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Research Article

Serum lactate dehydrogenase activity in COVID-19 patients at admission and discharge from an isolation center: Suggestion as metabolic checkpoint

Onifade A.A., Rahamon S.K. and *Arinola O.G

Department of Immunology, College of Medicine, University of Ibadan, Nigeria

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Abstract

Coronavirus disease 2019 (COVID-19) infection is a multi-organ disease. Reports have shown that elevated activity of lactate dehydrogenase (LDH) is associated with poor outcomes in patients with viral infections. Changes in LDH activity from admission through discharge were determined in patients with SARS-CoV-2 infection with a view to determining its prognostic properties. Serum activity of LDH was determined in sixty COVID-19 patients, at admission and at discharge from the isolation centre, and sixty controls using ELISA. The mean serum LDH activity was significantly higher in COVID-19 patients at admission and at discharge compared with the controls. However, the mean LDH activity was slightly lower in COVID-19 patients at discharge compared with the activity at admission. It could be concluded from the study that LDH activity is elevated in COVID-19 patients and changes in its activity during the course of COVID-19 management could provide clinical information on patient's response to therapy.

Key Words: Inflammation, Innate Immunity, Lactate dehydrogenase, SARS-CoV-2

INTRODUCTION

Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) has been reported to be mild in few infected individuals, but a notable proportion of patients with the severe form of the disease deteriorated remarkably due to multiple organ failure and death (Arinola *et al*., 2021a).Despite avalanche of reports, the pathogenesis of COVID-19 is still not completely understood. Elevated levels of biomarkers of inflammation, cardiac and muscle injury, liver and kidney function, and coagulation have been reported in patients with COVID-19 (Arinola *et al*., 2020, Arinola *et al*., 2021b, Alonge *et al*., 2022, Akinkunmi *et al*., 2021). Therefore, the need for the identification of biomarkers with prognostic properties in the management of COVID-19 cannot be overemphasized.

Lactate dehydrogenase (LDH) is an enzyme involved in energy production. It converts lactate to pyruvate. LDH is present in almost all the cells of the body with highest levels in the heart, liver, lungs, muscles, kidneys, and blood cells (Lu *et al*., 2018). The enzyme serves as a general indicator of acute or chronic tissue damage and hence, considered an inflammatory marker (Sepulveda, 2013).Elevated LDH activities have been reported during acute and severe lung damage and in other interstitial lung infections (Mcfadden and Oliphant, 199i;Sepulveda, 2013). Considering the close relationship between LDH organ distribution with COVID-19 pathogenesis and organ involvement, it is thus, of clinical importance to determine the level of serum LDH as a prognostic factor of COVID-19. Zhang *et al*. (2020) reported that elevated LDH activitysignifies tissue hypo-perfusion and the extent of COVID-19, hence, may affect prognosis. This was further buttressed by the report of Huang *et al*. (2020) which showed that LDH is a potential prognostic biomarker in patients with COVID-19.

Elevated LDH level is an indicator of cell injury and the product of enhanced glycolytic activity due to hypoxia (Lu *et al*., 2018),the latter being associated with COVID-19 (Nitsure*et al*; 2020).The abundance of LDH in many cells and its iso-enzymatic tissue specificity facilitated its significant diagnostic properties in many diseases including myocardial infarction, liver diseases, haemolytic anaemia, and various types of myopathies (Lu *et al*., 2018). In addition, elevated LDH levels have been associated with increased morbidity and mortality in multiple diseases (Ballas 2013; Dennison *et al*., 2013).Glucose deprivation and hypoxia affect immune cell function and in-turn the outcome of SARS-CoV-2 infection (Taylor and Scholtz, 2022) and expression of hypoxia markers has been reported to be elevated in COVID-19 with necrotic fractions (Nitsure *et al*., 2020).Similarly, several reports have shown that haemolysis and inflammation are not uncommon in patients with COVID-19 (Assiri *et al*., 2013; Arinola *et al*., 2021c, Arinola *et al*., 2021d; Arinola *et al*., 2022), these thus, suggest that elevated LDH activity may contribute to the severity and mortality of COVID-19. The report of Huang *et*

**Author for Correspondence:* Tel: +2348023451520 E-mail: *drarinolaog64@yahoo.com*

al. (2020) showed that high serum LDH positively predicts adverse outcomes in patients with critical COVID-19.

Due to paucity of information on LDH activities in adult Nigerians with COVID-19, we evaluated the serum activities of LDH in COVID-19 patients at admission and discharge with a view to determining its prognostic properties. This will also, reflect the extent of organ damage in COVID-19 patients, and reveal if the damage persists from the day of admission to the day of discharge.

MATERIALS AND METHODS

Subjects and methods

Ethical approval (UI/EC/20/0283) was obtained from the University of Ibadan/University College Hospital Joint Institutional Research Ethics Committee before the commencement of this study. Also, written informed consent was obtained from each study participant.

Thirty (30) newly diagnosed adults with COVID-19 were enrolled into this study from amongst the patients on isolation at the Infectious Diseases Center (IDC), Ibadan, Nigeria, and were followed up till discharged. The COVID-19 cases were confirmed by the detection of SARS-CoV-2 nucleic acid using real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay in nasal and pharyngeal swab specimens following recommended guidelines. Whilst on admission, patients were commenced on a cocktail of medications; oral chloroquine or hydroxychloroquine for three days; oral zinc for three weeks; azithromycin for three days; Vitamin C for three weeks and Vitamin D was added to the protocol in patients with respiratory symptoms along with those whose oxygen saturation falls below 95% in room air. Chest physiotherapy was commenced, and antibiotics listed in the antibiogram for community pneumonia in Nigeria was administered via the intravenous route for a minimum of 48 hours (and later changed to oral medications) if patients had clinical features of chest infection. Subcutaneous Enoxaparin (Clexane) 40 mg daily or twice daily and Dexamethasone with doses ranging from 6mg to 10mg daily depending on the weight of the patient were added to the regime of medications in patients with lung infiltration on plain radiograph or on computerized tomography of the chest. All the patients were well hydrated and served a high-protein diet except when contraindicated. In addition, the physiotherapist carried out breathing exercises, conducted physical workout and aerobic exercises for patients twice weekly whilst indoor games were provided as the needs arose. Patients and control with co-morbidities such as liver disease, heart disease, anemia, muscle trauma, history of bone fractures, cancers, and infections were excluded. Pregnant females were also excluded. These strict criteria accounted for the low number of participants.

Twenty (20) gender-and age-matched apparently healthy adults recruited from amongst the staff and students of the University College Hospital, Ibadan, Nigeria served as controls. SARS-CoV-2 infection status of the controls was confirmed using a rapid diagnostic kit. Veinous blood (5 ml) was obtained from each participant and dispensed into sample bottles without anti-coagulants. Serum was obtained from the blood after contraction and centrifugation at 4000 rpm for 10 minutes. LDH activity was determined spectrophotometrically at 340 nm as described by the manufacturer (Fortress Diagnostics Lmt, UK). Independent Student's t-test was used to determine the differences in the mean LDH activities between COVID-19 patients and the controls. P-value < 0.05 was considered statistically significant.

RESULTS

The characteristics of the COVID-19 patients are presented in Table 1. Most of the COVID-19 patients were non-civil servants (artisans, self-employed and non-governmental companies), mostly females and aged between 10-65 years. There was no mortality among the patients and the hospital stay was between 3–21days (Table 1). The mean ± SD of serum LDH in COVID-19 patients at admission or at discharge compared with control is presented in Table 2. The result in Table 2 shows that the mean serum LDH level in COVID-19 patients at admission or at discharge was significantly higher compared with the controls. Also, the mean LDH activity in COVID-19 patients at admission was non-significantly higher compared with the level in COVID-19 at discharge (Table 2).

Table 1:
Characteristics of COVID-19 patients and controls

Variables	COVID-19 patients (n, %)	Controls (n, %)
Age		
15-35 years	18 (61.0)	13 (64.0)
>35 years	12 (39.0)	7 (36.0)
Gender		
Male	20 (65.0)	12 (60.0)
Female	11 (35.0)	8 (40.0)
Occupation		
Non-civil servant	26 (85.0)	18 (90.0)
Civil servant	5 (15.0)	2 (10.0)
Mortality	0	Not Applicable
Co-morbidity		
Hypertension	18 (60.0)	0
Peptic Ulcer	2 (7.0)	0
Diabetes mellitus	10 (33.0)	0
Length of hospitalization		
≤10 days	23 (76.0)	Not Applicable
11-21days	4 (14.0)	Not Applicable

Table 2:
Mean serum LDH activities in COVID-19 patients at admission and discharge compared with the controls

	At admission	At discharge	Controls	P1	P2	P3
LDH (U/L)	261.59 ± 66.16	242.10 ± 99.67	164.06 ± 15.72	0.000 *	0.000 *	0.459

*Significant at P < 0.05, LDH = Lactate dehydrogenase, P1 = P-value for comparison between COVID-19 patients at admission and the controls, P2 = P-value for comparison between COVID-19 patients at discharge and the controls, P3 = P-value for comparison between COVID-19 patients at admission and at discharge

DISCUSSION

COVID-19 is characterised by increased energy requirement that is associated with elevated metabolic rate (He *et al*., 2020), increased glucose uptake (Smith *et al*., 2021), and elevated levels of respiratory burst factors (Arinola *et al*., 2021c). Reports have shown that elevated LDH activity is a common feature in COVID-19 patients, and it is associated with poor clinical outcome (Huang *et al*., 2020). Although elevation in LDH activity has been associated with various infectious and chronic diseases, its activity could also be suggested as a supportive tool in diagnosing COVID-19 and could be of clinical importance in monitoring responses to therapy.

LDH, a cytosolic enzyme, plays important role in aerobic glycolysis. Its plasma level increases when the cell membrane is damaged and it is thus, used as an indicator of cell injury and necrosis (Huang *et al*., 2020, Zhang *et al*., 2020). Severe infection is linked with cytokine-mediated tissue damage and LDH release (Martinez-Outschoorn *et al*., 2011). The observed elevated LDH activity in COVID-19 patients in this study corroborates the report of Huang *et al*. (2020) which showed that elevated LDH activity is a common feature in COVID-19 patients. Our observation could be due to hypoxia, cytokine storm and necrosis which are key features in COVID-19 patients (Nitsure *et al*., 2020; Zhang *et al*., 2020). However, it will be of clinical importance if the LDH isozyme whose activity is elevated is identified. Farhana and Lappin (2022) showed that the activity of LDH isozyme 3, which is present in lung tissue, is elevated in patients with COVID-19.

Cell immune function is hampered by glucose deprivation and hypoxia (Taylor and Scholz 2022). Also, immune response to COVID-19 has been shown to be impaired by hypoxia or over-expression of hypoxia-regulating factors. As with other viral infections, T cells responses are essential for viral clearance, provision of effective memory, and mediation of viral variants recognition (Moss *et al*., 2022). However, effective response by effector T cells is highly dependent on aerobic glycolysis via LDH activity. Aerobic glycolysis in T cells is regulated by the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Besides its metabolic function, GAPDH is also involved in regulation of mRNA translation (Chang *et al*., 2013). Dennison *et al*.(2013), in an in vitro study, showed that T cells proliferation is reduced during glucose deprivation. Also, when T-lymphocytes are glucose-deprived, GAPDH binds IFN-gamma (IFNγ) mRNA thereby preventing its translation and its subsequent production (Chang *et al*., 2015; Renner *et al*., 2015). IFN-γ is pivotal in anti-viral immunity. It could therefore be suggested that elevated LDH activity in COVID-19 patients contributes to dampened T-cell functions and increased susceptibility to SARS-CoV-2 infection.

Altered metabolism is increasingly being recognized as an important hallmark of COVID-19 (Arinola *et al*., 2021e, Onifade *et al*., 2022). During hypoxia, there is a switch from oxidative metabolism to glycolytic metabolism. This switch facilitates significant reduction in cellular oxygen available for ATP production. It has been shown that hypoxia and its associated glycolytic metabolism contribute to an immune suppressive microenvironment (Taylor and Scholz 2022). Therefore, LDH activity could partially reflect glycolytic activities. It has been shown that patients with elevated LDH activity benefit less from immunotherapy but might benefit more from a combination of checkpoint LDH inhibitors and glycolysis inhibitors (Van-Wilpe *et al*., 2020). At present, no glycolysis inhibitor has been approved in clinical practice. However, several glycolysis inhibitors are being developed with a number of them undergoing evaluation in preclinical and early clinical trials in cancer therapy (Gottfried *et al*., 2013; Katt and Cerione, 2019). The reports of Gottfried *et al*. (2013) and Lacroix *et al*. (2018) showed that diclofenac, a non-steroidal anti–inflammatory drug, exhibits glycolytic inhibitory properties and demonstrated positive effect on response to checkpoint inhibitors in mice. Future studies that will assess the therapeutic value of glycolysis inhibitors and checkpoint LDH inhibitors in severe COVID-19 patients with elevated LDH activity are suggested.

Elevated LDH activities have been associated with poor outcomes in patients with COVID-19 (Nitsure *et al*., 2020; Zhang *et al*., 2020). The observed slight reduction in LDH activity in COVID-19 patients at discharge compared with the activity at admission might suggest reduction in haemolysis, cell damage and inflammation that are associated with COVID-19 (Arinola *et al*., 2021b; Arinola *et al*., 2022). It is not known if our present observation was as a result of intervention and would require further investigations. Therefore, further investigations would be required to understand the clinical interplay between changes in LDH activities during the course of COVID-19 management and patient's response to therapy.

Despite the significant differences in the activities of LDH in COVID-19 patients and control, it was observed that the values were within the normal reference range in participants with COVID-19 and the controls (105 - 333 IU/L) (Carty *et al*., 2017). This probably indicates that majority of COVID-19 patients in our study had mild COVID-19. This observation corroborates previous reports which classified COVID-19 in patients from the same IDC as mild (Alonge *et al*., 2022, Arinola *et al*., 2022).

CONCLUSION

LDH activity is elevated in COVID-19 patients and changes in its activity during the course of COVID-19 management could provide clinical information on patient's response to therapy. Further studies investigating the possible use of LDH inhibitors as metabolic checkpoint in the management of COVID-19 patients are suggested.

Declarations
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