ASSOCIATION OF LIVER FUNCTION DERANGEMENTS WITH DISEASE SEVERITY IN COVID-19 PATIENTS

Asma Hayat, Syed Raza Jaffar*, Numan Majeed, Shabana Abbas, Jawaria Asghar, Nadia Tayyab

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To determine the association of liver function derangement with disease severity in COVID-19 patients
Study Design: Cross sectional study.
Place and Duration of Study: Study was conducted in department of Pathology, Army Medical College and Pak Emirates Military Hospital, Rawalpindi, from Apr 2020 to May 2020.
Methodology: Data collection was done for the 96 patients having RT-PCR positive for COVID-19 admitted in the hospital over a period of one month. Patients with history of liver disease were excluded from the study. Patients were categorized as mild, moderate or severe according to the symptoms as well as the location of the patient in different wards. Patients in regular wards were considered as mild, while those in High dependency unit (HDU) were considered to have moderate disease. Whereas patient admitted to intensive care unit (ICU/ITC) were considered as cases with severe disease.
Results: A total of 96 patients were included in the study, out of which 90 were males and 6 were females. Bilirubin levels were significantly deranged in all groups with *p*-value of <0.001 and same the case for alanine aminotransferase (ALT) (*p*-value<0.001), Alkaline Phosphatase (ALP) (*p*-value<0.006) and albumin (*p*-value <0.001). Post Hoc analysis of the significant parameters showed that levels of total bilirubin, ALT and ALP although increased with severity of disease but were found to be non-significantly associated with mild to moderate disease status.
Conclusion: Majority of the Patients with COVID-19 had deranged liver function test, irrespective of disease severity status.

Keywords: Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), COVID-19, Liver function Tests (LFTs), SARS-CoV-2, Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

INTRODUCTION

The Novel corona virus was identified in December 2019, in the Wuhan province of China and was named as 2019-nCoV by the World health organization[1]. Later in February 2020, after establishing the taxonomic classification of the virus it was named as SARS-CoV-2 and the disease caused by the virus was termed as COVID-19[2]. This outbreak caused severe respiratory disorder in the patients and was declared as a pandemic and a public health emergency by WHO on January 30th, 2020.

The toll of the infected people are increasing rapidly globally, 12.1 million cases worldwide have been reported half of which (7.1 million) have been recovered while the mortality is reported to be 4,550,157 (4.53%). Pakistan is ranking 24th globally in terms of new cases being reported while a total mortality of 1.46 million[3].

Seven human coronaviruses have been identified so far, of which severe acute respiratory syndrome (SARS)-CoV, middle east respiratory syndrome (MERS)-CoV and the newest, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), not only had similar epidemic pattern,, but also share 50% common genome sequencing[4].

This family of viruses is known to have both respiratory as well as gastrointestinal manifestations in not only human but also in some animal species as well[5]. Recent studies have

Correspondence: Dr Asma Hayat, Associate Professor of Pathology, Army Medical College Rawalpindi Pakistan
Email: asma.hayat72@gmail.com
Received: 11 Jun 2020; revised received: 26 Jul 2020; accepted: 27 Jul 2020

shown that almost half of the patients show varying level of liver disease Although some studies have shown that biopsy from liver of COVID-19 patientsare indicative of hepatic injury but it is not yet completely understood that it is either due to viral activity itself or is drug induced[6].

Other possible explanation behind liver damage as depicted by our study could be high level of positive end expiratory pressure which could contribute towardshepatic congestion[7]. Furthermore, the available evidence relating liver damage and COVID-19 infection is also suggestive of finding similar to our results with some showing up to 14-53% derangements in liver enzymes[8].

Many drugs, especially antiviral drugs, involved in management of COVID-19 patients may cause hepatic damage; preliminary assessment of liver status of the patient may warrant the clinicians regarding use of these drugs[9]. We planned this study to evaluatethe extent of liver damage due to COVID-19 infection in various levels of severity of disease.

METHODOLOGY

A cross-sectional study was planned in known COVID-19 patients. It was conducted in department of Pathology, Army Medical College and Pak Emirates Military Hospital, Rawalpindi, from 2nd April 2020 to 28th May 2020.

Ethical approval was sought from the institute (ERC/ID/31). Patients with known COVID-19 RT-PCR test considered as target population. The study included only admitted cases with COVID-19 for initial inclusion. Patients with known liver disease were excluded from the study. All patients gave informed consent for inclusion into the study. Consecutive sampling was done during the period of study. We included a total of 96 known admitted COVID-19 patients for further work up. In order to maintain patient confidentiality, the identity of patient was not disclosed to anyone by allocating a specific identification number instead of patient's names or data. Patients were followed for at least one month to see their final outcome.

Patients were categorized based upon their admission status as following:

Mild COVID-19 disease (Patients in regular wards were considered as mild), moderate COVID-19 disease (patient admitted in High Dependency Unit (HDU) and Severe COVID-19 disease- Patients (admitted to ICU/ITC)

Other outcome measures after a period of one month from the date of admission were discharged, expired and still under treatment. Three ml of venous blood specimens were obtained from patients in Vacctue TMGel tubes by trained staff nurse under aseptic measures, were sealed and sent to lab as per procedure in vogue. The samples were analyzed for liver enzymes including ALT, ALP and long with total bilirubin and albumin levels, using Roche Cobas 6000 based on spectrophotometric technique. Serum ferritin levels were assayed on Roche Cobas e411 by electrochemiluminescence. We used SPSS version 23 for calculation of descriptive and inferential statistics. Mean and SD were calculated for numeric data while frequencies and percentages were calculated for qualitative data. ANOVA was applied to test the association of parameters under observation with disease severity followed by Post HOC Tukey's test to assess differences between the groups for candidate laboratory parameters. We also used Pearson's Correlation to assess the change in levels according to severity of the disease using serum ferritin as a marker of severity of disease.

RESULTS

A total of 96 patients were included in the study, out of which 90 were males and 6 were females. Mean age of the study participants was reported to be 43.2 ± 13.1 years while group wise distribution of gender and age is as shown intable[1]. Patient's outcome of disease was categorized on the basis of patients discharged, expired and under treatment after a period of one month from the date of admission. The mortality reported in our patients was 3.1% (table-II). Table-III

demonstrate the differences for total bilirubin, ALT, ALP, albumin and ferritin between mild, moderate and severe disease. High ALT and ferritin values in moderate group may be due to wider SD. Post Hoc analysis of the significant parameters showed that levels of all the parameters were significantly associated with all the stages of disease except total bilirubin and ALP, inferring that the change in the levels of both albumin and ALT is significantly associated with disease progression, while in Albumin; it was significant only in mild cases (table-IV) Pearson's correlation suggested that a highly significant positive change was observed in all analytes, except albumin, which showed an inverse relationship with disease severity, observed with respect to serum ferritin levels (table-V) Few patients had co-morbidities like diabetes, hypertension, history of ischemic heart diseases, but none of the participants had any history of either hepatitis or any other hepatic disorder.

Table-I: Basic demographic data of different groups of disease.

	Mild	Moderate	Severe
Male	64 (66.7%)	19 (19.8%)	7 (7.3%)
Female	3 (3.1%)	-	3 (3.1%)
Age (mean ± SD)	38.79 ± 9.97	51.84 ± 13.33	67.17± 5.27

Table-II: Outcome of patients in different categories in our patient population.

	Mild	Moderate	Severe
Recovered and discharged	58 (60.54%)	19 (19.8%)	7 (7.3%)
Undertreatment	9 (8.4%)	-	-
Expired	-	-	3 (3.1%)

Table-III: Result of ANOVA along with Mean and SD for the analytes of interest.

	Mild	Moderate	Severe	*p*-value
Bilirubin (µmol/mL)	8.47 ± 4.34	9.95 ± 5.1	16 ± 6.39	<0.001
Alanine transferase (ALT) (U/L)	38.8 ± 24.48	88.7 ± 144.5	42 ± 26.02	0.006
Alkaline phosphatase (ALP) (U/L)	83.46 ± 22.4)	78.32 ± 21.67	202 ± 173.61	<0.005
Albumin (g/L)	45.46 ± 3.93	36.82 ± 9.89	29 ± 4.24	<0.001
Ferritin (ng/mL)	148.22 ± 82.96	492.48 ± 411.3	756.5 ± 435.36	<0.001

DISCUSSION

Our principal findings indicate COVID-19 infections affect liver functions and positive acute phase reactants in severity wise manner. We do know that COVID-19 is clinically characterized by symptoms resembling viral pneumonia in general which may later on evolves to respiratory failure. The reasoning behind the liver enzyme and protein derangements could be the presence of viral entry receptor i.e., Angiotensin converting enzyme 2 (ACE2) which havewide distribution in the human body. The SARS-CoV-2 receptors after affecting immune system could

Table-IV: Post HoC analysis of significant parameters.

Analyte	Condition	Comparative Condition	*p*-value
Bilirubin (µmol/mL)	Severe	Moderate	<0.001
		Mild	<0.001
	Moderate	Mild	0.607
ALP (U/L)	Severe	Moderate	0.007
		Mild	0.005
	Moderate	Mild	0.881
ALT (U/L)	Moderate	Moderate	0.541
		Mild	0.015
	Moderate	Mild	0.104
Albumin (g/L)	Severe	Moderate	0.160
		Mild	<0.001
	Moderate	Mild	0.004

Table-V: Pearson correlation in different parameters.

		Ferritin
Bilirubin (µmol/mL)	Pearson Correlation	0.367
	Sig. (2-tailed)	<0.001
ALT (U/L)	Pearson Correlation	0.250
	Sig. (2-tailed)	0.015
ALP (U/L)	Pearson Correlation	0.285
	Sig. (2-tailed)	0.005
Albumin (g/L)	Pearson Correlation	- 0.600
	Sig. (2-tailed)	<0.001

cause other systematic manifestations involving lungs, kidney and pancreas along with liver as we have shown in our data[10,11]. Some studies have described the vasculitis as the cause of underlying organ damage in severely ill COVID-19 patients. According to these studies, this damage is caused by the activation of inflammatory cytokines leading to activation of complement system as well as pro-inflammatory cytokines[12,13]. Another study suggested hypoxia-reperfusion dysfunction as a mechanism of hepatic injury, proposing that hypoxia induces cellular death and infiltration of inflammatory cells leading to further insult to the liver due to the response of inflammatory cascade activation[14]. Although exact mechanism of liver insult due to COVID-19 is still not completely understood, but some explanations regarding the mechanism of such an insult to the liver are being proposed. Cholangiocytes exhibit angiotensin converting enzyme-2 receptors and as this virus use this route to enter the cell, it causes direct cell injury byinduction of immune response[15,16].It may also cause microvascular steatosis along with mild portal and lobular activity.

In this study we found that the severity of the disease increases with increase in age of the patients. Our findings are quitesimilar to those reported by Garg *et al* who reported that the number of hospitalized cases due to COVID-19 increase with the increase in age[17].

A highly significant raise in levels of total bilirubin, alanine transferase and a significant decline in albumin levels, while ALP levels showing mild association are consistent with the findings of other studies[18]. There are some studies that suggest a significant change in ALP levels as well, but majority suggest otherwise. Another study suggested that 15% of the patients of corona patients had 3 times of the upper reference limit of total serum bilirubin, while no increase in ALP.

The study also suggested that the patients with deranged LFT's had significantly higher odds of progressing COVID infection. Study by Chen *et al* suggested that in a cohort of 799 patients, amongst which 113 were non-survivors COVID cases, ALT was markedly increased[19]. Zhang *et al* suggested that although there was no obvious sign of jaundice, but all the patients had significantly higher bilirubin levels[20].

Our study has few limitations which need to be acknowledged: Firstly, the COVID-19 is evolving disease with much to be explored in days or months to come. Secondly, the sample size was smalland the conduct was cross-sectional and only final outcome was studied in prospective manner over one month. It is therefore a possibility that a type-2 statistical error could be the reason behind the post hoc comparisons in our study. Another limitation is the gender differences which could be for real due to the effect of protective immunity among females, so a comment upon females could not be justified.

Provided a small-scale cross-sectional design the study has important clinical implications: This seems to be the pioneer local study from Pakistan which has attempted to highlight the associated liver damage among patients with COVID-19 infection. Underlying hepatic damage with severity of disease merits caution while therapeutic intervention and the same is recommended while treating patients with variety of medication. Being a preliminary cross-sectional analysis, the study has also been able to highlight the need of bigger trials in our locality to identify the need of conducting liver function tests especially with severe disease. More so the study opens up new dimension to the multifactorial nature of the COVID-19 infection which can help treating physicians to plan optimal treatment approach.

CONCLUSION

Liver functions derangement is common in patients with COVID-19 infection, irrespective of severity status. All COVID-19 patients should be evaluated for any liver injury to rationalize the use of antivirals and other hepatotoxic drugs which may help to prevent aggravation of

hepatic injury and improve the outcome. Regular monitoring of liver function should also be done to avoid any hepatic complications.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Rolling updates on coronavirus disease (COVID-19): World Health Organization; 2020. Available from: https://www.who.int/ emergencies/diseases/novel-coronavirus-2019/events-as-they-happen
2. Fan Z, Chen L, Li J, Cheng X, Yang J, Tian C, et al. Clinical Features of COVID-19-Related Liver Functional Abnormality. Clin Gastroenterol Hepatol 2020; 18(7): 1561-66.
3. COVID-19 coronavirus pandemic 2020 Available from: https://www.worldometers.info/coronavirus/
4. Niu P, Shen J, Zhu N, Lu R, Tan W. Two-tube multiplex real-time reverse transcription PCR to detect six human coronaviruses. Virol Sin 2016; 31(1): 85-88.
5. Dong Y, Liang X, Yu X. Prognostic value of the dynamic changes in extra vascular lung water index and angiopoietin-2 in severe multiple trauma patients with acute respiratory distress syndrome. Chinese Crit Care Med 2019; 31(5): 571-76.
6. Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, et al. COVID-19: abnormal liver function tests. J Hepatol 2013; S0168-8278(20): 30218.
7. Bangash MN, Patel J, Parekh D. COVID-19 and the liver: little cause for concern. Lancet Gastroenterol Hepatol 2020; 5(6): 529-30.
8. Zhang C, Shi L, Wang FSJ, Hepatology. Liver injury in COVID-19: Management and challenges. Lancet Gastroenterol Hepatol 2020; 5(5): 428-30.
9. Boeckmans J, Rodrigues RM, Demuyser T, Piérard D, Vanhaecke T. COVID-19 and drug-induced liver injury: a problem of plenty or a petty point? Arch Toxicol 2020; 94(4): 1367-69.
10. Gorbalenya AE, Baker SC, Baric RS, et al. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5, 536-44.
11. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020; 8(4): 420-22.
12. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020; 18(4): 844-47.
13. Lin L, Lu L, Cao W, Li T. Hypothesis for potential pathogenesis of SARS-CoV-2 infection–a review of immune changes in patients with viral pneumonia. Emerg Microbes Infect 2020; 9(1): 727-32.
14. Feng G, Zheng KI, Yan QQ, Rios RS, Targher G, Byrne CD, et al. COVID-19 and liver dysfunction: current insights and emergent therapeutic strategies. J Clin Transl Hepatol 2020; 8(1): 18.
15. Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A, et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. Bio Rxiv 2020; 2020: 931766.
16. Cai X, Ren M, Chen F, Li L, Lei H, Wang XJBT. Blood transfusion during the COVID-19 outbreak. Blood Transfus 2020; 18(2): 79-82.
17. Pititto BD, Ferreira SR. Diabetes and COVID-19: more than the sum of two morbidities. Rev Saude Pub 2020; 54(1): 54-60.
18. Ponti G, Maccaferri M, Ruini C, Tomasi A, Tomris O. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci 2020; 5(1): 1-1.
19. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. Bio Med J 2020; 368(1): 1-2.
20. Zhang Y, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q. Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single center in Wuhan city, China. Liver Int 2020.