

Effect of COVID-19 the Liver Function Test, Plasma D-dimer, Serum Ferritin and Renal on Function Test (Urea and Creatinine)

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ABSTRACT:

BACKGROUND:

The assessment of the level of some biochemical parameters (liver function test, renal function test, serum ferritin and plasma D-dimer) revealed that the Covid-19 infection affected the level of them. The study aimed to demonstrate the biochemical changes of liver and renal functions tests, serum ferritin and plasma D-dimer in Iraqi patients with Covid-19 disease.

PATIENTS AND METHODS:

A retrospective cross sectional study conducted in Isolation centers for Covid-19 patients in Baghdad and AL-Najaf AL-Ashraf Governorate from July 2020 to February 2021. Forty five males and 76 females were included in this study. The exclusion criteria were patients below eighteen years old age, pregnant women, and subjects with hematological diseases, cardiovascular disease, liver diseases and renal diseases. All parameters measured by automated method by Beckman coulter Au480 chemistry analyzer.

RESULTS:

Covid-19 infection was more in females than males and it is more in post middle age (41-50 years old). In the current study, most of the biochemical parameters (AST, ALT, urea, serum ferritin, AST/ALT ratio <1 and plasma D-dimer) increased through the period of Covid-19 infection of them. AST, ALT, urea and AST/ALT ratio <1 were more increased in females. The age was significantly negative and positive correlated with the activity of AST and urea concentration respectively. The gender was significantly positive correlated with AST/ALT ratio. There was no correlation between plasma D-dimer and serum ferritin with other biochemical parameters except for AST activity which was significantly positive correlated with serum ferritin.

CONCLUSION:

Most of the biochemical parameters (AST, ALT, urea, plasma D-dimer and serum ferritin) were affected by Covid-19 infection which indicated organs damage. The age and gender were affected the activity and level of some biochemical parameters (AST and Urea were significantly negative and positive correlated with age respectively and AST/ALT ratio was significantly positive correlated with gender).

KEYWORDS: COVID-19, Liver Function Test, Plasma D-dimer, Serum Ferritin and Renal Function Test.

INTRODUCTION:

It is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The median incubation period of SARS-CoV-2 is ~5 days (ranging from 2 to 14 days), and people who develop symptoms do so within ~12 days of infection (ranging from 8 to 16 days) [1]. A sizable portion of person-to-person virus transmission may occur before infected individuals develop symptoms (presymptomatic) [2]. A fraction of infected individuals never develops symptoms (asymptomatic); yet may contribute substantially to disease transmission [3]. Plasma D-dimer is

a specific product of the degradation of fibrin clots that results from the action of 3 enzymes: (a) thrombin, generated from the activation of the coagulation cascade that converts fibrinogen into fibrin clots; (b) activated factor XIII that cross-links fibrin clots by means of covalent bonds between fibrin monomers; and (c) plasmin, the ultimate enzyme of fibrinolysis that degrades cross-linked fibrin [4]. Serum ferritin is present in most tissues as a cytosolic protein, although a mitochondrial form has recently been described and nuclear localization and functions have been proposed. Serum ferritin is a 24-subunit protein that is composed of two types of subunits, termed H and L. The H refers to the original isolation of isoforms of serum ferritin from human heart, which are rich in

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the H subunit, or to its electrophoretic migration as the heavier of the two subunits. The L refers to serum ferritin isolated from human liver, which is rich in a lighter subunit [5]. The liver, located in the right upper quadrant of the body and below the diaphragm is responsible for several functions including primary detoxification of various metabolites, synthesizing proteins, and producing digestive enzymes. The liver also has a significant role in metabolism, regulation of red blood cells (RBCs) and glucose synthesis and storage. Typically, when reviewing LFTs, the discussion includes alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR) and albumin. These tests can be helpful in determining the area of hepatic injury, and the pattern of elevation can help organize a differential diagnosis [6].

METHODOLOGY

A retrospective cross sectional study, conducted in isolation centers for COVID-19 patients at Nursing Home Private Hospital in Baghdad Medical City and at AL-Sadder Teaching Hospital in AL-Najaf AL-Ashraf governorate. Study period was extended from July 2020 to February 2021. The eligible population of this study was all patients diagnosed by PCR and chest CT scan as cases of Covid-19, admitted to the isolation centers for COVID-19 hospitals during the study period. Convenient sampling used to select the study group from the sample frame, with assistance of medical and health staff; all eligible patients presented or admitted to the wards during the study period were selected.

Data was collected in regarding to demographic, clinical data and laboratory parameters from

2652 patients medical records using standardized data collection form from isolation centers for COVID-

19 patients (1550 patients' records, only 66 were included in this study from AL-Sadder Teaching Hospital in AL-Najaf AL-Ashraf and 1102 patients' records, only 55 were included in this study from Nursing Home Private Hospital in Baghdad).

Liver function test (AST, ALT, ALP, TSB, AST/ALT ratio), urea, creatinine, serum ferritin and plasma D-dimer all were measured for each participant.

Blood was collected and parameters were measured by the medical staff in laboratories at AL-Sadder Teaching Hospital in AL-Najaf AL-Ashraf and at Nursing Home Private Hospital in Baghdad.

A bout 5 ml of blood samples were obtained from veins for COVID-19 patient's. Each blood sample was divided into two parts:

The first part: 2ml of whole blood retained in sodium citrate tubes and separated by centrifugation at 3000 rpm for 10 min for obtaining plasma to measure plasma D-dimer, the assay were applied by automated method by using Beckman coulter AU480 chemistry analyzer.

The second part: 3ml retained in gel tubes for 30 min at room temperature, and separated by centrifugation at 3000 rpm for 10 min, the resulting serum is divided into aliquot in Eppendorf tubes for biochemistry measurements of serum ferritin, Urea, Creatinine, AST, ALT, ALP and total serum bilirubin, the assay were applied by automated method by using Beckman coulter AU480 chemistry analyzer.

The instruments used in this study are listed with the producing company and the country of origin in the table below

No.	Equipment	Company	Origin
1	Centrifuge	Kubota	Japan
2	Beckman coulter AU480	Beckman coulter	USA
3	Incubator	Gallenkamp	England
4	Micropipette (0.5-10 u L), (20-200u L)	Biobasic	Canada
5	Stop Watch	Kienzle	Germany
6	Gel tube	AFCO	Jordan
7	Sodium citrate tube	AFCO	Jordan
8	Eppendorf tube (1.5ml)	AFCO	Jordan
9	Pipette tips	AFCO	Jordan

Statistical analyses were performed using the SPSS version 20.0 software program (SPSS). The data were normally distributed. Pearson Correlation. Descriptive data are presented as mean \pm standard deviation for continuous variables and number (%) for categorical variables. A $p < 0.05$ was considered as statistically significant.

RESULTS:

By class interval classification according to age of study group, 4.1% of patients from 20 to 30 years, 11.6% from 31 to 40 years, 28.1% from 41 to 50 years, 26.4% from 51 to 60 years, 15.7% from 61 to 70 years, 10.7% from 71 to 80 years and 3.3% from 81 to 90 years, of them 37.2% was male and 62.8% was female. The mean \pm SD of age was 54.14 ± 14.48 year. Distribution of study group by age and gender is shown in (table 1).

Table 1: Distribution of the Study Group by Socio-Demographic Characteristics (Age and Gender).

Gender	Group	Freq.	%
Gender	Female	76	63
	Male	45	37
Age range(years)	20 to 30	5	4.1
	31 to 40	14	11.6
	41 to 50	34	28.1
	51 to 60	32	26.4
	61 to 70	19	15.7
	71 to 80	13	10.7
	81 to 90	4	3.3
	Mean \pm SD		54.14 ± 14.48

The mean \pm SE of AST of study group was 48.803 ± 4.062 U/L, the mean \pm SD ALT was 59.832 ± 5.320 U/L, The mean \pm SE of T.B. was 0.9567 ± 0.2770 mg /dl, The mean \pm SE of ALP was 85.526 ± 4.391 , The mean \pm SE of Urea was 60.395 ± 6.003 mg/dl, The mean \pm SE of

Creatinine was 1.0 ± 0.241 mg/dl, The mean \pm SE of serum Ferritin was 803.195 ± 64.842 ng/ml , The mean \pm SE of plasma D-dimer was 18.780 ± 4.097 and The mean \pm SE of AST/ALT ratio was $1.067 \pm .519$ as shown in Table 2.

Table 2: Distribution of Study Group by Biochemical Parameters.

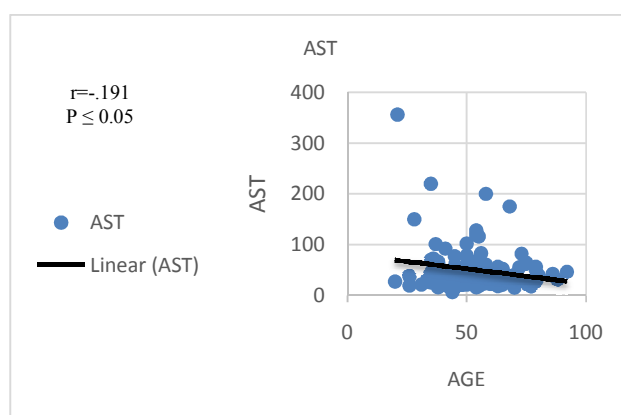
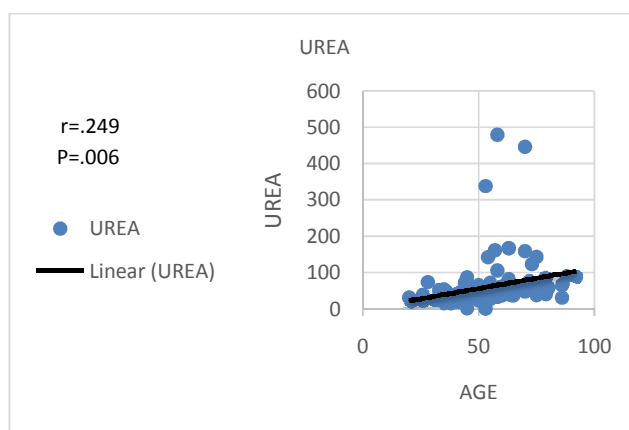
Parameter	Mean \pm St Error of Mean
AST (U/L)	48.803 ± 4.062
ALT (U/L)	59.832 ± 5.320
TSB (mg/dl)	0.9567 ± 0.2770
ALP (U/L)	85.526 ± 4.391
Urea (mg/dl)	60.395 ± 6.003
Creatinine (mg/dl)	1.0 ± 0.241
Serum Ferritin (ng/ml)	803.195 ± 64.842
Plasma D-dimer (mg/l)	18.780 ± 4.097
AST/ALT	$1.067 \pm .519$

The AST is negatively significantly negative correlated with age in this study, with p value ≤ 0.036 as shown in figure (3.1), Urea is significantly correlated with age with p value \leq

0.006 as shown in figure (3.2), there is no significant correlation between age and other parameters. Correlation of the age with parameters in this study shown in table 3.

Table 3: Correlation of Age with Parameters.

Parameter	r- value	p- value
AST	-.191-	.036*
ALT	-.137	.134
T.B	.000	.997
ALP	.046	.613
UREA	.249	.006**
CREATININE	-.049	.594
Ferritin	.009	.919
DIMER	.128	.163
AST/ALT	.082	.373

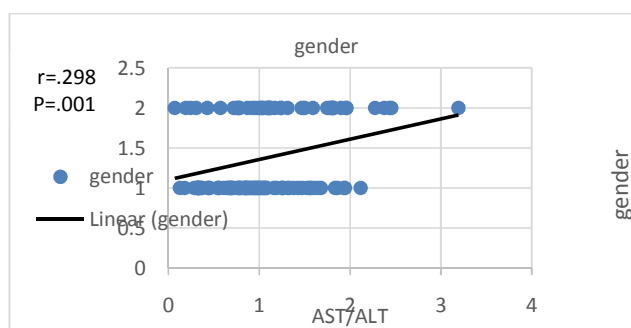
**Figure 1: Shows the correlation between age and AST.****Figure 2: Shows the correlation between age and urea.**

The AST/ALT ratio is significantly correlated with gender with p value ≤ 0.001 as shown in figure (3.3). There is no significant correlation between gender and other parameters according to r value and p value (r=-

.072, -.142, .100, -.024, .019, -.087, -.091, .101 for AST, ALT, T.B., ALP, Urea, Creatinine, ferritin and D-dimer respectively). Correlation of the gender with parameters in this study is shown in table 4.

Table 4: Correlation of the Gender with Parameters.

Parameter	r- value	p- value
AST	-.072	.430
ALT	-.142	.122
T.B	.100	.277
ALP	-.024	.795
UREA	.019	.837
CREATININE	-.087	.344
Ferritin	-.091	.318
DIMER	.101	.269
AST/ALT	.298	.001**

**Figure 3: Shows the correlation between gender and AST/ALT ratio.**

There is no significant correlation between ALP, Urea, Creatinine, ferritin and AST/ALT plasma D-dimer and other parameters according to r value and p value ($r = -.049, -.043, -.017, -.021, .110, -.012, -.004, .002$ for AST, ALT, T.B., this study is shown in table 5.

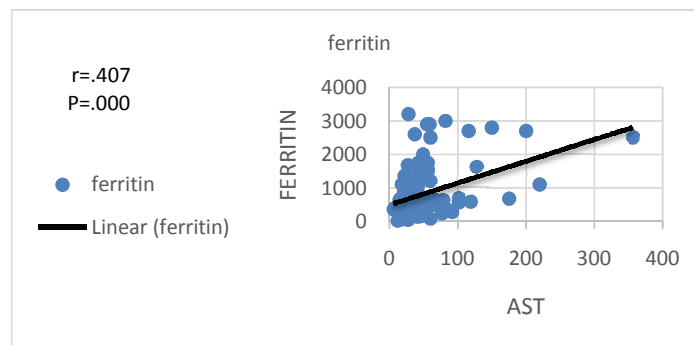
Table 5: Correlation the D-dimer with other Parameters.

Parameter	r- value	p- value
AST	-.049	.592
ALT	-.043	.638
T.B	-.017	.856
ALP	-.021	.819
UREA	.110	.229
CREATININE	-.012	.898
ferritin	-.004	.966
AST/ALT	.002	.982

There was significantly positive correlation between ferritin and AST with p value ≤ 0.000 . There is significant no correlation between ferritin and other parameters according to the p value and r value ($r = .164, -.060, .058, .053, -.020, -.004, .098$ for AST, ALT, T.B., ALP, Urea, Creatinine, D-dimer and AST/ALT ratio respectively) $p \geq 0.05$. Correlation of the ferritin with other parameters in this study is shown in table 6.

Table 6: Correlation of the ferritin with other Parameters.

Parameter	r- value	p- value
AST	.407	.000**
ALT	.164	.072
T.B	-.060	.514
ALP	.058	.525
UREA	.053	.561
CREATININE	-.020	.824
DIMER	-.004	.966
AST/ALT	.098	.283

**Figure 4: Shows the correlation between ferritin and AST.****DISCUSSION:**

Distribution of the Study Group by Socio-Demographic Characteristics (Age and Gender)

In the current study, analysis of such demographic characteristics of patients indicates that females were infected more than males (63% were females and 37% were males). The highest of those (28.1%) are post middle age 41-50 years old, of them 10.45% were males and 17.64% were females. These findings are similar to the study conducted by Ding et al. (2021). Jie et al. (2020) conducted a study revealed that the angiotensin converting enzyme inhibitor gen (ACE2 gene) is located on the X chromosome; therefore, female individuals should have higher ACE2 levels, which might be the reason they are more susceptible to SARS-CoV-2 infection in comparison to males^[7,8].

Such age-gender-dependent differentiations imply that females need to strengthen their vigilance to take more effective self-protecting measures to stay away from the virus.

Distribution of Study Group by Biochemical Parameters

In the current study, analysis of biochemical parameters revealed that most of parameters (AST, ALT, UREA, serum Ferritin, plasma D-dimer and AST/ALT ratio) will be elevated during Covid-19. This is similar to the study conduct by Benedé-Ubieto et al (2021)^[9]. Another study similar to this study conducted by

Tenugu et al (2020), revealed that plasma D-dimer was elevated in the hospitalized patients with Covid-19. Therefore, these parameters can be used to aid the diagnosis of the disease and to guide treatment and follow up^[10].

Distribution of Study Group by Biochemical Parameters and their gender

Regarding the biochemical parameters distribution according to gender, current study depicts that the differences in parameters activity (AST, ALT and urea high in female, AST/ALT ratio<1 more in females) these parameters depend on patient gender, while the other parameters have same indicators in both genders. Regarding to plasma D-dimer, similar study revealed that no significant difference in median plasma D-dimer values between genders was noticed^[10]. Regarding liver function tests, a study conducted by Benedé-Ubieto et al (2021)^[9], revealed that only AST/ALT ratio value showed significant correlation with gender while ALT, AST and ALP activities did not show significant correlation with gender. This study agrees with the current study regarding to AST/ALT ratio, but disagree regarding to ALT, AST and ALP activities. Regarding urea and creatinine, a study conducted by Xiang in 2021, revealed that both urea and creatinine were elevated and there is no difference in urea nitrogen between female and male patients, this

study disagrees with the current study. Regarding serum ferritin, a study conducted by Carubbi et al 2021, revealed that male displayed significantly higher levels of serum ferritin and so that disagrees with current study regarding gender. Such findings present an evidence that these parameters might differ depending on gender^[11].

Correlation of Age with Biochemical Parameters. In the current study, analysis of biochemical parameters and their relation with age indicates that the (AST, Urea) have highly significant relation with age respectively, while there was no significant relation with other parameters. This can be interpreted as increasing age might affect renal function more than other organs.

Qu et al (2020)^[12], conducted a study revealed that liver function test abnormality is more common in older patients, this disagrees with the current study in which AST is significantly negative correlated with age and other liver function tests (ALT, ALP and TSB) are not affected. Regarding urea and creatinine, urea is significantly correlated with age while no correlation between creatinine and age, a study conducted by Xiang et al 2021, revealed that both urea and creatinine are higher in older patients than in younger patients^[13], this agrees with the current study in regarding urea and disagrees regarding creatinine. Regarding to serum ferritin and plasma D-dimer, a study conducted by Huang et al (2020) agrees with the current study. Another study conducted by Sharp and Ghodke in 2020, revealed that plasma D-dimer is significantly correlated with age so that disagrees with the current study^[14].

Correlation of Gender with Biochemical Parameters

In the current study, analysis of biochemical parameters and their relation with gender indicated that the AST/ALT ratio is significantly related with gender while no significant relation with other parameters. Regarding liver function test, a study conducted by Hoang et al (2020)^[15], is similar to the current study in that the AST/ALT is significantly related with gender while disagree with current study as it revealed that AST, ALT, ALP and TSB activities were statistically significantly higher in male compared to female. Regarding urea and creatinine, a study conducted by Xiang et al 2021, disagrees with the current study, which revealed that the levels of urea nitrogen and creatinine were higher in males than those in females. Regarding serum ferritin and plasma D-dimer, another study conducted by Sharp and

Ghodke in 2020, revealed that the level of plasma D-dimer was higher in females than those in males and so females are at a higher risk of developing thrombotic disorders than males, so that disagrees with the current study^[16].

Correlation of plasma D-dimer with other Biochemical Parameters

The current study revealed that there was no significant correlation between plasma D-dimer and other parameters according to p-value results, a study revealed that increased aminotransferases (AST and/or ALT), associated with elevated level of plasma D-dimer, and so disagrees with the current study^[15].

Correlation of serum ferritin with other Parameters

In the current study, analysis of such comparative differences indicates that there is a highly significant relationship between serum ferritin and AST, this is similar to the study conducted by Ram et al (2020) which revealed that a positive correlation was found between serum ferritin with AST, ALT, ALP and total bilirubin, so disagrees with the current study regarding ALT, ALP and total bilirubin. In the current study, there was no significant relation between ferritin and other parameters according to p-value results^[17]. Huang in (2020), revealed that both serum ferritin and plasma D-dimer increased in COVID-19 patients and considered them as good marker for severity and prognosis^[14].

CONCLUSION:

Covid-19 disease has an impact on liver function tests, renal function tests, serum ferritin and plasma D-dimer. There is a significant association between gender and Covid-19 infection among the study group. AST and urea are correlated significantly with age among the study group and AST/ALT ratio is significantly correlated with gender. Ferritin is significantly correlated with AST. There was a significant difference in the activity of liver enzymes and ratio between males and females (AST, ALT, and AST/ALT ratio <1 higher in female) and in the level of some biochemical parameters like blood urea.

RECOMMENDATIONS:

Further studies in different settings in pediatric and teenagers age group are needed to further elaboration of the association between Covid-19, severity of disease, prognosis and mortality. Iraqi Ministry of Health should consider the implementation the use of plasma D-dimer, serum ferritin, urea, and creatinine, CBC, liver function test (especially AST, ALT, and LDH),

CRP and IL6 as markers for Covid-19 severity, prognosis and mortality and to daily measured these parameters throughout infected period.

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