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Research Article

Assessment of Hepatic Enzyme Derangements in Patients with Covid-19

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Abstract: About half of the patients with Covid-19 have deranged hepatic enzymes at presentation. The goal of the study was to identify specific patterns of abnormalities in enzymes so that clinical treatment and therapeutic methods can be improved for affected individuals. This understanding is crucial for improving patient outcomes and creating individualized treatment schedules. A total of 182 RT-PCR-confirmed Covid-19 cases were enrolled and different biochemical variables were compared among patients with varying degrees of disease severity. Data with abnormal distribution were described as median (minimum-maximum) and analyzed with the Mann-Whitney U test and the Kruskal-Wallis test. Multivariate binary regression analysis was applied to find the predictors associated with disease severity. The mean age of patients was 56.46 ± 15.60 years. Median AST levels in 182 patients were more than ALT at admission (52.45 vs. 46.35 U/L. Most of the subjects with the deranged hepatic enzyme at presentation had minimal elevations 1-2X upper limit of normal (ALT 74.8%, AST 77.0%, TBIL 98.3%). An increase of ≥ 5 times the upper limit of normal was observed in 7 (3.8%) and 5 (2.7%) patients for AST and ALT, respectively. Non-survivors were older, had higher median levels of AST 70 U/L vs. 47 U/L, LDH 855 vs 470 (for both p-value = 0.0001), and had a longer hospital stay compared to discharged groups. In multivariate analysis, advanced age, raised level of LDH and extended hospital stay showed a significant association with mortality. Liver dysfunction is commonly observed in hospitalized subjects and may be linked to severe disease.

Keywords: Alanine Aminotransferase, Covid-19, Total Bilirubin, Liver Damage, Aspartate Aminotransferase.

1. INTRODUCTION

Since its eruption from Wuhan, China, Coronavirus disease (Covid-19) is linked with considerable morbidity and mortality [1]. Covid-19 exhibits a wide range of clinical presentations extending from lack of overt signs or only mild symptoms in 81% to critical disease in 5% of the patients [2]. Although primarily manifested as pulmonary infection, liver impairment is a highly reported complication of Covid-19. Around 14–76% of Covid-19 patients had raised concentrations of hepatic enzymes, i.e., alanine aminotransferase (ALT) or aspartate aminotransferase (AST) [3] Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative pathogen in Covid-19. It shares about 79.6% sequence with SARS-CoV-1targets the Angiotensin-Converting Enzyme 2 (ACE2) receptor to enter the body of the host. ACE2 is expressed in various cells, including hepatocytes, cholangiocytes in the biliary epithelium, and alveolar cells in the lungs. In the lungs, the virus binds to ACE2 on alveolar cells, leading to inflammation and respiratory complications, which can result in conditions like pneumonia and acute respiratory distress syndrome (ARDS) [4]. In recent times, 60% of SARS patients had extensive liver damage [5]. SARS-CoV-2 may likewise have hepatotoxic effects because it belongs to the same family and can attach directly to ACE-2 receptors found on hepatocytes and cholangiocytes. Due to the lack of prior immunity and the incomprehensible nature of the disease, clinical assessment, and patient management protocols are

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perplexing yet evolving. Deranged liver enzymes are prominently identified as an extra-pulmonary clinical manifestation of Covid-19 infection reported by at least one-third of the Covid-19 infected subjects. This research was done to determine how individuals with Covid-19 were affected by hepatic dysfunction in terms of disease severity and prognosis.

2. METHODOLOGY

This cross-sectional observational study was carried out at the Department of Pathology, Benazir Bhutto Hospital, Rawalpindi, from June 8th, 2020 to December 30th, 2020. One hundred and eighty-two, RT-PCR-confirmed Indoor Covid-19 patients were enrolled by consecutive sampling. Patients were followed up to the final clinical outcome. Liver Function Tests (LFTs), including, Total Bilirubin (TB), Aspartate Aminotransferase (AST) Alanine Aminotransferase (ALT), and Lactate Dehydrogenase (LDH) were assayed on an Automatic Chemistry Analyzer Beckman Coulter Au - 480 at presentation, and peak values during hospital stay were noted. Subjects with a known history of hepatic dysfunction were not enrolled in the study. Liver dysfunction and reference ranges for increased aminotransferases were defined according to the guidelines of the European Association for the Study of the Liver [6]. Baseline data inclusive of gender, age, and duration of hospital stay were collected. Subjects were grouped into mild, moderate, and severe according to Clinical Management Guidelines for COVID-19 Infections v3 Document Code: 12-03 Date: July 2nd, 2020, Pakistan [7]. The patient's age, sex, hepatic enzyme, ALT, AST, TB, LDH, and ferritin were assessed at admission. All the variables were compared among patients with varying degrees of disease severity (Figure 1).

2.1. Statistical Analyses

Normally distributed parameters were presented as means ± SD and analyzed with an independent sample t-test and One-way ANOVA. Data with abnormal distribution were analyzed with the nonparametric tests and nominal data were analyzed with the Chi-square test. Multivariate regression was used to find the association between hepatic dysfunction, disease severity, and mortality. Mild or moderate/severe were dependent variables, and response variables included: ALT, AST, TB, LDH, ferritin, and hospital stay. The odds ratio (OR) with a 95% confidence interval (CI) was measured for each variable. Graph pad prism and SPSS version 25 were used to analyze the data. A p-value < 0.05 was considered significant.

3. RESULTS

The mean age of the participants was 56.46 ± 15.60 years (17-92). The majority of the patients, 119 (65.4%), were male, and 77(42.3%) were more than 60 years of age. The median hospital stay for severe patients was ten days, and a predominantly high percentage of severe patients (84.2%) succumbed to death compared with the subjects with a mild and moderate degree of 'disease' (Table1).

3.1. Distribution of Laboratory Parameters at Presentation

Median concentrations of AST, ALT, TB, LDH, and ferritin were significantly higher in the severely infected group compared to the mildly and

Table 1. Comparison of study variables according to disease severity.

Admission characteristics	Data (n = 182)	Mild (n = 47)	Moderate (n = 78)	Severe (n = 57)	p-value
Age (years)≠	59 (17-92)	46.53±16.82	55.49±12.64	65.96±12.58	0.0001
Sex (male)	119 (65.6%)	30 (25.2%)	49 (41.2%)	40 (33.6%)	0.501
ALT U/L*	47 (12-1209)	27.0 (12-70)	54 (14-157)	71 (12-1209)	0.0001
AST U/L*	53 (16.5-724.7)	33 (17-109)	54 (24-131)	88 (31-630)	0.0001
TB mg /dl*	0.45 (0.3-8.0)	0.5 (0.3-1.40)	0.6 (0.3-2.3)	0.6 (0.3-8.0)	0.025
LDH *	484 (170-3922)	429 (169-619)	498 (220-936)	856 (344-3922)	0.0001
Ferritin*	440 (15-1000)	126 (15-620)	399 (31-1000)	738 (46-1000)	0.001
Hospital stay*	5 (1-46)	1 (1-24)	6 (1-28)	11 (1-46)	0.0001
Mortality	66 (36.3%)	5 (10.6%)	13 (16.7%)	48 (84.2%)	0.0001

*Kruskal Wallis test ≠ One Way ANOVA

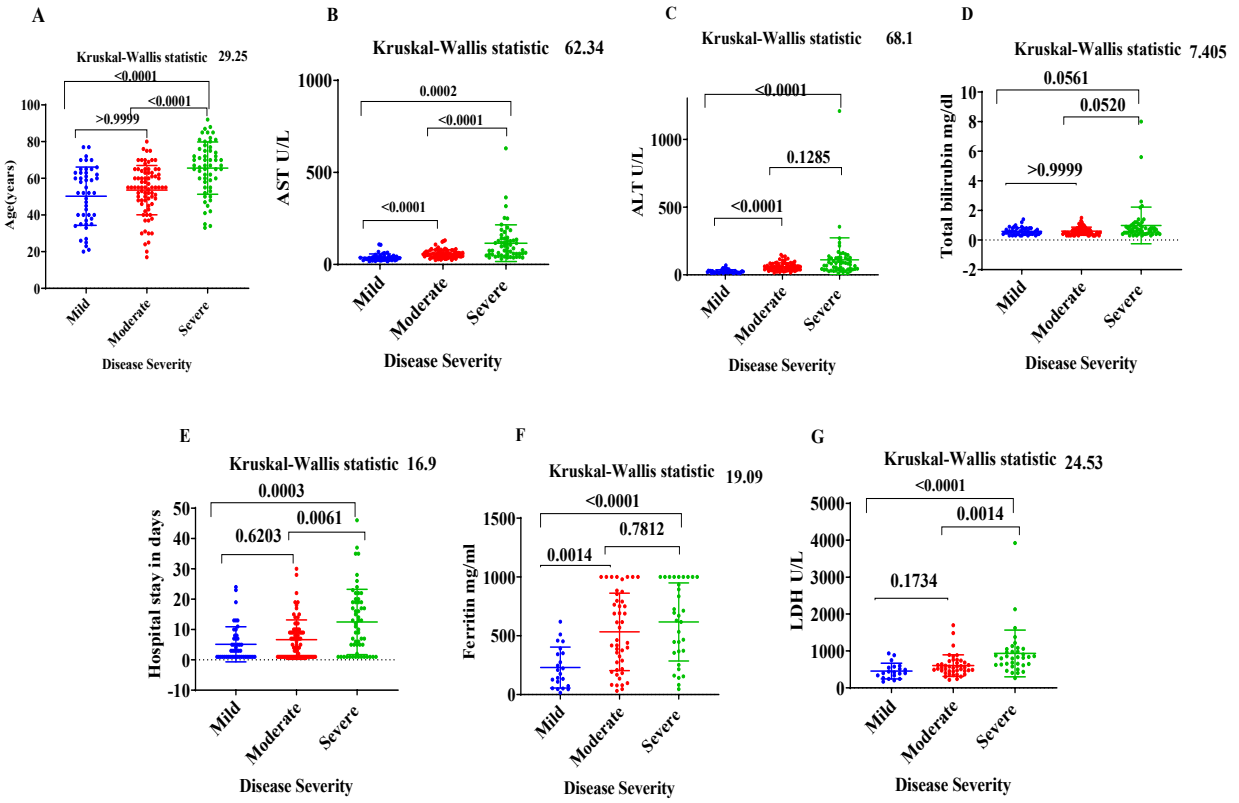

Fig. 1. Post hoc comparisons of A (age), B (Aspartate transaminase), C (Alanine transaminase), D (Total bilirubin), E (Hospital stay), F (Ferritin), G (Lactate dehydrogenase) with disease severity.

moderately affected group. At admission, median AST concentrations were higher than ALT (58 vs. 47 U/L). Covid-19 patients were classified into different groups based on the liver transaminase level. A higher number of patients have raised levels (≥ 40) of AST 126 (69.2%) compared to elevations in ALT 104 (57.1%). Elevations in total bilirubin were rare. Only 3 (1.5%) have ≥ 2 upper limits of normal (ULN) bilirubin. In the majority of patients, elevations in hepatic enzymes were up to 1-2 ULN, while an increase of more than five times ULN was observed in 7 (3.8%) and 5 (2.7%) patients for AST and ALT, respectively (Appendix 1). In comparison to subjects with normal aminotransferases, patients with elevated transaminase activity were significantly older, had elevated ferritin (p-value = 0.001), and LDH concentrations (p-value = 0.003), as well as a longer hospital stay (p-value = 0.032). No significant change in bilirubin levels was seen between the two groups (Appendix 2). Out of 57 critically ill patients, 43 (75.4%) succumbed to death, and 14 survived. Nonsurvivors were older (67 vs. 50 years; p = 0.0001) and had longer hospital stays (4 vs. 9 days; p = 0.001) compared to the survivors. Additionally, non-survivors had significantly elevated levels of AST median (min-max) (69.7 vs 46.9 U/l, p = 0.0001); LDH (870 vs 470 U/l, p = 0.0001) and bilirubin (0.5 vs 0.7, p = 0.038) (Appendix 3).

3.2. Correlation of AST with LDH and Ferritin

The relationship between AST and other markers of disease severity was evaluated. AST showed a highly significant correlation with ALT and LDH throughout the hospital stay (r = 0.731), (r = 0.503), (p-value = 0.0001), whereas the correlation between AST and ferritin was moderate (Figure 2).

3.3. Univariate and Multivariate Regression Analysis

In multivariate regression analysis, age, LDH, and longer hospital stay showed a significant association with disease severity (Table 2). AST is a nonspecific marker of liver damage. When the multivariate regression model was further adjusted for LDH, AST lost its significance indicating the multi-organ source of AST.

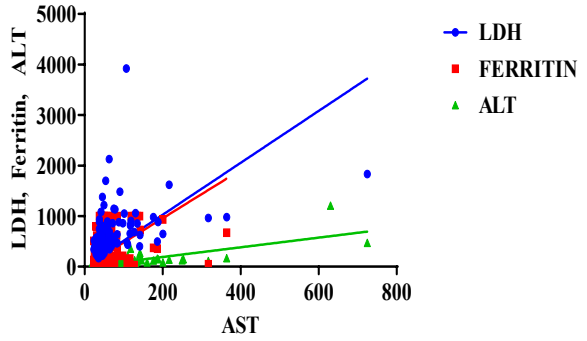

Fig. 2. Correlation of AST with ALT, LDH, and ferritin.

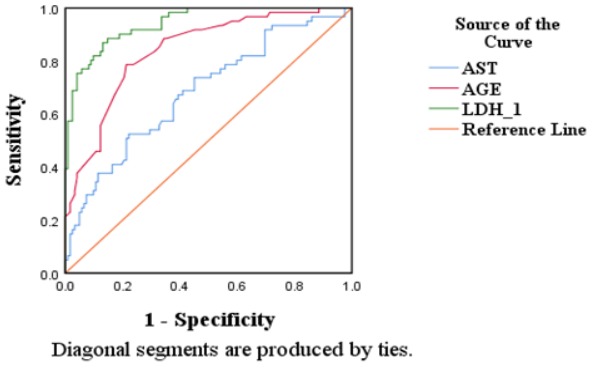

Fig. 3. ROC curve analysis for Age, AST, and LDH.

3.4. Receiver Operator Curve (ROC) Analysis

ROC curves were performed for the variables that showed a significant association with disease severity in multivariate regression analysis. LDH, with a cutoff value of 615 U/l, was the most significant predictor (specificity of 80.0% and sensitivity of 83.0%). Age with a cutoff value of 59.5 years had a high sensitivity (75.6%) and specificity (71.7%), and AST with a cutoff value of 59 U/l (sensitivity 60.0% and specificity 61.30%) remained significant as shown in Fig (3) and Table (3).

4. DISCUSSION

Recently, several international studies reported some degree of liver damage, mainly manifested as the raised concentration of aminotransferases in patients with Covid-19. Our results concur with recent literature, which claims that severely infected subjects are more likely to develop elevated transaminases than mild or moderately infected patients [3, 7-9]. In the current study, high levels > 40 U/L of AST and ALT have been observed in 69.4% and 57.4% of patients with Covid-19. This percentage is higher than observed in a study conducted on 1099 Chinese patients, where raised concentrations (> 40 U/L) of AST and ALT were found in 39.4% and 28.1% of the subject respectively [10]. Similarly, in a study including 554 Turkish participants, elevated transaminases at presentation were observed in 27.6% of the patients [11]. In an American study conducted on 5700 patients, AST and ALT levels

Table 2. Univariate and multivariate regression analysis.

Univariate regression analysis			**Model 1**		**Model 2**	
Variables	OR (95%CI)	p-value	OR (95% CI)	p-value	OR (95%CI)	p-value
Age(years)	1.112 (1.074, 1.152)	0.0001	1.113 (1.071, 1.157)	0 .0001	1.150 (1.065, 1.243)	0 .0001
Sex	1.69 (0.875, 3.263)	0.118				
ALT U/L	1.004 (0.998, 1.004)	0.156				
ASTU/L	1.012 (1.004, 1.019)	0.002	1.019 (1.004, 1.033)	0.012	0.993 (0.981, 1.006)	0.307
TB mg/dl	1.128 (0.803, 1.847)	0.354				
Hospital Stay	1.008 (1.005, 1.011)	0.0001	1.075 (1.023, 1.130)	0.004	1.084 (1.004, 1.172	0.040
LDH					2.009 (2.005, 2.014)	0.0001

Abbreviations: OR = odds ratio,
Model 1 variables entered in step 1 Age, sex, ALT, AST, TB, and hospital stay.
Model 2 Age, AST, hospital stay and LDH

Table 3. ROC curve analysis for Age, AST, and LDH.

Parameters	**AUC (95% CI)**	**Cuttoff-value**	**Sensitivity**	**Specificity**	**p-value**
LDH	0.880 (0.809-0.951)	615 IU/L	80.0%	83.0%	0.0001
Age	0.789 (0.709-0.888)	59.5 years	75.6%	71.7%	0.0001
AST	0.684 (0.579-0.789)	59 U/L	60.0%	61.3%	0.006

were raised in 58.4% and 39.0% of the subjects, respectively [12]. In another study including 1827 US patients, elevated concentrations of AST and ALT were present at admission in 66.9% and 41.6% of the patients, respectively [13]. In research conducted on deceased and recovered patients, higher concentrations of transaminases and total bilirubin were observed in nonsurvivors than in survivors [14].

The phylogenetic resemblance of SARS-CoV-2 to SAR-CoV and MERS supports the evidence of liver dysfunction in subjects with Covid-19. Previously, elevated levels of transaminases and liver impairment have been observed in critical SARS-CoV and MERS-infected patients [15-17]. The clinical data have shown that hepatic damage in most patients with Covid-19 is manifested as mild elevation (usually < 3 × ULN AST/ALT) accompanied by slightly higher bilirubin levels. Additionally, in the current study, like many others, a more frequent elevation in AST than ALT is observed [10, 12, 18]. Among the several proposed hypotheses for liver dysfunction in Covid-19, one potential mechanism is the direct cytopathic effect of the virus on biliary epithelium or hepatocytes through the upregulated expression of ACE2 receptors. Single-cell RNA-sequencing of ACE2 receptors in liver cells has shown the highest release (60%) in cholangiocytes, followed by the lowest in (3%) of ACE2 mRNA (3%) in hepatocytes and absent in other liver cell types [4, 19]. In contrast to greater ACE receptor expression in cholangiocytes, Covid-19 infection is characterized by a hepatocellular pattern of liver injury that is manifested as elevated concentrations of ALT and AST. Jaundice or some degree of cholestasis is not commonly observed even in intensive care units (ICU) patients. Additionally, viral RNA in the liver cells of dead patients was not confirmed by PCR [20]. The other possible pathophysiology implicated in hepatic injury in critically ill patients is the hyperactive immune-mediated response related to an excessive uncontrolled release of interleukins (cytokine storm), eventually culminating in multi-organ failure and acute respiratory distress syndrome.

In the current study, AST showed a significant correlation with LDH and ferritin. The upraised concentration of AST with the concomitant rise in LDH levels mirrors tissue damage linked with many disorders, including liver and lung ailments. Additionally, LDH is essentially involved in anaerobic respiration and is usually found raised under hypoxic conditions in the liver, i.e., hepatic congestion. The increased values of ferritin are indicative of the hazardous role of immune-mediated inflammation in liver injury [21]. Abnormal liver function at presentation in infection is suggestive of the fact that liver damage is not the result of medical intervention but rather a multifactorial phenomenon.

Moreover, endothelial cells of both small and large arteries have ACE expression. ACE2 (1-7) is produced by the vascular endothelium [22, 23]. In the vasculature, the ACE2/ angiotensin- (1-7)/ MAS axis has antithrombotic, antiproliferative, and vasodilatory actions. SARAS-CoV2 RNA has been found in the endothelia of many small vessels [24]. Critical Covid-19 patients had considerably higher plasma D-Dimer levels [25]. Disseminated intravascular coagulation (DIC) is a common occurrence in the early stages of the infection. Additionally, coagulation factors are produced by the liver cells, any damage to the liver will impact the coagulation process negatively.

5. CLINICAL SIGNIFICANCE

Monitoring of liver dysfunction in the early stage of Covid-19 infection could identify severe cases and contribute towards better management of the patients.

6. CONCLUSIONS

Hepatic dysfunction in Covid-19 disease at presentation is mainly manifested as mildly raised hepatic transaminases. Liver damage in subjects with Covid-19 appears to be a complex phenomenon including direct cytopathic of the virus, hyperactive immune-mediated response, and hypoxia generated by respiratory distress. Older age and raised lactate dehydrogenase at presentation can be used as predictors of severe disease in patients with Covid19.

7. ETHICAL APPROVAL STATEMENT

The Ethical Review Board, Rawalpindi Medical University, approved the project (83/IREF/ RMU/2020).

8. ACKNOWLEDGMENTS

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9. AUTHOR CONTRIBUTION

All authors contributed equally.

10. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Appendix 1. Distribution of laboratory parameters at admission.

Ferritin (ng/mL)	492.88 (15-1000)
Lactate dehydrogenase	688(169-3922)
Bilirubin	
Normal	173 (94.5%)
1–2 IN	7 (3.8%)
2–3 IN	1 (0.5%)
3-5 IN	1(0.5%)
≥ 5ULN	1(0.5%)
Alanine Aminotransferase	
Normal	78 (42.6%)
1–2 IN	58 (31.7%)
2–3 IN	26 (14.2%)
3-5 IN	15 (8.2%)
≥ 5 ULN	5 (2.7%)
Aspartate Aminotransferas	
Normal	56 (30.6%)
1–2 ULN	84 (45.9%)
2–3 ULN	20 (10.9%)
3-5 ULN	15 (8.2%)
≥ 5ULN	7 (3.8%)

Appendix 2. Comparison of subjects with a normal and raised level of hepatic enzyme.

Parameters	**AST-ALT ≤ 40 (n=44)**	**AST-ALT ≥ 40 (n=138)**	**p-value**
Age(years)	52.39±16.05	57.75±15.28	0.047
Sex (male)	26 (62%)	93 (66%)	0.591
ALT U/L	21 (12-39)	57 (12-1209)	0.0001
AST U/L	30 (17-40)	61(21-630)	0.0001
TB mg /dl	0.5 (0.3-1.40)	0.6(0.3-8.0)	0.124
LDH	419 (170-936)	624 (220-3922)	0.003
Ferritin	246 (45-791)	592 (15-100)	0.001
Hospital stay	3 (1-24)	7 (1-46)	0.032
Mortality	6 (14.2%)	52 (37.14%)	0.003

Appendix 3. Comparison of variables between discharged and deceased.

Variable	**Discharged N=124 (68.1%|)**	**Deceased N= 58 (31.9%)**	**p-value**
Age	50.33 ± 14.11	67.23±11.86	0.0001
Sex	77 (62.1%)	42 (72.4%)	0.186
ALT U/L*	45.5 (12-270)	49 (17-1209)	0.116
AST U/L *	46.9 (16-317)	69.7 (21-630)	0.0001
TB mg /dl*	0.5 (0.3-8)	0.7 (0.3-2.60)	0.075
LDH *	470 (170-1622)	870 (265-3922)	0.0001
Ferritin*	357 (15-100)	671 (82-1000)	0.001
Disease severity	14/57 (24.6%)	43/57 (75.4%)	0.0001
Hospital stay*	4 (1-35)	9 (1-46)	0.001

* Mann Whitney U test