World Journal of Medical Case Reports
2023; 4(1): 14-21
http://www.sciencepublishinggroup.com/j/wjmcr
doi: 10.11648/j.wjmcr.20230401.13

Research Article

The Trajectories of Hepato-Biliary Indices Following Exposure to SARS-CoV-2

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To cite this article:

Kelachi Thankgod Wala, Collins Amadi, Stephenson Lawson, Emmanuel Mustapha Owamagbe, Nkeiruka Joyce Amadi. The Trajectories of Hepato-Biliary Indices Following Exposure to SARS-CoV-2. *World Journal of Medical Case Reports*. Vol. 4, No. 1, 2023, pp. 14-21.
doi: 10.11648/j.wjmcr.20230401.13

Received: March 27, 2023; **Accepted**: April 12, 2023; **Published**: April 24, 2023

Abstract: *Background*: The impact of SARS-CoV-2 on the hepato-biliary system is conflicting within the existing literature. Previous studies on the subject have mostly been documented among Caucasians using retrospectively-acquired data from patients with several confounding variables. Hence, the current study evaluated the trajectories of hepato-biliary biochemical indices among SARS-CoV-2-infected Nigerians who had no background confounding factors. *Methods*: This was a prospectively-designed longitudinal study conducted within Southern Nigeria among patients with RT-PCR-confirmed mild SARS-CoV-2 infection. All eligible participants were serially monitored/followed up before, during, and after mild SARS-CoV-2 infection using clinical/laboratory parameters to determine the impact of the virus on the hepato-biliary system. Specimen acquisition, laboratory workflow, and data management were all carried out using standardized protocols. *Results*: Among 152 studied, 46.1% had mild SARS-CoV-2 infection 5–10 days (Mean=7.5; SD:=2.19) after exposure with male predominance. Cough, malaise, and loss of taste/smell were the most predominant clinical manifestations among the confirmed mild cases. During the follow-up period, an increasing trend of hepato-biliary indices of the cholestatic pattern (with only total bilirubin and GGT reaching statistically significant threshold) in parallel with inflammatory markers (CRP, di-dimer and the neutrophil to lymphocyte ratio) was observed between 2-12 days following mild SARS-CoV-2 infection. However, no relationship was established between these cholestatic and inflammatory markers among the mild SARS-CoV-2-infected patients ($p>0.05$). *Conclusion*: Mild SARS-CoV-2 infection is associated with altered hepato-biliary biochemical indices of cholestatic pattern independent of SARS-CoV-2-induced inflammatory events. Incorporating hepato-biliary assessment during the initial evaluation and the use of non-hepatotoxic therapeutics during treatment is highly recommended.

Keywords: SARS-CoV-2, COVID-19, Hepato-Biliary Indices, Inflammatory Markers

1. Introduction

Since the emergence of the current novel 2019 coronavirus disease (COVID-19) pandemic triggered by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), its exact pathophysiologic basis has remained poorly characterized within the existing literature [1]. A cardinal unique feature of COVID-19 is its characteristic distortions of various physiologic organ systems among those infected [1, 2]. This is particularly more evident within those organ systems

possessing the angiotensin-converting enzyme 2 (ACE2) receptors, the well-documented biologic receptor for the etiologic agent of the COVID-19 disease [3].

Besides the organ system of the respiratory tract, other organ systems including the central nervous, cardiovascular, renal, gastrointestinal tract including hepato-biliary tract have all been documented with abundant research evidence to express the ACE2 receptors which enable the direct pathologic effect of the SARS-CoV-2 on these organ systems [2, 3]. Consequently, several variants of altered hepato-biliary events, including hepatocellular and cholestatic patterns, have been documented among patients with COVID-19 [4-12].

Some investigators have suggested that the hepato-biliary manifestation of SARS-CoV-2 is strongly associated with the direct cytopathic effect of the virus on the abundant ACE2 receptors within the hepato-biliary system [6]. However, some experts have contrary views and had suggested that the hepato-biliary manifestations could be related to the overwhelming immune-inflammatory events common during SARS-CoV-2 infection [5, 13-17]. Others have also implicated therapeutic interventions initiated during the management of the infection [4, 5].

Hence, the relationship between SARS-CoV-2 and its effect on the hepato-biliary system has remained controversial within the existing literature and thereby requires further investigation.

Hence, the current study evaluated the impact of SARS-CoV-2 on the hepato-biliary system using relevant markers among apparently healthy subjects who were followed up before, during, and after SARS-CoV-2 infection following report of exposure to close relatives who had clinical and laboratory evidence of COVID-19 disease.

2. Materials and Methods

2.1. Study Design and Populations

This was a community-based observational prospectively-designed longitudinal study conducted during January-December, 2022 in Rivers State, South of Nigeria. The study was aimed to evaluate the dynamics of hepato-biliary biochemical indices among patients with mild COVID-19 disease who were followed up during home quarantine following contact (>15 min face-to-face or physical contact such as handshakes, etc) with close relatives diagnosed with COVID-19 disease. During the follow-up period, these contacts were consequently diagnosed with COVID-19 using a real-time reverse-transcription polymerase chain reaction (RT-PCR) test from a nasopharyngeal swab and were subsequently managed conservatively with non-hepatoxic medications and monitored using clinical/laboratory parameters during home treatment.

2.2. Ethical Considerations

Approval was obtained from the Research Ethics Committee of Rivers State Hospital Management Board (RSHMB) before commencement. The study was conducted per the principles embodied within the World Medical Association's Helsinki Declarations.

2.3. Sample Size Determination

The calculated minimum sample size required for this study is 76 comprising 76 adult males and 76 adult females matched for age, body mass index, time of recruitment, and illness severity. The sample size was determined using a formula for sample size determination for cross-sectional studies for defined characteristics in a population >10,000 using a 0.015% prevalence of COVID-19 in Nigeria as documented by Nas and colleagues [18]. Though the result from the sample size calculation was 0.230, to improve the power of the study, we enrolled 300% of this value; that is 76 (0.230 x 300% = 76) inclusive of a projected 10% non-compliance rate.

2.4. Eligibility Criteria

2.4.1. Inclusion into the Study Was Meeting All of the Following Criteria

1. Adults aged 21-40 years of age enjoying relative normal/stable health status and having laboratory evidence of normal hepato-biliary status/functions before COVID-19 diagnosis; 2. Existence of positive exposure to a close relative with RT-PCR-confirmed COVID-19 disease within a 10-day window before COVID-19 diagnosis; 3. Subjected to contact tracing, quarantined, and monitored using clinical and laboratory parameters including hepato-biliary parameters before COVID-19 diagnosis; 4. Consequently diagnosed with mild COVID-19 disease using RT-PCR from a nasopharyngeal swab specimen within 10 days following exposure to a close relative known to have RT-PCR-confirmed SARS-CoV-2 infection; 4. Subsequently isolated at home, managed with non-hepatotoxic medications, and monitored using clinical and laboratory parameters within 3 days of RT-PCR-confirmed COVID-19 diagnosis; 5. Having complete and relevant daily clinical/laboratory parameters for at least a week during the home management.

2.4.2. Exclusion from the Study Is Based on Having at Least One of the Following Criteria

1. Aged <21 and above 40 years of age at the time of contact tracing/monitoring; 2. Diagnosed with pre-symptomatic, moderate, severe, or critical COVID-19 disease including progression beyond mild disease; 3. Pregnant at the time of contact tracing/monitoring before the COVID-19 diagnosis; 4. The presence of any pre-existing comorbidities at the time of recruitment; 5.

On any medications within the last 4 weeks known to negatively influence hepatobiliary status before enrollment and contact tracing/monitoring; 6. Having previous or current hepato-biliary disease; 7. Evidence/history of being a present/past habitual alcoholic or tobacco consumer.

The comorbidities of interest included being of age ≥65 years; having cardiovascular disease, hypertension, chronic lung disease, asthma, sickle cell disease, HIV/AIDS, diabetes, cancer, obesity, or chronic kidney disease; being a cigarette smoker; being a transplant recipient, and receiving

immunosuppressive therapy.

The hepatobiliary disorders include acute/chronic liver disease, viral hepatitis, toxic hepatitis, drug-induced hepatitis, infective hepatitis (hepatitis A, B, and C), alcoholic hepatitis, gallstones, cholangitis (autoimmune/infective), and any inborn errors of metabolism affecting the hepato-biliary status/normal physiology. Drugs of interest influencing hepato-biliary disorders were chlorambucil, carbamazepine, chlorpromazine, cytotoxic drugs, erythromycin, halothane, isoniazid, methyldopa, nitrofurantoin, phenothiazines, acetaminophen overdose, salicylate overdose, statins, and valproate.

2.5. Infection Prevention and Control Measures

Adequate infection prevention and control measures as recommended by the Nigeria Center for Disease Control were strictly adhered to during data acquisition, specimen collection, and laboratory analysis [19].

2.6. Data Acquisition

Following the COVID-19 diagnosis, close relatives who are close contacts of those already diagnosed were counseled during contact tracing about the study and to obtain written informed consent. Consented close relatives were further evaluated to determine their eligibility status. Those who certified the eligibility criteria were subsequently enrolled in the study and all data from them were obtained in four series as follows:

First series: The baseline socio-demographic, clinical, and laboratory data were obtained from consented close relatives to confirm baseline negative COVID-19 status (using rapid antigen and RT-PCR test), normal hepato-biliary/inflammatory status, and urinalysis findings.

Second series: Those with negative COVID-19 status and normal hepatobiliary parameters were further evaluated every 2 days while on home quarantine using clinical/laboratory parameters including RT-PCR test.

Third series: Those with eventual RT-PCR-confirmed mild COVID-19 during the second series were offered conservative home management (including occasional 500mg oral acetaminophen that was administered pro re nata during fever manifestation) and monitored physically every 2 days using clinical/laboratory parameters including RT-PCR test for at least a week.

The clinical data obtained upon diagnosis included duration of contact tracing before diagnosis, duration from contact tracing to the onset of symptoms, duration from contact tracing to COVID-19 diagnosis, duration of exposure before clinical manifestations, clinical manifestation at diagnosis, oxygen saturation at diagnosis, disease severity at diagnosis, duration of progression from pre-symptomatic/mild disease to either mild, moderate or severe disease, duration from COVID-19 diagnosis to clinical recovery, duration from COVID-19 management to clinical recovery, clinical outcome following management, etc. Laboratory parameters included liver function test [Total and conjugated bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) enzyme activities] including other biochemical, hematological, coagulation, and inflammatory parameters.

2.7. Referral Protocol

Those who progressed from mild COVID-19 were eventually referred to a dedicated treatment center (Eleme COVID-19 treatment center, Port Harcourt, Nigeria) for further management.

2.8. Specimen Management and Laboratory Analysis

Nasopharyngeal swabs were collected based on recommended guidelines. The RT-PCR analysis on the swab was conducted at the Rivers State University Teaching Hospital (RSUTH) molecular laboratory. Random ten milliliters (10mls) of whole blood (equal aliquots transferred into lithium, ethyl di-amine tetra-acetic acid [EDTA], plain, and sodium citrate specimen tubes), and 5mls of urine (collected into sterile tubes) were obtained at each visit during the first, second, and third series of data collection. Serum (following clotting and full retraction) and plasma were isolated from whole blood via centrifugation and aliquots were stored frozen at -70 degrees until analyzed.

All laboratory analyses were carried out in the RSUTH Chemical Pathology laboratory and the side laboratory at the Eleme COVID-19 treatment center. Heparinized plasma was analyzed for plasma sodium, potassium, bicarbonate, and chloride on an ion-selective electrode chemistry analyzer (SFRI 6000, SFRI Diagnostics, Berganton, France) including the analyses for urea, creatinine, albumin, total protein, total/conjugated bilirubin, ALT, AST, ALP, GGT including creatine kinase (CK) on an automated chemistry analyzer (BS200, Mindray, Shenzhen, China). EDTA whole blood was analyzed for hemoglobin (Hb) concentration, full blood count (FBC)/FBC differentials, red blood cell count (RBC), and platelet counts on an automated hematology analyzer (BC10, Mindray, Shenzhen, China). Plain-tube processed serum was analyzed for pro-calcitonin, D-dimer, and ferritin on an automated immunoassay analyzer (Mini Vidas, Biomerieux, France) including the analyses for CRP using a CRP analyzer (HEALES, Shenzhen, China). Sodium-citrated plasma was analyzed for fibrinogen and prothrombin time (PT) using a coagulation analyzer (COA04, Biobase, China).

Urine biochemistry was analyzed Son an automated urine analyzer (Combilyzer-13, Human Diagnostics, Germany).

2.9. Data Definitions

The clinical spectrum of COVID-19 disease was categorized as asymptomatic/pre-symptomatic, mild, moderate, severe or critical infection/illness [20].

Pre-symptomatic/asymptomatic COVID-19 infection: Individuals who test positive for SARS-CoV-2 using an RT-PCR from a nasopharyngeal swab but who have no symptoms that are consistent with COVID-19.

Mild illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath and dyspnea.

Moderate illness: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO_2) ≥94% on room air at sea level. Severe illness: Individuals who have SpO_2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%.

Critical illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Close relatives were defined as members of immediate households such as spouses (wife/husband), fathers, mothers, sisters, brothers, daughters, and sons.

2.10. Data Management / Statistical Analysis

Data were managed and analyzed using the Statistical Package for Social Sciences software version 25. Continuous data were initially evaluated for conformity to a normal distribution using Shapiro-Wilk tests and those found not to be of the normal pattern were logarithmically transformed before analysis and summarized using means ± standard deviations; the comparison was made with the independent student t-test or analysis of variance (ANOVA), where necessary. The categorical data were summarized using proportions with counts/percentages; the comparison was made with the chi-square test or Fisher's exact test and Yate's continuity correction was applied, where necessary. Crude/adjusted linear regression models were used to determine the magnitude of the relationship between variables at 95% Confidence Intervals (CI). A p-value difference <0.05 was taken as a statistically significant threshold.

3. Results

Table 1 depicts the baseline socio-demographic, clinical, and laboratory variables of all the studied cohorts (n=152) before SARS-CoV-2 infection. All the clinical/laboratory data were found to be normal before infection including confirmation of a negative SARS-CoV-2 status (Table 1).

As shown in Table 2, among the entire 152 study cohorts evaluated, 70 (46.1%) were confirmed to mild SARS-CoV-2 infection within 7.57±2.19 days (range 5 – 10 days) from exposure to the time of RT-PCR confirmation. The males predominated among those with RT-PCR-confirmed disease compared to the females (Table 2). Cough, malaise, and loss of taste/smell were the most predominant clinical manifestations, however, while the males had higher proportions of those with cough and malaise, the females predominated among those with loss of taste/smell (Table 2).

During the follow-up following mild SARS-CoV-2 infection, an increasing trend of hepato-biliary indices of the cholestatic pattern was observed with changes only in TBil and GGT values reaching statistical significance, in parallel with an increasing trend of CRP, di-dimer, and the neutrophil to lymphocyte ratio between day 2 and 12 among those with mild SARS-CoV-2 infection ($p<0.05$) (Panel D1 & D2; Table 3).

Although other hepato-biliary indices (CBil, ALT, AST, and ALP) showed increasing trend patterns, they did not reach statistical significance during the follow-up period (Panel D1; Table 3).

Two (2) of the mild cases eventually progressed to moderate COVID-19 during the follow-up period around days 12 and 14 and were referred to a dedicated treatment center (Eleme COVID-19 treatment center) (Panel B; Table 3).

In Table 4, no relationship was established between the hepato-biliary indices with increasing trend trajectory during the follow-up period and the inflammatory markers ($p>0.05$).

Table 1. *First series of data acquisition: Baseline socio-demographic, clinical, and laboratory characteristics before mild COVID-19 diagnosis.*

Variables	Study cohorts, n=152 Mean ± SD / n (%)	Range	Inference
A. Socio-demographics variables			
Age, years	28.33 ± 2.23	21 - 40	Young adults
Sex (Male; Female)	76 (50); 76 (50)	NA	NA
Occupation: (Employed; Unemployed)	112 (73.7); 40 (26.3)	NA	NA
ED: Primary; secondary; tertiary	0 (0%); 5 (3.3); 147 (96.7)	NA	NA
Marital status: Married; single	60 (39.5); 92 (60.5)	NA	NA
Residential Area: Urban; Rural	148 (97.4); 4 (2.6)	NA	NA
Religion: Christian; Moslem	149 (98.0); 3 (2.0)	NA	NA
B. Clinical variables			
BMI, kg/m^2 (Normal: 18.5 – 24.9)	24.97 ± 3.04	19.6 – 24.7	Normal/NAD
AT, ^oC (RI: 36.0 – 37.2)	36.34 ± 1.07	36.3 – 36.8	Normal/NAD
SBP, mmHg (Normal: <120)	118.44 ± 4.67	105 - 118	Normal/NAD
DBP, mmHg (Normal: <80)	78.36 ± 2.88	70 - 79	Normal/NAD
HR, bpm (Normal: 60 - 100)	72.22 ± 3.46	70 - 92	Normal/NAD
RR, brpm (Normal: 12 - 20)	22.21 ± 1.23	12 - 17	Normal/NAD
SpO_2, % (Normal: ≥95)	96.87 ± 2.19	95 - 98	Normal/NAD
C. Laboratory variables			
SARS-CoV-2 qRT-PCR test outcome			
qRT-PCR: Negative; Positive	(n=152; 100%); 0 (0%)	NA	Normal/NAD
Hepato-biliary			
Plasma TBil, umol/L (RI: 5 - 17)	8.44 ± 1.11	7.10 – 15.30	Normal/NAD

Variables	Study cohorts, n=152 Mean ± SD / n (%)	Range	Inference
Plasma CBil, umol/L (RI: 0 - 5)	2.31 ± 0.88	1.10 – 3.44	Normal/NAD
Plasma AST, IU/L (RI: 5 - 40)	21.33 ± 2.77	9.00 – 27.00	Normal/NAD
Plasma ALT, IU/L (RI: 5 - 40)	19.44 ± 2.64	7.00 – 23.00	Normal/NAD
Plasma ALP, IU/L (RI: 40 - 150)	56.77 ± 3.43	42.00 – 64.00	Normal/NAD
Plasma GGT, IU/L (RI: 10 - 45)	15.66 ± 1.83	11.00 – 21.00	Normal/NAD
Plasma Albumin, g/L (RI: 35 - 50)	42.12 ± 2.41	38.00 – 43.00	Normal/NAD
PT, s (RI: 10 - 14)	11.22 ± 1.08	10.11 – 12.50	Normal/NAD
Inflammatory			
Serum PCT, ug/L x 10^2 (RI: ≤5.0)	2.33 ± 1.06	1.63 – 2.97	Normal/NAD
Serum CRP, nmol/L (RI: 2.8 – 49.0)	6.73 ± 1.74	3.44 – 7.98	Normal/NAD
Serum ferritin, pmol/L (RI: 67 - 674)	169.14 ± 4.71	167.31 – 244.11	Normal/NAD
Serum D-dimer, ug/L FEU (RI: ≤500)	154.31 ± 5.66	107.63 – 233.41	Normal/NAD
Neutrophil to lymphocyte Ratio (RI: 1- 3)	1.94 ± 0.65	1.04 – 2.66	Normal/NAD
Electrolytes**/Urea/Creatinine	NAD	NA	Norma/NAD
Urinalysis	NAD	NA	Normal/NAD
Viral hepatitis screen	NAD	NA	Normal/NAD

*Statistically significant; SD: standard deviation; NA: not applicable; ED: educational status; BMI: body mass index; AT: axillary temperature; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate; SpO_2: oxygen saturation; RT-PCR: real-time reverse transcription polymerase chain reaction; RI: reference interval; TBil: total bilirubin; CBil: conjugated bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; PT: prothrombin time; PCT: procalcitonin; CRP: C-reactive protein; FEU: fibrin-equivalent unit; NAD: no abnormality detected; **Electrolytes (sodium, potassium, chloride, bicarbonate)

Table 2. *Second series of data acquisition: SARS-CoV-2 qRT-PCR test outcome and clinical parameters obtained at the point of mild COVID-19 diagnosis ≤10 days following exposure.*

Variables	Entire study cohorts, n = 152	Male cohorts, n=76	Female cohorts, n=76	p-value
	n (%)	n (%)	n (%)	
A. SARS-CoV-2 qRT-PCR status				
Negative test	80 (52.6%)	34 (42.5%)	46 (57.5%)	0.064
Positive test with >mild disease	2 (1.3%)	1 (50%)	1 (50%)	NA
Positive test with only mild disease	70 (46.1%)	41 (58.6%)	29 (41.4%)	0.041*
B. Positive cases with mild SARS-CoV-2 infection	n=70	n= 41	n=29	
	Mean ± SD/n (%)	Mean ± SD/n (%)	Mean ± SD/n (%)	
1. Clinical variables at diagnosis of mild disease				
Duration of exposure before recruitment, days	2.33 ± 1.11	2.08 ± 1.06	2.13 ± 1.01	0.160
Duration of exposure before symptom onset, days	5.88 ± 1.24	5.73 ± 1.16	5.63 ± 1.13	0.231
Duration of exposure before diagnosis, days	7.57 ± 2.19	7.33 ± 1.17	7.45 ± 1.18	0.206
Clinical manifestations at diagnosis				
Fever, AT ≥37.5°C	26 (37.1%)	14 (53.8%)	12 (46.2%)	0.058
Cough	52 (74.3%)	37 (71.1%)	15 (28.9%)	<0.001*
Sore throat	21 (30.0%)	13 (61.9%)	8 (38.1%)	<0.001*
Malaise	49 (70.0%)	27 (52.1%)	21 (42.9%)	0.076
Headache	15 (21.4%)	8 (53.3%)	7 (45.7%)	0.061
Muscle ache	23 (32.9%)	11 (47.8%)	12 (52.2%)	0.087
Nausea (± vomiting or diarrhea)	19 (27.1%)	9 (47.4%)	10 (52.6)	0.121
Loss of taste/smell	36 (51.4%)	15 (41.6%)	21 (58.5%)	0.029*

*Statistically significant; SD: standard deviation; NA: not applicable; AT: axillary temperature; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate; SpO_2: oxygen saturation; qRT-PCR: real-time reverse transcription polymerase chain reaction

Table 3. *Third series of data acquisition: Follow-up clinical/laboratory data after mild COVID-19 diagnosis.*

Variables	Day 2-4	Day 5-7	Day 8-10	Day 11-12	Day 12-14	p trend
	n=40	n=40	n=40	n=40		
	Mean ± SD/n	Mean ± SD/n	Mean ± SD/n	Mean ± SD/n	Mean ± SD/n	
A. Follow-up status						
Lost to follow-up, n	0	0	2	0	0	NA
On follow-up, n	70	70	63	43	11	NA
B. SARS-CoV-2 RT-PCR test						NA
Followed-up test, n	70	70	63	43	11	NA
Negative/clinical recovery, n	0	5**	20**	32**	9**	NA
Positive, n	70	65	43	11	2***	NA
C. Clinical						
BT, °C	36.14 ± 1.17	36.18 ± 1.26	36.15 ± 1.10	36.12 ± 1.11	NA	0.120
SBP, mmHg	130.66 ± 5.26	130.76 ± 5.34	129.91 ± 4.92	129.84 ± 4.81	NA	0.214
DBP, mmHg	84.77 ± 3.30	83.65 ± 3.28	83.93 ± 3.21	82.82 ± 2.84	NA	0.110

Variables	Day 2-4 n=40 Mean ± SD/n	Day 5-7 n=40 Mean ± SD/n	Day 8-10 n=40 Mean ± SD/n	Day 11-12 n=40 Mean ± SD/n	Day 12-14 Mean ± SD/n	p trend
HR, bpm	85.87 ± 4.09	84.33 ± 4.44	83.21 ± 4.21	78.55 ± 3.71	NA	0.059
RR, brpm	24.89 ± 1.57	25.65 ± 1.41	25.83 ± 1.23	26.11 ± 1.49	NA	0.281
SpO_2, %	96.11 ± 2.06	95.88 ± 2.07	95.62 ± 1.94	95.96 ± 1.97	NA	0.464
D. Laboratory						
1. Hepato-biliary						
Plasma TBil, umol/L	10.75 ± 1.20	11.33 ± 1.39	15.65 ± 1.66	17.75 ± 2.09	NA	0.041*
Plasma CBil, umol/L	2.67 ± 0.65	3.87 ± 0.91	4.01 ± 1.02	4.16 ± 1.01	NA	0.176
Plasma AST, IU/L	24.20 ± 2.63	31.64 ± 2.91	36.20 ± 2.63	39.11 ± 3.01	NA	0.095
Plasma ALT, IU/L	23.19 ± 2.77	27.31 ± 2.86	31.19 ± 3.11	35.22 ± 3.55	NA	0.103
Plasma ALP, IU/L	88.91 ± 4.07	105.41 ± 5.63	121.91 ± 4.86	139.91 ± 5.13	NA	0.095
Plasma GGT, IU/L	41.42 ± 2.76	74.88 ± 3.86	91.42 ± 3.34	146.52 ± 3.54	NA	<0.001*
Plasma albumin, g/L	38.24 ± 2.09	37.18 ± 2.71	36.24 ± 2.65	36.11 ± 1.62	NA	0.087
PT, s	12.36 ± 1.78	13.17 ± 1.92	13.26 ± 1.81	13.82 ± 2.20	NA	0.162
2. Inflammatory						
Serum PCT, ug/L x 10^2	9.87 ± 2.04	12.88 ± 2.51	13.06 ± 2.43	13.92 ± 2.61	NA	0.067
Serum CRP, nmol/L	54.43 ± 3.65	95.62 ± 4.22	116.41 ± 4.69	128.55 ± 4.34	NA	<0.001*
Serum ferritin, pmol/L	701.23 ± 44.17	711.23 ± 44.17	718.66 ± 45.06	720.01 ± 45.18	NA	0.074
Serum D-dimer, ug/L	609.31 ± 13.66	781.16 ± 13.28	852.15 ± 13.92	916.21 ± 16.71	NA	<0.001*
NLR	4.67 ± 1.34	5.83 ± 1.61	6.78 ± 1.88	6.96 ± 1.91	NA	<0.001*
3. Electrolytes/urea/creatinine	NAD	NAD	NAD	NAD	NA	NA

*Statistically significant; **Excluded from statistical analysis; ***Progressed to moderate disease and referred to a dedicated treatment center; SD: standard deviation; NA: not applicable; BT: body temperature; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate; SpO_2: oxygen saturation; qRT-PCR: real-time reverse transcription polymerase chain reaction; RI: reference interval; TBil: total bilirubin; CBil: conjugated bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; PT: prothrombin time; PCT: procalcitonin; CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio; NAD: no abnormality detected

Table 4. *Correlation between hepato-biliary and inflammatory indices among those with mild SARS-CoV-2 infection.*

	INFLAMMATORY INDICES		
	CRP, nmol/L	D-dimer, ug/L FEU	NLR
	β: p-value	β; p-value	β; p-value
HEPATO-BILIARY INDICES			
Crude linear regression			
TBil, µmol/L	0.203; 0.068	0.211; 0.150	0.209; 0.117
GGT, IU/L	0.232; 0.131	0.254; 0.166	0.237. 0.145
Sex-adjusted linear regression			
TBil, µmol/L	0.213; 0.121	0.192; 0.133	0.179; 0.211
GGT, IU/L	0.221; 0.362	0.217; 0.095	0.208; 0.092

β: regression coefficient; FEU: fibrin equivalent units; TBil: total bilirubin; GGT: gamma-glutamyl transferase; CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio

4. Discussion

4.1. Principal Findings

Since the evolution of COVID-19, controversies have existed among experts on the influence of SARS-CoV-2 on hepato-biliary parameters/functions. In the current study, we evaluated the influence of SARS-CoV-2 on hepato-biliary indices in patients who were recruited before infection following exposure to a known close relative with RT-PCR-confirmed infection. Those exposed and recruited were subsequently followed up after developing the disease to evaluate the trajectories of hepato-biliary indices. Unlike in previous similar studies, we had prospectively recruited only those with normal hepato-biliary parameters/function in addition not to have any previous or present episodes of hepato-biliary conditions or on any medications/toxicants known to influence hepato-biliary parameters/functions before the SARS-CoV-2 infection.

Following analysis, 46.1% were confirmed to have mild SARS-CoV-2 infection following exposures to close relatives with RT-PCR-confirmed SARS-CoV-2 infection. During the follow-up of those with mild SARS-CoV-2 infection, an increasing trend trajectory of hepato-biliary indices of the cholestatic pattern (with only TBil and GGT reaching statistically significant thresholds) in parallel with some inflammatory markers (CRP, di-dimer, and the neutrophil to lymphocyte ratio) was observed between day 2 and 12. However, no statistically significant relationship was observed between GGT and these inflammatory markers among the mild SARS-CoV-2 infected patients in both crude and adjusted linear regression models.

4.2. Relationship Between Current Findings and the Existing Literature

In a recent similar study documented among the Chinese COVID-19 in-patients, hepato-biliary indices were

compared between different time points about SARS-CoV-2 shedding, using days 3-7 before the first detection of viral shedding as the reference baseline, the authors observed that ALT, AST, and ALP abnormal rates and levels did not show any significant dynamic changes during the full period of viral shedding but that of TBil and GGT significantly increased [21]. Based on their observations, the authors concluded that SARS-CoV-2 does not directly lead to elevations in ALT and AST but may result in elevations in cholestatic indicators (GGT and TBil) due to direct effect of the virus on the abundant ACE2 receptors expressed on cholangiocytes during the early stage of the viral infection [11]. That report concurs with the findings of the present study; however, that study was limited by its retrospective design and the recruitment of patients on various medications and comorbid conditions that may have influenced the hepato-biliary indices [21]. In another similar study conducted among another subset of Chinese COVID-19 patients, the authors observed no changes in hepato-biliary indices during the early stage of the non-severe (mild/moderate) infection, but clinically significant changes were observed in severe infection [11]. These discordant findings may be related to the limited sample size (n=12) population evaluated by the authors of this other Chinese study [11].

4.3. Mechanistic Considerations

Since the advent of SARS-CoV-2 infection, various mechanisms by several investigators have been adduced for the hepato-biliary effects of SARS-CoV-2 in the literature including direct cytopathic effects from viral replication in hepatocytes, immuno-Inflammatory effects, ischemia, and hypoxic-reperfusion dysfunction due to respiratory failure, iatrogenic due to drug-induced/ventilation effects, and exacerbation/re-activation of pre-existing hepato-biliary conditions [10, 22-25]. Moreover, current epidemiologic data have demonstrated that ACE2 receptors are predominantly expressed on the cholangiocytes compared to the hepatocytes (2.6% versus 59.7%) and these cholangiocytes are known to play major roles in immune response and liver regeneration during various viral infection-induced hepato-biliary insults [10, 22].

Therefore, considering the hepato-biliary effects of the cholestatic pattern observed among our study cohorts independent of immune-inflammatory involvement and the very fact we had excluded those likely to have been on any therapeutics/toxicants with hepato-biliary effects and also those with pre-existing hepato-biliary diseases, the most likely pathophysiologic mechanism of the hepato-biliary effects of the SARS-CoV-2 among our studied cohorts could be the direct cytopathic effects of the viral agent on the cholangiocytes as previously described [21].

4.4. Relevance to Clinical Practice and Future Research

With the evidence of SARS-CoV-2-induced hepato-biliary influence in the current study, the incorporation of clinical and laboratory-oriented hepato-biliary assessment must be instituted during the initial evaluation of those infected and the utilization of less hepatotoxic therapeutics during management should be prioritized. Hence, it is essential to further explore the exact mechanism of the SARS-CoV-2-induced hepato-biliary injury in more elaborate studies with a large sample population among those with moderate/severe SARS-CoV-2 infection.

4.5. Strength and Limitations

The study was strengthened by its prospective design using population-based data. Yet, the study was limited by some factors which are areas for improvement in future studies. As with most observational studies, its findings do not infer a cause-effect implication but associations. The study was also limited by the smaller sample size population.

5. Conclusion

The current has provided evidence that SARS-CoV-2 infection, even of a mild degree, may be associated with altered hepato-biliary indices of cholestatic pattern independent of SARS-CoV-2-induced inflammatory events. Hence, the incorporation of hepato-biliary assessment during the initial evaluation of those infected and the utilization of less hepatotoxic therapeutics during management is highly recommended.

Statement of Ethics

The ethical approval of the study was obtained from the Research Ethics Committee of RSHMB following the review of the study protocols and the study was subsequently executed in compliance with the principles embodied in the Helsinki Declaration.

Disclosure Statement

The authors have no conflict of interest to declare.

Author Contributions

All the authors were involved substantially in the concept and design of the study, data acquisition, analysis and interpretation of the data, drafting the article, revising the article critically for its intellectual content, and in the final approval of the version to be published.

Data Availability

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (CA) upon reasonable request.

Acknowledgements

The authors appreciate the efforts of all the medical doctors, nurses, and laboratory scientists at the Rivers State Eleme COVID-19 treatment center and the COVID-19 Molecular Laboratory at the Rivers State University Teaching Hospital (RSUTH) for all their professional assistance during the conduct of the study.

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