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RESEARCH ARTICLE - MEDICAL TECHNIQUES

Estimation of Some Biomarkers in Recovered COVID19 Patients

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Abstract

A novel pandemic COVID-19 occurred in 2019. Most studies across the world are concerned with this outbreak. In this prospective study, a certain biomarker was estimated as a predicting factor in 150 recovered COVID-19 patients and group of 50 healthy controls. The serum creatinine, Aspartate aminotransferase, Interlukin-24, prothrombin time, and fibrinogen were measured using different techniques. The results revealed that the level of AST in recovered patients exceeded that of the healthy control group with a highly significant difference. There was also a highly significant increase in the level of IL-24 in recovered patients in comparison with that in healthy control, while both prothrombin time and fibrinogen levels were lower in recovered patients compared to healthy control. We conclude that the variation in the level of parameters of interest may reflect a prognostic view in recovered COVID-19 patients.

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1. Introduction

A number of pneumonia cases with no known etiology were reported in Wuhan (Hubei), China, around the end of 2019 [1]. A vast percentage of COVID-19 infections are asymptomatic, but they can also result in fulminant pneumonia, acute respiratory distress syndrome (ARDS), multiple organ failure, and death [2]. A sizable number of COVID-19-related cases of viral pneumonia that spread quickly and fatally occurred in December 2019. The infection was quickly disseminated over the world by tourists, causing considerable media coverage and public concern [3]. The patients all shared the experience of visiting a grocery market in Wuhan where live animals and shellfish are sold. Due to the presence of spikes of glycoprotein on its envelope, the Corona Virus Disease 2019 (COVID-19) RNA has the classic crown-like look under an electron microscope [4]. The four CoV genera that are assumed to represent avian species are (I) -coronavirus (alpha CoV), (II) -coronavirus (beta CoV), (III) -coronavirus (deltaCoV), and (IV) -coronavirus (gamma CoV) [5]. According to a molecular assay, there are more than 100 million people worldwide (from more than 210 countries) that have a confirmed SARS-CoV-2 infection as of February 2021, 13–14 months after the virus was originally described. COVID-19 has been implicated in more than 2 million fatalities [6]. The World Health Organization (WHO) declared a public health emergency in late January 2020 and described it as a pandemic in March 202[7] as a result of the infection spreading since the initial reports of COVID-19, which resulted in more than 81.552 cases in China and growing cases (> 1.4 million) worldwide, the present worked we estimate some parameters that we expected to change in COVID19 recovered patients and it is changes may led to certain complication.

2.Material and Methods

2.1. study subjects and study plan

This study was conducted in a Baghdad teaching hospital from July to September 2021. All patients who recovered subjects had clinical symptoms of COVID-19 with a previous history of PCR positive and negative RT-PCR COVID-19 will be followed.

2.2. Patients constant

Approval and patient consent were taken from Baghdad teaching hospital and the patients before sample taking.

Nomenclature			
ARDS	Acute respiratory distress syndrome	PT	Prothrombin Time
AST	Aspartate aminotransferase	WHO	World Health Organization

2.3. *Recovered Patient groups*

A total of 150 patients were included in this study group (94 female and 56 male) within the period more than a month of recovery from COVID-19 infection, The infection was confirmed by real time PCR technology for detection of the causative agent Corona virus, 150 patient group have COVID19 clinical symptoms infection there were a medical examination done by physician, their age ranged from 15 –65years.

2.4. Control groups

Fifty healthy people were selected as a control group (25 females and 25 males) between the ages (15-65) years and they do not have a history of COVID-19.

2.5. Blood sample collection

In this study 150 samples (serum, plasma) were collected with sterile precautions, 150 Sample from recovered patients group and 50 the control group, 5 ml of blood sample was obtained from each patient and healthy control by vein puncture using disposable syringe, 3 ml transported to a non-heparinized blood collecting gel tube and then was centrifuged at 3500rpm for 10 min, Anticoagulant-free blood serum.
And other two ml were put in in tubes containing sodium citrate (3.2%) and then mixed with whole blood (1.968) with the substance, and then were centrifuged at 1500rpm for 15 min. all samples were kept at -20°C until preparation for analysis.

2.6. Serum samples

Use a Serum separator tube (SST) and allow samples to clot for two hours at room temperature -20 c^o before centrifugation for 10 minutes at 3500rpm. Remove serum using a Pasteur pipette, divide it into Eppendorf tubes then label after collection of the whole blood, and assay immediately and store samples at -20 c^o [8].

2.7. Plasma samples

When whole blood is collected in tubes that are treated with an anticoagulant (3.2%) sodium citrate tubes (light blue tops). centrifuge for 15 minutes at 1500rpm. Remove plasma using a Pasteur pipette, divide it into Eppendorf tubes then labeled after collection of the whole blood, and assay immediately and store samples at -20 c^o [8].

2.8. Methods

Serum creatinine to detect renal function and ALT enzyme were estimated using biochemical analyzer (full-automation) Flexor-EL-80 (South Africa). Full-automation biochemical analyzer. Prothrombin time and fibrinogen were estimated using a coagulation device (semi-automation) Spain React (Spain).
Interleukin 24(IL-24) in human serum was measured by an ELISA test.
This assay employs sandwich enzyme immunoassay technology. Standards and samples are pipetted into the wells using a technique developed by CUSABIO Kit Company (CSB-E15840h). the optical density absorbance at 450 nm in a microplate reader.

2.9. Statistical analysis

Statistical analysis was done according to percentages to compare between samples using SPSS V.25 using T-test and ($P \leq 0.05$).

3. Results and Discussion

The present work was arranged to evaluate certain biomarkers (serum creatinine, Aspartate Aminotransferase, Interleukin 24, Prothrombin time and fibrinogen) in recovered COVID19 patients and in healthy control group.

3.1. Distribution of sample study according to Gender and Age groups in recovered patients

The current study shows significance differences (P=0.0483) between female patients which had a high percent (26.00%) and (16.67%) at the age range (30-50) and >50 year, and significance differences (P= 0.0441) of recovered male patients which have a high percent (18.67%) and (7.33%) at the age range (30-50) and<30 year as shown in Table 1.
The current study showed that the incidence of female's number (62.67%) is higher than that of males (37.33%), as shown in Table 1, and this might be due to the randomness of the sample. Age is a risk factor for a more severe disease outcome, because of the generally decreased function of the immune system among older people. At old age, the adaptive immunity would become exhausted, ineffective, and almost deleterious innate response [9].The present study differs from the result of another study in Iran (10), which found that the percentage of age in the range (10-19 and 50-59) are (1.3 % and 20.9 %) respectively.

Table 1 Comparison of study sample according to Gender and Age groups in recovered patients

Age groups	Female	Male	Total	P-value
	No (%)	No (%)		
<30 yr.	30 (20.00%)	11 (7.33%)	41 (27.33%)	
30-50 yr.	39 (26.00%)	28 (18.67%)	67 (44.67%)	*P=0.0483
>50 yr.	25 (16.67%)	17 (11.33%)	42 (28.00%)	**P=0.0441
Total	94 (62.67%)	56 (37.33%)	150 (100%)	
P-value	* 0.0483	*0.0441	*0.0372	

* (P≤0.05)

3.2 level of biomarkers in recovered patients and healthy control

The results in a Table 2 revealed a highly significant difference among the various interested parameters concerned in the current study.

Table 2 Comparison among biomarkers in recovered patients and healthy control

Parameters	Mean ± SE Patients(recovered) N=150	Mean ± SE healthy(control) N=50	T-test	P-value
Serum Creatinine(mg/dl)	1.125 ±0.05	0.755 ±0.02	0.112 **	0.0001
AST (U/l)	39.58 ±0.98	32.63 ±0.67	2.458 **	0.0001
IL-24 (pg./ml)	335.12 ±10.68	215.54 ±6.51	26.063 **	0.0001
Prothrombin time. (sec)	9.77 ±0.20	12.56 ±0.18	0.539 **	0.0001
FIB (mg/dl)	223.05 ±5.92	291.52 ±5.66	16.404 **	0.0001

** (P≤0.01).

These results revealed elevated creatinine in recovered patients, in comparison with healthy control, and the current results agreed with a study in China [11].

3.3. Level of Serum Creatinine in patients and control

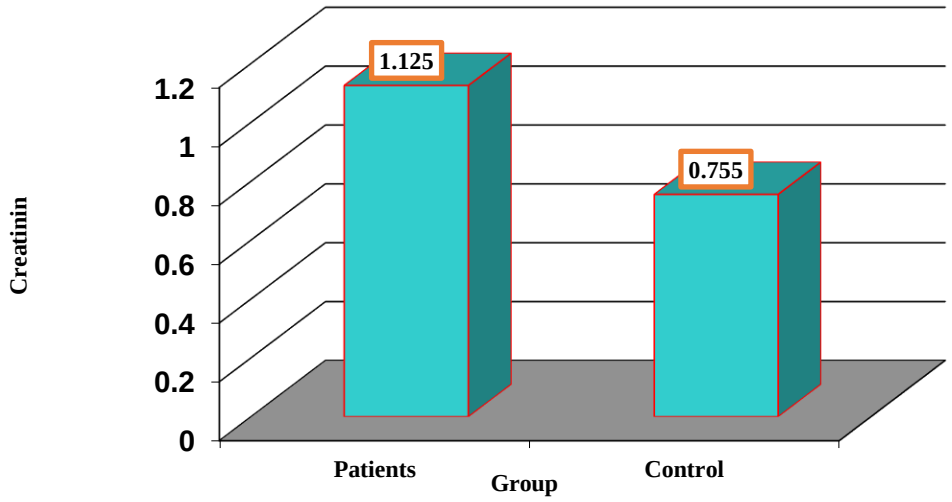

Fig 1. Comparison between patients and control in Serum Creatinine levels

In fact, the serum creatinine level is within the normal range, and it will be not considered a negative indicator, serum creatinine is a commonly used as an indicator for the detection of renal function, Creatinine elevated in recovered patients were attributed to advanced age, lifestyle, and renal tubular dysfunction reflects of complication direct viral infection because renal impairment is common in COVID-19, The of use treatment (corticosteroids)during infection lead abnormal renal function [12, 13]
On the other hand, an increase in the level of Aspartate aminotransferase (AST) in serum of recovered patients were (39.58) exceeding that for healthy control (32.63), as shown in the Fig. 2.

3.4. Levels of AST in patients and control

These results agreed with other studies in Egypt done by [14], China done by [15], and [16].
Aspartate aminotransferase (AST) is a helpful screening tool which is an effective modality to detect hepatic dysfunction and heart [17]. The mechanisms underlying liver impairment in COVID-19 as a result of direct viral infection of hepatocytes immune-related injury (cytokines storm) which can lead to the damage of the liver cells and myocardial injury and also due to drug hepatotoxicity. There is also suggestion that

the virus may bind to cholangiocytes through the Angiotensin converting enzyme receptor -2 receptor to dysregulate the liver function [18 - 20].

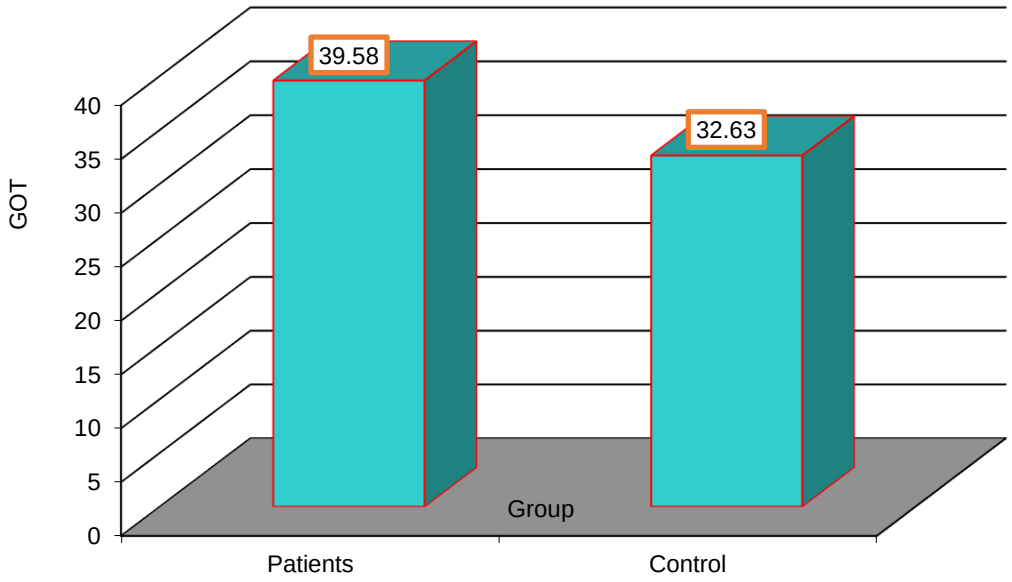

Fig 2. Comparison between patients and control in AST/GOT

3.5. Levels of IL-24 in patients and control

Fig. 3 shows an increased level of Interleukins -IL24 in the serum, of recovered patients were (335.12) compared with the control group were (215.54), as shown in the Fig. 3.

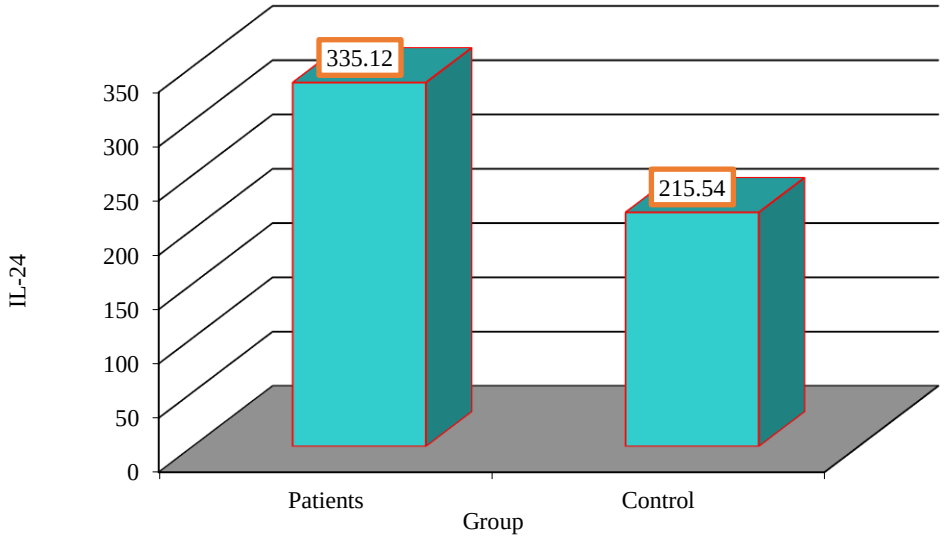

Fig 3. Comparison between recovered patients and control in IL-24

Senescence of the immune system in the elderly has been termed “inflammaging”, which refers to increased levels of tissue and circulating proinflammatory cytokines in the absence of an immunological threat (21). The compositional changes of immune cells in recovery status associate with age [22].

Il-24 mechanisms of immune dysregulation, strong up-regulation of IL-24 in lungs post-infection and human lung epithelial cells induce IL-24 production [23]. COVID-19 RNA is present in diverse epithelial and immune cells, Interleukin (IL)-24 is a member of the IL-20 family of cytokines and is produced by various types of cells such as CD4 T cells [24] Which reflects increased IL-24.

IL-24 plays an essential role in the pathogenesis of proinflammatory autoimmune disorders involved in the pathogenesis of allergic lung because IL-24 produce from bronchial epithelial cells [24].

3.6. Levels of prothrombin Time *in patients and control*

From the data in Table 2, it was found that the results of hematological parameters show high significance (P=0.0001) of recovered patients in comparison to healthy control in parameters (prothrombin time, fibrinogen). As shown in the figure (4-and 5) respectively.
These results were approved in this study showed a decrease in the level of prothrombin time in plasma, recovered group patients were (9.77) and compared to the control group were (12.56), as shown in the Fig. 4.

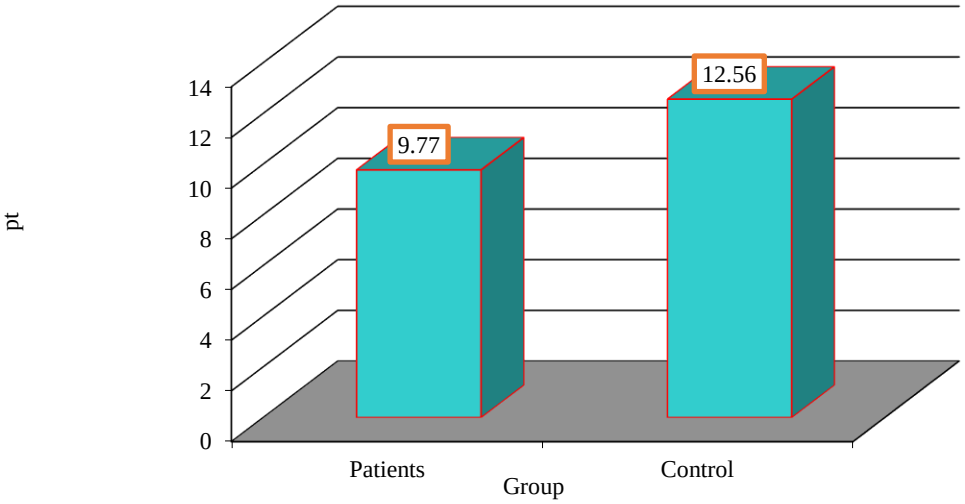

Fig 4. Comparison of prothrombin times between patients and control

The current findings conflict with those of other research conducted by [25] in China, [26] in Bangladesh, and [27] in Italy.
According to these findings, thrombi development was brought on by a change in coagulation function because prothrombin time levels were lower in recovered individuals. Lower prothrombin time levels were found in recovered patients and were linked to age-related changes in coagulation function, lifestyle factors, antiviral medications, and liver-related COVID-19 problems [28]. One explanation could be that the various types and concentrations of cytokines, which cause varying degrees of coagulation problems, have increased, such as IL-6 and other cytokines [27].

3.7. levels of FIB in patients and control

The decreased level of fibrinogen (FIB) in plasma, in the recovered group was (223.05) and in the control group was (291.52), as shown in the Fig. 5.

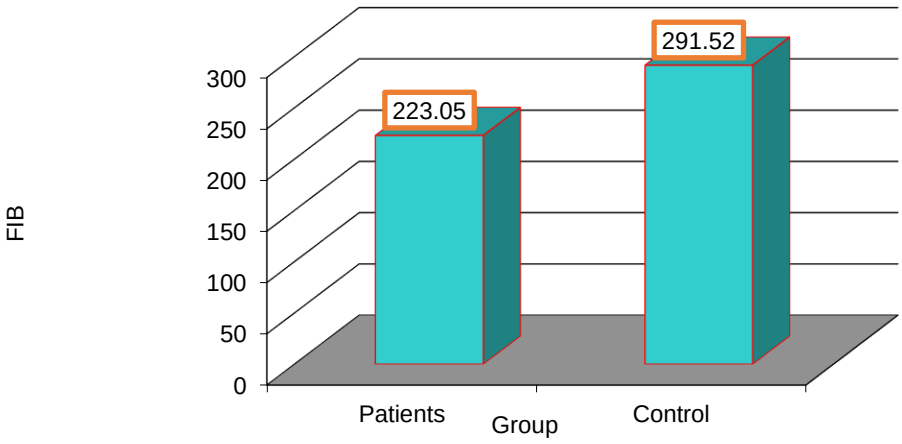

Fig 5. Comparison between patients and control in Fibronogen

The current findings conflict with two investigations conducted in Taiwan and Japan (29, 30). These findings explained why recovered patients' fibrinogen levels were lower than those of the healthy control group. Hepatocellular damage can lower fibrinogen levels by impairing the liver's ability to produce fibrinogen or by excessively stimulating the breakdown of clots and consumption of fibrinogen. This can lead to decreased acquired fibrinogen deficiency, which is an indirect side effect of aspirin treatment for infection.
Following infection, the injured tissue releases tissue factors into the blood and engages the exogenous coagulation system, leading to widespread micro thrombus formation and coagulation factor depletion [33]. Last but not least, a disturbance in physiological equilibrium causes endothelial inflammation, which increases the risk of thrombosis and atherosclerosis [34].

4. Conclusion

The Study conclude that the variation in the level of an interesting parameter may reflect a prognostic view in recovered COVID-19 patients.

Conflict of Interest

The authors hereby declare no conflict of interest.

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