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Hepatitis C and Typhoid Co-epidemics in COVID-19 Era in Lahore, Pakistan: A Cross-sectional Research to Rule out Probable Relation

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ORIGINAL ARTICLE

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ABSTRACT

Background: Infectious diseases including typhoid, tuberculosis, malaria, dengue, and hepatitis are widespread in Pakistan because of population growth, poverty and a lack of healthcare infrastructure. Their co-infection with COVID-19 can further worsen the disease.

Objectives: To determine the prevalence of typhoid and HCV co-infection with COVID-19 in patients from Lahore Pakistan.

Methods: The cross-sectional survey collected demographic data and blood specimens from 199 COVID-19 hospitalized patients at a private hospital in Lahore. D-dimer, ferritin, and CRP levels were determined qualitatively, as well as typhoid and HCV quick diagnostic tests. The Chi-square and Pearson correlation tests were used for statistical analysis.

Results: Gender distribution revealed that 55% of COVID-19 patients were male and 45% were female. CRP and D-dimer levels were increased in 132 (66.33%) patients, whereas serum ferritin levels were increased in 92 (46.23%) individuals. Typhoid was detected in 71 out of 199 patients, with 13 (18.3 percent) patients testing positive for IgM and 58 (81.7 %) patients testing positive for both IgM and IgG. D-dimer, CRP, and ferritin levels were elevated in COVID-19 patients with typhoid co-infection. Additionally, they were positively linked with age in COVID-19 patients with concurrent typhoid illness, with statistical significance ($p<0.05$). Similarly, 45 (22.6 %) of patients tested positive for HCV, although the results were not statistically significant ($p>0.05$).

Conclusion: Typhoid was frequent among COVID-19 patients. Patients' age, D-dimer, ferritin, and CRP levels increased concurrently with disease severity and were strongly linked with one another, despite low HCV seropositivity in COVID-19 patients.

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Introduction

Over the past two decades, viruses have emerged as significant disease-causing agents. The World Health Organization (WHO) has classified viral outbreaks as severe threats to community health due to their recurring emergence [1]. On December 31, 2019, a pneumonia outbreak of unknown cause was reported in Wuhan, China. On January 7, 2020, the China CDC identified a

new strain of the virus, later named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. WHO officially named the disease caused by this virus COVID-19 (Coronavirus Disease 2019) [3]. Within days, cases were reported in Japan, Thailand, and South Korea. Within three months, approximately 10,000 cases and 150 fatalities were recorded [4].

Two strains of similar viruses had previously been identified but lacked human-to-human transmission history. Severe acute respiratory syndrome-associated coronavirus was mainly transmitted by horseshoe bats in southern China, while Middle East Respiratory Syndrome Coronavirus (MERS-CoV), identified in Saudi Arabia in 2012, spread through camels [5]. Coronaviruses are a large family of viruses that cause diseases in humans and animals, ranging from respiratory infections to fever [6]. Shortly after its emergence, SARS-CoV-2 rapidly spread worldwide and was declared a pandemic [7]. The virus is highly contagious, spreading via respiratory droplets during sneezing and coughing, with an incubation period of 2 to 14 days [8]. Symptoms vary from asymptomatic to severe, including fever, cough, pneumonia, and acute respiratory distress syndrome. Currently, no specific medication or vaccine exists; only supportive treatments are available to alleviate disease severity [9]. Preventive measures such as personal protective equipment, frequent hand washing, social distancing, and self-isolation for 14 days are recommended [10][11]. Diagnosis relies on reverse transcription polymerase chain reaction (RT-PCR) tests using throat or nasopharyngeal swabs. Disease severity can be assessed using symptoms, risk factors, and chest CT scans showing pneumonia-like characteristics [12].

Studies indicate that co-infection can influence the progression of COVID-19. While pre-existing severe diseases may exacerbate COVID-19 outcomes, conditions such as cancer, cardiac diseases, hypertension, diabetes, liver diseases, and lung issues are linked to poor prognosis when co-infected with SARS-CoV-2 [13][14][15]. Older patients with medical conditions are particularly vulnerable to severe COVID-19 outcomes [16]. The clinical similarities between COVID-19 and other illnesses complicate laboratory diagnoses. In countries like Pakistan, with high prevalence of infectious diseases like typhoid, malaria, dengue, TB, and hepatitis, co-infections may overlap with COVID-19 due to overpopulation, poverty, and inadequate healthcare facilities [17]. Following a typhoid epidemic in Lahore, studies investigated the prevalence of typhoid co-infection in COVID-19 patients. Given Pakistan's high hepatitis C virus (HCV) prevalence, co-infections of COVID-19 and HCV were also explored.

Typhoid co-infection with SARS-CoV-2 presents challenges, as both diseases independently cause overlapping symptoms such as fever, respiratory, and gastrointestinal issues, alongside inflammatory responses in laboratory findings [18]. Low lymphocyte levels, common in COVID-19 patients, increase susceptibility to other infections, potentially leading to severe complications in up to 50% of cases [19]. Differential diagnosis is critical, especially in regions with high infectious disease prevalence [17].

Hepatic diseases also pose risks when co-infected with SARS-CoV-2. Patients with liver issues often exhibit elevated liver enzyme levels, and other coronaviruses have been shown to impact both the respiratory tract and liver [20][21]. The abundance of ACE2 receptors in liver tissues makes it highly susceptible to SARS-CoV-2 pathogenicity [23]. Studies confirm that COVID-19 can cause liver damage in patients with a history of viral hepatitis [24][25]. Co-infection with SARS-CoV-2 triggers a cytokine storm via ACE2 receptor binding in hepatocytes, elevating inflammatory markers like IL4 and IL6. This leads to liver damage and hypoxia in affected individuals [26].

Sometimes, patients infected with SARS-CoV-2 exhibit no symptoms of respiratory distress syndrome, suggesting the infection's multifaceted nature [27]. To predict the presence and severity of the disease, various biomarkers such as C-reactive protein (CRP), D-dimer, and ferritin are utilized [28]. These biochemical parameters are directly linked to disease presence and progression, with a positive correlation to its severity [29].

C-reactive protein (CRP), a plasma protein produced by the liver in response to IL-6, serves as a biochemical indicator of inflammation. As an acute-phase reactant, elevated CRP levels often signal disease severity. Retrospective studies have consistently demonstrated a strong association between CRP levels and COVID-19, making it a highly sensitive predictor of disease presence and progression. CRP is thus considered crucial for identifying and monitoring COVID-19 patients [30][31][32].

D-dimer levels are another significant marker associated with disease presence and severity. In COVID-19 patients, inflammatory responses triggered by pneumonia and severe hypoxia enhance coagulation activation, leading to fibrinolysis and potential multi-organ dysfunction [33]. Retrospective studies frequently report elevated D-dimer levels in COVID-19 patients. Moreover, a correlation between D-dimer and CRP levels has been observed in inflammatory conditions, linking both biomarkers to disease occurrence and severity [29]. Increased D-dimer levels are also associated with poor outcomes in COVID-19 cases.

Ferritin, the storage form of iron in the body, plays a role in immune dysregulation and can contribute to cytokine storms during inflammation [34]. Studies have shown that elevated ferritin levels, often accompanied by cytokine storms, can lead to fatal outcomes in COVID-19 [35]. Diabetic individuals, who typically have higher serum ferritin levels, may face severe complications if infected with SARS-CoV-2 [36]. Elevated ferritin levels in severe disease cases underscore their role as an inflammatory biomarker, reflecting the disease's condition and severity [37].

Methods

Setting and Duration: Demographic data and blood samples were collected from Umer Shoaib Surgical hospital from hospitalized patients, and this study was performed at the University of Management and Technology, Lahore. Our study period was of four months i.e. from May to August 2021.

Sample Size: In this cross-sectional study, 220 COVID-19-positive participants were selected, 21 with missing data or inadequate sample were excluded.

Inclusion Criteria: All COVID-19 active patients were hospitalized, and all COVID-19 confirmed patients with demographic characteristics, including age and gender.

Exclusion Criteria: Patients who tested negative for COVID19 and those whose inadequate samples were hemolyzed and improperly labeled were excluded from the study.

Sample Technique: About 4 ml clotted blood sample was taken in yellow gel tubes from COVID-19 active patients with written informed consent. Samples were centrifuged at 7000 rpm for 7 minutes, and the serum was separated. 21 samples were excluded, as mentioned above, not fulfilling our inclusion criteria. For the screening purpose, the immunochromatographic technique was used for both the tests and side-by-side positive and negative controls were run. Samples were thawed and mixed well before use. Then with the help of the dropper, 30 µl of serum sample was dispensed onto the cassette, and one drop of buffer was added immediately over it. Time was noted and results were recorded within the first 10 minutes. Typhoid IgM, IgG, IgM and IgG, and HCV analysis were done through the same rapid testing immunochromatographic diagnostic kits. Edan test kits (90.01.54.DDIM06-10) were used to analyze serum D-dimer qualitatively. Lab Kits (LKSGDTT03) were used for the qualitative and semi-quantitative analysis of CRP in human blood. The Calbiotech, Inc, (CBI) Ferritin ELISA Kits (FR248T) were used to analyze ferritin in human blood quantitatively.

CRP (Slide Agglutination test): First, allow the samples and reagents to achieve room temperature. Take the test slide and add 50-microliter serum samples and one drop of each control (positive and negative) into separate circles over the slide. Mix the latex reagent thoroughly and add one drop of this reagent next to the sample circle. Using a stirrer, mix both drops of sample and latex reagent over the entire surface of the slide. Now using the rotator, rotate the slide at 80-100r.p.m for 2 minutes. Note down the results within two minutes.

D-Dimer Rapid Testing: Allow the samples and reagents to reach room temperature. Open the test cassette and use within one hour of opening seal. Place the cassette

over a level surface. Hold the dropper in vertical position and take two drops of the sample of interest carefully. Apply the drops over the sample application pad on the test cassette. Now add 1 drop of buffer over the sample. Start the timer until the purple line appears in the test zone. Wait for 15 minutes and note the result before 20 minutes.

Ferritin ELISA: Allow the kit and reagent to reach room temperature. Gently shake all the reagents and solutions well before use. Take holder and place the required number of coated strips on it. Now take 25microliter of ferritin sample and controls into each well. Now add 100µl microliter of biotin reagent into each well. Shake the plate gently for 30 sec. Now cover the plate and keep it into the incubator for half an hour at room temperature. Remove the liquids from each well and add wash buffer to remove excess reagents and proteins. Add 100 microliter of Anti-ferritin enzyme reagent into each well. Again, cover the plate and incubate the plate at room temperature for half-hour. Remove liquids from each well and use wash buffer to remove excess reagents and proteins. Put 50 µl of stop solution to each well and incubate for 15 minutes at 25℃ temperature. Carefully shake the plate for 20 seconds. Finally, read the absorbance at 450nm on the ELISA reader within 10 minutes of adding stop solution to the plate. Table 1 shows normal levels of biochemical parameters included in the study.

Table 1: Normal ranges of biochemical parameters

	Normal Ranges
CRP	Up to 0.3 mg/dL
D-dimer	<500 ng/mL
Ferritin	13-400 ng/mL

Results

Among 199 cases of COVID-19 patients, 110 (55.3%) were male, and 89 (44.7%) were female, Figure 1. Patients were divided into five age groups: ≤24 years (16 cases, 8.04%), 25–36 years (46 cases, 23.1%), 37–48 years (54 cases, 27.14%), 49–60 years (48 cases, 24.12%), and >60 years (35 cases, 17.6%), Figure 2.

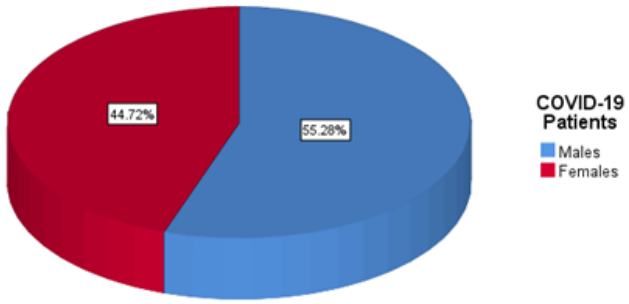

Figure 1: Gender distribution of COVID-19 patients in the population of Lahore

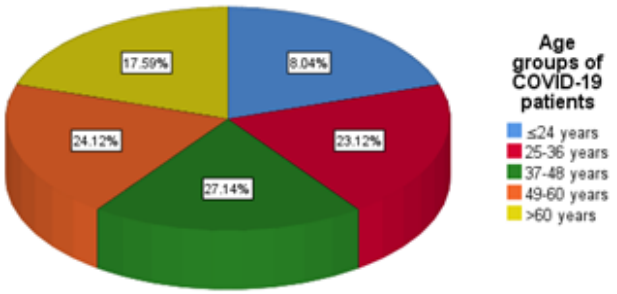

Figure 2: Age distribution of COVID-19 patients in the population of Lahore

The study analyzed inflammatory markers (D-dimer, CRP, and ferritin) and serological markers (typhoid IgM, typhoid IgM and IgG, HBV, and HCV) in COVID-19 positive males and females. Elevated D-dimer levels were observed in 67.3% of males and 65.2% of females, while elevated CRP levels were found in 69.1% of males and 62.9% of females. Elevated ferritin levels were present in 46.4% of males and 46.1% of females. For typhoid IgM, 6.4% of males and 6.7% of females tested positive, while 27.3% of males and 31.5% of females were positive for both typhoid IgM and IgG. HBV positivity was seen in 4.5% of males and 3.3% of females, while HCV positivity was observed in 26% of males and 22% of females. Statistical significance was determined using the Chi-Square test, with a significance level of <0.05, Table 2.

Table 2: Distribution of different biochemical parameters in COVID-19 patients in the population of Lahore

	COVID-19 Patients	Normal levels	Elevated levels	*p*- value
D-dimer	**Males**	36 (32.7%)	74 (67.3%)	0.755
	Females	31 (34.8%)	58 (65.2%)	
CRP	**Males**	34 (30.9%)	76 (69.1%)	0.36
	Females	33 (37.1%)	56 (62.9%)	
Ferritin	**Males**	59 (53.6%)	51 (46.4%)	0.967
	Females	48 (53.9%)	41 (46.1%)	
Typhoid IgM	**Males**	103 (93.6%)	7 (6.4%)	0.915
	Females	83 (93.3%)	6 (6.7%)	
Typhoid IgM & IgG	**Males**	80 (72.7%)	30 (27.3%)	0.518
	Females	61 (68.5%)	28 (31.5%)	
HBV	**Males**	105(95.4%)	5(4.5%)	0.302
	Females	85(96.6%)	3(3.3%)	
HCV	**Males**	81(74%)	29(26%)	0.526
	Females	69(78%)	20(22%)	

Table 3: Gender based comparison of elevated inflammatory markers in typhoid co-infection in COVID-19 patients of Lahore

	COVID-19 Patients	Typhoid Positive	Typhoid Negative	*p*- value
Elevated D-Dimer Levels	**Males**	21 (28.40%)	53 (71.6%)	0.018
	Females	28 (48.3%)	30 (51.7%)	
Elevated CRP Levels	**Males**	28 (36.80%)	48 (63.2%)	0.268
	Females	26 (46.40%)	30 (53.60%)	
Elevated Ferritin Levels	**Males**	18 (35.3%)	33 (64.7%)	0.124
	Females	21 (51.2%)	20 (48.8%)	

Plasma D-dimer levels in typhoid positive and negative males and females who were also COVID-19 positive were been observed. In typhoid positive male patients 21 (28.40%) had elevated plasma D-dimer levels while in typhoid negative male patients 53 (71.6%) had elevated plasma D- dimer levels. Also, in typhoid positive female patients 28 (48.3%) had elevated plasma D-dimer levels while in typhoid negative female patients 30 (51.7%) had elevated plasma D- dimer levels. Statistical analysis showed significant results (*p*-value= 0.018). Serum CRP levels in typhoid positive and negative males and females who were also COVID-19 positive were been observed. In typhoid positive male patients 28 (36.80%) had elevated serum CRP levels while in typhoid negative male patients 48 (63.2%) had elevated serum CRP levels. In addition, in typhoid positive female patients 26 (46.40%) had elevated serum CRP levels while in typhoid negative female patients 30 (53.60%) had elevated serum CRP levels. Statistical analysis showed non-significant results (*p*-value= 0.268). Serum ferritin levels in typhoid positive and negative males and females who were also COVID-19 positive were also been observed. In typhoid positive male patients 18 (35.3%) had elevated serum ferritin levels while in typhoid negative male patients 33 (64.7%) had elevated serum ferritin levels. Similarly, in typhoid positive female patients 21 (51.2%) had elevated serum ferritin levels while in typhoid negative female patients 20 (48.8%) had elevated serum ferritin levels. Statistical analysis showed non-significant results (*p*-value= 0.124). Level of significance was tested using CHI Square and level of significance was <0.05, Table 3.

Table 4: Different biochemical parameters in different age groups in COVID-19 patients of Lahore

	COVID-19 Patients	Normal levels	Elevated levels	*p*- value
D-Dimer	**≤ 24**	7 (43.8%)	9 (56.3%)	0.552
	25 - 36	11 (23.9%)	35 (76.1%)	
	37 - 48	20 (37%)	34 (63%)	
	49 - 60	17 (35.4%)	31 (64.6%)	
	> 60	12 (34.3%)	23 (65.7%)	
CRP	**≤ 24**	9 (56.3%)	7 (43.8%)	0.129
	25 - 36	14 (30.4%)	32 (69.6%)	
	37 - 48	20 (37%)	34 (63%)	
	49 - 60	17 (35.4%)	31 (64.6%)	
	> 60	7 (20%)	28 (80%)	
Ferritin	**≤ 24**	14 (87.5%)	2 (12.5%)	0.032
	25 - 36	20 (43.5%)	26 (56.5%)	
	37 - 48	32 (59.3%)	22 (40.7%)	
	49 - 60	24 (50%)	24 (50%)	
	> 60	17 (48.6%)	18 (51.4%)	
Typhoid IgM	**≤ 24**	15 (93.8%)	1 (6.3%)	0.035
	25 - 36	39 (84.8%)	7 (15.2%)	
	37 - 48	53 (98.1%)	1 (1.9%)	
	49 - 60	44 (91.7%)	4 (8.3%)	
	> 60	35 (100%)	0 (0%)	
Typhoid IgM & IgG	**≤ 24**	14 (87.5%)	2 (12.5%)	0.001
	25 - 36	40 (87%)	6 (13%)	
	37 - 48	40 (74.1%)	14 (25.9%)	
	49 - 60	30 (62.5%)	18 (37.5%)	
	> 60	17 (48.6%)	18 (51.4%)	
HBV	**≤ 24**	16(0%)	0(0)	0.441
	25 - 36	45(97.8%)	1(2.1%)	
	37 - 48	51(94.5%)	3(5.5%)	
	49 - 60	47(97.9%)	1(2.1%)	
	> 60	32(91.4%)	3(85.7%)	
HCV	**≤ 24**	13(81.2%)	3(18.8%)	0.909
	25 - 36	33(71.7%)	13(28.3%)	
	37 - 48	42(77.7%)	12(22.2%)	
	49 - 60	35(72.9%)	13(27%)	
	> 60	27(77.1%)	8(22.9%)	

The study examined inflammatory and serological markers across five age groups of COVID-19 patients. Elevated D-dimer levels were most prevalent in the 25–36 age group (76.1%) and least common in those ≤24 years (56.3%). Elevated CRP levels were highest in patients >60 years (80%) and lowest in those ≤24 years (43.8%). Ferritin levels were most frequently elevated in the 25–36 age group (56.5%) and least in those ≤24 years (12.5%). Typhoid IgM positivity was rare across all groups, highest in the 25–36 age group (15.2%), and absent in those >60 years. Typhoid IgM and IgG positivity was highest in patients >60 years (51.4%) and lowest in those ≤24 years (12.5%). HBV positivity was minimal, peaking at 5.5% in the 37–48 age group, while HCV positivity was highest in the 25–36 age group (28.3%) and lowest in those ≤24 years (18.8%).

Statistical significance for these findings was determined using the Chi-Square test with a significance level of <0.05, Table 4.

The analysis of co-infection with HCV and COVID-19 in patients with elevated inflammatory markers (D-dimer, CRP, and ferritin) revealed that males had a slightly higher proportion of co-infection compared to females. Among patients with elevated D-dimer, 25% of males and 21% of females had co-infection. For elevated CRP levels, 28% of males and 23% of females had co-infection. Similarly, with elevated ferritin, 28% of males and 26% of females showed co-infection. However, statistical analysis using the Chi-square test indicated that these differences were not significant ($p>0.05$). When analyzing D-dimer levels across different age groups in patients with only COVID-19 and those with HCV co-infection, it was observed that in the ≤24 age group, 2 out of 9 patients (22%) with elevated D-dimer levels had HCV co-infection, while 7 (78%) had only COVID-19. In the 25-36 age group, 6 out of 35 (17%) were co-infected, while 29 (83%) had only COVID-19. Among 37-48-year-olds, 7 out of 34 patients (21%) were co-infected, while 27 (79%) had only COVID-19. In the 49-60 age group, 9 out of 32 patients (28%) were co-infected, while 23 (72%) had only COVID-19. In patients aged >60, 7 out of 23 (30%) had co-infection, while 16 (70%) had only COVID-19. The Chi-square test showed non-significant results ($p>0.05$), Table 5.

The study compared inflammatory markers (D-dimer, CRP, and ferritin) in typhoid-positive and typhoid-negative COVID-19 patients across age groups. Typhoid-negative patients generally exhibited higher proportions of elevated D-dimer and CRP levels across all age groups, except in older groups (49–60 and >60 years), where the differences narrowed. Elevated ferritin levels showed a similar trend, with typhoid-negative patients typically presenting higher rates in younger and middle age groups, while proportions were equal in patients >60 years. Statistical analyses confirmed significant differences for D-dimer ($p=0.023$), CRP ($p=0.004$), and ferritin ($p=0.037$) between typhoid-positive and negative groups across the studied ages, Table 6.

Similarly, for CRP levels, in the ≤24 age group, 1 out of 7 patients (14%) with elevated CRP had co-infection, while 6 (86%) had only COVID-19. Among the 25-36 age group, 7 out of 32 patients (22%) were co-infected, while 25 (78%) had only COVID-19. For the 37-48 age group, 10 out of 34 (29%) were co-infected, while 24 (71%) had only COVID-19. In the 49-60 age group, 8 out of 31 (26%) had co-infection, while 23 (74%) had only COVID-19. In the >60 age group, 8 out of 28 (29%) were co-infected, while 20 (71%) had only COVID-19. Again, the Chi-square test results were non-significant ($p>0.05$).

For ferritin levels, none of the 2 patients in the ≤24 age group had HCV co-infection. In the 25-36 age group, 6 out of 25 patients (24%) were co-infected, while 19 (76%) had only COVID-19. Among 37-48-year-olds, 6 out of 22 patients (27%) were co-infected, while 16 (73%) had only COVID-19. In the 49-60 age group, 7 out of 25 (28%) were co-infected, while 18 (72%) had only COVID-19. For patients >60 years, 6 out of 18 (33%) had co-infection, while 12 (67%) had only COVID-19. The Chi-square test showed non-significant results ($p>0.05$). The level of significance was tested using the Chi-square test with $p<0.05$, Table 7.

Table 5: Gender based comparison of elevated inflammatory markers in COVID-19 patients with HCV co-infection of Lahore

	COVID-19 Patients	HCV Positive	HCV Negative	*p*- value
Elevated D-Dimer Levels	**Males**	19(25%)	56(75%)	0.529
	Females	12(21%)	56(79%)	
Elevated CRP Levels	**Males**	21(28%)	55(72%)	0.556
	Females	13(23%)	43(77%)	
Elevated Ferritin Levels	**Males**	14(28%)	36(72%)	0.845
	Females	11(26%)	31(74%)	

Table 6: Age based comparison of elevated inflammatory markers with typhoid co-infection in COVID-19 patients of Lahore

	COVID-19 Patients	Typhoid Positive	Typhoid Negative	*p*- value
Elevated D-dimer levels	**≤ 24**	2 (22.2%)	7 (77.8%)	0.023
	25 - 36	9 (25.7%)	26 (74.3%)	
	37 - 48	12 (35.3%)	22 (64.7%)	
	49 - 60	15 (48.4%)	16 (51.6%)	
	> 60	11 (47.8%)	12 (52.2%)	
Elevated CRP levels	**≤ 24**	2 (28.6%)	5 (71.4%)	0.004
	25 - 36	7 (21.9%)	25 (78.1%)	
	37 - 48	13 (38.2%)	21 (61.8%)	
	49 - 60	17 (54.8%)	14 (45.2%)	
	> 60	15 (53.6%)	13 (46.4%)	
Elevated Ferritin levels	**≤ 24**	1 (50%)	1 (50%)	0.037
	25 - 36	6 (23.1%)	20 (76.9%)	
	37 - 48	9 (40.9%)	13 (59.1%)	
	49 - 60	14 (58.3%)	10 (41.7%)	
	> 60	9 (50%)	9 (50%)	

Table 7: Age based comparison of elevated inflammatory markers with HCV co-infection in COVID-19 patients of Lahore

	COVID-19 Patients	HCV Positive	HCV Negative	*p*- value
Elevated D-Dimer levels	**≤ 24**	2(22%)	7(78%)	0.212
	25 - 36	6(17%)	29(83%)	
	37 - 48	7(21%)	27(79%)	
	49 - 60	9(28%)	23(72%)	
	> 60	7(30%)	16(70%)	
Elevated CRP levels	**≤ 24**	1(14%)	6(86%)	0.462
	25 - 36	7(22%)	25(78%)	
	37 - 48	10(8%)	24(71%)	
	49 - 60	8(26%)	23(74%)	
	> 60	8(21%)	20(71%)	
Elevated Ferritin levels	**≤ 24**	0	2(100%)	0.374
	25 - 36	6(24%)	19(76%)	
	37 - 48	6(27%)	16(73%)	
	49 - 60	7(28%)	18(72%)	
	> 60	6(33%)	12(67%)	

Discussion

When samples from the Lahore district were divided by gender, it was shown that males were more likely (55.3 %) to be COVID-19 positive than females. The findings were not statistically significant (p >0.05). Recent research conducted in 2020 in the Vehari district found that males were more likely to be COVID-19 positive (71.3 %) than females, consistent with our findings (28.7 %). These investigations concluded that females in these two regions have some degree of immunity to COVID-19 [38]. Additionally, another study confirmed these findings, demonstrating that males have a worse COVID-19 outcome than females [39]. Patients were also divided on the basis of different age groups and it was seen that middle-aged group patient i.e. from 25-36 years (23.1%), 37-48 years (27.1%) and from 49-60 years (24.1%) more positive COVID-19 results. Similarly, a study conducted in the Vehari district also showed the same results, with the majority of the patients (51.70%) were between the ages of 31-60 years [40].

In COVID-19 positive males, the serum CRP levels were seen more 76 (69.1%) than COVID-19 positive females

who had 56 (62.9%) elevated levels of serum CRP. Similarly, a study showed that the males with severe COVID-19 had significantly higher CRP levels than females, regardless of age or comorbidities [41]. Similarly, another study has also represented similar results in which males showed significantly elevated levels of serum CRP than females. Moreover, when CRP levels were seen across different age groups in the same study it was observed that patients from age <60 years and age ≥60 years showed elevated CRP levels, which was following our study, which depicted a substantial increase in serum CRP levels above 37 years [24]. Serum CRP levels were also seen elevated in COVID-19 patients with typhoid in our study. The results were non-significant (p> 0.05). A study has also shown that serum CRP levels are raised in hospitalized typhoid patients [42].

Elevated serum D-dimer levels were also seen in both COVID-19 positive males and females. Males 74 (67.3%) showed more elevated serum D-dimer levels than females 58 (65.2%). The results were non-significant (p>0.05). A recent study also showed the same results and presented elevated D-dimer levels more in males than females [43]. In our study, serum D-dimer levels were also seen elevated in COVID-19 patients with typhoid. The results were significant (p< 0.05). A study has also shown that serum D-dimer levels are raised up in the febrile phase of typhoid [44].

Elevated ferritin level was seen in COVID-19 positive males 46.4% more than females 46.1% (p> 0.05). A study has also represented the same results that males show more elevated levels of ferritin in COVID-19 patients as compared to females [45].

Typhoid positivity was detected at a higher rate in COVID-19 positive men (33.6 percent) than in COVID-19 positive females (33.6 %) (38.2 %). According to research done in Bangladesh, males are also more susceptible to typhoid and are more prone to suffer than females [46].

Another study discovered that 230 patients tested positive for typhoid out of 443 COVID-19 patients. Additionally, it was shown that COVID-19 men were more likely to be typhoid positive 164 (71.3 %) than COVID-19 females 66 (28.7 %) [39].

Additionally, the prevalence of typhi dot IgM positivity was determined independently from that of typhidot IgM and IgG positivity. Only 13 (18.3 %) COVID-19 patients had positive typhidot IgM antibodies, whereas 58 (81.7 %) had positive typhidot IgM and IgG antibodies. On the other hand, research from the Vehari district reported 164 (71.3%) COVID-19 individuals with typhidot IgM positivity, which was inconsistent with our findings [39].

In our study, 49 (22.6 %) of 199 individuals had co-infection with COVID-19 and HCV. In contrast to our findings, a study done in New York City, America, found that only 0.1 % of COVID-19 patients had co-infection with HCV and SARS-CoV-2. Another study done in Pennsylvania found that 3% of HCV and SARS-Cov-2 co-infection patients occurred [47]. No other related study was found that reported the co-infection of HCV and COVID-19.

When we compared serum CRP levels in HCV-positive, and HCV-negative patients, patients with co-infection, had less elevated CRP levels while patients with only COVID-19 diseases had higher levels of CRP. Significance was tested by CHI square, but the results were non-significant. A study reported on Serum CRP levels in HCV patients also reported that CRP levels do not elevate in HCV infection [48]. This implies that serum CRP levels are not positively associated with HCV infection. It may not affect the condition or, if it does, the effect will be diminished in HCV infection. Similarly, there is no correlation between blood D-dimer levels and hepatitis C, and the results were non-significant. When serum Ferritin levels were examined between patients with co-infection and patients with just COVID-19 infection, serum Ferritin levels were significantly higher in patients with only COVID-19 disease than in patients with co-infection. A study on ferritin levels and their relationship to HCV infection discovered that they became higher in chronic HCV infection [49].

Our study has several limitations. To begin, there is a small sample size of 199 cases. Based on the restricted sample size of this study, it is impossible to anticipate any outcomes. In addition, the research did not have an equal number of males and females, which may be caused a biased conclusion. Furthermore, the rapid test kits may provide false positive or negative findings, and the results are not entirely dependable.

Additionally, to determine whether there is a link between COVID-19 and viral hepatitis, the sample size should be increased, patients should be monitored regularly, other liver abnormalities should be monitored, and treatment strategies associated with COVID-19 and their outcomes

should be considered. Other tests for liver functions can also be studied with liver scans for further studies. Patient follow-up and post-COVID 19 complications can also be reviewed.

Conclusion

Males are more susceptible to COVID-19 infection, and plasma D-dimer, serum CRP, and ferritin levels are increased and positively linked with illness severity. COVID-19 individuals are prone to co-infection with typhoid, and their D-dimer, CRP, and ferritin levels are likewise elevated and strongly associated. Plasma D-dimer, serum CRP, and ferritin levels are all favorably linked with age in COVID-19 patients with typhoid co-infection. Early diagnosis of COVID-19 individuals with typhoid infection is crucial for guiding treatment decisions and reducing morbidity and death. Additionally, when inflammatory biomarkers (CRP, ferritin, and D-dimer) were compared between patients with co-infection with HCV and COVID-19 and those with just SARS-CoV-2 infection, these were discovered that biomarkers do not have a substantial correlation with viral hepatitis. This study demonstrates a general prevalence of viral hepatitis in a random population, and it is necessary to monitor and routinely examine these individuals.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR	1
	Design of the work, OR	1,3
	Data acquisition, analysis, or interpretation	1,4
2. Drafting or reviewing	Draft the work, OR	1
	Review critically for important intellectual content	2,3,4
3. Final approval	Approve the version to be published	1,2,3,4
4. Accountable	Agree to be accountable for all aspects of the work	1,2,3,4

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The UMT Ethics Review Committee approved the study. All participants gave their consent before enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

[1]. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Napoli RD. Features, Evaluation, and Treatment of Coronavirus (COVID-19) [Updated 2023 Aug 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK554776/

[2]. Hafeez A, Ahmad S, Siddqui SA, Ahmad M, Mishra SJE. A review of COVID-19 (Coronavirus Disease-2019) diagnosis, treatments and prevention. 2020;4(2):116-25.

[3]. She J, Jiang J, Ye L, Hu L, Bai C, Song YJC, et al. 2019 novel coronavirus of pneumonia in Wuhan, China: emerging attack and management strategies. 2020;9(1):1-7.

[4]. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Mil Med Res. 2020;7(1):1-10.

[5]. Markotić A, Kuzman IJCmj. The third coronavirus epidemic in the third millennium: what's next? Croat Med J. 2020;61(1):1-4.

[6]. Das A, Ahmed R, Akhtar S, Begum K, Banu SJGR. An overview of basic molecular biology of SARS-CoV-2 and current COVID-19 prevention strategies. Gene Rep. 2021:101122.

[7]. Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, Verma V. COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLoS Pathog. 2020;16(8):e1008762.

[8]. Lotfi M, Hamblin MR, Rezaei NJCca. COVID-19: Transmission, prevention, and potential therapeutic opportunities. Clin Chim Acta. 2020;508:254-66.

[9]. Güner HR, Hasanoğlu İ, Aktaş FJTJoms. COVID-19: Prevention and control measures in community. Turk J Med Sci. 2020;50(SI-1):571-7.

[10]. Yesudhas D, Srivastava A, Gromiha MM. COVID-19 outbreak: history, mechanism, transmission, structural studies and therapeutics. Infection. 2021;49(2):199-213.

[11]. Fasogbon BM, Ademuyiwa OH, Bamidele OP, Wahab

IE, Ola-Adedoyin AT, Alakija O. Positive therapeutic role of selected foods and plant on ailments with a trend towards COVID-19: A review. Prev Nutr Food Sci. 2021;26(1):1-11.
[12]. Watkins J. Preventing a covid-19 pandemic. BMJ. 2020;368:m810. doi: 10.1136/bmj.m810.
[13]. Zhang J, Wang X, Jia X, Li J, Hu K, Chen G, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. Clin Microbiol Infect. 2020;26(6):767-72.
[14]. Singh AK, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. Diabetes Metab Syndr. 2020;14(4):303-10.
[15]. Paudel SS. A meta-analysis of 2019 novel corona virus patient clinical characteristics and comorbidities. Research Square. 2020. doi: 10.21203/rs.3.rs-21831/v1.
[16]. Sanyaolu A, Okorie C, Marinkovic A, Patidar R, Younis K, Desai P, et al. Comorbidity and its impact on patients with COVID-19. SN Compr Clin Med. 2020;2(8):1069-76.
[17]. Rana MS, Usman M, Alam MM, Ikram A, Salman M. Overlapping clinical manifestations of COVID-19 with endemic infectious diseases in Pakistan: A looming threat of multiple lethal combinations. Infect Ecol Epidemiol. 2021;11(1):1873494..
[18]. Ayoubzadeh SI, Isabel S, Coomes EA, Morris SK. Enteric fever and COVID-19 co-infection in a teenager returning from Pakistan. J Travel Med. 2021;28(3):taab019.
[19]. Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? J Microbiol Immunol Infect. 2020;53(4):505-12.
[20]. Bertolini A, van de Peppel IP, Bodewes F, Moshage H, Fantin A, Farinati F, et al. Abnormal liver function tests in patients with COVID-19: Relevance and potential pathogenesis. Hepatology (Baltimore, Md). 2020;72(5):1864-72.
[21]. Schattenberg JM, Labenz C, Wörns MA, Menge P, Weinmann A, Galle PR, et al. Patterns of liver injury in COVID-19 - a German case series. Uni Euro Gastro J. 2020;8(7):814-9.
[22]. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5(5):428-30.
[23]. Leuzinger K, Roloff T, Gosert R, Sogaard K, Naegele K, Rentsch K, et al. Epidemiology of severe acute respiratory syndrome coronavirus 2 emergence amidst community-acquired respiratory viruses. J Infect Dis. 2020;222(8):1270-1279. Erratum in: J Infect Dis. 2021;223(4):734-5.
[24]. Mi J, Zhong W, Huang C, Zhang W, Tan L, Ding L. Gender, age and comorbidities as the main prognostic factors in patients with COVID-19 pneumonia. Am J Transl Res. 2020;12(10):6537-48.
[25]. Kumar MP, Mishra S, Jha DK, Shukla J, Choudhury A, Mohindra R, et al. Coronavirus disease (COVID-19) and the liver: a comprehensive systematic review and meta-analysis. Hepatol Int. 2020;14(5):711-22.
[26]. Ahmad A, Ishtiaq SM, Khan JA, Aslam R, Ali S, Arshad MI. COVID-19 and comorbidities of hepatic diseases in a global perspective. World J Gastroenterol. 2021;27(13):1296-310.
[27]. Li Y, Shi J, Xia J, Duan J, Chen L, Yu X, et al. Asymptomatic and symptomatic patients with non-severe coronavirus disease (COVID-19) have similar clinical features and virological courses: a retrospective single center study. Front Microbiol. 2020;11:1570.
[28]. Farasani A. Biochemical role of serum ferratin and d-dimer parameters in COVID 19 diagnosis. Saudi J Biol Sci. 2021;28(12):7486-90.
[29]. Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z. Plasma CRP level is positively associated with the severity of COVID-19. Ann Clin Microbiol Antimicrob. 2020;19(1):18.
[30]. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020;57(6):389-99.
[31]. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - A systematic review. Life Sci. 2020;254:117788.
[32]. Ghahramani S, Tabrizi R, Lankarani KB, Kashani SMA, Rezaei S, Zeidi N, et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. Eur J Med Res. 2020;25(1):30.
[33]. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 2020;92(7):791-6.
[34]. Cheng L, Li H, Li L, Liu C, Yan S, Chen H, et al. Ferritin in the coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. J Clin Lab Anal. 2020;34(10):e23618.
[35]. Gandini O, Criniti A, Ballesio L, Giglio S, Galardo G, Gianni W, et al. Serum ferritin is an independent risk factor for Acute Respiratory Distress Syndrome in COVID-19. J Infect. 2020;81(6):979-97.
[36]. Deng F, Zhang L, Lyu L, Lu Z, Gao D, Ma X, et al. Increased levels of ferritin on admission predicts intensive care unit mortality in patients with COVID-19. Med Clin (Engl Ed). 2021;156(7):324-31.
[37]. Vargas-Vargas M, Cortés-Rojo C. Ferritin levels and COVID-19. Rev Panam Salud Publica. 2020;44:e72.
[38]. Feld J, Tremblay D, Thibaud S, Kessler A, Naymagon L. Ferritin levels in patients with COVID-19: A poor predictor of mortality and hemophagocytic lymphohistiocytosis. Int J Lab Hematol. 2020;42(6):773-9.

[39]. Aslam MS, Shabeer S, Majeed R, Asghar F, Jafri SR, Khan H. Prevalence of Typhi DOT IGM positive results in COVID-19 patients at primary and secondary health care hospitals in Punjab, Pakistan. Prof Med J. 2021;28(06):779-83.
[40]. Haitao T, Vermunt JV, Abeykoon J, Ghamrawi R, Gunaratne M, Jayachandran M, et al. COVID-19 and sex differences: mechanisms and biomarkers. Mayo Clin Proc. 2020;95(10):2189-2203.
[41]. Qin L, Li X, Shi J, Yu M, Wang K, Tao Y, et al. Gendered effects on inflammation reaction and outcome of COVID-19 patients in Wuhan. J Med Virol. 2020;92(11):2684-92.
[42]. Barrett FC, Knudsen JD, Johansen IS. Cases of typhoid fever in Copenhagen region: a retrospective study of presentation and relapse. BMC Res Notes. 2013;6:315.
[43]. He X, Yao F, Chen J, Wang Y, Fang X, Lin X, et al. The poor prognosis and influencing factors of high D-dimer levels for COVID-19 patients. Sci Rep. 2021;11(1):1830.
[44]. Ohnishi K, Nakamura-Uchiyama F, Komiya N. Plasma D-dimer levels in patients with typhoid fever. Southeast Asian J Trop Med Public Health. 2007;38(5):911-2.
[45]. Shoeb F, Hussain I, Afrin G, et al. Hyperinflammatory conditions, gender differences and mortality in Indian COVID-19 patients. medRxiv; 2021. doi: 10.1101/2021.01.19.21250134.
[46]. Dewan AM, Corner R, Hashizume M, Ongee ET. Typhoid fever and its association with environmental factors in the Dhaka Metropolitan Area of Bangladesh: a spatial and time-series approach. PLoS Negl Trop Dis. 2013;7(1):e1998.
[47]. Reddy KR. SARS-CoV-2 and the Liver: Considerations in Hepatitis B and Hepatitis C Infections. Clin Liver Dis (Hoboken). 2020;15(5):191-4.
[48]. Shah S, Ma Y, Scherzer R, Huhn G, French AL, Plankey M, et al. Association of HIV, hepatitis C virus and liver fibrosis severity with interleukin-6 and C-reactive protein levels. AIDS. 2015;29(11):1325-33.
[49]. Uchino K, Tateishi R, Fujiwara N, Minami T, Sato M, Enooku K, et al. Impact of serum ferritin level on hepatocarcinogenesis in chronic hepatitis C patients. Hepatol Res. 2016;46(4):259-68.