Evaluation of hepatic enzymes activities in COVID-19 patients in Kurdistan region of Iraq.

Rundk Hwaiz[1,4], Badraldin Hamad[2], Mohammed Merza[3,4*], Shirin Hama Salih[1], Younis Smael[2], Mustafa Mohammed[5], Harmand Hama[6]

[1]Department of Clinical Analysis, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq

[2]Department of Pharmacology, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq

[3]Department of Clinical Biochemistry, College of Health Sciences, Hawler Medical University, Erbil, Kurdistan, Iraq

[4]Department of Medical Analysis, Tishk International University, Erbil, Kurdistan, Iraq

[5]Ministry of Health/Corona Centre, Kurdistan, Iraq

[6]Department of Biology, Tishk International University, Erbil, Kurdistan, Iraq

Abstract

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 (TBIL) 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. In conclusion, we found that most of the patients with SARS-CoV-2 have abnormal hepatic enzyme activities and that is related to virus replication in the liver.

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 10453 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 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 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.

Materials and Methods

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 WHO. The standard symptoms of COVID-19 patients include, fever, a radiograph of chest showing consolidation of the thorax 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 as shown in Table 1.

Table 1. *The distribution of COVID-19 patients according to age.*

Age (year)	Number of cases (n)	Ratio %
Under 20	15	20.2
21-30	16	21.6
31-40	17	22.9
41-50	15	20.2
51-60	10	13.5
Above 60	1	1.67

Data collection

The data collected from the patients with SARS-CoV-2 in the first day of admission and then analyzed for hepatic functions.

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 lab (ORF1ab) and the Nucleocapsid protein (N) were amplified and examined. The corresponding sequences for ORF1ab were 50-CCCTGTGGGTTTTACACTTAA-30 (F) ,5 0- AC G AT T GT GC AT C AGCT GA-3 0 (R) , a n d 5 0 - C Y 3 - C C G T C T G C G G T A T G T G G AAAGGTTATGG-BHQ1-30 (probe), and those for N were 50-GGGGAACTTCTCCTGCTAGAAT-30 (F), 50-CAGACATTTTGCTCTCA AGCTG-30 (R), and 50-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-30

(probe). Samples ran in triplicate with positive and negative control. The reconfirmed samples were identified as positive for SARS-CoV-2 by the central laboratory in Erbil city.

Liver function tests

Liver function tests included Alanine Amino Transferase (ALT), Aspartate Amino Transferase (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.

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 less than 0.05 was considered to indicate statistical significanceand 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.

Results

The study compromised 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.

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. Our results show that most of the patients have abnormal liver function test at admission date as shown in Table 2. Majority of patients with SARS-CoV-2 are male with no previous liver diseases, as shown in Table 3. About 74.3% of patients with abnormal liver function tests were male and about 54.2% were female, as shown in Table 4.

Table 2. *The rate of abnormal hepatic enzymes outcomes.*

	ALT	AST	ALP	TBili
Total cases (n)	72	70	59	74
Abnormal cases (n)	25	28	12	39
Ratio (%)	34.7	40.0	20.3	52.7

Table 3. *The incidence of COVID-19 in male vs. female.*

	Male	Female
Number of cases (n)	39	35
Ratio %	52.7	47.3

Table 4. The incidence of abnormal liver function test in male and female.

	Male	**Female**
Number of cases	29	19
Ratio %	74.3	54.2

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 65.8% of patients were symptomatic Table 5. Of 70 patients, 46 had symptoms including, fever, cough, sore throat, loss of taste and smell. Regarding body temperature, 46 patients had abnormally high body temperature with average of 38.5 °C.

Table 5. The percentage of symptomatic vs. asymptomatic patients.

	Symptomatic	**Asymptomatic**
Number of cases	46	24
Ratio %	65.8	34.2

CRP, IL-6 and IL-1 are inflammatory biomarkers that indicate infection with pathogen; our results showed that IL-6 and CRP are elevated in patients with COVID-19, 189 pg/ml and 1.5 mg/dl respectively, as shown in Table 6.

Table 6. Measurement of inflammatory markers in COVID-19 patients.

Parameters	Number of cases (n)	Abnormal cases (n)	Mean ± SE	Ratio	P-value
IL-6	32	24	231.1 ± 35.7	75	≤ 0.05
CPR	50	27	4.9 ± 1.0	54	≤ 0.05

Discussion

Our results showed that more than 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. 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, 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. 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 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 31-40 years, 22.9%. Our result showed the higher incidence of COVID-19 in men than in women (1.5 fold), and this could attribute to the hormonal difference. Testosterone which is known to suppress immune system while oestrogens is known to promote immune system and this could be the reason that the women have stronger immune response to fight bacteria and viruses [17]. 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 [18,19]. Moreover, a study showed that the mortality rate among men infected with SARS-CoV-2 is higher than in women [17]. The prevalence of SARS-CoV-2 among 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.

Conclusion

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 SARS-CoV-2 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.

Acknowledgement

Authors' Contribution

RH supervised the project performed the tests, recorded the patient's data, analyzed data and wrote the manuscript. BH, MYM, MGM 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.

Competing interests

We do not have any conflicts of interest associated with this publication.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020; 382: 727-733

2. Khot WY, Nadkar MY. The 2019 Novel Coronavirus Outbreak - A Global Threat. J Assoc Physicians India 2020; 68: 67-71.

3. Ministry of Health KRoI: Kurdistan Region Dashboard Coronavirus (COVID-19). 2020.

4. Zhang Y, Geng X, Tan Y, Li Q, Xu C, Xu J. New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. Biomed Pharmacother 2020; 12: 110195.

5. Pirola CJ, Sookoian S. COVID-19 and ACE2 in the liver and gastrointestinal tract: Putative biological explanations of sexual dimorphism. Gastroenterology.

6. Youssef M, Hussein M, Attia AS, Elshazli R, Omar M, Zora G. COVID-19 and Liver Dysfunction: a systematic review and meta-analysis of retrospective studies. J Med Viro. 2020

7. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol 2020; 5: 428-430.

8. Fan Z, Chen L, Li J, Cheng X, Yang J, Tian C. Clinical Features of COVID-19-Related Liver Functional Abnormality. Clin Gastroenterol Hepatol 2020; 18: 1561-1566.

9. Piano S, Dalbeni A, Vettore E, Benfaremo D, Mattioli M, Gambino CG. Abnormal liver function tests predict transfer to intensive care unit and death in COVID-19. Liver Int 2020.

10. Olry A, Meunier L, Delire B, Larrey D, Horsmans Y, Le Louet H. Drug-Induced Liver Injury and COVID-19 Infection: The Rules Remain the Same. Drug Saf 2020 43: 615-617.

11. Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y. COVID-19: Abnormal liver function tests. J Hepatol 2020.

12. Chakraborty C, Sharma AR, Bhattacharya M, Sharma G, Lee SS, Agoramoorthy G. COVID-19: Consider IL-6 receptor antagonist for the therapy of cytokine storm syndrome in SARS-CoV-2 infected patients. J Med Virol 2020.

13. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, Hlh Across Speciality Collaboration UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020; 395: 1033-1034.

14. Hagi T, Nakamura T, Iino T, Matsubara T, Asanuma K, Matsumine A. The diagnostic and prognostic value of interleukin-6 in patients with soft tissue sarcomas. Sci Rep 2017; 7: 9640.

15. Song J, Park DW, Moon S, Cho HJ, Park JH, Seok H, Choi WS. Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions. BMC Infect Dis 2019; 19: 968.

16. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill 2020; 25: 2000180.

17. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. Front Public Health. 2020; 8: 152.

18. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016; 16: 626-638.

19. Klein SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg 2015; 109: 9-15.

*Correspondence to:

Mohammed Merza

Department of Clinical Analysis

College of Pharmacy

Hawler Medical University

Erbil

Kurdistan