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Original Research Article

Evaluation of liver function test abnormalities in patients having COVID-19 according to severity of disease

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ABSTRACT

Background: COVID-19 infection affects all major organs of body in addition to lungs. Damage done to liver varies from being hepatocellular, obstructive or mix pattern. The aim of the study was to evaluate abnormalities of LFTs, in COVID-19 patients according to disease severity in our population.
Methods: A cross sectional study, conducted at GTTH and Surgimed Hospital Lahore, on 100 patients who presented with COVID-19 in between October 2020 and September 2021. According to Symptoms, patients were marked as having mild, moderate or severe disease. Bilirubin, Albumin, ALP, ALT, AST, GGT, LDH and prothrombin time were checked at admission and subsequently. Data was analyzed using SPSS v23. Laboratory values were computed in each severity category and SD values, odds ratio, 95% confidence intervals were studied in logistic regression models. P value<0.05 were considered statistically significant.
Results: Among studied patients, 66% were male, 34% were female, mean age of 44.68±9.36 and range of 29-68 years. 29% were with mild disease, 40% with moderate disease, and 21% with severe disease level. 80% cases with rural background had moderate/severe disease levels while only 14.3% cases with severe levels were from urban areas. Only direct bilirubin showed a statistical significance with p value<0.05 in all severity groups. Other LFT's didn't show any significance between different severity groups.
Conclusions: Among Liver function markers only bilirubin was related to COVID-19 disease and proportional to severity of disease.

Keywords: SARS-CoV-2, COVID-19, Alanine aminotransferase, Liver function tests, Alkaline phosphatase, Reverse-transcriptase polymerase chain reaction

INTRODUCTION

Coronaviruses belong to a family of viruses, Coronaviridae, that may cause respiratory or intestinal diseases in humans as well as animal species. These viruses target the upper respiratory tract in majority of cases, and cause mild, moderate or severe illness, ranging from simple cold to pneumonia.[1] Up till now seven human coronaviruses types have been discovered. Out of these, three are known for epidemics i.e.; SARS-CoV

severe acute respiratory syndrome, MERS-CoV Middle east respiratory syndrome, and now the latest one, SARS-CoV-2 Severe acute respiratory syndrome coronavirus-2.[2]

In December 2019, pneumonia cases of unidentified cause, started to spread in Chinese city, Wuhan, which is now known as Novel SARS-CoV-2, with over 468 million confirmed cases and over 6 million deaths have globally.[3] COVID-19 , as it is abbreviated, has been declared a pandemic by WHO, and it has led to hundreds and thousands of hospitalizations and deaths worldwide. Although, most of the COVID-19 cases are labelled as mild disease, more extreme cases may lead to septic shock, respiratory failure or multiple organ failure.[4] As Covid-19 disease and this virus is evolving, it is bound to spread more, so further Biochemical/Clinical features need to be searched so as to improve knowledge of the virus, and to update diagnostic and therapeutic capabilities, so as to reduce its impact by controlling morbidity and mortality.

Evidence of damage of COVID-19 on body organs like heart, kidneys and brain is increasingly being reported with a significant number of COVID-19 patients showing varying levels of hepatic involvement.[5] In a recent study it was observed that SARS-CoV-2 virus binds to angiotensin-converting enzyme 2 (ACE-2) abundantly present on cholangiocytes in addition to Respiratory tract and cardiovascular system, leading to systemic inflammatory response ultimately causing Cholangiocyte malfunction and Hepatic injury.[6]

Almost seven trials have reported a rise of levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in a range of 14% to 53% among COVID-19 patients.[7] In a study done to evaluate liver-biopsy samples of COVID-19 patients who died of this disease, revealed mild level of lobular and portal activity and moderate level of microvesicular steatosis, confirming that this virus infection is responsible for the hepatic damage.[8] In a study from Pakistan, analysis of various liver functions showed that total bilirubin, ALT and ALP increased with increasing disease severity but were non-significantly associated with mild to moderate level of disease status.[9]

However, more data is needed to comprehensively analyze various liver markers/enzymes in our population and clinical pattern of hepatic dysfunction among patients suffering from COVID-19 infection.

The aim of the study was to evaluate the clinical course and liver function test abnormalities in patients suffering from COVID-19 admitted to our hospital and to further categorize it according to severity of disease. With better knowledge of demographics and pattern of liver damage, more targeted therapies and management plans can be designed, which may help prevent further liver injury in COVID-19 patients.

METHODS

Study design and participant criteria

This study had a cross-sectional design, and was done on patients admitted in Ghurki Trust Teaching Hospital and Surgimed Hospital, Lahore, Pakistan which are tertiary referral Hospitals. From October 2020 to September 2021. A total of 100 patients having COVID-19 based upon National COVID-19 guidelines were identified.[10] These were the patients who had at-least one abnormal liver function test result throughout hospital stay. Patients with prior history of any liver disease, hepatitis and alcohol intake were excluded from the study. This study received approval by the ethics committee of GTTH. Informed consent was obtained and from all patients/relatives. In order to maintain patient confidentiality, instead of patient's name a specific ID was initiated. Patients were followed for one month with serial tests and to see the final outcome.

Confirmation of COVID-19

Real-time reverse transcription PCR was done to confirm COVID-19 infection. Open reading frame 1ab (ORF1ab) was targeted by two pairs of primers while amplifying and examining the nucleocapsid protein (N). Every sample was run under Pathologist's supervision, in triplicate with positive and negative controls. This diagnostic criterion was designed as per recommendations given by National Health Services Regulations and Coordination Guidelines, Pakistan.

Liver test evaluation

Abnormal liver function test was defined as, elevation of any of the following liver enzymes: total bilirubin (TBIL)>1.2 mg/dl, ALT>40 U/l, AST>40 U/l, alkaline phosphatase (ALP)>135 U/l and gamma-glutamyltransferase (GGT)>49 U/l.

3 ml of venous blood samples were collected from patients in Vacctue TM Gel tubes by technicians who were trained for sample collection and ensuring aseptic measures. Samples were taken to laboratory as per hospital laboratory protocol. They were analyzed for all liver enzymes like ALT, AST, GGT, ALP, total bilirubin, direct and indirect bilirubin, and serum albumin levels, using spectrophotometric technique as is marketed by Roche Cobas 6000. Serum ferritin level and other tests were done on Roche Cobas by electrochemiluminescence.

Severity of COVID-19

According to the National guidelines for COVID-19 infection in Pakistan, all PCR positive patients were classified into having either mild, moderate or severe cases based on symptoms, clinical examination and results of chest radiography.[10]

Mild disease

Symptoms consistent with COVID-19 while maintaining Oxygen saturation ≥94%, without any compromise of hemodynamics, or need for oxygen. Chest X-ray findings either minimal or absent.

Moderate disease

Oxygen saturation between 90-94% (hypoxia) or chest X-ray findings showing infiltrates in <50% of the lung fields, while manifesting no or minimal complications due to disease.

Severe disease

Clinical signs and symptoms of pneumonia plus, any of the following: SpO2≤90% on room air; severe respiratory distress; respiratory rate >30/min; chest X-ray showing infiltrates consistent with COVID in >50% of lung fields.

Statistical analysis

SPSS (version 23) was used for data analysis and calculation of inferential and descriptive statistics. Mean values and standard deviation were calculated for numeric data while frequencies and percentages were calculated for qualitative data. Multivariable logistic regression was applied to study association between liver function test abnormalities and severity of disease. P value<0.05 was considered significant.

RESULTS

A total of 100 patients were included in this study, having COVID-19 infection and liver function abnormalities. Disease severity was categorized into various groups according to above mentioned protocol. Among different groups of COVID-19 patients, more than half, 66 (66%) were male while 34 (34%) were female, with a mean age of 44.68±9.36 ranging from 29-68 years. Patient's status in terms of severity of COVID-19 disease is shown in Table 1 and Figure 1. It shows that 29% of cases were with mild level disease, 40% had moderate level, and 21% presented with severe level of disease. The mortality rate in our study was 10% and 90% of patients did recover after proper treatment. Stratification of demographic characteristics of patients with different groups of disease further revealed that more than half of the females 58.8% had moderate symptoms of COVID-19 disease (respiratory rate >24/min, breathlessness, Sp02 90% to ≤93% on room air) but this gender disparity wasn't statistically significant.

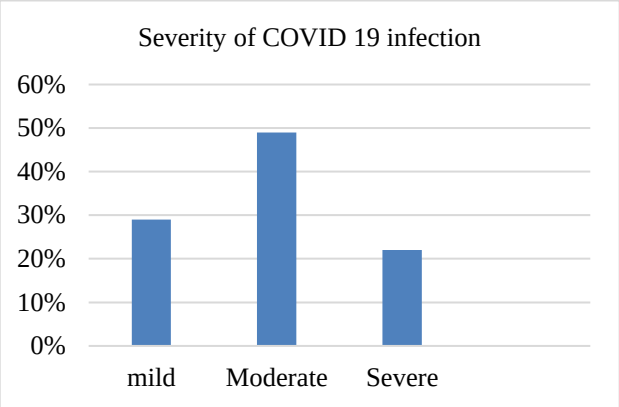

Figure 1: Frequency of mild, moderate and severe disease patients.

Similarly, most of the patients were either young or of middle age group but again it wasn't statistically significant. In our study, habitat/residential background had a significant association with the severity of disease as 80% cases with moderate and severe level of disease were coming from rural areas while only 14.3% cases with severe disease were from urban areas. Table 2 shows comparison of biochemical profile of patients between mild, moderate, and severe disease categories. Out of all checked LFT (albumin, prothrombin, bilirubin, direct and indirect bilirubin, ALT, AST, GGT, alkaline phosphatase, LDH) only the direct bilirubin showed to have statistically significance as p value<0.05 in average level between mild, moderate, and severe disease patients. Severe disease had a high level of DB as 1.53 as compared to moderate 1.17 and mild level of 1.35. The other LFT's didn't show any significant statistical difference between different groups of disease p value>0.05.

Table 1: Distribution of demographic profile of patients according to the severity of COVID-19 disease (n=100).

Characteristics	Categories	Mild	Moderate	Severe	P value
Gender	Male	21 (31.8)	29 (43.9)	16 (24.2)	0.370
	Female	8 (23.5)	20 (58.8)	6 (17.6)	
Age (years)	29-40	10 (25.6)	19 (48.7)	10 (25.8)	0.565
	41-52	10 (25)	22 (55.0)	8 (20.0)	
	>52	9 (42.9)	8 (38.1)	4 (19.0)	
Residence	Rural	6 (20.0)	12 (40.0)	12 (40.0)	0.016
	Urban	23 (32.9)	37 (52.9)	10 (14.3)	

Table 2: Comparison of biochemical profile of patients according to a different group of COVID-19 disease.

Parameters	Mild	Moderate	Severe	P value
Hemoglobin (Hb)	13.31±2.14	13.31±2.18	13.00±1.72	0.829
Total leucocyte count (TLC)	17.03±4.34	17.16±4.15	19.05±3.75	0.156
Platelet thrombocyte (PLT)	323.83±139.53	309.37±139.88	383.45±150.18	0.128
Neutrophil count (NEU)	79.90±6.92	79.71±6.96	75.91±9.62	0.113
Prothrombin time (PT)	11.62±2.90	12.86±3.00	12.59±3.80	0.230
Bilirubin (BIL)	2.17±0.64	1.93±0.60	2.20±0.81	0.173
Direct bilirubin (DB)	1.35±0.46	1.17±0.50	1.53±0.60	0.022
Indirect bilirubin (IDB)	0.79±.25	0.73±0.31	0.85±0.34	0.242
Alanine transferase (ALT)	97.28±24.83	91.53±24.33	97.82±39.04	0.573
Alkaline phosphatase (ALP)	429.06±121.44	375.57113.64	383.05±223.92	0.284
Aspartate aminotransferase (AST)	73.28±33.03	67.80±24.04	76.68±40.00	0.492
Gamma-glutamyl transferase (GGT)	394.86±150.45	356.49±154.12	430.68±223.16	0.224
Lactate dehydrogenase (LDH)	324.17±150.90	311.69±162.94	281.18±154.82	0.619
Ferritin (FER)	353.17±225.11	355.18±241.11	288.91±160.92	0.474
C-Reactive protein (CRP)	59.83±26.98	55.22±26.30	54.86±27.83	0.727
Interleukin-6 (IL-6)	2.86±1.22	2.95±1.43	3.17±1.04	0.691

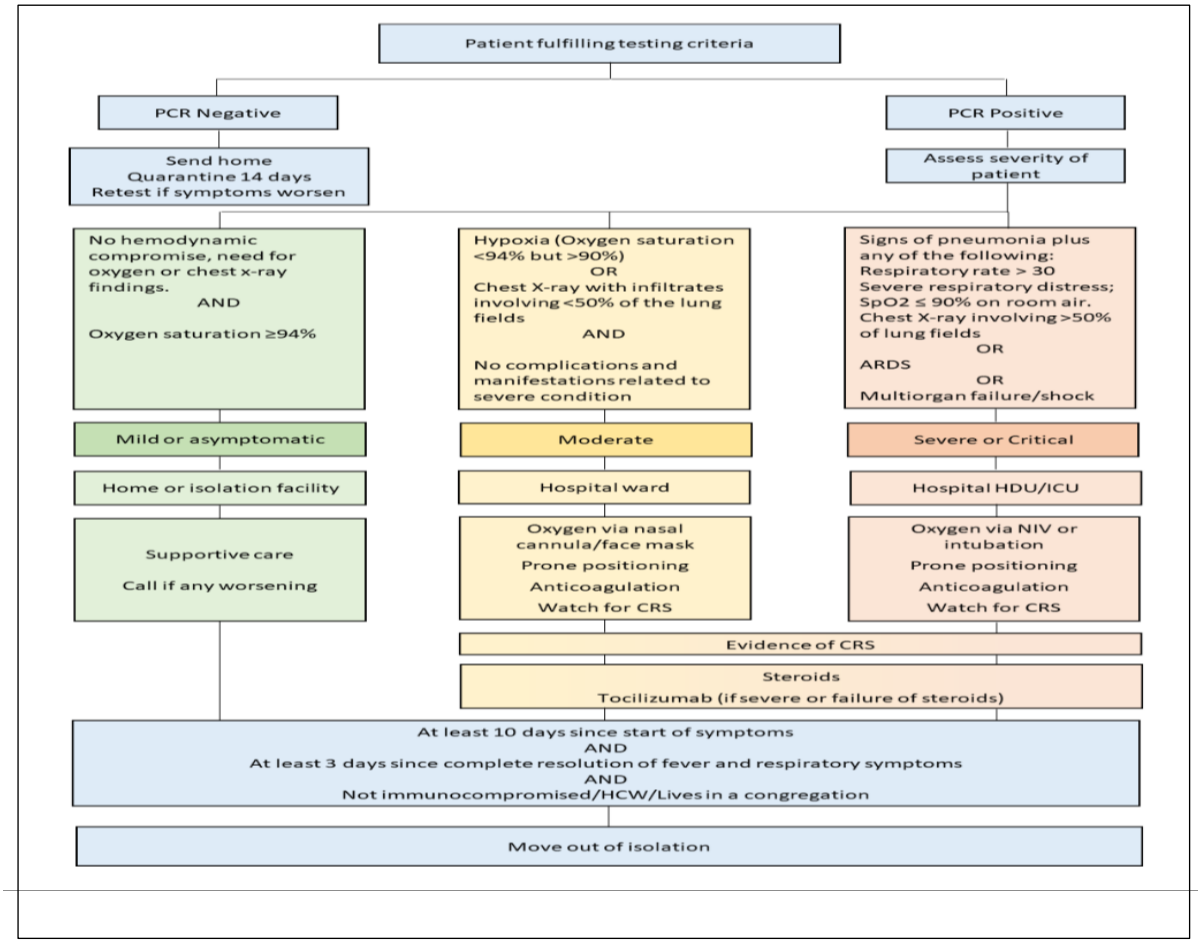

Figure 2: Summary algorithm of COVID management.

Source: National action plan for COVID-19 infection in Pakistan. In: Ministry of National Health Services. Available at: https://www.nih.org.pk/wp content/uploads/2020/03/COVID-19-NAP-V2-13-March-2020.

DISCUSSION

In present study, we studied the clinical, epidemiological and outcome features of 100 patients presenting with COVID-19 infection and altered liver enzyme levels from Tertiary care Hospital of Lahore, Pakistan.

Previous studies, on this subject have focused only on the features of severe cases of COVID-19 but we have analyzed all three categories of disease i.e., mild, moderate and severe. Overall, in our cohort of patients, elevation of liver enzymes was mild, while no liver failure was observed on follow-up. However, contrary to patients having normal liver test values at presentation, those having abnormal liver function test results had a higher risk of developing severe COVID-19 infection. Baseline liver function test abnormalities, which are used for predicting degree of severity of illness, especially Bilirubin level as has been observed in our study.

Because of nature of coronaviruses in terms of constant mutations, concerns have risen on adaptive mutations and enhanced virulence of SARS-CoV-2 virus during transmission between humans.[11] Constant search is needed to find out modes of entry of virus in humans and its impact on various systems of body. Major findings of our study were that COVID-19 infection affects liver function and positive acute phase reactants as per severity of disease.

Although COVID-19 is clinically identified by features which resemble simple viral pneumonia, which may or may not progress to respiratory distress, but the mechanism of liver enzymes derangements may be the viral entry receptor i.e., angiotensin converting enzyme 2 (ACE2) which are widely present in various organs of human body including cholangiocytes.

A recent study showed that COVID-19 virus binds to ACE2 receptors on cholangiocytes, thus gaining access to liver which then leads to hepatic damage, which may also explain the liver test dysfunction in patients of our study.[12] It causes injury to hepatic cells by induction of immune response 6, and also cause microvascular steatosis along with mild portal and lobular activity.[13] Some studies have shown that vasculitis is the main cause of multiple organ damage in severe category COVID-19 patients. According to these studies, hepatic dysfunction and damage by virus is due to the activation of inflammatory cytokines which then lead to activation of complement system and release of pro-inflammatory cytokines.[14,15,20]

In another study, it was suggested that hypoxia-reperfusion dysfunction is also a possible mechanism of liver injury, and it is suggested that cellular death induced by hypoxia and infiltration of inflammatory cells due to activation of inflammatory cascade further leads to hepatic damage.[16,21] Although exact mechanism that how virus attacks and damages liver is still not completely understood, but these are some explanations regarding various mechanisms of insult to the liver, and same applies in our cohort of patients.

Garg et al and Hayat et al reported that the number of hospitalized cases due to COVID-19 increase with the increasing age i.e.; more patients are in older age groups.[9,17] This finding was in absolute contrast to findings in our study where majority of patients were either young or in middle age groups and their relation to severity of disease was also non-significant. Majority of patients being young to middle age and from rural background may be a probable cause of this contrast to other data.

Zhang et al in his study from Wuhan China suggested that although there were no signs of jaundice in his study population, but all the patients had high bilirubin levels.[18] These findings are in close proximity to our findings that Bilirubin is the major marker which is elevated in all the disease categories of COVID-19 infection in comparison to ALT, AST, AP, LDH and GGT. Also, this is the earliest liver marker to be raised in Covid infection. In another Chinese study by Chen et al, which was done on 799 patients, it was suggested that ALT was markedly increased as compared to other parameters.[19] Here it is pertinent to mention that in this particular study 113 patients couldn't survive, while our study has much less mortality and ALT wasn't significantly raised.

There are few limitations of our study, which must be mentioned. Firstly COVID-19 is an emerging disease and due to advent of recent mutant variants of the virus, it must be studied according to the virus variant. Secondly, Females as a gender were less in our cohort of patients which creates a gender Bias in statistical analysis. Further, sample size was not large enough to give any recommendation.

Still then, this small-scale study with cross-sectional design has few important clinical implications. This was one of few local studies from Pakistan which has shown concomitant hepatic damage among patients with COVID-19 infection. Underlying liver damage becomes of paramount importance when treatment is being offered with medications who have Hepatotoxic potential. Being a preliminary cross-sectional analysis, our study highlights the need of bigger trials, to identify the need of conducting liver function tests especially with severe disease, while planning optimal treatment approach.

CONCLUSION

In this study we reported, the epidemiological, clinical and diagnostic features of patients presenting with COVID-19 infection and deranged liver enzymes. Age and gender didn't have any particular implication while rural background had significance. Bilirubin, especially direct Bilirubin was found elevated early and in all three stages of disease and increased with severity of disease.

Attention should be paid to deranged liver enzymes in COVID 19 patients, especially where drugs with hepatotoxic potential are to be used. In depth studies are needed to find out SARS COV 2 infection mechanism and hepatic damage.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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