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Some Biochemical Changes in COVID-19 Egyptian Patients

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Background: Coronavirus disease causes a broad range of clinical manifestations, including minor symptoms, pneumonia, and/or multiple organ failure. Hematological testing and biochemical indicators are used to stratify and prognosticate COVID-19 patients, both adult and pediatric. These indicators are linked to unfavorable outcomes, including the development of multisystem organ failure, the requirement for mechanical breathing or critical care, and maybe even death.

Aim: Our study aimed to study some biochemical changes in COVID-19 Egyptian patients, which include: D-dimer, ferritin, C-reactive proteins (CRP), liver enzymes (ALT and AST), kidney function (creatinine and urea), complete blood count (Hb, RBCs, PLT), differential leucocytic counts (WBCs, lymphocytes, neutrophils, monocytes), and TUBB and CIP2A proteins as biomarkers for potential progression to critical patients.

Subjects and Methods: This study included eighty (80) individuals of both sexes whose age ranged from 40 to 90) years, divided into two categories, Group I (control healthy): This group included twenty healthy individuals. They had no history of COVID-19 infection or any other diseases that might interfere with the studied parameters. Group II (COVID-19 Patients): this group included sixty (60) COVID-19 patients.

Results: Our results showed that biochemical markers such as D-dimer, ferritin, and C-reactive proteins (CRP) are important for severity determination of the condition of the patient suffering from COVID-19 infection. Also, the CIP2A and TUBB were increased in the COVID-19 severe case form, but the TUBB is more accurate and specific than the CIP2A. D-dimer, ferritin, CRP, and TUBB are important for severity determination of the condition of the patient suffering from the COVID-19 infection. TUBB and CIP2A were determined to be promising parameters that may be used for the prediagnosis of COVID-19 patients.

Keywords: SARS-COV-2 , Biochemicals, COVID-19 , TUBB, CIP2A.

1. Introduction

SARS-CoV-2, or severe acute respiratory syndrome coronavirus 2, is the virus that causes COVID-19.It is a infectious illness. Wuhan, China had contracted the COVID-19 virus before the end of 2019. The virus is an enveloped, positive-polarity RNA virus that belongs to the Coronaviridae family.. [1]. Conversely, as demonstrated by the occurrences of SARS and Middle East respiratory illnesses (MERS), prior coronavirus diseases are highly infectious and have little pathogenicity. [2]. COVID-19 symptoms, including fever, coughing, dyspnea, and loss of taste and smell, can manifest two to fourteen days following the virus's incubation phase. virus and usually transmits with droplets. [3]. SARS-CoV-2 can cause a variety of clinical signs, ranging from asymptomatic infection to potentially fatal respiratory failure.. [4] COVID-19 is a multisystemic illness rather than a localized respiratory tract infection that might elicit hyperinflammation by stimulating the immune and inflammatory systems. [5]. Coronaviruses (CoVs) belong to the family Coronaviridae,the suborder Cornidovirineae, and the order Nidovirales. Letovirinae and Orthocoronavirinae are the two subfamilies that make up the family Coronaviridae. The genus Letovirinae includes the alphaletovirus. Based on phylogenetic study and genomic structure, Orthocoronaviridae is divided into four genera: Deltacoronavirus (δCoV), Gammacoronavirus (γCoV), Betacoronavirus (βCoV), and Alphacoronavirus (αCoV). In these genera, there are 17, 12, 2, and 7 distinct species.. [6]. Many domestic and wild animals are infected by coronaviruses; mammals are mostly infected by α- and βCoVs, whereas birds are primarily infected by α- and δCoVs.In 1960, the human coronavirus (HCoV) with the name B814 was initially discovered in hospitalized patients exhibiting symptoms of the common cold. [7].

Coronavirus (COVID-19) is a single, positive-stranded RNA virus enveloped in a lipid bilayer. [8]. When the lipid bilayer and host cell membrane combine, RNA is released into the cytoplasm, which triggers the translation of many viral proteins. New viruses that originate from cells are constructed from replicated RNA genomes and newly produced viral proteins.. [9] . Two proteins are linked to allow the corona virus to enterThe virus's peer is a glycoprotein called spike-protein (S-protein), which is produced on the viral envelope as a homotrimer. [10] S-proteins are made up of two subunits: While S2 organizes membrane fusion, S1 has a receptor-binding domain that attaches to receptors on host cells. The ACE2 human protein receptor is linked to the S-protein of this virus.. [11].

Adipose tissue, the kidney, the heart, and the lungs are rich sources of the human protein receptor ACE2. [12]. Coronavirus RNA can enter a cell by membrane fusion, which is made possible by the connection between S-protein and ACE2. the use of the connection between the two proteins as a target for illness and immunization. The COVID-19 virus enters host cells in a similar manner. Compared to SARS, COVID-19 accumulates more in the body.As a result, COVID-19 is more communicable and has a longer incubation period, while SARS has more severe symptoms. [13]. Test biomarkers have significant diagnostic and prognostic utility in COVID-19 risk classification. For hospitalized patients with severe COVID-19 infection, research is needed to develop a core set of test biomarkers that may be easily integrated into standard clinical practice to predict prognosis and outcome. [14].

PP2A Inhibitor of Carcinogenesis (CIP2A) As oncoprotein controls the stability of MYC and PP2A in cancer cells, it forms a "oncogenic nexus." The solid tumors for which CIP2A

expression and prognostic function are studied include prostate cancers, head and neck cancers, tongue cancers, bladder cancers, cervical carcinomas, colorectal carcinomas, gastric cancers, pancreatic cancers, brain cancers, breast cancers, lung cancers, ovarian carcinomas, renal cell carcinomas, and oral carcinomas[15].

The MTs, or microtubules, They are essential for numerous biological processes, such as MT-motor proteins (which move different cellular cargos), cell division (which divides a cell into daughter cells by assembling the mitotic spindle required for chromosome segregation to the spindle poles), and cell proliferation (which preserves cell shape and facilitates signal transduction). [16].

2. Subjects and Methods:

The current study included eighty (80) individuals of both sexes their age ranged (40-90) years. Samples were collected in the second wave of COVID-19 Eighty(80) individuals divided into two groups:

Group I (healthy control):

This group consist of twenty healthy individuals. They had no history of COVID-19 or any other diseases which may interfere with the studied parameters.

Group II (Patients): This group included sixty (60) COVID-19 patients.All individuals in this study diagnosed by the following: 1-Clinical manifestation: Among COVID-19 patients' most common symptoms are fever, tiredness, dyspnea, muscle aches, runny or congested noses, and dry cough. [17].

2-Computed tomography (C.T): The precise location of the infection in the chest can be determined by CT imaging, and lesions can be seen distributed throughout the lung's arterial bundles. [18]

3- Polymerase Chain Reaction in Real Time (RT-PCR): Real-time polymerase chain reaction (RT-PCR) is the recognized diagnostic method for COVID-19 diagnosis. [19]

2.1- Sampling:-

Seven ml venous blood was withdrawn from each individual; 2 ml of blood collected in K3EDTA tube for complete blood count (CBC), 1.8 ml of blood was drawn into a 3.2% sodium citrate tube, and the plasma was separated for the D-dimer measurement by centrifuging the tube at 2000 rpm for 10 minutes. After 3.2 ml of blood was drawn into a simple, anticoagulant-free tube, it was centrifuged for 10 minutes at 2000 rpm to separate the serum, which was then placed into a different tube and frozen. The blood was then put in a water bath at 37 ´C for 10 minutes to allow for coagulation. at -20 C to detect CRP, Ferritin, ALT, AST, Creatinine, Urea, TUBB and CIP2A.

2.2- Methods :-

2.2.1- Polymerase Chain Reaction in Real Time (RT-PCR):

With real-time PCR, the Corman et al., 2020 technique can be used to directly amplify the infection of COVID-19 in human samples such as nasal swabs, nasopharyngeal swabs (NPS), nasal wash, or aspirate. [20].

2.2.2-Determination of D-dimer: D-dimer was determined according to the method of Bick and Backer ,(1992) [21]

2.2.3- Determination of Ferritin: Ferritin was created using the technique of Miles et al, (1974) [22].

2.2.4- C- Reactive Protein (CRP) determination: C-Reactive Protein(CRP) was detected according to method of Hokama and Nakamura , (1987) [23].

2.2.5-Assessment of alanine Aminotransferase (ALT) Activity: The activity of alanin aminotransferase (ALT) was identified using the technique of ECCLS,(1989) [24][a].

2.2.6-Assessment of Aspartate aminotransferase (AST) activity: The activity of aspartate aminotransferase (AST) was identified using the technique of ECCLS,(1989) [24][b].

2.2.7-Creatinine detrmination: Creatinine was measured according to method of Bowers and Wong ,(1980) [25]

2.2.8-Urea Determination : Urea was measured according to the method of Fawcett and Scott , (1960) [26].

2.2.9-Complete blood counts (CBC): CBC was measured using an automated hematology analyzer was determined according to method of Dacie and Lewis ,(2001) [27]

2.2.10-Determination of Human β-tubulin: Human β-tubulin was determined using the technique of Hyman et al ,(1991) [28]using Sandwich-ELISA.

2.2.11-Determination of Human Phosphatase PP2A Cancer Inhibitor(CIP2A) Human Phosphatase PP2A Cancer Inhibitor (CIP2A) was measured according to the method of Niemelä Minna ,(2012) [29] using Sandwich-ELISA.

3. Statistical analysis:

The statistical analysis was completed using t-test for comparing between the two group (control healthy and Covid-19 infected group).The results of comparing levels of the different studied variables among different group was significant when the level of significant lower than that of ($P < 0.05$). All statistical analysis was made using the SPSSPC+ computer program. [30].

4. Results and Discussion:

Wide-ranging symptoms, from little to none at all to severe pneumonia, which may result in fatalities or acute respiratory distress syndrome,can be caused by the inflammatory sickness COVID-19.[31]. COVID-19 is characterized by pulmonary inflammation, which harms the liver, gastrointestinal systems, and brain system. [32]. It may also result in additional symptoms like a cough and fever. [33].

4.1- COVID-19 detection by Polymerase Chain Reaction in Real-Time (RT-PCR): Our RT-PCR results, which are observed in Table (1), are significantly different from those of the Covid-19 patient group and the healthy control group. The Covid-19 patient group's RT-PCR results were positive, however the healthy control group saw negative results. Our results agree with those of Noh et al. (2017), who indicated that due to its rapid detection, high sensitivity, and specificity, the polymerase chain reaction (PCR) approach is the "gold standard" for the identification of some viruses. For this reason, real-time reverse transcriptase-PCR (RT-PCR) is particularly recommended for the identification of SARS-CoV-2 since it is a rapid and accurate qualitative test. [34] . Furthermore RT-PCR exhibits suitable sensitivity for early infection diagnosis. As a result, the primary technique to be used to identify the cause of COVID-19 and SARS-CoV-2 is the real-time RT PCR assay.[35]

Table(1):RT-PCR results between control healthy and Covid-19 patients group

	Group	N	Result
RT-PCR	1.0	20	Negative
	2.0	60	Positive

4.2-Effect of COVID-19 on the D-dimer, ferritin and CRP levels:

4.2.1D-dimer :

D-dimer was found to be 611.25±138.31 ng/ml in covid-19 patients group which was significantly increased by 568.03% than that of control healthy group 91.50±22.06 ng/ml ($P<0.001$) table (2):.. Our results are in agreement with Yesupatham et al , [36] who reported that the D-dimer levels in groups 1, 2, and 3 were 1259.37±258.9 ng/ml, 2632.60±472.6 ng/ml, and 229.53±18.4 ng/ml (p-value<0.001), respectively, which increased with the severity of the disease. A total of 3636 cases (79.6%) had increased D-dimer levels, with mean values of 617.7 ±297.8 ng/ml (range 209.6–1680 ng/ml).. [37]. The D-dimer value ranged from 890 mg/L to 5640 mg/L overall, with a COVID-19 median value of 2240 mg/L. patients[38]. D-dimer levels above normal suggest a higher chance of atypical blood coagulation. Higher mortality risk from community-acquired pneumonia is connected to increased D-dimer concentrations. [39]. This outcome is credited to Increases in D-dimer may be an indirect sign of an inflammatory response when a virus infection progresses to sepsis and causes coagulation dysfunction.. An imbalance between coagulation and fibrinolysis in the alveoli may be caused by inflammatory cytokines, which could activate the fibrinolysis system and increase D-dimer levels.. [40].

4.2.2-Ferritin:

Ferritin level was found to be 597.25±144.83 ng/ml in covid-19 patients group which was significantly increased by 438.31% than that of the control group (110.95±21.60 ng/ml) ($P<0.001$) table (2):.. Our results are in agreement with Yesupatham et al , who found that the

ferritin levels in groups 1, 2, and 3 were 528.58±45.03 ng/ml, 511.48±74.4 ng/ml, and 256.89±51.8 ng/ml (p-value <0.007), respectively. which also elevation with the disease's severity [36] . Other predictors of poor result contain the serum levels of ferritin and lactate dehydrogenase(LDH) [41].Where The high death rate and intensive care unit admission linked to hyperferritinemia, which is a result of excessive inflammation from an infection, indicate which patients are high-risk and should be targeted for therapeutic intervention to decrease inflammation. [42] .Our results cleared that, the Ferritin level showed a higher level in Covid-19 patients group as its level reached to 597.25 ng/ml while, in the control group was 110.95 ng/ml. This results attributed to , Serum ferritin, a characteristic of hemophagocytic lymphohistiocytosis, a known viral infection consequence, is strongly associated with a bad prognosis in COVID-19 patients, and elevated ferritin levels are more common in individuals with compromised lung lesions. [43].

4.2.3- C-Reactive protein (CRP):

In the group of COVID-19 patients, CRP was determined to be 70.39±16.10 mg/ml, and by 1970, it had dramatically grown.table (2): 29% less than 3.40±0.69 mg/ml in the healthy control group (P<0.001). Our findings concur with those of Kaftan et al., who stated that the Mann Whitney test was employed to compare the two study groups' median laboratory parameter values. The CRP level at admission was a sensitive and early predictor of COVID-19 severity, according to a comparison of the D-dimer, CRP, and ferritin levels of COVID-19 positive individuals to those of the negative outcomes, which showed substantial (P: <0.01, <0.01, and 0.03 correspondingly) rises [44]. The lung lesion and CRP level correlated positively on tomographic pictures. As part of an extensive panel of biological studies conducted upon entry [45].

Table (2): Ddimer, Ferritin and CRP level between control healthy and Covid-19 patients group

	Group	N	Mean Std. Deviation	t-value	Precentage %	P-value
D-dimer(ng/ml)	Control healthy	20	91.50±22.06	16.67	568.03	<0.001***
	Covid-19 patients	60	611.25±138.31			
Ferritin(ng/ml)	Control healthy	20	110.95±21.60	14.89	438.30	<0.001***
	Covid-19 patients	60	597.25±144.83			
CRP(mg/ml)	Control healthy	20	3.40±0.69	18.52	1970.29	<0.001***
	Covid-19 patients	60	70.39±16.10			

*** = significant at (P < 0.001).

Inflammatory disease or during infectious states, CRP levels can activate the classical complement cascade of the immune system and modified the activity of phagocytic cells, confirming the function of CRP in the opsonization of pathogens and living or dead cells [46].Although the precise impact of CRP in COVID-19 is yet unknown, it has been noted that measuring it can help with early pneumonia identification [47]. and estimate of severe pulmonary infectious diseases [48]. The results of CRP showed a higher level in Covid-19 patients group as its level reached to 70.39 mg/ml while, in the control group was 3.40 mg/ml

Our results were in line with other studies that suggested the admission CRP level was a sensitive and early indicator of COVID-19 severity. [49] .

4.3-Effect of COVID-19 on the liver enzymes activities:

ALT was found to be 46.32±19.62 U/L in covid-19 patients group which was significantly increased by 126.5% than 20.45±4.32 U/L in control group (P<0.01).and AST was found to be 45.52±15.52 U/L in covid-19 patients group which was significantly increased by 93.7% than that of the control group (23.50±3.47 U/L) (P<0.01) table (3). Our results agreement with those of Qu et al., whose investigation included 266 COVID-19 patients, of whom 235 were mild cases and 31 were severe cases. In contrast to mild cases, severe patients had higher activities of (ALT) and (AST) and urea nitrogen (BUN) (all p<0.01). Binary logistic regression analysis also ALT [OR=2.680 (1.036–6.934), p=0.042] as independent factor of COVID-19 patients severity[50].

All research participants had elevated liver function test parameter values, and among critical (ICU) patients, the levels of three of these parameters—AST, ALT, and ALP—were higher (56.9±57.7 U/L, 58.5±63 U/L, and 114.6±60 U/L, respectively) [51]. We also demonstrate a noteworthy rise in S.AST during the previous six months. group compared with the ≤3 months group and a significant increase in the>6 months group compared with the ≤6 months group. Significant increases in ALT were also observed in groups ≤6 months and >6 months after healing compared with group ≤3 months [52]

Table (3): AST and ALT activities between control healthy and Covid-19 patients group

	Group	N	Mean Std.Deviation	t-value	Precentage %	P-value
ALT(U/L)	Control healthy	20	20.45±4.32	5.82	126.50	<0.01**
	Covid-19 patients	60	46.32±19.62			
AST(U/L)	Control healthy	20	23.50±3.47	6.26	93.70	<0.01**
	Covid-19 patients	60	45.52±15.52			

** = Significant at (P < 0.01)

4.4- Effect of COVID-19 on the kidney functions :

Creatinine was found to be 1.34±0.38 mg/dL in covid-19 patients group which was significantly increased by 61.45% than that of the control group (0.83±0.11 mg/dL) (P<0.01), and Urea was found to be 50.48±19.33 mg/dL in covid -19 patients group which was significantly increased by 87.66% than that of the control group (26.90±4.87 mg/dL) (P<0.001) table (4):. Our results agreement with those of Qu et al. (2021), whose study included 266 COVID-19 patients, of whom 235 were mild cases and 31 were severe cases. Severe patients showed greater levels of the aminotransferases alanine (ALT), aspartate (AST), and urea nitrogen (BUN) (p<0.01) compared to moderate patients [50]. Additionally, research revealed that the virus had no discernible impact on renal function for any particular cause [53].However, a different study demonstrates a strong link between COVID-19 infection and renal insufficiency [52].

Table (4): Creatinien and urea level between control healthy and Covid-19 patients group

	Group	N	Mean Std. Deviation	t-value	Precentage %	P-value
Creatinine(mg/dL)	Control healthy	20	0.83±0.11	5.96	61.44	< 0.01**
	Covid-19 patients		1.34±0.38			

		60				
Urea(mg/dL)	Control healthy	20	26.90±4.87	5.37	87.65	< 0.01**
	Covid-19 patients	60	50.48±19.33			

** = Significant at (P < 0.01)

4.5- Effect of COVID-19 on haematological parameters:

Hemoglobin (Hb) was found to be 11.94±1.75 g/dL in covid-19 patients group which was insignificantly different than that of the control group (12.50±1.42 g/dL) and We observed that the mean RBCs count of covid 19 patients group was 4.27±0.57 x103cells/µl which was insignificantly different than that of the control group (4.41±0.38 x103cells/µl) table(5).

According to the results of our investigation, there was no discernible difference in the two groups' hemoglobin and red blood cell counts. In contrast, The RBCs, Hb, and Hct values quickly decreased as the disease progressed. When compared to the control healthy group, the MCV of only the moderate COVID-19 patient group significantly dropped. (P=0.015) [54]. Additionally, compared to the control group, the COVID-19 patients had significantly lower median values for hemoglobin concentration (Hb), red blood cell count (RBCs count), haematocrit (Hct), mean cells hemoglobin (MCH), and mean cells hemoglobin concentration (MCHC).r ($p = 0:041$, $p \leq 0:01$, $p \leq 0:01$, and $p \leq 0:01$, in that order) [55]. A considerable percentage of COVID-19 patients experienced anemia, according to an analysis of their erythrocyte properties. In 38,2% of COVID-19 cases, hemoglobin levels dropped[56]. Taneri et al.'s metaanalysis revealed that COVID-19 patients' mean hemoglobin levels had dropped [57].

The platelets (PLT) count of the COVID-19 patients group was found to be 190.48±41.83 x103cells/µl; this was considerably lower (-20.27%) than the control group's (238.90±42.33 x103cells/µl) (P<0.01) table (5). Our findings concur with those of Elderdery et al., who found that The median platelet counts of COVID-19 patients were substantially lower than those of controls. ($p \leq 0:01$). [55]. In COVID-19 patient groups, PLT parameter was found to be lower ($P < 0.001$). [54]. When admitted to the hospital, thrombocytopenia is found in 25.1% of COVID-19 patients.20 Additionally, thrombocytopenia has been linked in a number of studies to both patient survival and the COVID-19 infection's severity. According to a meta-analysis, individuals with severe Compared to those with a milder form of the disease, COVID-19 patients typically have lower platelet counts.19 Numerous studies have revealed that the platelet count of patients who do not survive is considerably lower than that of those who do[58].

White Blood Cell count (WBCs) was found to be 7.01±2.04 x103cells/µl in covid 19 patients group and 7.78±1.44 x103cells/µl in control group, between the two groups, there is no statistically significant difference., We detected a mean lymphocytic count was found to be 15.02±2.57 x103cells/µl in covid 19 patients group which was significantly decreased by (-56.21%) than that of the control group (34.30±4.34 x103cells/µl) (P<0.001), We detected a mean neutrophil count was found to be 76.89±4.13 x103cells/µl in covid 19 patients group which was significantly increased by (36.81%) than that of the control group (56.20±4.48 x103cells/µl) (P<0.001) and the mean monocyte level was found to be 5.47±1.58 % in covid 19 patients group which was significantly decreased by (-31.63%) than that of the control

group (8±1.49 %) (P<0.01) table (5). Our finding results with those of Elderdery et alfound that there was no statistically significant difference between the two groups' median values for the total white blood cell count and basophil count. Nonetheless, the patient group's lymphocyte count dramatically dropped in comparison to the control group. (p value<0.001), and its neutrophil count increased significantly (p value<0.001). [55]. The patients group with mild COVID-19 infection had a lower WBC count than the other groups (P = 0.264). Additionally, it was demonstrated that, in comparison to the control group, the WBC count of patients assigned to the moderate, severe, and died groups increased dramatically. All patient groups had considerably greater neutrophil counts, which increased in direct proportion to the illness's severity. Along with a large rise in neutrophils, all patient groups had significantly decreased lymphocyte counts. Numerous studies have also confirmed that the differential count and evaluation of white blood have a major impact on both predicting the disease's severity and confirming the COVID-19 diagnosis.. [54]. Compared to healthy individuals, COVID-19 patients typically have decreased leukocyte counts. On the other hand, leukocyte levels in COVID-19 individuals with severe disease are greater during observation (7.44 vs 4.40 × 109/L; P < 0.001) than in those with mild-moderate disease. [59]. Also we found that the mean monocyte level was 5.47±1.58 % in COVID-19 patients group which was significantly decreased by (-31.63%) than that of the control healthy group (8±1.49 %) (P<0.001) table (5). Which is consistent with Rostami-Far et al who stated that Additionally, all patient groups observed a significant decrease in the number of monocytes. [54]. In contrast no significant difference between both groups regarding Monocytes count as median monocytic count was 8:1±3:7 vs 8:1±3:97 in patients with COVID-19 and healthy controls respectively[55].

Table (5): Hematological parameters levels between control healthy and Covid-19 patients group

	Group	N	Mean Std. Deviation	t-value	Precentage %	P-value
HB(g/dL)	Control healthy Covid-19 patients	20 60	12.50±1.42 11.94±1.75	1.29	- 4.5	>0.05 NS
RBCs(x10^{3}cells/µl)	Control healthy Covid-19 patients	20 60	4.41±0.38 4.27±0.57	1.01	- 3.17	> 0.05 NS
PLT(x10^{3}cells/µl)	Control healthy Covid-19 patients	20 60	238.90±42.33 190.48± 41.83	4.47	- 20.26	< 0.01 **
WBCs(x10^{3}cells/µl)	Control healthy Covid-19 patients	20 60	7.78±1.44 7.01±2.04	1.55	- 9.89	> 0.05 NS
Lymphocytes (x10^{3}cells/µl)	Control healthy Covid-19 patients	20 60	34.30±4.34 15.02±2.57	24.14	- 56.2	< 0.001***
Neutrophil (x10^{3}cells/µl)	Control healthy Covid-19 patients	20 60	56.20±4.48 76.89±4.13	19.01	36.81	< 0.001***
Monocytes(%)	Control healthy Covid-19 patients	20 60	8.00±1.49 5.47±1.58	6.29	- 31.62	< 0.01 **

*** = significant at (P < 0.001). ** = significant at (P < 0.01). NS = Non-significant at (P > 0.05)

4.6- Effect of COVID-19 on biochemical Markers (TUBB and CIP2A):

TUBB was found to be 136.22±30.89 pg/ml in covid-19 patients group which was significantly increased by 185.88% than its value in the control group (47.65±7.70 pg/ml) (P<0.001) table (6): also, based on the results of ROC curve analysis, TUBB was a significant predictor of Covid-19(AUC=1, P value<0.001). At the suggested cut off value (>59), TUBB gave a sensitivity of 100%, specificity of 95%, PPV of 98.4% and NPV of 100%. Table (7), figure (1). and the mean CIP2A level was found to be 1.45±0.37 in covid 19 patients group and 1.32±0.28 in control healthy group, without a significant statistical difference between both groups Table (6):also based on the results of ROC curve analysis CIP2A wasn't a significant predictor of Covid 19 with AUC of 0.586, P value of > 0.05). At the suggested cut off value (>1.15), CIP2A gave a sensitivity of 73.33%, specificity of 30%, PPV of 75.9% and NPV of 27.3% Table (7), figure (2).

The coronavirus spike (S) protein, which mediates host cell attachment and viral entrance [61], is used by the SARS-CoV-2 cell to attack a host cell. Membrane receptor proteins, namely the ACE2 receptor, are bound by the S protein. TUBB production is boosted by intracellular microtubules, which are linked to β-tubulin. also, This study was indirectly confirmed by the effectiveness demonstrated in anti-microtubule clinical trials. COLCORONA (Colchicine Coronavirus SARS-COV2), a drug that inhibits the mitotic spindle poles in human cells by targeting microtubules, has been selected for the phase 3 trial of the COVID-19 treatment.. [62]. Also there was no significant difference between both groups regarding the mean CIP2A level also we found that CIP2A wasn't a significant predictor of Covid 19 based on the results of ROC curve analysis with AUC of 0.586, P value > 0.05. So we found that TUBB had a significantly higher AUC value than CIP2A (P value<0.001), indicating that TUBB was a significantly better diagnostic marker of Covid 19 Compared to matching normal tissues, CIP2A mRNA and protein levels were elevated in colorectal cancer (CRC) tissues. Additionally, overexpression of CIP 2A protein is caused by IL 10, an inflammatory marker that is linked to COVID-19 but not specifically to the illness.It is a useful independent prognostic indicator for figuring out disease-free survival (DFS) and overall survival (OS). [63].

Also there was no sufficient data about CIP2A in progression of COVID 19 infections but had major role in progression of solid tumors and heamatolgical malignancy [64].In our results the tubulin level showed a higher level in Covid-19 patients group as its level reached to136.22, while, in the control healthy group was 47.65.As actin filaments are key players in every stage of infection, this finding that coronaviruses employ cellular filaments in the most complicated way may apply to COVs. Microtubules (MTs) are required for the internalization of viruses and, subsequently, at multiple stages in the establishment of the viral replication site. [65].

Table (6): Tubbulin and CIP2A level between control health and Covid-19 patients group

	Group	N	Mean Std. Deviation	t-value	Precentage %	P-value
TUBB(pg/ml))	Control healthy	20 60	47.65±7.70	12.64	185.87	<0.001***
	Covid-19 patients		136.22±30.89			
CIP2A(ng/ml)	Control healthy	20 60	1.32±0.28	1.52	9.85	> 0.05 NS
	Covid-19 patients		1.45±0.37			

Table (7): Diagnostic performance of TUBB for the early prediction of Covid-19

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC
TUBB	>59	100	95	98.4	100	1.000
CIP2A	>1.15	73.33	30	75.9	27.3	0.586

PPV: Positive predictive value,NPV:Negative predictive value, AUC: Area under the curve

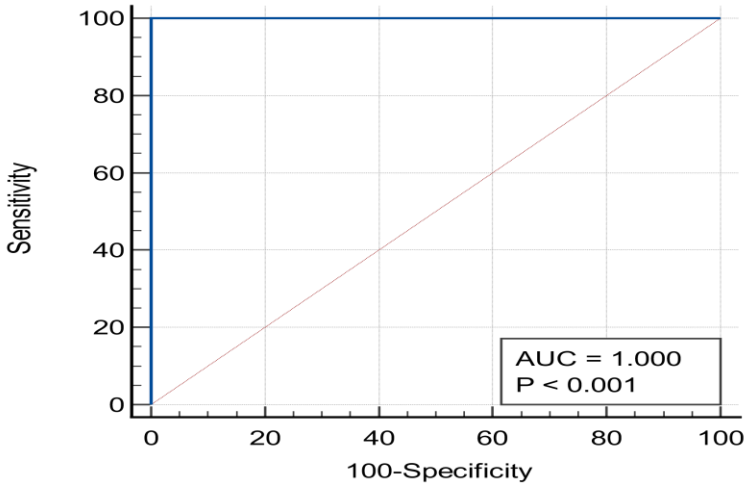

Figure (1): ROC curve of TUBB for the early prediction of Covid 19

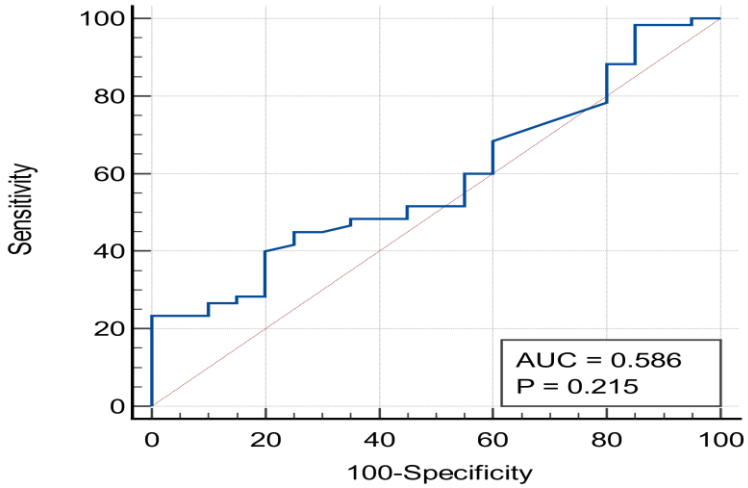

Figure (2): ROC curve of CIP2A for the early prediction of Covid 19

The comparison between both ROC curves of TUBB and CIP2A revealed that TUBB had a significantly higher AUC value than CIP2A (P value<0.001), indicating that TUBB was a significantly better diagnostic marker of Covid 19 than CIP2A

5. CONCLUSION:

Ferritin, CRP, and D-dimer are effective biomarkers for predicting COVID-19 death. Additionally we were unable to establish significant relationships between numerous other biomarkers in detecting the coronavirus disease's severity. During the hospital stay in our study, acute renal damage and hypernatremia developed, both of which were demonstrated to be fatal.. These findings are significant and can serve as guidelines for treating people with the disease in this location or determining the severity of the disease.The tubulin and CIP2A increased in the severe form of COVID-19 and can be used as a guide for determination of severity and The effectiveness of COVID-19 treatment. but the tubulin is more accurate and specific. than the CIP2A.

6- Conflicts of interests :

There are no conflicts to declare.

References

[1] Tabassum,T.,Rahman,A.,Araf,Y.,Ullah,M.A.,&Hosen,M.J.(2021).Prospective Selected biomarkers in COVID-19 diagnosis and Treatment.Biomarkers in Medicine ,15(15) 1435–1449.

[2] Das, B., Bhatia, S. Y., & Pal, P. M.(2021).Evaluation of the Role of Routine Laboratory Biomarkers in COVID-19 Patients:Perspective from a Tertiary Care Hospital in India . Indian journal of clinical biochemistry : IJCB, 36(4), 473–484

[3] Balci A, Yildiz E and Selendili O,(2021) , New markers in the differentiation of Covid 19 disease and pneumonia:neutrophil and platelet lymphocyte ratio. J Contemp Med , 11(4):478-482

[4] Bahceci I, Yildiz IE,Duran OF,Soztanaci US,Kirdi Harbawi Z,et al,(2022),Secondary bacterial infection rates among patients with COVID-19. Cureus . 14(2),e22363;1-8

[5] Samprathi M and Jayashree M, (2020) , Biomarkers in COVID-19: an up-to-date review.Front Pediatr.2020, 8:607647;1-12

[6] Zhou Zhijian, Ye Qiu and Xingyi Ge,(2021), The taxonomy, host range and pathogenicity of coronaviruses and other viruses in the Nidovirales order, Animal Diseases 1:5;1-28.

[7] Tyrrell D and Bynoe M,(1965), Cultivation of a novel type of common-cold virus in organ cultures. Br. Med. J. 1965, 1,1467–1470

[8] Roujian Lu, Xiang Zhao, Juan Li, Peihua Niu, Bo Yang, et al,(2020) ,Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395, 565–574.

[9] Pan Qin, En-Zhong Du,Wen-Ting Luo,Yong-Le Yang, Yu-Qi Zhang, et al, (2019), Characteristics of the life cycle of porcine delta coronavirus (PDCoV) in vitro: replication kinetics, cellular ultrastructure and virion morphology and evidence of inducing autophagy,Viruses,11,455,1-14

[10] Fang Li , (2016) , Structure, function and evolution of coronavirus spike proteins,Ann.Rev,Virol,3,237-261
[11] Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang,2020), A pneumonia outbreak associated with a new coronavirus of probable bat. origin.Nature,579,270-273
[12] Anthony J. Turner, Julian A. Hiscox and Nigel M. Hooper, (2004), ACE2: from vasopeptidase to SARS virus receptor, TRENDS in Pharmacological,Sciences , 25,291–294
[13] Zhaoqian Su and Yinghao Wu, (2020) A multiscale and comparative model for receptor binding of 2019 novel coronavirus and the implications of its life cycle in host cells, BioRxiv,1 – 24
[14] Battaglini D , Lopes-Pacheco M, Castro-Faria-Neto H ,Pelosi1 P, and Rocco P. R.M.,(2022),Laboratory Biomarkers for Diagnosis and Prognosis in COVID-19 Frontiers in Immunology;13:1-11
[15] Pradip De , Jennifer Carlson , Brian Leyland-Jones and Nandini Dey , (2014), Oncogenic nexus of cancerous inhibitor of protein phosphatase 2A(CIP2A): an oncoprotein with many hands, Oncotarget,5(13);4581 – 4602
[16] Hammond, J. W., Cai, D., & Verhey, K. J., (2008)., Tubulin modifications and their cellular functions. Current opinion in cell biology, 20(1), 71–76
[17] Baj J,Karakuła-Juchnowicz H,Teresiński G,Buszewicz G,Ciesielka M,et al ,(2020),COVID-19: Specific and Non-Specific Clinical Manifestations Symptom:The Current State of Knowledge. J Clin Med. ;9(6):1753-1775
[18] Xu X, Yu C, Qu J, Zhang L, Jiang S, et al ,(2020), Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2. Eur J Nucl Med Mol Imaging; 47(5):1275-1280
[19] Beltrán-Corbellini Á., Chico-García J.L., Martínez-Poles J., Rodríguez-Jorge F.and Alonso-Cánovas A.,(2020), Reply to letter: Acute-onset smell and taste disorders in the context of Covid-19: A pilot multicenter PCR-based case control study. Eur. J. Neurol .27(9):e34
[20] Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., et al, (2020). Detection of 2019 novelcoronavirus (2019-nCoV) by real-time RT-PCR.Euro surveillance : bulletinEuropeen sur les maladies transmissibles = European communicable disease bulletin , 25(3), 2000045;1-8
[21] Bick R L. and Backer W F. ,(1992), Diagnostic efficacy of the D-dimer assay in disseminated intravascular coagulation(DIC),Thrombosis Research,65(6):785-790
[22] Miles L. E., Lipschitz D. A., Bieber, C. P. and Cook , J. D. (1974) Measurement of serum ferritin by a 2- site immunoradiometric assay,61(1):209-224
[23] Hokama Y and Nakamura RM , (1987) , C-Reactive protein: current status and future perspectives. J Clin Lab Anal;1:15-27
[24][a] Eccls. (1989). Determination of the catalytic activity concentration in serum of L- alanine aminotransferase (EC 2.6.1.2, ALAT). . Clin Chem Mitt,20, 204-211
[24][b]- ECCLS.(1989),Determination of the catalytic activity concentration in Serum on L-aspartate aminotransferase(EC2.6.1.1,AST) Clin chem,20,204-211
[25] Bowers LD and Wong ET,(1980), kinetic serum creatinine assay.Ⅱ. A critical evaluation and review. Clinincal Chemistry;26(5);555-561
[26] Fawcett J. K. and Scott J. E. (1960), A rapid and precise method for the determination of Urea,J Clin Pathol,13:156-169
[27] Dacie John V ,Lewis Mitchell ,Barbara Jane Bain and Imelda Bates,(2001),Dacie and Lewis's PracticalHaematology,ChurchillLivingstone;9thedition:652 pages
[28] Hyman A, Drechsel D, Kellogg D, Salser S,Sawin K,et al.(1991). Preparation of modified tubulins. Methods Enzymol 196,478–485.

[29] Niemelä Minna ,(2012), Identification and characterization of CIP2A as a novel oncogenic inhibitor of PP2A, Annales Universitatis Turkuensis D 1032:1-94
[30] Douglas Stauber, (2017), What's New in SPSS Statistics 25 & Subscription"SPSS StatisticsI BM Data Science Community".community.ibm.com.Retrieved 2021-06-30
[31] Merad M.,and Martin J.C.,(2020), Author Correction : Pathological inflammation in patients with COVID-19:a key role for monocytes and macrophages,Nat .Rev.Immunology,20:448,355-362
[32] Liu Zhonghua ,Bing Xing and Z Zhi,(2020),Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China ,41,145–151
[33] Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C et al,(2020), Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany, N Engl J Med 2020; 382:970-971
[34] Noh, J. Y., Yoon, S. W., Kim, D. J., Lee, M. S., Kim, J. H., et al. (2017).Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR. Archives of virology, 162(6), 1617–1623
[35] Shen, M., Zhou, Y., Ye, J., Abdullah Al-Maskri, A. A., Kang, Y., Zeng, S., & Cai, S , (2020), Recent advances and perspectives of nucleic acid detection for coronavirus. Journal of pharmaceutical analysis, 10(2), 97–101.
[36] Yesupatham ST,Mohiyuddin S A,Arokiyaswamy S,Brindha H,Anirudh V et al.,(2022), Estimation of Ferritin and D-Dimer Levels in COVID-19 Patients. Journal of Clinical and Diagnostic Research. 2022 Jan, Vol-16(1): BC12-BC15
[37] Kumari, A.,Ranjan, A.,Nishant, P.,Sinha,S &Sinha, R. K,(2023), Cross-sectional study to describe the severity, bio-chemical associations, and final outcomes of COVID-19-associated rhino-orbital-cerebral mucormycosis in a tertiary hospital of East India . Indian journal of ophthalmology,71(5),2193–2198
[38] Sukrisman L and Sinto R.,(2021), Coagulation profile and correlation between D- dimer, inflammatory markers, and COVID-19 severity in an Indonesian national referral hospital. J Int Med Res. 2021 Nov;49(11):1-11
[39] Querol-Ribelles J.M., Tenias J.M. and Grau E.,(2004),Plasma d-dimer levels correlate with outcomes in patients with community-acquired pneumonia.Chest;126(4):1087 1092.
[40] Tang N, Bai H, Chen X, Gong J, Li D and Sun Z.,(2020), Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost;18(5):1094-1099
[41] Ruan Q, Yang K, Wang W, Jiang L and Song J., (2020),Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan,China. Intensive Care Med;46(5):846-848
[42] Carcillo JA, Sward K, Halstead ES, Telford R, Jimenez-Bacardi A, et al.,(2017), ASystemic Inflammation Mortality Risk Assessment Contingency Table for Severe Sepsis. Pediatr Crit Care Med. ;18(2):143-150
[43] Fu S,Fu X,SongY,Li M,Pan P,et al ,(2020),Virologic and clinical characteristics For prognosis of severe COVID-19:a retrospective observational study in Wuhan China.medRxiv , 4 (3) 1-29.
[44] Kaftan AN, Hussain MK, Algenabi AA, Naser FH, Enaya MA et al , (2021), Predictive Value of C-reactive Protein, Lactate Dehydrogenase, Ferritin and D-dimer Levels in Diagnosing COVID-19 Patients: a Retrospective Study. Acta Inform Med. 2021 Mar;29(1):45-50
[45] Wang G, Wu C, Zhang Q, Wu F, Yu B, et al, (2020), C-reactive protein level may predict the risk of COVID-19 aggravation Open Forum Infectious

Diseases.2020;7(5);1-5.
[46] Ballou S P and Kushner I,(1992),C-reactive protein and the acute phase Response Adv Intern Med. ;37:313–336.
[47] Warusevitane A, Karunatilake D, Sim J, Smith C and Roffe C., (2016), Early diagnosis of pneumonia in severe stroke: Clinical features and the diagnostic role of C-reactive protein. PLoS One;11(3):e0150269;1-11.
[48] Marnell L, Mold C and Du C T W., (2005), C-reactive protein: Ligands, receptors and role in inflammation. Clin Immunol;117(2):104-111
[49] Chen W, Zheng K I, Liu S, Yan Z, Xu C and Qiao Z , (2020) , Plasma CRP level positively associated with the severity of COVID-19. Ann Clin Microbiol Antimicrob.2020;19(1):1824
[50] Qu, J., Zhu, H. H., Huang, X. J., He, G. F., Liu, J. Y., et al,. (2021). Abnormal Indexes of Liver and Kidney Injury Markers Predict Severity in COVID-19 Patients . Infection and drug resistance, 14, 3029–3040
[51] Tsegay YG, Bitew M, Workneh T, Atlaw A, Aragaw M, et al , (2022),.The level of liver and renal function biomarker abnormalities amonghospitalized COVID-19 patients in Ethiopia.Cold Spring Harbor Laboratory.Press.2022:1-17
[52] Noori Ali, Roaa; Fadhil Alasadi, Yusor; Isam Jameel, Zahraa (2021) Estimation some Kidney function parameters among COVID-19 Patients in Kerbala province. Archivos Venezolanos de Farmacología y Terapéutica, 2021, vol. 40, núm. 7, ISSN: 0798- 0264:p 743-749
[53] Israa H. Ibrahim, Mena Y. Abd, Murtadha Matai Al-Fatlawi, and Murtadha Matai Al-Fatlawi.,(2023), Renal function, Liver function, and lipid profile state in subjects after healing from COVID-19 infection .J Popl Ther Clin Pharmacol 30(17):809-814.
[54] Rostami-Far Z, Rahmani K, Mansouri K, Khadem Erfan M B, Shaveisi-Zadeh F,et al, (2022), Complete Blood Count as an Indicator of COVID-19 Disease Severity. J Clin Res Paramed Sci. 2022;11(2):e133320:1-6
[55] Elderdery, A. Y., Elkhalifa, A. M. E., Alsrhani, A., Zawbaee, K. I., Alsurayea, S. M., et al, (2022), Complete blood count alterations of COVID-19 patients in Riyadh, Kingdom of Saudi Arabia. Journal of Nanomaterials, 2022, [6529641]Volume 2022, Article ID 6529641, 6 pages,1-6
[56] Huang I and Pranata R.,(2020), Lymphopenia in severe coronavirus Disease –2019 (COVID- 19):systematic review and meta-analysis. J.Intensive Care;8:36.
[57] Taneri PE,Gómez-Ochoa SA,Llanaj E,Raguindin PF,RojasLZ,et al ,(2020) , Anemiaand iron metabolism in COVID-19:a systematic review and meta-analysis;Eur J Epidemiol ; 35(8):763-773
[58] Sun S, Cai X,Wang H,He G,Lin Y, Lu B,et al.,(2020),Abnormalities Of Peripheral Blood System In Patients With COVID-19 In Wenzhou, China. Clin Chim Acta [Internet]. 2020;507(April):174– 180
[59] Thirumalaisamy P. Velavana and Christian G. Meyer. ; (2020); Mild versus severe COVID-19: Laboratory markers., International Journal of Infectious Diseases.;95 ;304–307
[60] Aminpour,M.,Hameroff, S & Tuszynski,J. A,(2022),How-COVID-19 Hijacks-Cytoskeleton: TherapeuticImplications. Life (Basel,Switzerland) ,12(6),814:1-14
[61] Shang J, Ye G, Shi K, Wan Y, Luo C, et al,(2020), Structural basis of receptor recognition by SARS-CoV-2. Nature ;581:221-224
[62] Deftereos S.G., Giannopoulos G., Vrachatis D.A., Siasos G.D., Giotaki S.G, et al, (2020), Effect of Colchicine vs Standard Care on Cardiac and Inflammatory Biomarkers and Clinical Outcomes in Patients Hospitalized with Coronavirus Disease 2019: The GRECCO-19 Randomized Clinical Trial. JAMA Netw. Open. 2020;3:e2013136:1-29
[63] Chen, W., Liang, J. L., Zhou, K., Zeng, Q. L., Ye, J. W., & Huang, M. J. ,(2020, Effect of

CIP2A and its mechanism of action in the malignant biological behavior of colorectal cancer. Cell communication and signaling : CCS, 18(1), 67:1-16

[64] Zhang N, Wang S, Wong C.,et al,(2022), Proteomics research of SARS-CoV-2 and COVID-19 disease. Medical Review. 2022;2(4): 427-445.

[65] Wen Z., Zhang Y., Lin Z., Shi K. and Jiu Y.,(2021), Cytoskeleton A crucial key in host cell for coronavirus infection. J. Mol. Cell Biol;12(12):968–979

[66] Myari A, Papapetrou E, Tsaousi C.,et al,(2022), Diagnostic value of white blood cell parameters for COVID-19: Is there a role for HFLC and IG? Int J Lab Hematol;44(1):104-111.

[67] Wei, T., Li, J., Cheng, Z., Jiang, L., Zhang, J., Wang, H., & Zhou, L. (2023). Hematological characteristics of COVID-19 patients with fever infected by the Omicron variant in Shanghai: A retrospective cohort study in China. Journal of clinical laboratory analysis, 37(1), e24808:1-8

[68] Luo X, Zhou W, Yan X, Guo T, Wang B, et al,(2020), Prognostic Value of C-Reactive Protein in Patients With Coronavirus 2019. Clin Infect Dis;71(16):2174-2179