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Evaluation of hepatic enzymes activities in COVID-19 patients

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**A B S T R A C T**

**SARS-CoV-2 or Coronavirus disease 2019 (COVID-19) outbreak which caused by the severe acute respiratory syndrome, has rapidly spread over the world. The exact mechanism how this virus will affect the liver remained elusive. The aim of this study was to evaluate the liver function in patients with severe acute respiratory syndrome coronavirus 2 and potential causes of hepatic enzymes disease in these patients. Clinical characteristics and laboratory findings were collected from patients with COVID-19 who were admitted to the corona center in Erbil city/Kurdistan region of Iraq, from March 10 to July 10, 2020. Serum was collected from patients with COVID-19 and liver enzyme tests were measured. Liver alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBL) were analyzed in these patients. Of the 74 patients, 25 (34.7%) had abnormal ALT activity, 28 (40%) had abnormal AST activity, 12 (20.3%) had abnormal ALP activity, and 39 (52.7%) had abnormal total bilirubin P-value < 0.05. The inflammatory biomarkers CRP and IL-6 in COVID-19 patients with abnormal liver function test (4.9 ± 1.0 mg/dl) and (231.2 ± 35.7 pg/ml) respectively. The levels of both biomarkers were statistically significantly higher than COVID-19 patients with normal liver function test (2.1 ± 0.5 mg/dl) and (21.1 ± 0.5 mg/dl) respectively, P-value < 0.05. However, CRP and IL-6 were not statistically significant different between male and female COVID-19 patients P-value < 0.05. In conclusion, we found that most of the patients with SARS-CoV-2 have abnormal hepatic enzyme activities and that is might related to virus replication in the liver.**

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**1. Introduction**

Coronaviruses are members of the subfamily Coronavirinae in the family Coronaviridae and the order Nidovirales [1]. So far, three epidemic viruses, severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV and the current emergence of a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have been recognized. Currently, the latter has become a threat to public health, giving rise to a pandemic outbreak, which is termed as 2019-nCoV or COVID-19 [2]. To date 17 of July 2020, there are 10,453 confirmed cases in Kurdistan region of Iraq, with a mortality rate of 3.7%, included 2652 cases in Erbil city [3].

As SARS-CoV-2 attack alveolar cell in the lungs resulting in the severe inflammation [4]. Evolving to respiratory failure, SARS-CoV-2 infection is commonly manifested by dry cough, fever, fatigue, and headache. The viral is known to attach to and enter cells via angiotensin converting enzyme 2 (ACE2) receptors via endocytosis. The receptor is abundant in human body and is more likely to be the initial step triggering systemic illness that involves damage of vital organs, for instance, liver dysfunction. SARS-CoV-2 is shown to cause various degree of liver injury. It is common in around one third of the infected individuals [5].

Liver dysfunction can occur as a complication of covid-19 infection or it can be due to the adverse drug reaction of the medicines used to control the infection. Hospitalized patients with or without liver disease

**Abbreviations:**

ACE: Angiotensin converting enzyme; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; COVID-19, Coronavirus disease 2019; MERS, Middle East respiratory syndrome; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TBili, Total bilirubin.

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before are likely to be subjected to liver injury. In moderate-severe covid-19 male cases admitted to hospitals, a study showed that there is a remarkable rise in the liver enzymes, AST and ALT. However, there is an inconsiderable increase in bilirubin level [6,7].

Hence, the aim of this study was to assess the hepatic enzyme activities in patients with COVID-19 on the day of admission to hospital in Erbil city in order to have more understanding about pathogenesis of liver disease and for better managing liver damage in patients with COVID-19.

2. Material and method

The present study comprised all patients who diagnosed with SARS-CoV-2 with no history of liver diseases and were admitted in Corona center in Erbil city/Iraq, from March 10 to July 10, 2020, and excluded such patients with pre-existing liver diseases. All patients fulfilled the criteria for SARS-CoV-2 that has been set up by the World Health Organization (WHO). The common symptoms of COVID-19 patients include, fever, a radiograph of chest showing consolidation of the lungs and a close contact with an individual to whom severe acute respiratory syndrome had been previously diagnosed. A positive viral culture and PCR were used to confirm the diagnosis. Our study comprised of 74 patients, 39 males and 35 females with age ranged from 7 to 65.

2.1. Data collection

The data collected from the patients with SARS-CoV-2 in the first day of admission and then analyzed for hepatic functions. The recruitment was hospital based; subjects (covid-19 volunteer patients) gave verbal consent to participate in this study. Inclusion criteria; patients diagnosed with covid-19 from all departments including symptomatic and asymptomatic patients. Exclusion criteria; any patient with history of liver disease was excluded in this study.

2.2. Laboratory examination

The suspected patients infected with SARS-CoV-2, were referred to Corona centre in Erbil city. The swabs were taken from patient’s throat and nose, in order to confirm the existence of SARS-CoV-2. The samples then were identified by real-time reverse transcription PCR. Two pairs of primers targeting the open reading frame 1ab (ORF1ab) and the nucleocapsid protein (N) were amplified and examined. The corresponding sequences for ORF1ab were 50'-CCCTGTGGGTTTTA-CACTTAA-30 (F), 50'-ACGA TTGTGCATCAGCTGA-30 (R), and 5’-CY3-CAGTCTGGGTTTTA-30 (F), 50’-ACGA TTGTGCATCAGCTGA-30 (R), and -CY3-

2.3. Liver function tests

Liver function tests included alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) and total bilirubin (TBili). We examined these variables from the first day that the patient admitted to hospital. The normal ranges of hepatic enzymes were 5–40 U/L for ALT, 5–37 U/L for AST 35–140 U/L for ALP, and 0.2–1 mg/dl for TBili.

2.4. Statistical analysis

Univariate analysis was used to compare patients with normal and abnormal serum hepatic enzyme activities, and statistical evaluations were performed. A P-value of < 0.05 was considered to indicate statistical significance and n represents the total number of patients. Statistical analysis was performed by using of IBM SPSS Statistics software version 20 and data were reported as mean ± SE unless otherwise indicated.

3. Results

The study comprised of 74 patients diagnosed with SARS-CoV-2, 39 males and 35 females from different ethnic background and different age ranged from 7 to 65 years and the mean age was 34.09 years old (Table 1).

3.1. Hepatic chemistries elevation for COVID-19 patients

In order to investigate the liver function, we determined the ALT activity as a marker for hepatocyte function. Of the 74 patients with SARS-CoV-2, elevated ALT was observed in 25 patients (34.7%), 28 (40%) had elevated AST, 12 (20.3%) had elevated ALP, while 39 (52.7%) had abnormal TBili, as shown in Table 2.

3.2. Clinical features abnormalities for COVID-19 patients

Patients also tended to have clinical features of SARS-CoV-2, like cough, headache, sore throat and fever. Around 67.5% of patients were symptomatic Table 3. Levels of abnormal hepatic function were statistically significant in COVID-19 patients compared to COVID-19 patients with normal hepatic function, ALT, AST, ALP, and bilirubin (67.66 ± 6.44, 73.32 ± 4.45, 242.96 ± 6.69, and 2.10 ± 0.15) respectively, P value < 0.05. Our results show that patients with COVID-19 had abnormal liver function test at admission date as shown in Table 2. Majority of patients with SARS-CoV-2 were male with no previous liver diseases (52.7%) and (47.3%) were female as shown in Table 3. About 74.3% of patients with abnormal liver function tests were male and about 54.2% were female. Of 70 patients, 50 had symptoms including, fever, cough, sore throat and shortness of breath. Regarding body temperature, 46 patients had abnormally high body temperature with average of 38.5°C. The majority of patients were presenting symptoms with a P-value < 0.05 as shown in Table 3. In addition, we observed that asymptomatic patients with abnormal LFT were significantly lower than symptomatic patient with abnormal LFT P-value < 0.05 as shown in Table 4.

3.3. Inflammatory biomarkers in patients with COVID-19

CRP and IL-6 are inflammatory biomarkers that indicate infection with pathogen; our results showed that IL-6 and CRP are elevated in patients with COVID-19, 231.1 ± 35.7 pg/ml and 4.9 ± 1.0 mg/dl respectively, P-value < 0.05, as shown in Table 5. In order to observe the status of patient with abnormal LFT according to inflammatory markers level, we compared CRP and IL-6 in abnormal and normal LFT. The levels of CRP and IL-6 elevated in COVID-19 patients, however, the levels of CRP and IL-6 inCOVID-19 patients with abnormal LFT was significantly higher than with those of normal LFT P-value < 0.05, as shown in Table 5. In addition, our result showed that there were no significant difference between male and female in inflammatory biomarkers CRP and IL-6 male (3.43 ± 1.38), female (1.45 ± 0.73) with Pvalue 0.626 and male (371.45 ± 36.181), female (384.01 ± 51.567).

Table 1

The distribution of COVID-19 patients according to age.

| Age (year) | Number of cases (n) | Ratio/%
|-----------|---------------------|------|
| Pediatric < 15 | 8 | 10.8 |
| 16–20 | 7 | 9.4 |
| 21–30 | 16 | 21.6 |
| 31–40 | 17 | 22.9 |
| 41–50 | 15 | 20.2 |
| 51–60 | 10 | 13.5 |
| >60 | 1 | 1.67 |

Table 2

The distribution of COVID-19 patients according to admission date.
Previous studies have shown that the antiviral drugs which with previous study, which demonstrated that ACE is abundantly of SARS-CoV-2 into the liver cells. Moreover, it has been shown that ACE differences between patients with symptoms and without symptom in and clinical features, we found there were no statistically significant blood [10,11]. In our study, the liver enzyme activities are abnormal and COVID-19 and that lead to increase the liver enzyme activities in the received by the COVID-19 patients may lead to liver damage during and least common ALP abnormality 52.7%, 34.7%, 40.0% liver enzyme activities. Notably, TBili is most common and less common

### Table 2

The rate of abnormal hepatic enzymes outcomes.

| Parameter | Total cases | Normal cases | Mean ± SE | Percentage of abnormal LFT | Abnormal cases | Percentage of abnormal LFT | Mean ± SE | P-value |
|-----------|-------------|--------------|-----------|---------------------------|---------------|---------------------------|-----------|---------|
| ALT       | 72          | 47           | 15.80 ± 1.12 | 65.3% | 25 | 34.7% | 67.66 ± 6.44 (U/L) | <0.05 |
| AST       | 70          | 42           | 18.43 ± 1.04 | 60% | 28 | 40% | 73.32 ± 4.45 (U/L) | <0.05 |
| ALP       | 59          | 47           | 62.33 ± 2.90 | 79.7% | 12 | 20.3% | 242.96 ± 6.09 (U/L) | <0.05 |
| TBili     | 74          | 35           | 0.51 ± 0.02  | 47.3  | 39 | 52.7% | 2.10 ± 0.15 (mg/dl) | <0.05 |

Normal cases indicate the COVID-19 patients with normal liver function test.
Abnormal cases indicate the COVID-19 patients with abnormal liver function test.

### Table 3

The prevalence of normal and abnormal liver function test in male and female with percentage of number of cases with or without symptoms.

| Parameter | Male | Female | Male | Female | Yes | No | Number of cases (n) | Percentage | Mean ± SE | P-value |
|-----------|------|--------|------|--------|-----|----|---------------------|------------|-----------|---------|
| ALT       | 39   | 35     | 29   | 19     | 50  | 24 | 60%                 | 52.7%      | 15.80 ± 1.12| <0.05   |
| AST       | 22   | 44%    | 16   | 48.5%  | <0.05 |     |                     |            | 18.43 ± 1.04| <0.05   |
| ALP       | 31   | 75.6%  | 10   | 24.4%  |     |    |                     |            | 62.33 ± 2.90| <0.05   |

with P-value 0.626 and 0.167 respectively, Table 6. In order to investigate the relation between the inflammatory biomarkers IL-6 and CRP and clinical features, we found there were no statistically significant differences between patients with symptoms and without symptom in both inflammatory biomarkers, P-value > 0.05 Table 7.

### Table 4

Clinical characteristic of COVID-19 patients with abnormal liver function test.

| Parameter | Abnormal LFT | Percentage | Normal LFT | Percentage | P-value |
|-----------|--------------|------------|------------|------------|---------|
| Asymptomatic (n = 33) | 17 | 51.5% | 16 | 48.5% | <0.05 |
| Symptomatic (n = 41) | 31 | 75.6% | 10 | 24.4% |     |

### 4. Discussion

Our results showed that >60% of COVID-19 patients have abnormal liver enzyme activities. Notably, TBili is most common and less common ALT and AST and least common ALP abnormality 52.7%, 34.7%, 40.0% and 20.3% respectively Table 2. ACE2 is located on the liver cells and is the target for SARS-CoV-2, could be the reason that facilitates the entry of SARS-CoV-2 into the liver cells. Moreover, it has been shown that ACE bind easily to the spike protein of SARS-CoV-2 [5]. Our result is in line with previous study, which demonstrated that ACE is abundantly secreted by biliary epithelial cells but the abnormality is less common in ALP [6,8,9]. Previous studies have shown that the antiviral drugs which received by the COVID-19 patients may lead to liver damage during COVID-19 and that lead to increase the liver enzyme activities in the blood [10,11]. In our study, the liver enzyme activities are abnormal and the abnormality could be caused by SARS-CoV-2, due to the fact that the liver function test has done on the day or the next day of patient’s admission to hospitals, who didn’t receive any medication yet.

The liver damage caused by virus as demonstrated by previous study [11]. The virus invades the host and the immune responses by releasing the inflammatory mediators like chemokines and cytokines [12,13]. Our results showed the overwhelming of inflammatory biomarkers like CRP and IL-6, by 75% and 54% respectively Table 5, indicating the host responses to inflammation that caused by SARS-CoV-2. In addition, the high body temperature in the patients is due to the cytokines storm [13]. It is worthy to mention that IL-6 is one of the biomarker for sepsis and septic lung injury [14,15]. Notably, our results showed that most of patients had markedly high IL-6 and CRP. In addition, most of the patients with abnormal LFT, have high levels of IL-6 and CRP and this might be that IL-6 and CRP related to the severity of inflammation and abnormal LFT Table 5. The levels of inflammatory biomarkers in COVID-19 patients in different gender were not statistically significant. The serum levels of inflammatory biomarkers are effectively evaluating disease severity [13,14,15].

Nearly, two third of patients were symptomatic and one third of the patients in our study were asymptomatic, and it has been shown that asymptomatic carriers are a potential risk factor for transition of SARS-CoV-2 which may delay controlling this virus [16]. Our result showed that COVID-19 patients with abnormal liver function test have most of the sign and symptoms. In total of 48 cases abnormal LFT, 31 cases were symptomatic. Alexander et al 2020 demonstrated that hepatic abnormality related to severity of SARS-COV-2 [17]. Our result showed that the incidence of COVID-19 among male individuals was higher than in female individuals 52.7% and 47.3% respectively. Moreover, the abnormally high hepatic enzymes observed in male individual than in female individual, 74.3% and 54.2% respectively. In addition, the prevalence of COVID-19 was highest in age ranged between 31 and 40 years, 22.9%. Our result showed the higher incidence of COVID-19 in men than in women (1.5 fold), and this could suppress immune system while estrogens is known to promote immune system and this could be the reason that the women have stronger immune response to fight bacteria and viruses [18]. Our study is in line with other studies that have done on other types of coronaviruses like, Middle East Respiratory Syndrome (MERS), which showed that males are more susceptible to the coronavirus [19,20]. Moreover, a study showed that the mortality rate among men infected with SARS-CoV-2 is higher than in women [18]. The prevalence of SARS-CoV-2 among

### Table 5

Inflammatory markers in COVID-19 patients with abnormal liver function test.

| Parameter | No of cases (n) | Abnormal LFT cases (n) | Mean ± SE | Percentage | Normal LFT cases (n) | Mean ± SE | Percentage | P-value |
|-----------|----------------|------------------------|-----------|------------|---------------------|-----------|------------|---------|
| IL-6      | 32             | 24                     | 23.1 ± 55.7 (pg/ml) | 75% | 8 | 128.4 ± 6.2 pg/ml | 25% | <0.05 |
| CRP       | 50             | 41                     | 4.9 ± 1.0 mg/dl | 82% | 9 | 2.1 ± 0.5 mg/dl | 18% |     |

*P value indicates statistical significance of IL-6 and CRP in COVID-19 patients with abnormal and normal LFT.
meddle aged and men individual could be due to the fact that this group of people have more activities and contacts which is lead to that they may be more prone to be infected with SARS-CoV-2. Recent study mentioned that direct cytotoxicity from active viral replication of SARS-CoV-2 in the liver immune-mediated liver damage due to the severe inflammatory response/systemic inflammatory response syndrome (SIRS) in COVID-19, hypoxic changes induced by respiratory failure, vascular changes due to coagulopathy, endothelitis or cardiac congestion from heart failure, drug-induced liver injury and exacerbation of underlying liver disease [17]. The incidence of elevated liver transaminases (ALT and AST) in COVID-19 patients might related with virus replication in liver.

In summary, abnormal hepatic enzymes are common among COVID-19 patients. Liver damage may be caused by SARS-CoV-2. Moreover, this study is the first study that conducted in Iraq to elucidate the effect of drugs and duration of receiving drugs on hepatic enzyme activities. However, it could be better if we test the effect of drugs and duration of receiving drugs on hepatic enzyme activities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors’ contributions

RH supervised the project performed the tests, recorded the patient’s data, analyzed data and wrote the manuscript. MYM, BH, SHH, MGM, KM and HH performed tests, recorded the patient’s data, analyzed data and wrote the manuscript. All authors have read and approved the final version of the manuscript.

Table 7

| Parameter | Symptomatic | Percentage | Asymptomatic | Percentage | P-value |
|-----------|-------------|------------|--------------|------------|---------|
| CRP       | 26          | 52%        | 24           | 48%        | 0.559   |
| IL-6      | 16          | 50%        | 16           | 50%        | 0.548   |

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