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Patterns of Hepatobiliary Pathologies and Their Relationship with Markers of Inflammation in COVID-19 patients

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ABSTRACT

***Background*: Altered hepatobiliary status has been reported in association with COVID-19 which has been linked, with limited evidence, to the exaggerated COVID-19-induced hyper-inflammatory responses. Hence, the current study evaluated hepatocellular status and its association with indicators of inflammation among COVID-19 patients.**

***Methods*: This study was conducted among the RT-PCR-confirmed and treatment-naïve COVID-19 patients in Port Harcourt, South-south Nigeria. Pre-medical/surgical data were retrieved retrospectively from archived patients' case notes, medical review charts, nurses' vital signs/medication sheets, and laboratory records at the center using validated data acquisition templates. All retrieved data were analyzed using standard protocols.**

***Results*: Among the 396 studied, 16.7% (n=66) had hepatobiliary pathologies. The majority of those with hepatobiliary pathologies had severe COVID-19 (93.3%). Patients with severe COVID-19 and concurrent hepatobiliary pathologies were mostly males and of older age. Cholestatic-specific pathology was the most common pattern observed among the general cases with hepatobiliary pathologies and among those having specific mild, moderate, and severe COVID-19. Among those with severe COVID-19, significant positive relationships were observed between markers of inflammation (Proclacitonin/C-reactive protein/D-dimer) and cholestatic-specific hepatobiliary markers (ALP/G-GT/TBil/CBil) ($p<0.05$), but not with the hepatocellular-specific markers (ALT/AST) ($p>0.05$). In contrast, no significant relationship existed between the relevant markers of inflammation and all the cholestatic/hepatocellular markers among those with mild and moderate COVID-19 ($p>0.05$).**

***Conclusion*: Hepatobiliary pathologies, mostly of cholestatic patterns, are frequent among the studied COVID-19 patients and were associated with inflammatory markers among those with severe disease. Hence, hepatobiliary evaluation should be prioritized, especially among those with severe COVID-19.**

Keywords: COVID-19, hepatobiliary pathology, markers of inflammation, severe COVID-19.

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I. Introduction

Since the evolution of the ongoing COVID-19 pandemic induced by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), its definite pathophysiologic basis continues to be the focus of intense research [1]. A common feature of the disease is the alterations of several organ systems among those infected [2]-[4]. This is particularly more pronounced among those organ systems enriched with the angiotensin-converting enzyme 2 receptors (ACE2) known to be the biological viral receptor for SARS-CoV-2 [4]. Besides the respiratory system, other organ systems including the brain, the heart, the kidney, the gastrointestinal tract including the liver have all been documented to express the ACE2 receptors which enable the pathologic effect of SARS-CoV-2 [2]-[4]. Several patterns of hepatobiliary pathologies, including hepatocellular and cholestatic patterns, have been documented and reported among patients diagnosed with COVID-19 since the ongoing pandemic [5]-[11].

The mechanism underlying the COVID-19-induced hepatobiliary pathologies is conflicting in the literature, though several investigators have suggested the involvement of the hyper-inflammatory responses inherent during the disease [6]-[11]. The hyper-inflammatory response is a cardinal feature of the disease [12]-[15]. However, a large number of previous studies on this subject have been documented among western populations while including patients with various pre-existing comorbid conditions and those on various medications/toxicants known to have negative influences on normal hepatobiliary functions [6]-[10]. Hence, the current study evaluated the pattern of hepatobiliary pathologies and their relationship with markers of inflammation among COVID-19 patients of Nigerian origin who had no pre-existing comorbid conditions and nil past/present history of being on any hepatobiliary-influencing medications/toxicants at the time of recruitment.

II. Materials and Methods

A. Study Design, Location, and Populations

The present study was an observational retrospective cross-sectional-designed study including adult patients with confirmed COVID-19 disease who were managed at the Eleme COVID-19 treatment center in Port Harcourt, Southern Nigeria from 2020 to 2022. The treatment center, with an attached laboratory that is fully equipped with automated chemistry/hematology analyzers, is one of the three treatment centers set up by the Nigerian Rivers State Government for patients diagnosed with COVID-19. Patients are usually referred to the treatment center following confirmation of COVID-19 in one of the several molecular laboratories offering real-time reverse transcription polymerase chain reaction (RT-PCR) tests in Rivers State including the COVID-19 molecular laboratory at the Rivers State University Teaching Hospital (RSUTH).

B. Ethical Considerations

The study was approved by the Research Ethics Committee of the Rivers State Government Hospital Management Board and was conducted in compliance with the Declaration of Helsinki.

C. Study Materials

Pre-medical/surgical intervention data (demographic, clinical, and laboratory) of mild, moderate, and severe cases of RT-PCR-confirmed SARS-CoV-2 infected patients who were managed at the Eleme COVID-19 treatment center in Port Harcourt, Nigeria between 2020 and 2022.

D. Eligibility Criteria

The eligibility status for inclusion included (1) Those aged ≥18 years of age; (2) Those with positive RT-PCR test from a properly collected nasopharyngeal swab specimens; (3) Those with relevant demographic, clinical, and laboratory data. The criteria for exclusion included:

1) Those with evidence of having been on any medications/toxicants known to significantly influence normal hepatobiliary physiology before COVID-19 diagnosis;
2) Those with any prior history of self, home, or hospital medical/surgical interventions before presentations;
3) Those with any pre-existing comorbid conditions especially hepatobiliary pathologies before COVID-19 diagnosis.

E. Data Acquisition

Relevant data were those obtained at presentation before any form of medical/surgical intervention in the treatment center from case notes, medical charts, nurses' vital signs charts, medication charts, laboratory records, and electronic records in the treatment center by well-trained research assistants using validated data collection templates. Demographic data included age, sex, occupation, education levels, marital status, residential area, religion, and smoking/alcohol consumption status. Clinical data included respiratory rate, pulse rate, blood pressure, body mass index, oxygen saturation, comorbid conditions, disease severity, and outcome (discharged home, intensive care admission or treatment, and mortality). Laboratory parameters included biochemical parameters [plasma sodium, chloride, potassium, bicarbonate, urea, creatinine, albumin, and total protein (TP)], hematologic parameters [hemoglobin, total white blood cell count (TWBC), and differentials including neutrophils, lymphocytes, monocytes, eosinophil, and basophil counts], inflammatory markers [pro-calcitonin, C-reactive protein (CRP), ferritin, and liver function test parameters [total bilirubin (TBil), conjugated bilirubin (CBil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (G-GT) enzyme activities].

F. Specimen Acquisition and Laboratory Activities

During the period under evaluation, all specimens were obtained immediately upon presentation at the treatment center and analyzed in the side laboratory by well-experienced analysts. Heparinized plasma was analyzed for plasma sodium, potassium, bicarbonate, and chloride on an ion-selective electrode chemistry analyzer (SFRI 6000, Berganton, France) including analysis for urea, creatinine, albumin, and total protein on an automated chemistry analyzer (BS200, Mindray, Shenzhen, China). EDTA whole blood was analyzed for Hb concentration, FBC, 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). The plain tube-derived serum was analyzed for CRP using a CRP analyzer (HEALES, Shenzhen, China). Prothrombin time and plasma fibrinogen levels were determined in citrated plasma on a coagulation analyzer (COA04, Biobase, China). Two levels of commercial control materials were interspersed during each analytical run to monitor accuracy/precision.

G. Data Definitions

The clinical spectrum of COVID-19 disease at presentation was categorized as mild, moderate, and severe disease as previously documented [16].

- 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 (SpO2) ≥94% on room air at sea level.

- Severe illness

Individuals who have SpO2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%. The patterns of hepatobiliary pathologies were categorized as hepatocellular, cholestatic, or mixed variants as previously described [6].

- Hepatocellular pathology

Those with raised plasma ALT and/or AST activities more than three times the upper limit unit of normal (ULN);

- Cholestatic pathology

Those who had raised plasma ALP/GGT activities with or without raised total plasma bilirubin (TBil) level; and

- Mixed hepatobiliary pathology

Those who had a combination of both plasma ALT/AST activities elevated more than three times the ULN and ALP/GGT activities with or without TBil twice the ULN.

H. Data Management/Statistical Analysis

Data were managed/analyzed using the Statistical Package for Social Sciences software version 25. Continuous data were first evaluated for conformity to a normal distribution using both visual (histogram/probability plots) and statistical (Kolmogorov-Smirnov/Shapiro-Wilk tests) parameters. Continuous data with non-normal distribution were log-transformed before analysis and summarized using means ± standard deviations; the comparison was made with the independent student t-test or analysis of variance, where necessary. Categorical data were summarized using counts/percentages; the comparison was made with the chi-square test or Fisher's exact test and the Yate's continuity correction was applied, where necessary. Crude/adjusted linear logistic regression models were used to determine the relationship between continuous data at 95% confidence intervals. A p-value difference of less than 0.05 was deemed statistically significant.

III. Results

During the studied period (2020-2022), 678 RT-PCR-confirmed COVID-19 patients presented in the treatment center of whom 396 met the eligibility status to be included in the current study.

Of the total number (n=396) of cases studied, 52.3% (n=207), 43.9% (n=174), and 3.8% (n=15) presented with mild, moderate, and severe COVID-19 variants, respectively (Table I).

Upon evaluation of hepatobiliary status of all the eligible 396 cases, 16.7% (n=66) were found to have hepatobiliary pathologies while 83.3% (n=330) had normal hepatobiliary status (Table I). Based on COVID-19 severity, hepatobiliary pathologies were documented among 6.3% (n=13), 22.4% (n=39), and 93.3% (n=14) of those with mild, moderate, and severe COVID-19 variants, respectively ($p<0.05$) (Table I). Based on hepatobiliary-specific pathologies, 53.0% (n=35), 28.8% (n=19), and 18.2% (n=12) had cholestatic, hepatocellular, and mixed pathologies, respectively ($p<0.05$) (Table I). Cholestatic-specific hepatobiliary pathology was the most common documented among all the cases with hepatobiliary pathology (n=35; 53.0%) and was also the most pronounced among those with mild (n=6; 46.3%), moderate (n=20; 51.3%), and severe (n=9; 64.3%) COVID-19 variants ($p<0.05$) (Table I).

In Table II, those with hepatobiliary pathologies were older with male predominance, and had higher plasma creatinine, total white cell count, neutrophil count, all hepatocellular/cholestatic markers (ALT, AST, ALP, G-GT, TBil, and CBil) of hepatobiliary pathologies, markers of inflammation (PCT, CRP, and D-dimer) but lower prothrombin time, albumin, total protein, lymphocyte count, platelet count, and oxygen saturation compared to those with normal hepatobiliary functions ($p<0.05$).

In Table III, those with severe COVID-19 who had hepatobiliary pathologies were relatively older with male predominance, and had higher plasma creatinine, total white cell count, neutrophil count, cholestatic-specific markers (ALP, G-GT, TBil, and CBil) of hepatobiliary pathologies, inflammation markers (PCT, CRP, and D-dimer) but lower prothrombin time, albumin, total protein, lymphocyte count, platelet count, and oxygen saturation compared to those with mild/moderate COVID-19 who had hepatobiliary pathologies ($p<0.05$).

No relationship was established between the significant markers of inflammation (PCT, CRP, and D-dimer) and all the cholestatic/hepatocellular markers (ALT, AST, ALP, G-GT, TBil, and CBil) of hepatobiliary pathologies among those with mild/moderate COVID-19 variants (Panels 1 & 2; Table IV). However, positive relationships were observed between the markers of inflammation (PCT, CRP, and D-dimer) and cholestatic-specific markers (ALP, G-GT, TBil, and CBil) but not with the hepatocellular-specific markers (ALT and AST) of hepatobiliary pathologies (Panel 3; Table IV).

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TABLE I: STRATA OF STUDIED POPULATION (N=396) BY COVID-19 SEVERITY, HEPATOBILIARY STATUS AND PATTERNS OF HEPATOBILIARY PATHOLOGIES

		CATEGORIES OF COVID-19 SEVERITY			
	Overall cases	Mild cases	Moderate cases	Severe cases	p-values
Variables	n (%)	n (%)	n (%)	n (%)	
Categories of study population	n=396 (100)	n=207 (52.3)	n=174 (43.9)	n= 15 (3.8%)	<0.001*
Hepatobiliary status, n (%)					0.003*
Without HB pathologies	330 (83.3)	194 (93.7)	135 (87.6)	1 (6.7)	
With HB pathologies	66 (16.7)	13 (6.3)	39 (22.4)	14 (93.3)	
Patterns of HB pathologies, n (%)					0.016*
Hepatocellular	19 (28.8)	4 (30.8)	14 (35.9)	1 (7.1)	
Cholestatic	35 (53.0)	6 (46.2)	20 (51.3)	9 (64.3)	
Mixed	12 (18.2)	3 (23.0)	5 (12.8)	4 (28.6)	

*Statistically significant; HB: hepatobiliary

TABLE II: COMPARATIVE ANALYSIS OF CLINICAL/LABORATORY VARIABLES BETWEEN THOSE WITH AND WITHOUT HEPATOBILIARY PATHOLOGIES

	HEPATOBILIARY STATUS OF STUDY COHORTS, n=396			
Variables	Without HB Pathologies, n=330	With HB Pathologies, n=66	p-value	All cases, n=396
	Mean ± SD/n	Mean ± SD/n		Mean ± SD/n
Age, years	41.07 ± 6.19	42.98 ± 5.15	0.040*	42.20 ± 6.71
Males/Females	220/110	44/22	0.016*	264/132
BMI, kg/m^2	28.01 ± 4.16	28.18 ± 4.65	0.410	27.64 ± 4.27
Body temperature, ^{o}C	36.23 ± 3.36	36.82 ± 3.74	0.439	36.71 ± 3.66
SBP, mmHg	136.19 ± 6.13	136.86 ± 6.41	0.365	137.36 ± 6.24
DBP, mmHg	83.88 ± 4.07	84.41 ± 4.51	0.159	84.93 ± 4.22
HR/minute	81.08 ± 4.12	81.70 ± 4.33	0.445	82.46 ± 4.18
RR/minute	20.71 ± 2.26	21.01 ± 2.06	0.379	21.11 ± 2.34
Plasma sodium, mmol/L	133.83 ± 5.66	134.10 ± 5.77	0.066	134.16 ± 5.84
Plasma chloride, mmol/L	96.94 ± 4.29	97.01 ± 4.46	0.502	96.45 ± 4.32
Plasma bicarbonate, mmo/L	24.13 ± 2.53	23.91 ± 2.37	0.090	24.06 ± 2.72
Plasma urea, mmol/L	5.70 ± 1.21	6.03 ± 1.40	0.134	5.93 ± 1.38
Plasma creatinine, µmol/L	95.16 ± 5.35	98.86 ± 5.76	0.022*	97.67 ± 5.66
RPG, mmol/L	7.30 ± 2.31	7.33 ± 2.55	0.147	7.75 ± 2.42
Plasma ALT activity, IU/L	29.53 ± 2.74	144.76 ± 6.84	<0.001*	59.85 ± 4.32
Plasma AST activity, IU/L	34.46 ± 2.82	176.44 ± 7.63	<0.001*	63.67 ± 4.65
Plasma ALP activity, IU/L	65.13 ± 3.59	168.13 ± 8.76	<0.001*	96.39 ± 4.24
Plasma G-GT activity, IU/L	24.8. ± 2.16	171. ± 9.22	<0.001*	73.22 ± 4.06
Plasma TBil, ug/L	23.31 ± 2.33	47.14 ± 4.07	<0.001*	47.28 ± 2.15
Plasma CBil, ug/L	3.23 ± 1.04	12.73 ± 1.66	<0.001*	6.02 ± 1.25
Prothrombin time, seconds	10.94 ± 2.36	16.11 ± 2.86	<0.001*	13.71 ± 2.50
Plasma albumin, g/L	38.86 ± 3.58	35.54 ± 3.67	0.020*	35.63 ± 3.22
Plasma total protein, g/L	65.82 ± 5.68	63.94 ± 5.77	0.046*	66.57 ± 5.11
Hb concentration, g/L	112.13 ± 6.44	111.88 ± 6.44	0.116	112.74 ± 6.19
Serum pro-calcitonin, µg/L	1.94 ± 1.03	3.86 ± 1.57	<0.001*	2.76 ± 1.41
Serum CRP, nmol/L	126.34 ± 8.03	145.51 ± 8.48	<0.001*	133.42 ± 7.91
Serum ferritin, pmol/L	933.04 ± 17.14	954.65 ± 18.83	0.061	854.16 ± 17.63
Plasma fibrinogen, g/L	7.56 ± 1.24	8.01 ± 1.56	0.073	7.63 ± 1.35
D-Dimer, (µg/L FEU)	901.44 ± 80.54	978.65 ± 82.81	0.017*	943.53 ± 81.92
Total WBC x 10^9/L	14.96 ± 2.75	15.73 ± 2.61	0.041*	15.23 ± 2.52
Neutrophil count x 10^9/L	13.96 ± 2.36	15.84 ± 2.44	0.016*	13.91 ± 2.33
Lymphocyte count x 10^9/L	1.52 ± 0.29	1.31 ± 0.34	0.030*	1.51 ± 0.26
Monocyte count x 10^9/L	0.88 ± 0.19	0.90 ± 0.22	0.145	0.83 ± 0.17
Eosinophil count x 10^9/L	0.33 ± 0.10	0.35 ± 0.12	0.097	0.29 ± 0.08
Basophil count x 10^9/L	0.17 ± 0.17	0.18 ± 0.20	0.058	0.17 ± 0.14
Platelet count x 10^9/L	145.16 ± 8.23	141.53 ± 8.43	0.040*	149.87 ± 8.78
Red cell count x 10^{12}/L	4.32 ± 1.27	4.44 ± 1.31	0.104	4.67 ± 1.46
Oxygen saturation, %	94.03 ± 5.65	93.36 ± 5.78	0.033*	94.67 ± 5.41

*Statistically significant; HB: hepatobiliary; SD: standard deviation; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; RR: respiratory rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; G-GT: gamma-glutaryl transferase; TBil: total bilirubin; CBil: conjugated bilirubin; Hb: hemoglobin; CRP: C-reactive protein; FEU: fibrin-degradation unit; WBC: white cell count

TABLE III: COMPARATIVE ANALYSIS OF THE CLINICAL/LABORATORY CHARACTERISTICS OF CASES WITH HEPATOBILIARY PATHOLOGIES (N=66) BY COVID-19 SEVERITY

	CATEGORIES OF HB PATHOLOGIES BY COVID-19 SEVERITY, n=66			
Variables	Mild COVID-19 cases with HB Pathologies, n=13	Moderate COVID-19 cases with HB Pathologies, n=39	Severe COVID-19 cases with HB Pathologies, n=14	p-value
	Mean ± SD/n	Mean ± SD/n	Mean ± SD/n	
Age, years	39.61 ± 5.02	41.74 ± 5.20	49.82 ± 5.37	<0.001*
Males/Females	8/5	27/12	9/5	0.001*
BMI, kg/m^2	27.84 ± 4.12	28.13 ± 4.07	29.66 ± 4.79	0.204
Body temperature, ^{o}C	36.56 ± 3.12	36.73 ± 3.17	36.92 ± 3.23	0.218
SBP, mmHg	129.13 ± 5.65	135.88 ± 5.80	136.65 ± 6.07	0.094
DBP, mmHg	85.73 ± 4.58	86.91 ± 4.66	88.61 ± 4.87	0.057
HR/minute	79.86 ± 4.04	82.71 ± 4.16	84.16 ± 4.69	0.065
RR/minute	20.56 ± 2.54	22.63 ± 2.71	23.94 ± 2.92	0.058
Plasma sodium, mmol/L	136.74 ± 6.04	137.84 ± 6.89	138.11 ± 6.90	0.117
Plasma chloride, mmol/L	96.95 ± 4.51	97.57 ± 4.67	98.03 ± 4.67	0.164
Plasma bicarbonate, mmo/L	24.91± 3.71	23.67 ± 3.92	21.06 ± 3.98	0.121
Plasma urea, mmol/L	5.96 ± 1.44	6.07 ± 1.41	8.63 ± 2.03	0.062
Plasma creatinine, µmol/L	95.37 ± 6.06	103.92 ± 5.91	125.68 ± 5.73	<0.001*
RPG, mmol/L	7.76 ± 2.60	6.75 ± 2.42	5.96 ± 2.07	0.074
Plasma ALT activity, IU/L	138.84 ± 6.53	145.06 ± 6.90	149.63 ± 6.96	0.068
Plasma AST activity, IU/L	149.74 ± 7.72	195.42 ± 7.75	222.04 ± 8.11	0.091
Plasma ALP activity, IU/L	176.56 ± 8.87	186.13 ± 8.94	191.51 ± 9.14	0.041*
Plasma G-GT activity, IU/L	148. ± 9.01	183.44 ± 9.90	223.04 ± 10.02	<0.001*
Plasma TBil, ug/L	39.57 ± 4.34	56.43 ± 4.45	69.91 ± 4.86	<0.001*

TABLE III: COMPARATIVE ANALYSIS OF THE CLINICAL/LABORATORY CHARACTERISTICS OF CASES WITH HEPATOBILIARY PATHOLOGIES (N=66) BY COVID-19 SEVERITY *(CONT)*

Variables	Mild COVID-19 cases with HB Pathologies, n=13	Moderate COVID-19 cases with HB Pathologies, n=39	Severe COVID-19 cases with HB Pathologies, n=14	p-value
Plasma CBil, ug/L	6.81 ± 1.73	7.44 ± 2.45	8.16 ± 2.70	0.044*
Prothrombin time, seconds	15.23 ± 2.89	16.75 ± 2.92	19.97 ± 2.98	<0.001*
Plasma albumin, g/L	36.78 ± 3.75	35.66 ± 3.61	33.81 ± 3.24	<0.001*
Plasma total protein, g/L	63.96 ± 5.43	61.46 ± 5.37	60.20 ± 5.24	0.106
Hemoglobin concentration, g/L	110.77 ± 7.59	109.64 ± 6.43	108.66 ± 6.31	0.310
Serum pro-calcitonin, µg/L	2.56 ± 1.54	3.07 ± 1.79	3.87 ± 1.96	0.030*
Serum CRP, nmol/L	137.64 ± 8.72	141.33 ± 9.02	162.34 ± 9.16	<0.001*
Serum ferritin, pmol/L	934.34 ± 18.56	938.57 ± 18.64	973.94 ± 18.73	0.079
Plasma fibrinogen, g/L	7.20 ± 1.66	7.12 ± 1.41	6.92 ± 1.34	0.260
D-Dimer, (µg/L FEU)	906.67 ± 81.71	1,.158 ± 84.93	1,245.80 ± 85.07	<0.001*
Total WBC x 10^9/L	15.88 ± 2.84	17.04 ± 3.22	19.61 ± 3.63	<0.001*
Neutrophil count x 10^9/L	13.91 ± 2.01	15.81 ± 2.35	17.27 ± 2.55	<0.001*
Lymphocyte count x 10^9/L	1.43 ± 0.22	1.34 ± 0.35	1.30 ± 0.19	<0.001*
Monocyte count x 10^9/L	0.88 ± 0.15	0.89 ± 0.23	0.90 ± 0.07	0.114
Eosinophil count x 10^9/L	0.33 ± 0.09	0.32 ± 0.05	0.26 ± 0.03	0.301
Basophil count x 10^9/L	0.16 ± 0.14	0.15 ± 0.11	0.14 ± 0.08	0.454
Platelet count x 10^9/L	142.87 ± 8.64	138.06 ± 8.63	127.51 ± 8.34	<0.001*
Red cell count x 10^{12}/L	4.16 ± 1.54	4.02 ± 1.36	3.94 ± 1.20	0.068
Oxygen saturation, %	94.96 ± 5.95	93.23 ± 5.63	91.06 ± 5.41	<0.001*

TABLE IV: LINEAR REGRESSION ANALYSIS BY COVID-19 SEVERITY OF INFLAMMATORY MARKERS WITH HEPATOBILIARY PARAMETERS AMONG COHORTS WITH HB PATHOLOGIES, N=66

	INFLAMMATORY MARKERS					
	Crude linear regression analysis			Adjusted linear regression analysis***		
	PCT levels	CRP levels	D-dimer levels	PCT levels	CRP levels	D-dimer levels
Variables	β; p-value	β; p-value	β; p-value	β; p-value	β; p-value	β; p-value
1. Mild COVID-19, n=13						
Plasma ALT activity, IU/L	0.167; 0.116	0.102; 0.102	0.214; 0.098	0.089; 0.334	0.078; 0.245	0.168; 0.109
Plasma AST activity, IU/L	0.186; 0.127	0.190; 0.173	0.217; 0.104	0.101; 0.246	0.089; 0.341	0.144; 0.269
Plasma ALP activity, IU/L	0.136; 0.220	0.165; 0.145	0.226; 0.088	0.110; 0.201	0.104; 0.190	0.098; 0.153
Plasma G-GT activity, IUL/L	0.147; 0.200	0.191; 0.160	0.234; 0.110	0.090; 0.345	0.095; 0.199	0.113; 0.226
Plasma TBil, ug/L	0.107; 0.119	0.115; 0.121	0.206; 0.092	0.087; 0.209	0.076; 0.341	0.123; 0.177
Plasma CBil, ug/L	0.205; 0.098	0.178; 0.104	0.199; 0.087	0.114; 0.110	0.087; 0.240	0.154; 0.111
2. Moderate COVID-19, n=39						
Plasma ALT activity, IU/L	0.230; 0.115	0.244; 0.086	0.253; 0.103	0.166; 0.230	0.201; 0.117	0.190; 0.167
Plasma AST activity, IU/L	0.208; 0.150	0.210; 0.102	0.223; 0.079	0.177; 0.163	0.176; 0.122	0.180; 0.101
Plasma ALP activity, IU/L	0.184; 0.334	0.167; 0.240	0.178; 0.301	0.106; 0.415	0.099; 0.289	0.110; 0.399
Plasma G-GT activity, IUL/L	0.155; 0.410	0.231; 0.330	0.323; 0.066	0.085; 0.432	0.200; 0.394	0.226; 0.115
Plasma TBil, ug/L	0.234; 0.201	0.144; 0.340	0.140; 0.446	0.183; 0.316	0.112; 0.478	0.089; 0.521
Plasma CBil, ug/L	0.181; 0.322	0.201; 0.129	0.224; 0.446	0.134; 0.366	0.170; 0.200	0.155; 0.507
3. Severe COVID-19, n=14						
Plasma ALT activity, IU/L	0.310; 0.078	0.298; 0.167	0.227; 0.122	0.228; 0.110	0.243; 0.201	0.130; 0.084
Plasma AST activity, IU/L	0.123; 0.233	0.107; 0.104	0.111; 0.444	0.106; 0.300	0.076; 0.567	0.101; 0.226
Plasma ALP activity, IU/L	0.367; 0.012*	0.548; <0.001*	0.674; <0.001*	0.323; 0.004*	0.511; <0.001*	0.577; <0.001*
Plasma G-GT activity, IUL/L	0.390; 0.006*	0.386; 0.036*	0.422; 0.014*	0.355; <0.001*	0.345; 0.010*	0.407; <0.001*
Plasma TBil, ug/L	0.349; <0.001*	0.324; 0.010*	0.355; <0.001*	0.322; <0.001*	0.317; <0.001*	0.333; <0.001*
Plasma CBil, ug/L	0.375; 0.033*	0.316; <0.001*	0.589; 0.017*	0.311; <0.001*	0.311; <0.001*	0.561; <0.001*

*Statistically significant; HB: hepatobiliary; PCT: pro-calcitonin; CRP: C-reactive protein; β: linear regression coefficient; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; G-GT: gamma-glutaryl transferase; TBil: total bilirubin; CBil: conjugated bilirubin; ***Adjusted for age, sex, plasma creatinine, prothrombin time, plasma albumin, plasma total protein, total white cell count, neutrophil count, lymphocyte count, platelet counts, and oxygen saturation

IV. DISCUSSION

A. Principal Findings

Altered hepatobiliary status has been reported in association with COVID-19 which has been linked, with limited evidence, to the hyper-inflammation response known to occur during the disease process. Hence, the current study evaluated patterns of hepatocellular pathologies and their association with indicators of inflammation among COVID-19 patients of African origin. However, previous studies on this subject were limited by the recruitment of patients with varied comorbid conditions and those on medications to negatively influence hepatobiliary functions. Consequently, the current study evaluated the hepatobiliary status of 396 treatment-naive patients with varying severity of COVID-19 who had no pre-existing hepatobiliary disorders nor on any medications/toxicants known to influence hepatobiliary functions.

In the current study, 16.7% had hepatobiliary pathologies with almost all of those with severe COVID-19 having hepatobiliary pathology. Additionally, those having severe COVID-19 concurrently with hepatobiliary pathologies were mostly males and of older age. Cholestatic-specific pathology was the most common recorded among the generality of cases with hepatobiliary pathologies and specifically among those having mild, moderate, and severe COVID-19. Among those with severe COVID-19, positive relationships were observed between markers of inflammation (pro-calcitonin/C-reactive protein/D-dimer) and cholestatic-specific markers (ALP/G-GT/TBil/CBil), but not with the hepatocellular-specific markers (ALT/AST). In Contrast, no relationship was established between the markers of inflammation and all the cholestatic/hepatocellular markers among those with mild and moderate COVID-19.

B. Comparison with Previous Studies

The current study findings compare to some recent study findings in the literature but do contrast with some others [5]-[10], [18], [19]. In a recent and similar study documented among 417 Chinese COVID-19 patients [6], the authors recorded a 5% incident rate of hepatobiliary pathologies at admission among their studied cohorts. As was also documented in that Chinese study [6], those with hepatobiliary pathologies were mostly the males and older

age groups who had more pronounced abnormal activities of markers of hepatobiliary cholestasis (increased G-GT activities). Increased G-GT activity has been reported to be a potential diagnostic marker for the pathology of the bile duct epithelial cells (cholangiocytes) and increased G-GT activity is frequently associated with severe COVID-19 [17]. Despite the differing incidence rates of hepatobiliary pathologies between the current study and that of the Chinese study, the vast majority of the report in that Chinese study [6] agrees with the current study. The discordant incidence rates may be related to differences in the study population characteristics. In that Chinese study [6], the authors evaluated both the pediatric and adult age groups while the current study evaluated only the adult population. Unlike the current study design, the authors of the Chinese study [6] did not also categorize the pattern of hepatobiliary pathologies by COVID-19 severity nor report on the relationship between the markers of inflammation and hepatobiliary pathologies at admission.

However, in another recent and similar retrospective observational cohort study of 1,827 COVID-19 patients documented in five major United States hospitals, the authors reported a significant association between abnormal liver tests and severe COVID-19, age, and the male sex [18]. Additionally, a recent meta-analysis of existing studies on this subject provided research evidence that inflammatory markers related to hepatobiliary pathology increase in severe forms of COVID-19 compared to mild forms of the disease [19]. The conclusions from these two previous reports [18], [19] are all consistent with findings from the current study.

C. Pathomechanisms

Research data indicate the abundance of more ACE2 receptors on the bile duct epithelial cells (cholangiocytes) compared to the hepatocytes. In addition, the bile duct epithelial cells play major roles in immune responses and liver regeneration during insults to the hepatobiliary tract [20], [21]. These characteristic features have led investigators to suggest that SARS-CoV-2 has a direct cytopathic effect on the bile duct epithelial cells via the ACE2 receptors which culminate in cholestatic-specific hepatobiliary pathologies, as documented in the current study [20]-[25]. Data also indicate that the hepatocellular-specific pattern of liver pathologies occurs early during SARS-CoV-2 infection which transitions to the cholestatic-specific pattern with worsening severity of the infection [25]. As previously documented, worsening COVID-19 severity is associated with marked hyper-inflammatory responses [12]-[15]. This may also explain the correlation documented between inflammatory markers and those of cholestatic-specific markers in the current study.

D. Relevance to Clinical Practice and Future Research

The study finding has significant clinical implications. During the management of severe COVID-19, most of the drugs used in clinical practice have variable effects on the hepatobiliary system and are mostly metabolized by this organ system. Hence, the evaluation of the hepatobiliary system during the management of severe COVID-19 and the administration of less hepatotoxic drugs should be prioritized.

E. Strength and Limitations

The strength of the current study lies in the recruitment of patients without pre-existing medical history, clinical, laboratory evidence of hepatobiliary disorders nor with any hepato-biliary-influencing comorbid conditions before RT-PCR-confirmed COVID-19. Nevertheless, the study was limited by a few factors which may be improved upon in further research. First, as observed in most observational studies, its findings do not infer cause-effect relationships, but mere associations.

Secondly, as a single health facility-based study, its conclusions may not reflect the larger population within the studied geographical zone. Lastly, few markers of inflammation were analyzed, which limits the conclusions to these analyzed makers. However, the study design can be extended to a larger panel of markers, if available.

V. Conclusion

Hepatobiliary pathologies, mostly of cholestatic patterns, are frequent among the studied COVID-19 patients which were associated with inflammatory markers and severe disease. Since most of the drugs used in clinical practice for severe COVID-19 treatment have variable effects on the hepatobiliary system and are also mostly metabolized by this organ system, it becomes clinically imperative to determine hepatobiliary status during severe COVID-19 management and the administration of any hepatotoxic drugs.

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.

Authors' 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.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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