ORIGINAL ARTICLE

Evaluation of Liver Function Tests (LFT) and C-reactive protein in COVID-19 (SARS Cov-2) positive patients diagnosed by Real-time PCR

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ABSTRACT

Background/Aim: Unpredicted social and economic consequences have resulted from the SARS-CoV-2 pandemic worldwide. Limited information is currently available addressing the COVID-19 infection impact on the RT-PCR Cycle threshold value trend, infection risk factors, impact on liver enzymes, etc.

Methods: From November 2020 to March 2021, a cross-sectional study was carried out in the Chemical Pathology also Molecular Biology divisions of the Pathology Department of Shalamar Hospital, Lahore.

Results: Males had a higher risk of contracting SARS-CoV-2 infection than females about 51%. 36.5 percent of total infected people fell within the 20–40 age range. Significant factors that affect the severity of COVID-19 include age and underlying comorbidities. The majority of the patients (78.3%) reported fever, 50.4% had a cough, and 50.1% had myalgias. Low Ct value of RT-PCR may be asignificant predictor of illness severity and risk of mortality, with p values of 0.001 and 0.003, correspondingly. Disease severity was substantially associated with CRP, AST, ALT, and bilirubin indirect. It was observed that the Deritis ratio and CRP were highly associated with the risk of mortality. These markers can therefore be used to evaluate a patient's status as it progresses toward a severe disease, liver damage from treatment, and death risk.

Conclusions: For doctors making patient management decisions, real-time PCR data and Ct values for SARS-CoV-2 may be useful. Age and comorbidities, among other risk variables, have been recognized as being associated with the likelihood of having a fatal illness. ALT, AST, Deritis ratio, and CRP are biochemical laboratory indicators that can be considered prognostic biomarkers for the development of severe disease and risk of mortality.

Keywords: SARS-CoV-2, COVID-19, Liver, Cough, Pandemic

INTRODUCTION

On March 11, 2020, the WHO proclaimed the SARS-CoV-2 pandemic. Following the flu in 1918, it was the fifth pandemic[1,2]. The COVID-19 coronavirus infection is currently a major public health concern[3,4]. Along with many other nations, Pakistan is trying to cope with a historic public health emergency[5]. The COVID-19 virus typically causes fever, myalgia, pneumonia, and a dry coughwhile it can also causeinjuryto the alveoli, which can result in progressive respiratory failure[6]. Of COVID-19 patients who had mild/asymptomatic disease about 81%, 14% progressed to severe disease and required hospitalization for pneumonia or respiratory distress, and only 5% of hospitalized patients required intensive care units[5]. According to current research, those who are older and who have compromised immune systems as a result of comorbid conditions are more susceptible to severe COVID-19 infections[7].

Real-time reverse transcription polymerase chain reaction (RT-PCR), which is the gold standard for diagnosing COVID-19, is used for the rapid detection of SARS-CoV and SARS-CoV-2[8,9]. Cycle threshold (Ct) values in RT-PCR represent the number of amplification cycles required for the target gene to reach a threshold level. This is an indirect way of counting the number of copies of virus RNA present in the sample. However, because a number of variables, such as the components in the sample matrix, or assay itself can alter the amplification efficiency, so, the Ct value is solely an approximate proxy for virus load[10].

It's interesting to note that besides the acute respiratory symptoms, patients with COVID-19 infection also exhibited varying degrees of liver injury or malfunction[11]. Liver participation in COVID-19 may be brought on by an uncontrolled immune response, a direct cytopathic action of the virus, medication-induced liver damage, or else sepsis. Type 2 alveolar cells have a large number of ACE2 receptors, and it is thought that the host's ACE2 receptors also play a role in how viruses enter cells. It's interesting to note that ACE2 receptors can also be found in the gastrointestinal tract (GIT), liver cholangiocytes, and vascular endothelium. Despite the presence of ACE2 receptors in cholangiocytes, the majority of patients experience increased transaminases[12].

Interleukin-6 (IL-6) causes the hepatocytes to produce the non-specific acute-phase protein (APP) C-Reactive Protein (CRP), which is a sensitive marker of infection, tissue damage,and inflammation. Although CRP expression is usually lower, it spontaneously and markedly rises in response to an acute inflammation. Elevated CRP may signify a viral or non-viral infection when present alone or in combination with other measures. Infection with COVID-19 is significantly affected by the inflammatory response, and also the severity of the infection is also increased by an inflammatory cytokine storm[13]. Humans are at the forefront of combating viruses like MERS, SARS, avian influenza H5N1, and H7N9 in this era of deadly viral respiratory infections[14].

The purpose of this study was to explain the impact of COVID-19 infection on LFT, CRP, and Deritis ratio, as well as to evaluate the relationship between Ct values and infection severity. These serve as markers for the identification of COVID-19 infection, as well as for giving information on treatment options and patient prognosis.

MATERIAL AND METHODS

A cross-sectional study of infected individuals at the Shalamar Teaching Hospital in Lahore was conducted. 345 individuals who tested positive for COVID-19 virus by RT-PCR between November 2020 and March 2021 were enrolled in this study with informed consent. All patients presenting in indoor and outdoor department of Shalamar Hospital were included in the study. Patients with any liver disorder e.g.; Hepatitis were included in the study. Anonymized patient data was used in this article with the legal representative's written informed consent. No personal information about any one person is included in the work.

C-Reactive Protein (CRP) and Liver function test (LFT): Serum was obtained by centrifuging blood samples at 5000 rpm for 10 minutes. On Cobas c 311, LFT and CRP were assessed using particle-enhanced immunoturbidimetric test and photometric methods, respectively. Abnormal LFT and CRP values were defined as those that were higher or lower than the specified ranges:bilirubin indirect (0.10 – 0.70 mg/dl), bilirubin direct (0.10 - 0.40 mg/dl), aspartate aminotransferase (AST) (5.00–40.00U/L), alanine aminotransferase (ALT) (5.00–40.00U/L), alkaline phosphatase (ALP) (40.00–135.00U/L), proteins total (6.30–

8.30g/dl), albumin (3.50–5.00g/dl), gamma G.T (GGT) (5.00–50.00U/L), deritis ratio calculated as AST/ALT (1.00), and CRP >5.00mg/dl.

Ethics: The study has been conducted under the authorization of Dental College; (SMDC-IRB/AL/60/2020).

Statistical analysis: Frequency and percentages were used to describe categorical data, whereas mean and SD were used for continuous variables. If the cell count was low, the Fisher exact test was used else the Chi-square test was used to compare categorical variables. The association between abnormal liver function test results and infection severity was investigated using one-way ANOVA. Statistical significance was defined as a p-value of 0.05. The statistical analysis software SPSS version 22.0 was used for all calculations.

RESULTS

The study comprised 345 COVID-19 patients who had their diagnoses confirmed by RT-PCR.The median Ct value RT-PCR was 15.5 (IQR10.4). 176(51%) of the patients were men, whereas 49% (N=169) were women. The median age of infected individuals were 47.5 years (SD 17.3).Patients were divided into four age groups to see which age group has high infection rate. 36.5% of the affected people were in the 20–40 age rangeand the least number of patients 1.40% were in age group <2. Other that this 32.2% patients were in age group 41-60, 27.90% in age group 61-80 and 2.0% in age group more than above 80.

The majority of the patients (78.3%), experienced symptoms of cough (50.4%) and myalgias (50.1%). Other symptoms include short breath (SOB) in 42%, fatigue 35.9%, loss of taste 35.6%, loss of smell 32.5% sore throat 24.3%, loss of appetite 23.8%, headache 19.1%, diarrhea 14.8%, nausea 10.4%, vomiting 9.6% nasal congestion 9.3%, reduced mobility 8.4%, reduced alertness 7.2% and delirium in 3.5% of patients.

According to WHO interim guidance from May 27, 2020, all participants were divided into mild, moderate, and severe groups based on symptoms. Majority of patients, 47.3%, fell into the mild category, followed by moderate patients 33.3%, asymptomatic individuals10.7%, and severe patients 8.7%. Out of 345 infected individuals, 117 had comorbidities; the majority of them had diabetes (15.4%), and hypertension (9.6%), whereas the other diseases are heart disease (5.8%), asthma (2.0%), chronic renal disease (1.6%), and malignancies (0.3%).

COVID-19 infection severity is associated with age, and existing comorbidities (tumors, diabetes, high blood pressure, heart and renal disease). Additionally, there is a significant association among the RT-PCR Ct value and the infection severity (p-value < 0.001) with IQR 8.43 in asymptomatic, 9.47 in mild group, 9.13 in moderate group and 10.02 in severe group.

However, the COVID-19 severity p-value of 0.175 does not take gender into account. 19(5.5%) of the 345 patients had died, while 326 (94.5%) were well again. 5.2% of patients who died, had existing comorbidities; 68.4% of female patients and 31.5% of male patients. Age, RT-PCR Ct, comorbidities, and mortality were all significantly correlated (p 0.001, 0.003, and <0.001). However, we did not find a gender disparity in infection mortality risk (p-value = 0.06) (Table 1).

Table 1: Gender, age and comorbidities association with COVID-19 infection severity

Severity	Asymptomatic (37)	Mild (163)	Moderate (115)	Severe (30)	P value
Gender					
Male	15	83	66	12	0.230
Female	22	80	49	18	
Age					
<20	2	3	0	0	
20-40	19	63	41	3	< 0.001
40-60	12	60	32	7	
60-80	3	35	38	20	
>80	1	2	4	0	
Comorbidities					
Diabetes	5	16	15	17	< 0.001
Hypertension	2	11	13	7	0.021
Cardiac Disease	1	3	4	12	< 0.001
Kidney Disease	0	0	1	4	< 0.001
Asthma	0	3	4	0	0.463
Tumor	0	0	0	1	0.012

Out of 345 COVID-19 infected individuals that were confirmed by RT-PCR, 31.1%, 45.6%, and 40.8% patients had deranged levels of AST, ALT, and Gamma G.T. Furthermore, in the majority of patients, low levels of albumin (35.9%) and total proteins (2.9%) were observed. In the small percentage of patients, other measures, such as bilirubin total, bilirubin indirect and bilirubin direct, were in abnormalrange (Figure1).

Deritis Ratio it is described as AST and ALT activity (AST/ALT). Exclusively 24.3% of patients had deritis ratio in normal range; 1.0, whereas 75.7% of individuals had a higher level. CRP increased in 78.9% of patients, and 21.1% had CRP levels that were within normal limits.

Asymptomatic, mild, moderate, and severe disease groups compared for bilirubin (total, indirect, direct), ALP, AST, ALT, proteins total, albumin, GGT, and CRP. The results of a one-way ANOVA demonstrate a significant relationship between illness severity and bilirubin indirect, AST, ALT, and CRP (Table 2).

Figure 1: LFT parameters in Covid-19 infected Patients. ALT & AST were increased in most of the patients whereas albumin was low in most patients. (Low: below the reference range, normal: in the reference range, High: above the reference range)
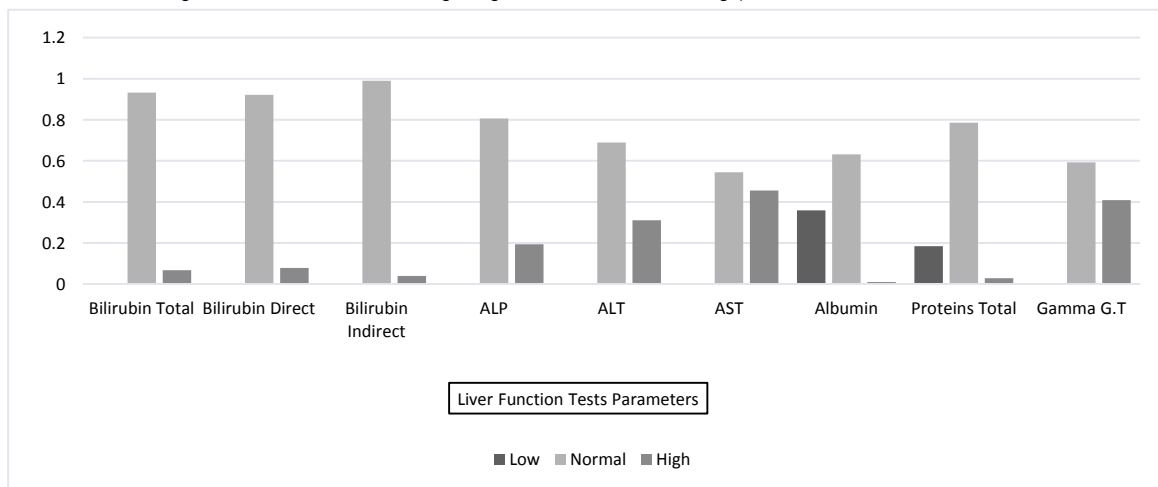
Table 3: ANOVA Results along with Mean ± SD of Liver Function Test and CRP

Liver Function Test	Asymptomatic	Mild	Moderate	Severe	P value
Bilirubin Total (mg/dl)	0.51 ± 0.23	0.68 ± 1.00	0.50 ± 0.35	1.24 ± 1.88	0.08
Bilirubin Indirect (mg/dl)	0.27 ± 0.15	0.24 ± 0.11	0.23 ± 0.10	0.46 ± 0.66	0.04
Bilirubin Direct (mg/dl)	0.24 ± 0.10	0.29 ± 0.44	0.26 ± 0.25	0.77±1.47	0.05
Alkaline Phosphatase (U/L) ALP	137.2 ± 82.9	94.6 ± 51.8	111.1 ± 54.21	120.8 ± 103.0	0.24
Alanine Amino Transferase (U/L) ALT	21.6 ± 9.83	26.3 ± 16.6	77.3 ±142.9	70.2 ± 32.0	0.04
Aspartate Amino Transferase (U/L) AST	21 ± 5.72	31.9 ± 13.1	106.0 ± 201.9	94.8 ± 69.1	0.03
Albumin g/dl	4.11 ± 0.40	4.73 ± 5.39	3.56 ± 0.60	3.56 ± 0.53	0.43
Proteins Total g/dl	7.01 ± 1.73	6.90 ± 0.63	6.88 ± 0.76	6.86 ± 0.77	0.96
Gamma G.T (U/L)	111.1 ± 164.7	63.1 ± 64.2	84.6 ± 73.7	54.1 ± 89.2	0.26
C- Reactive Protein (mg/dl) CRP	21.3 ± 0.0	91.7 ± 90.7	120.7 ± 107.6	145.7 ± 86.2	0.001

A significant relationship between the risk of mortality and the Deritis ratio and CRP; 70.3 (IQR 131.3) was also observed, with p values of 0.01 and 0.03, correspondingly.

DISCUSSION

This study provides an overview of the RT-PCR Ct; viral load of infection, medical history, infection severity, and additional blood chemistry such CRP, LFTs, and Deritis ratio in COVID-19 infection. The present investigation, males (51%) then females (49%) were infected with the virus; similar findings were seen in Jordan et al 2020's study. Males were likely to get SARS-CoV disease more than females, that is may be related to their more exposure to the virus, increased smoking behaviors, and consequent comorbidities[15,16,17]. In our study, the mean age of patients was 47.5 years, compared to 49.0-48.0 years in other studies, and 36.5% of the population was aged 20 to 40[17,18].

The viral load is high and the RT-PCR Ct value is low in patients with severe disease. RT-PCR, which is currently the only diagnostic test for COVID-19, is also recognized as the gold standard for the diagnosis of infection in COVID-19 patients. When detecting SARS-CoV-2 RNA, RT-PCR is a reliable, sensitive, and quick method[19]. The number of amplification cycles needed by the target gene to exceed the threshold level is denoted as the RT-PCR Ct value. Previous research suggested that the low Ct value of the SARS-CoV RT-PCR could be a significant contributor to the disease's severity and risk of mortality, which were both significant in our analysis with p values of 0.001 and 0.003, correspondingly[10].

The symptoms of COVID-19 infection can differ from asymptomatic to mild, moderate, or severe. High Ct values were strongly associated with risk of mortality and infection severity, according to our study. Since 47.20% of the cases in our study were mild infections, the majority of COVID-19 patients only experienced minor symptoms. Additionally, 10.70% of patients remained asymptomatic during the disease. According to data from China, 81% of Covid-19 infected individuals had mild or moderate disease, while 14% of them developed a severe viral infection[20]. Age or immunological condition may be the cause for this[21].

People over the age of 70 and those with existing comorbidities, such as diabetes, high blood pressure, cardiovascular, hepatic, pulmonary, renal disease, chronic lung disease, and malignancies, are included in the high-risk category with COVID-19 disease and associated factors with severe disease[15,22]. According to the CDC and Li et al., those who have hypertension are likely to develop severe COVID-19 infection (OR: 2.00; P =.003). Our investigation, also observed that patients with diabetes (15.40%), hypertension (9.60%), heart disease (5%), asthma (2%), renal disease (1.60%), and cancer (0.30%) are more at risk[17,22]. For doctors and public health services to combat the condition, identifying the risk factors that encourage the progression to severe COVID-19 is significantly vital.

In this study, the most prevalent clinical symptoms of infection were fever (76.3%), cough (50.4%), myalgias (50.1%), and short breath (42%). Headache (19.1%) and diarrhea (14.8%) were less frequent signs of illness. Our investigation supports the findings of Huang et al. and colleagues, who also stated that in their study, the prevalent symptoms were fever (98%), cough (76%), myalgias (44%), short breath (55%), and headache (8%), with diarrhea (3%), being the less prevalent[17,18].

Lower albumin (2.6 vs. 3.4g/dl, p 0.01) and higher levels of AST (28.1%) and ALT (39.4%), as well as deranged total bilirubin levels, were reported from previous studies[22-25].

However, we observed considerably elevated levels of the bilirubin indirect, transaminases; ALT 31.1% (p=0.04) and AST 45.60% (p=0.03) in our results. A low level of albumin was also observed in patients (35.90%), even though there was no relationship (p=0.431).

The cause of liver injury is still unknown, but research suggest that it may be brought on by cytotoxic reactions that occur during infection because the ACE2 receptor, which is thought to be the virus' proposed mechanism of entry, is present in cholangiocytes too, or by the toxicity of commonly used medicines in those infected individuals[22,26,27]. Deritis ratio; (ALT/AST) often falls below 1.0 and varies from 0.5 to 0.7[28]. In the majority of patients with COVID-19 infection in our results, it was on the higher side. We identified a significant relationship among elevated Deritis ratio and mortality of disease that was likewise noted in prior researches (p=0.018)[27].

Besides LFTs, the CRP levels, an indicator of inflammation and tissue damage (also known as an acute phase protein)[29,30], were also considerably higher in patients with a severe infection in our study (p=0.030).Zinellu et al. similarly noted higher CRP values in severe cases (p<0.05)[17,23,24,27]. These variables can be considered indicators of Death risk, cytokine storm development, and severe disease[22].

CONCLUSION

SARS-CoV-2 real-time PCR results that are qualitatively reported (as positiveor negative) are enough for diagnosis, although providing Ct values may be helpful for physicians when making patient care decisions. To support this, more research is still needed. Age, underlying comorbidities including diabetes and hypertension, and others have all been recognized as risk factors for the development of severe disease and mortality risk. Further research is required to see whether there is a connection between asthma and the severity of SARS-CoV-2. During infection, deranged LFTs are frequent and temporary. However, the laboratory measurements AST, ALT, Deritis ratio (AST/ALT), and CRP could be useful as markers that predict the development of severe disease and the risk of death.

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