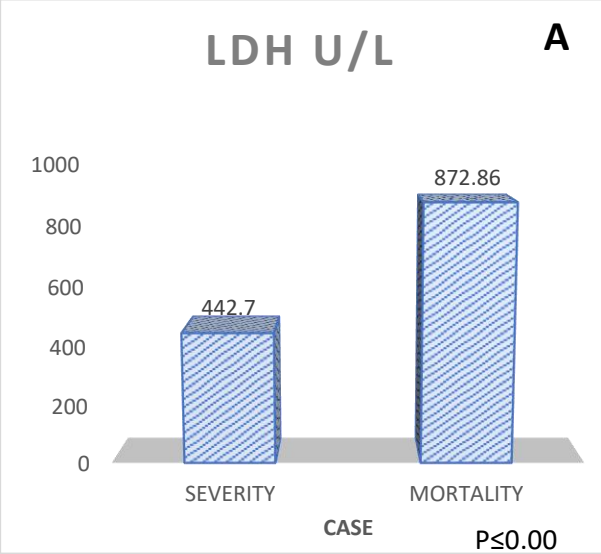
Figure (3) Sugar and renal failure markers in severity and mortality groups of paients with covid-19

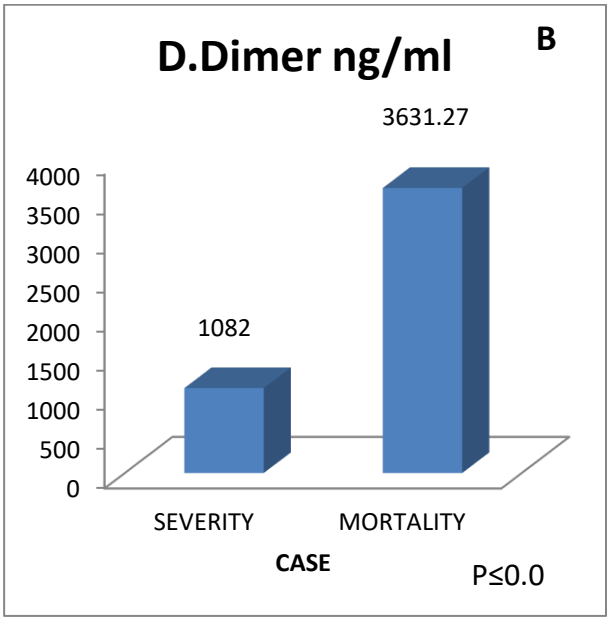


3-2-2-Lactate dehydrogenase and D.dimer markers

The both markers of lactate dehydrogenase LDH and D.dimer showed significantly differences between severity and mortality groups at levels P≤0.05. the level of LDH in severity groups was 442.7 U/L ± 125.58 Whereas its level in mortality groups was reached to 872.86 U/L ± 100.181 figure (4-A). the D. Dimer marker in severity groups was significantly an elevated and reached to 1082 ng/ml ± 798.1 and in mortality groups was reached to 3631.27 ng/ml ± 1225,76 figure (4-B)

Figure (4) Biochemical markers for covid-19 patients' mortality and severity (A = LDH, B = D-Dimer).





4. Discussion

Since January 2020 in China, The Corona virus disease is currently being caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (COVID – 19). Fever, fatigue, a hacking cough which doesn't produce mucus, and dyspnea are among the main signs and symptoms of this viral

disease,the role of various biomarkers in the development of COVID-19 and an evaluation of their levels in relation to disease severity [6].

Lymphopenia's effect on microbial infection

COVID-19 patients frequently exhibit lymphopenia, which is linked to both disease severity and death [5,6,15]. Multiple diseases include an interaction between immune homeostasis and microbes [2,3, 9]. (1,3) The well-known polysaccharide -D-glucan has a crucial structural role in the cell walls of fungi. Our research revealed that those with severe COVID-19 and decreased lymphocyte numbers were also more likely to be infected with microbes, showing that lymphopenic patients are more vulnerable to microbial infection [1, 16]. The results of our investigation are consistent with Chen, N.'s[17] demonstration that several microorganisms can be cultivated from a single patient. Overall, the results show that COVID-19 patients' microbial infection promotes the severity and course of their condition.

Neutrophils counts

We can hypothesize a close relationship between neutrophil overexpression in COVID-19 patients and lymphopenia, The defective lymphocytes in patients with COVID-19 may easily contract an infection due to faulty lymphocytes, which promotes neutrophil activation and recruitment in the blood of patients, It is understood that microbial infection can directly cause neutrophil recruitment to tissue locations. [10,18].

Interleukin-6 makers

IL-6 which is the main cause cytokine storms, inflammation is a major factor in COVID-19, and an inflammatory cytokine storm worsens the disease[4,5]. According to Wan et al. [6, 11], peripheral blood inflammatory factors like IL-6 may increase during COVID-19 infection. Cytokine storm, which a play vital role in the progression of COVID-19 disease that lead to serious complications and death, should be treated to increase treatment effectiveness and decrease mortality. [5,8].

Blood sugar and renal function

The results of blood sugar in presents study , the concentration of blood sugar were significantly increased in both groups of severity and mortality due to the hyperglycemia may be associated with increasing of the entrance point for this particular virus, SARS-CoV-2, is the binding of SARS-CoV-2 to human tissues via glycosylation of the ACE2 receptor [5]. Increased cellular COVID-19 disease severity and viral load may both increase as a result of penetration. Second, an increase in the viral source of energy and the onset of insulin resistance may be linked to hyperglycemia and poor prognosis [19]. Additionally, hyperglycemia raises inflammatory cytokines, which in COVID-19 develops in cytokine-storm syndromes and multi-organ failure [22]. That's because the pancreas' islet cells and acinar cells both express ACE2, damage to the islet cells may raise blood glucose levels [20],

The results of presents study showed, the blood urea concentration were an elevated in severity and mortality groups due substantial abnormality in the protein metabolism pathway causes severe types of COVID-19 infection to produce chronic kidney disease, and elevated blood urea levels upon admission are connected with severity [21].

lactate dehydrogenase LDH

During the breakdown of glucose, the enzyme LDH converts pyruvate into lactate. LDH secretion is a result of cell membrane

necrosis, which is indicative of viral infection or lung injury such that caused by pneumonia caused by SARS-CoV-2 [22]. The development of COVID-19 disease is closely associated with LDH levels[19]. A study indicated that mortality patients had much higher levels of LDH than severity patients did. LDH might function as a biomarker for severe illness [22]. LDH levels were substantially related with the severity of pneumonia, LDH is a biomarker that can be used to assess the severity of the COVID-19 infection as well as the tissue damage and inflammation that are increasing LDH levels [23].

D-dimer Markers

The lysis of cross-linked fibrin results in an increase in D-dimer levels, which indicate the activation of coagulation and fibrinolysis [14]. The severity and mortality categories were found to have grown considerably in this investigation. Early research has linked COVID-19 to haemostatic abnormalities, and one study found elevated D-dimer levels were higher in non-survivors than in survivors, indicating a potential role for D-dimer as a prognostic indicator and assist clinicians in keeping an eye on patients who are more likely to deteriorate quickly [13].

Elevated fibrinogen and D-Dimers are signs of procoagulant alterations [6,7]. A poorer result A progressive rise in the D-Dimers during the illness is associated with an increased risk of acute respiratory distress syndrome (ARDS) and mortality with notably higher levels in patients needing the ICU [3,4].

Ethical Approval and consent

All participant in this study were informed before to collected samples, and verbal agreement was obtained from each of them.

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