OPEN ACCESS

Scholars International Journal of Anatomy and Physiology
Abbreviated Key Title: Sch Int J Anat Physiol
ISSN 2616-8618 (Print) |ISSN 2617-345X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Association between Serum Total Bilirubin Level and Clinical Outcome of COVID-19 Infection: Protective Role of Bilirubin

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DOI: 10.36348/sijap.2023.v06i12.002 | **Received:** 25.10.2023 | **Accepted:** 01.12.2023 | **Published:** 06.12.2023

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Abstract

Background: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes covid-19 disease. The disease is a multisystem and mosaic disorder that demonstrates extra pulmonary manifestations involving liver and other organs. Elevated blood bilirubin levels have been associated with poor covid-19 disease outcome. However, discrepancies prevail in these findings. The study aimed to evaluate the association between serum total bilirubin and direct bilirubin levels and clinical outcome of covid-19 infection. ***Materials and methods***: This was a retrospective cross-sectional study. Data was obtained from medical records of patients admitted at Mount Kenya Hospital, Nyeri which was the designated county facility for diagnosis, treatment and management of covid -19 disease in Nyeri, Kenya. Data was obtained from patients' files admitted between 01/04/2021-30/09/2021. ***Inclusion criteria***: Positive covid -19 patients tested with real-time quantitative reverse transcription polymerase chain reaction test (rqRTPCR) and patients with serum total bilirubin and direct bilirubin levels done at admission. ***Exclusion criteria***: Positive rqRTPCR covid-19 patients with incomplete medical records, positive covid-19 patients tested using rapid antigen test, patients with comorbidities, and pregnant women. Categorization of liver injury based on bilirubin levels: severe liver injury >5× ULN, moderate liver injury 3–5x ULN and mild liver injury 1–2x ULN. Categorical data were presented as frequencies and the chi-square test was applied to test for associations. *P*-value < 0.05 was considered statistically significant. ***Results:*** There was no statistical significance between serum levels of total bilirubin (*P*=0.390), direct bilirubin (*P*=0.272) and covid-19 clinical outcome (survival or non-survival) and severity. ***Conclusion and Recommendations***: Serum total bilirubin and direct bilirubin levels has no significant association with covid-19 disease outcome and severity and thus are not reliable prognostic markers in our setting and in patients without preexisting comorbidities. The role of serum total bilirubin and direct bilirubin in prognosis of covid-19 be studied further to evaluate more mechanistic explanation.

Keywords: (SARS-CoV-2), covid-19 disease, rqRTPCR, liver injury.

1. INTRODUCTION

Coronavirus disease (covid-19) pandemic is the most recent global health crisis after the influenza pandemic of 1918. It has caused consequential global economic decline with loss of over 6 million lives with most of the deaths occurring in the early days of the pandemic when little was known about the virus. Covid-19 is largely associated with diffuse lung injury but the disease affects other organs including the liver which is the most commonly affected organ after the lung. Pandemics have a history of reemergence thus, there continues to be the need to define laboratory parameters that predict progression to severe forms of covid-19. Avid determination of the prognostic value of liver biochemical parameters in covid-19 disease is thus imperative and important guide in assigning status and risk stratification.

Bilirubin is the end product of the heme degradation process in mammals and it is the most potent endogenous antioxidant (Ali *et al*., 2017). At physiological levels, bilirubin exerts antioxidant effects such as scavenging reactive oxygen species (ROS) and repressing the action of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Some studies

link high serum bilirubin levels with development of ischemic strokes with poor functional outcome (Song *et al*., 2022). According to several clinical studies, the most frequent extra pulmonary manifestations of covid-19 is liver injury. Elevated bilirubin is defined as bilirubin above 1mg/dl, which was found to be elevated in a significant proportion of the Chinese patients with covid-19 (Papadopoulos *et al*., 2020). Elevated total bilirubin, as well as alanine transaminase and aspartate transaminase were found among the main liver parameter derangements in severe covid-19 disease patients, in contrast to non-severe patients, in China and Singapore (Ghahramani *et al*., 2020). Despite serum bilirubin levels derangement being common in covid-19 verification to its use as a clinical tool is inconsistent, with paucity of information in African populations. Therefore the aim of this study was to determine the association of serum total bilirubin level and clinical outcome of covid-19.

2. MATERIALS AND METHODS

2.1. Data source

The data was obtained from Mount Kenya Hospital, an annex of the main government hospital in Nyeri county, Kenya, the hospital was designated for covid-19 disease diagnosis, management and treatment. The hospital only admitted covid-19 disease confirmed cases. The data was extracted from the medical files of covid-19 in-patient cases between 01/04/ 2021-30/09/2021.During the study period the covid-19 positivity rate was at 25%, higher than the national rate of 17%.

2.2. Study design

We conducted a retrospective cross-sectional study and a census approach was used on all the 306 patient files admitted between 01/04/2021-30/09/2021.

2.3. Sample Size Calculation and Sampling Technique

Owing to inconsistency in manual record keeping, elemental bias and confounding variables that may have led to remarkable exclusion of the selected study population, a simple census technique was employed where all the 306 covid-19 patient files were used as the target population. For example, non-comorbidities patients and with positive polymerase chain reaction test were included in this study to lessen bias and establish consistency in the current study findings.

2.4. Methods and instruments of data collection

Data was collected from patient files retrieved from Mount Kenya Hospital inpatient registry of patients admitted between 01/04/2021- 30/09/2021. Data from eligible study participants was obtained and entered into password-protected researcher's data collection form, electronic excel sheet.

2.5. Techniques to minimize bias/errors

Use of census technique for sampling that enabled complete recruitment of all eligible study subjects. This approach also eliminated sampling error.

2.6. Ethical approval

Ethical approval was sought and granted by university of eastern Africa, Baraton (UEAB/ISERC/20/04/2023) and National Commission for science, technology and innovation (NACOsti) (Ref. no NACOSTI/P/23/25707). Request for waiver of informed consent was made to the Maseno university Ethical review board. As the study was of retrospective nature and the data analyzed was anonymous clinical data of the patients. Permission to access and collect data was sought and granted by Director of Medical services Nyeri County and Mount Kenya Hospital, Nyeri.

2.7. Inclusion criteria

1. Patients with positive covid-19 tested using real-time quantitative reverse transcription polymerase chain reaction test (rqRTPCR).
2. Patients with serum total bilirubin and direct bilirubin levels done at admission.

2.8. Exclusion criteria

1. Patients with positive covid-19 rqRTPCR whose medical records were incomplete
2. Patients with positive covid-19 tested using antigen rapid test
3. Patients with comorbidities
4. Pregnant women

The outcome variable of interest was survival or deceased at discharge.

2.9. Classification of serum bilirubin level abnormalities

Serum bilirubin level derangement was defined as the elevation of total bilirubin (TBIL) above the following range standards: 0-22 UMOL/L and direct bilirubin (DBIL) 0-6.8 UMOL/L in serum referring to Mount Kenya Hospital Nyeri laboratory range standards. There is still no consensus on classification of liver injury in covid-19 patients. However, in the context of the current study classification of liver injury based on serum bilirubin levels was as follows: patients with raised serum bilirubin >5× the upper limit normal (ULN) were categorized as severe liver injury; patients with raised serum bilirubin 3–5x ULN were categorized as moderate liver injury while those with raised serum bilirubin 1–2x ULN were categorized as mild liver injury.

2.10. Data analysis

Data was entered into statistical package for social sciences software, SPSS (version 26.0). Categorical data like liver injury classification based on serum levels of total bilirubin and direct bilirubin were

presented as frequencies and the chi-square test was applied to test for associations. All statistical analyses were conducted in SPSS (version 26.0) and $P< 0.05$ was considered statistically significant.

3. RESULTS

Chi-square test was carried out, serum total bilirubin and clinical outcome showed no significant statistical association P=0.390 (Table 1).

Table 1: Association between serum total bilirubin and clinical outcome

		OUTCOME		Chi-Square	P-value
		SURVIVORS	DECEASED		
TBIL	Normal	81 (75.7%)	26 (24.3%)	1.885	0.390
	Mild	4 (57.1%)	3 (42.9%)		
	Moderate	2 (100.0%)	0 (0.0%)		

Key: TBIL- total bilirubin

Of the total participants 75.7% of survivors had normal serum total bilirubin levels at admission compared to 24.3% of deceased participants (Figure 1).

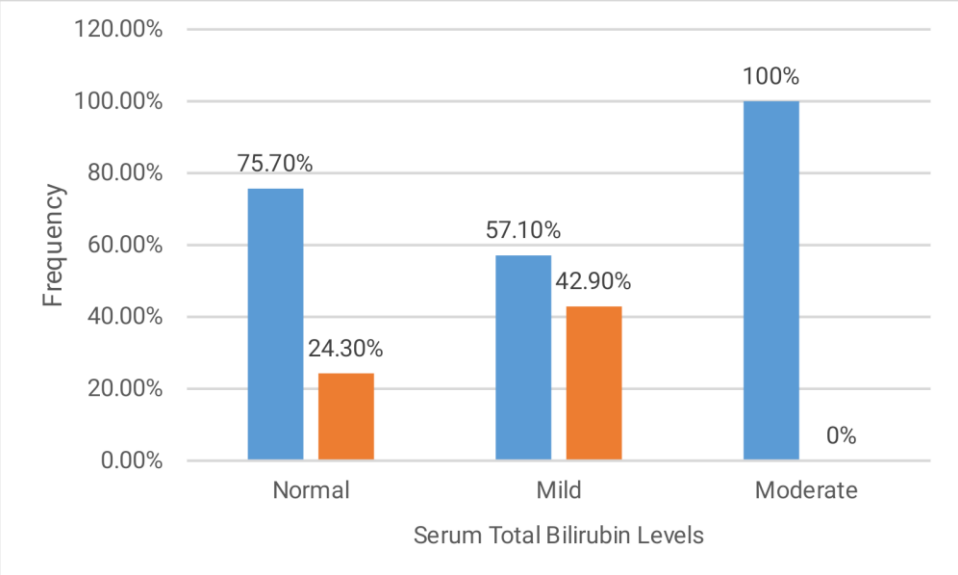

Figure 1: The proportion of serum total bilirubin levels in survivors vs. deceased

Chi-square test was carried out, serum direct bilirubin and clinical outcome showed no significant statistical association P=0.272 (Table 2).

Table 2: Association between serum direct bilirubin and clinical outcome

		OUTCOME		Chi-Square	P-value
		SURVIVORS	DECEASED		
DBIL	Normal	73 (76.8%)	22 (23.2%)	2.605	0.272
	Mild	12 (63.2%)	7 (36.8%)		
	Moderate	3 (100.0%)	0 (0.0%)		

Of the total participants 76.8% of survivors had normal serum direct bilirubin levels compared to 23.2% of deceased participants at admission (Figure 2).

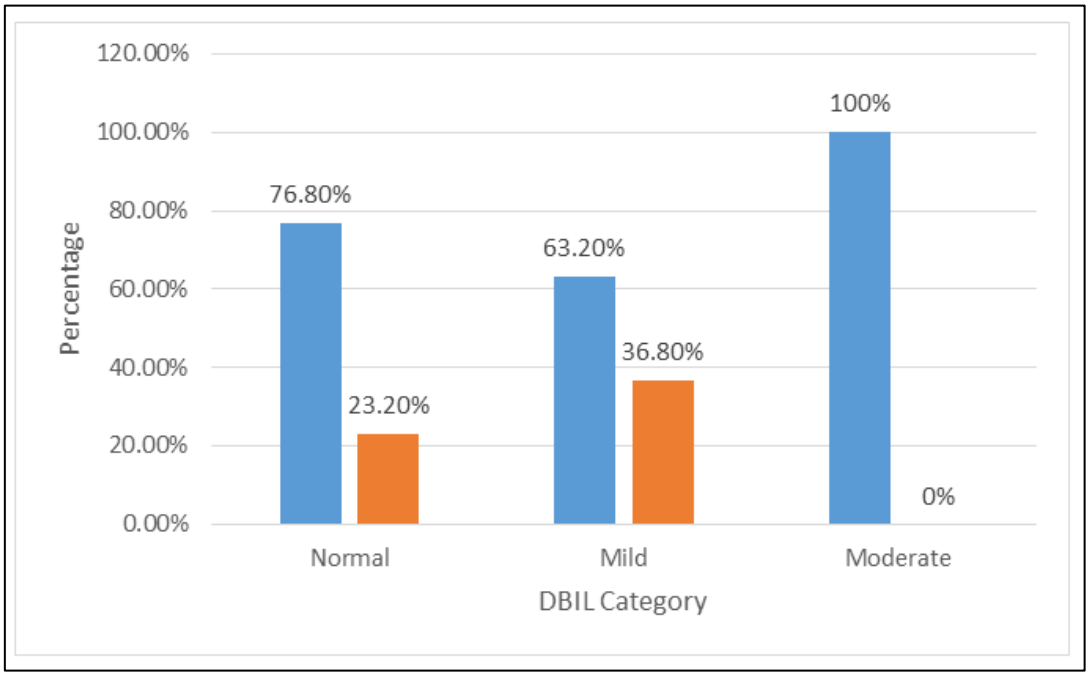

Figure 2: The proportion of serum direct bilirubin levels in survivors vs. deceased

Chi-square test was carried out, serum total bilirubin and severity of covid-19 showed no significant statistical association *P*=0.638 (Table 3).

Table 3: Association between serum total bilirubin and severity of covid-19

		COVID 19 SEVERITY		Chi-Square	P-value
		SEVERE	NON-SEVERE		
TBIL	Normal	82 (76.6%)	25 (23.4%)	0.899	0.638
	Mild	6 (85.7%)	1 (14.3%)		
	Moderate	2 (100.0%)	0 (0.0%)		

Chi-square test was carried out, serum direct bilirubin and severity of covid-19 showed no significant statistical association *P*=0.789 (Table 3).

Table 4: Association between serum direct bilirubin and severity of covid-19

		COVID 19 SEVERITY		Chi-Square	P-value
		SEVERE	NON-SEVERE		
DBIL	Normal	75 (78.9%)	20 (21.1%)	0.474	0.789
	Mild	14 (73.7%)	5 (26.3%)		
	Moderate	2 (66.7%)	1 (33.3%)		

4. DISCUSSION

For many years elevated systemic bilirubin levels have been considered an inauspicious sign in liver pathologies. Current data advocate that mildly raised bilirubin concentrations may provide powerful protective effects against oxidative stress-related diseases, including liver, cardiovascular and cancer diseases (Wagner *et al*., 2015; Gazzin *et al*., 2016) and this may be linked to reduced risk of total mortality. In this study, serum total bilirubin and direct bilirubin showed no statistically significant association with clinical outcome of covid-19 infection. Serum total bilirubin and direct bilirubin showed no significant statistical association with covid-19 severity. Bilirubin within normal levels exert antioxidant properties, with the ability to trap and break peroxyl radicals. In in vitro experiments, the antioxidant potency of bilirubin is optimized by presence of normal oxygen levels. Serum bilirubin levels may not correlate with poor prognosis but with the extent of oxidative-stress in the beginning of the disease process. This observation concurs with Elmunzer *et al*., (2021) in their retrospective study on various digestive manifestations of covid-19 that involved 1992 inpatients they found that even though gastrointestinal and hepatic anomalies including elevated total bilirubin are common they are usually mild and were not associated with worse clinical outcomes. According to a study done on bilirubin and ischemic stroke, high bilirubin level was related to the severity of oxidative-stress related disorders including liver diseases and

cerebral vascular accidents but it was not independently linked to the discharge outcome (Pineda *et al*., 2008). Bilirubin offers therapeutic advantages because it possesses antioxidant properties. According to Ali *et al*., (2017) bilirubin is the most powerful endogenous antioxidant and cushions against cell membrane lipid peroxidation. Pineda *et al*., (2008) highlighted high bilirubin levels in liver diseases and cerebral vascular accidents as an indicator of initial degree of oxidative-stress that was linked to disease severity but not to discharge outcome. This finding fails to concur with Russo *et al*., (2022) who reported a higher serum bilirubin level at hospital admission independently associated with the mortality risk during hospitalization. Equally, Liu *et al*., (2020) in a multicenter retrospective study reported high direct bilirubin levels in covid-19 patients in absence of pre-existing comorbidities was linked to severe disease and mortality. However, in this study, there was no association between serum total bilirubin or direct bilirubin levels and covid-19 clinical outcome or severity. A study done by Vitek *et al*., (2019) showed that bilirubin has a protective role whose relationship took shape in a linear manner as each unit rise of systemic bilirubin concentrations was connected to a decrease in cardiovascular and cancer risk.

Limitation/strengths of the study

The study was a single-centered cross-sectional study. The levels of liver enzymes were recorded at the time of admission only. Longitudinal approach may have helped monitor patterns in these parameters during the disease course. The study excluded patients with underlying comorbidities which was a main underlying confounder thus strengthening the study.

Conclusion and Recommendation

In this study, serum total bilirubin and direct bilirubin levels had no significant association with covid-19 disease outcome and severity and thus are not reliable prognostic markers in our setting and in patients without preexisting comorbidities. We recommend the role of serum total bilirubin and direct bilirubin in prognosis of covid-19 be studied further to evaluate more mechanistic explanation.

Acknowledgements

We greatly acknowledge the county government of Nyeri and Mount Kenya Hospital, Nyeri for allowing us access to the patients' data.

Conflict of interest: None declared

Funding: This study did not receive any funding

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