Baseline Electrolytes as Determinants of Severity Among Hospitalized Covid-19 Patients: A Case-Control Study

Kaleem Maqsood[*], Shaaf Ahmad[**], Azeem Saeed[***], Nabila Roohi[*]

*Institute of Zoology, University of the Punjab, Lahore
**King Edward Medical University/Mayo Hospital, Lahore
***Allama Iqbal Medical College/Jinnah Hospital, Lahore

***Abstract*- Background:** SARS-CoV-2, the virus that has spread globally, has infected hundreds of million persons and caused over 6.9 million deaths. It is frequently observed that COVID-19 patients experience disturbances in electrolytes homeostasis. **Aim/Objective:** The current study aimed to assess the serum electrolyte variations in patients with COVID-19 and their relationship to the severity. **Methodology:** Blood samples from 187 COVID-19 patients (mild=129 and severe=58) and 60 healthy individuals of the same age group as the study's control were collected. Serum was separated from collected blood samples to assess the levels of the electrolytes. The analysis was done using commercially available kits through a biochemistry analyzer, and obtained data were analyzed statistically through One-Way ANOVA using GraphPad Prism software. **Results:** Results from intergroup comparison showed that the levels of sodium and calcium were significantly ($P \leq 0.001$) low in patients' groups compared to controls. Potassium and magnesium levels in both the mild and severe patient groups also declined significantly ($P \leq 0.01$) compared to the control. However, phosphorous and chloride were not changed considerably in the patients.
Conclusion: It was concluded from the results that the COVID-19 infection has altered the serum electrolytes, which is linked with an enhanced risk of severity and mortality. Our results provide important markers of severity in COVID-19 patients, which may be used as promising clinical biomarkers for discriminatory diagnosis.
***Index Terms*-** Electrolytes; COVID-19; Severity.

I. INTRODUCTION

Coronavirus disease 2019 (COVID-19), a global health challenge, caused by a coronavirus strain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This coronavirus strain belongs to the Beta-coronavirus genus. Its emergence has profoundly impacted the lives of billions of individuals worldwide [1]. COVID-19 exhibits various clinical presentations, from individuals with no symptoms to those experiencing severe respiratory failure [2]. Patients can be classified into mild and severe types based on clinical signs. Mild cases typically involve either mild or no pneumonia.

On the other hand, severe cases exhibit various clinical indicators such as dyspnea, respiratory frequency of 30 or more breaths per minute, and lung infiltrates exceeding 50% within a 24 to 48-hour timeframe. In some instances, the condition may worsen, leading to respiratory failure, septic shock, and dysfunction of multiple organs [3]. Approximately 80% of COVID-19 infections manifest as mild or moderate cases, yet they remain contagious and have the potential to progress toward a severe or critical state. Given the ongoing spread of the virus, early detection of viral infection is crucial to minimize the number of individuals with mild or moderate symptoms and prevent disease exacerbation [4].

COVID-19 virus enters cells by attaching itself to angiotensin I-converting enzyme 2 (ACE2) [5]; ACE2 is a membrane protein and a crucial part of the renin-angiotensin system (RAS), which regulates electrolyte balance and blood pressure [6]. SARS-CoV-2 disrupts the regulatory function of ACE2 in the RAS, leading to electrolyte imbalances and increased blood pressure [7]. Additionally, it is common for COVID-19 patients to experience gastrointestinal symptoms like diarrhea and vomiting [8], which results in disturbed electrolyte homeostasis.

COVID-19 has been observed to affect multiple organs. Studies also indicate that more than 75% of patients hospitalized with COVID-19 experience some level of renal involvement throughout the disease [9]. Early studies on COVID-19 have provided evidence suggesting the presence of electrolyte disorders, including abnormalities in sodium, potassium, chloride, and calcium levels, upon patients' initial presentation [2, 10].

COVID-19 patients who develop hyponatremia, hypochloremia, or hypocalcemia often require more frequent admission to ICU, and experience higher mortality rates, and have more extended hospital stays [11]. Additionally, individuals with severe cases of COVID-19 are more likely to exhibit hypokalemia [12]. Furthermore, hypocalcemia is associated with enhanced mortality and complications [13]. These electrolyte disorders can potentially improve the susceptibility to fatal arrhythmias in individuals affected by COVID-19 [14]. Emerging research indicates a potential association between decreased sodium levels, known as hyponatremia, and the progress of severe COVID-19. It has been suggested that lower sodium in the blood may increase the risk and severity of the disease [15]. Furthermore, hyponatremia may serve as an indicator of more advanced stages of COVID-19.

The early recognition of COVID-19 patients at risk of increasing severity is crucial for better prognoses. Moreover, the similarity of initial clinical symptoms between confirmed and suspected cases poses challenges in distinguishing between them. Any misdiagnosis can have detrimental outcomes in controlling the spread of the epidemic. These electrolyte disturbances provide insights into potential pathophysiology underlying COVID-19, which could drive the exploration of novel prognostic and therapeutic opportunities.

II. MATERIALS AND METHODS

For this case-control study, we enrolled 247 participants who met the specified inclusion criteria. Among these individuals, 187 were infected with COVID-19 (Mild=129 and Severe=58) and were selected from Mayo Hospital and Ittefaq Hospital in Lahore. Additionally, we recruited 60 healthy individuals of similar age groups as controls from the University of the Punjab, Lahore. The Institute of Zoology, University of the Punjab, Lahore's ethical review committee approved this study.

Before enrollment, all participants were presented with detailed information about the study, and written consent was taken from each person. The study excluded subjects who had comorbidities and individuals who were smokers. A comprehensive proforma was designed to gather data on the patients' clinical symptoms and collect socio-demographic information from all participants.

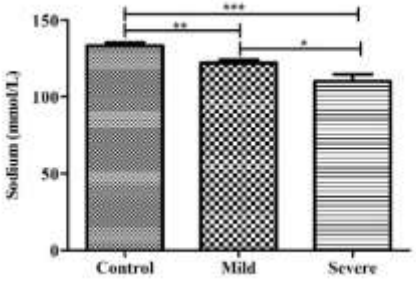
The blood sample collection process involved the assistance of a registered laboratory technician. Sterilized Becton Dickinson syringes were used to draw 5 ml of blood from each participant. Following the blood collection, the obtained blood samples were transferred into labeled red serum tubes containing clot activators. These tubes were then transported to the Physiology/Endocrinology laboratory of the Institute of Zoology at University of the Punjab, Lahore. Upon arrival, the vacutainers were kept at room temperature for thirty minutes to allow clot formation. Subsequently, the tubes were placed in a centrifuge and spun at 4000rpm for 15 minutes to separate serum. Obtained serum was carefully poured into labeled Eppendorf tubes and stored at -80°C till further analysis.

In the biochemical analysis, the concentration of the phosphorous, chloride, and calcium was measured by Monlab (Spain) kits. In contrast, sodium and potassium levels were measured by commercially available kits of Arena bioscience and magnesium with AMP diagnostics. The biochemical analysis was conducted in the Physiology/Endocrinology laboratory, Institute of Zoology, University of Punjab Lahore, using the biochemistry analyzer 5010 V5+ manufactured by Robert Riele KG.

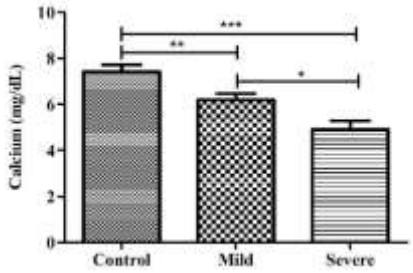
The statistical analysis of the obtained results was brought through One-Way ANOVA to identify variations between the groups at a significance level of $P \leq 0.05$. This data analysis was conducted using GraphPad Prism software. The results were presented in tabular and graphical forms, showing the mean ± SEM.

III. RESULTS

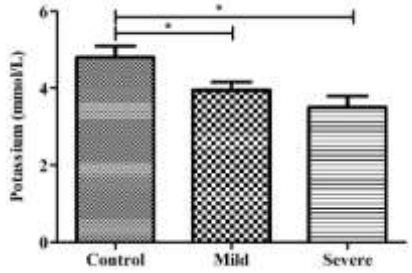
An intergroup comparison of mean±SEM values of serum electrolyte levels was conducted using One-Way ANOVA, comparing the levels between individuals having mild and severe patients with a control group.



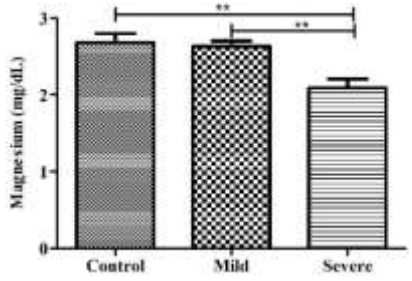
(Fig: A)



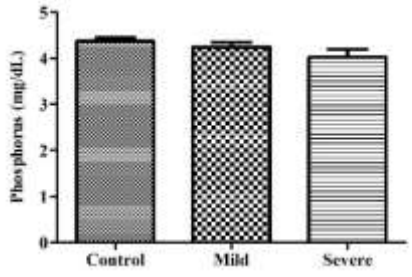
(Fig: B)



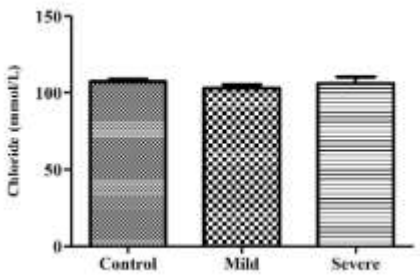
(Fig: C)



(Fig: D)



(Fig: E)



(Fig: F)

Figure (A-F): Intergroup comparison of serum electrolytes levels in control and patients with mild and severe COVID-19. *, ** and *** correspond to significance at P≤ 0.05, 0.01, and 0.001, correspondingly.

The results showed that serum sodium and calcium levels were significantly lower ($P \leq 0.001$) in the mild and severe patient compared to control. Additionally, the severe patient group had substantially lower sodium levels ($P \leq 0.05$) compared to the mild group (Table 1, Fig. A and B). However, potassium levels in both the mild and severe patient groups were significantly lower ($P=0.008$) compared to the control group (Table 1, Fig. C). The serum magnesium levels in both the mild and severe patient groups were significantly low ($P=0.0026$) compared to the control group. Among the patients' groups, the severe group showed a significant ($P \leq 0.01$) decline (Table 1, Fig. D). Among the electrolytes, phosphorous and chloride in the patients were not altered significantly (Table 1, Fig. E and F).

Table 1: Intergroup comparison of Mean ± SEM serum electrolytes of control and COVID-19 patients' groups.

Parameters	Mean ± SEM			*P-V*alue
	Control (n=60)	Mild (n=129)	Severe (n=58)	
Sodium (mmol/L)	133.40±1.85	122.00±2.23	110.20±4.59	< 0.0001
Calcium (mg/dL)	7.42±0.30	6.18±0.28	4.89±0.38	< 0.0001
Potassium (mmol/L)	4.79±0.29	3.95±0.21	3.51±0.28	0.0080
Magnesium (mg/dL)	2.68±0.12	2.63±0.07	2.09±0.12	0.0026
Phosphorus (mg/dL)	4.37±0.08	4.24±0.10	4.02±0.17	0.1498
Chloride (mmol/L)	107.30±1.49	103.10±2.33	106.10±4.34	0.3691

IV. DISCUSSION

COVID-19 poses an extraordinary threat to global healthcare systems. While several studies have discussed the clinical features of COVID-19 patients in various centers, comprehensive clinical characteristics of patients with disease severities are still limited. Our study focused on electrolyte alterations in COVID-19 patients with varying disease severities in Lahore, Pakistan.

Our research findings proved that hyponatremia was common among patients with severe COVID-19 compared to the mild group. However, in comparison to controls, both were hyponatremic. Patients diagnosed with hyponatremia exhibited a higher likelihood of experiencing a more severe illness in COVID-19. A previous study has indicated that the primary contributors to serum sodium disorders are enhanced electrolyte loss through the gastrointestinal tract and impaired kidney function [16].

Hu, Lv [17] revealed that intestinal electrolyte loss does not significantly influence the developing sodium imbalance in COVID-19 patients. Conversely, it is suggested that impaired kidney function may be associated with the reduced renal capability to regulate electrolyte levels in individuals with COVID-19 effectively.

A recent case series Ravioli, Niebuhr [18] and Yousaf, Al-Shokri [19] has documented syndrome of inappropriate antidiuretic hormone secretion or SIADH linked with COVID-19, reveals that patients having fever and hyponatremia was a significant prognostic factor for COVID-19. The assumed mechanisms of SIADH in COVID-19 disease contain the release of pro-inflammatory cytokines, ventilation-perfusion mismatch, depletion of intravascular volume, and various factors such as stress, pain, and emotions. Positive-pressure ventilation (PPV) is also a well-known factor that can lead to the development of hyponatremia and SIADH. This is likely due to the non-osmotic stimulation of ADH as the baroreceptors of pulmonary veins respond to a decrease in effective blood volume. Consequently, SIADH may contribute to the poor treatment outcomes of COVID-19 patients receiving PPV [18].

Our study also revealed a significant clinical value of hypocalcemia in diagnosing various severities of COVID-19 patients. Compared to individuals with moderate infections, those with severe cases of COVID-19 exhibited a higher likelihood of experiencing hypocalcemia. A study conducted by Yang, Ma [20] further demonstrated that abnormalities in serum calcium (Ca) and serum phosphorus (P) levels could act as indicators of the severity of COVID-19. Frater, Zini [21] proposed that Ca could improve diagnostic performances and early recognition of severe COVID-19 patients.

Furthermore, according to Yang, Ma [20], hypocalcemia exhibited the highest specificity among key clinical parameters. Kelly and Levine [22] proposed hypocalcemia can arise from various causes, including endocrine illnesses and side effects of medication. These causes may involve insufficient secretion of parathyroid hormone (PTH) or enhanced resistance to its action, deficiency of vitamin D, reduced dietary intake, hypomagnesemia, hypoproteinemia, and interactions with certain medications. Experiments of Nieto-Torres, Verdiá-Báguena [23] showed that the SARS-CoV- 2 E gene translates a transmembrane protein

having ion channel action permeable to Ca2+ and causes the disruption of Ca homeostasis.

Among COVID-19 patients, our study revealed that hypokalemia was a frequently observed electrolyte abnormality. The patients with severe infection were more hypokalemic than mild, as observations of Alfano, Ferrari [24] indicated that patients who encountered hypokalemia had considerably prolonged hospital stays compared to the control group.

COVID-19 patients were found to have about three predisposing risk factors for hypokalemia compared to the general population. Firstly, respiratory alkalosis caused by hypoxia-driven hyperventilation may contribute to transcellular shifts and increased intracellular potassium uptake. Secondly, anorexia resulting from prolonged use of face masks, ventilation helmets, or the severity of the illness can lead to reduced potassium intake. Thirdly, diarrhea, either due to medications or the viral cytopathic effect on gastrointestinal cells, has been identified as a contributing factor [25]. In an abstract from a Chinese article, hypokalemia was reported as a side effect of glucocorticoids in patients infected with SARS-CoV-1 [26]. These medications increase the urinary excretion of potassium by activating the mineralocorticoid receptors on kidney tubular cells [27].

Magnesium, a vital element in fundamental biochemical reactions, is crucial in various physiological functions and normal metabolism. In the context of COVID-19, potential causes of hypomagnesemia include reduced vitamin D absorption, sequestration of vitamin D in adipose tissue, or diminished capacity for vitamin D activation [28]. A study conducted in Iran demonstrated low serum levels of magnesium among intensive care unit (ICU) patients [29]. We also observed similar results in our study on COVID-19 patients in Lahore. Li, Luo [30] have suggested paying special attention to magnesium in COVID-19, COVID-19 disrupts the balance of magnesium in the body, and a magnesium deficit amplifies the risk of oxidative stress to endothelial cells.

Consequently, endothelial dysfunction occurs, leading to impaired fibrinolysis and an elevated risk of coagulation [30]. One contributing factor to hypomagnesemia in COVID-19 is the stress induced by the pandemic. The release of stress hormones such as catecholamines and corticosteroids during periods of heightened stress leads to the redistribution of magnesium from intracellular spaces to the extracellular space, resulting in increased urinary excretion of Mg2+. This initiates a vicious cycle, as the release of additional stress hormones perpetuates hypomagnesemia, exacerbating the condition [29].

A mild drop in phosphorous was observed among the patients compared to controls. Zhao, Li [31] demonstrated that serum phosphorous levels depend on dietary intake, renal glomerular function, tubular reabsorption, and intra-to-extracellular transfer of a phosphate.

According to Lippi, South [32], there were no significant chloride differences in blood between severe and mild COVID-19. The findings of our study were like his results.

To validate the findings, conducting studies involving a larger population with an extended follow-up period is necessary. While the diagnostic performance of electrolyte measurements has been improved, further research is needed to elucidate the underlying mechanisms responsible for these observed changes.

In conclusion, this study highlights the importance of closely monitoring serum electrolyte levels in COVID-19 patients. Specifically those having a severe infection and promptly initiating appropriate treatment. Such measures significantly contribute to enhancing patient prognosis. Given the ongoing global COVID-19 pandemic, it is strongly recommended that frontline healthcare professionals maintain a high level of awareness regarding the potential occurrence of electrolyte imbalances in managing COVID-19 patients.

ACKNOWLEDGMENT

The authors of the present investigations gratefully acknowledge Institute of Zoology, University of the Punjab for providing financial assistance.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

1. Nishiga, M., et al., *COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives.* Nature Reviews Cardiology, 2020. **17**(9): p. 543-558.
2. Huang, C., et al., *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.* The lancet, 2020. **395**(10223): p. 497-506.
3. Wu, Z. and J.M. McGoogan, *Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention.* jama, 2020. **323**(13): p. 1239-1242.
4. Zhou, X., et al., *Low serum calcium: a new, important indicator of COVID-19 patients from mild/moderate to severe/critical.* Bioscience reports, 2020. **40**(12).
5. Chen, L., et al., *The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2.* Cardiovascular research, 2020. **116**(6): p. 1097-1100.
6. Santos, R.A., A.J. Ferreira, and A.C. Simões e Silva, *Recent advances in the angiotensin-converting enzyme 2–angiotensin (1–7)–Mas axis.* Experimental physiology, 2008. **93**(5): p. 519-527.
7. Bernardi, S., et al., *High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage.* Nephrology Dialysis Transplantation, 2012. **27**(5): p. 1793-1800.
8. Wu, F., et al., *A new coronavirus associated with human respiratory disease in China.* Nature, 2020. **579**(7798): p. 265-269.
9. Pei, G., et al., *Renal involvement and early prognosis in patients with COVID-19 pneumonia.* Journal of the American Society of Nephrology, 2020. **31**(6): p. 1157-1165.
10. Guan, W.-j., et al., *Clinical characteristics of coronavirus disease 2019 in China.* New England journal of medicine, 2020. **382**(18): p. 1708-1720.
11. Tezcan, M.E., et al., *Baseline electrolyte abnormalities would be related to poor prognosis in hospitalized coronavirus*

disease 2019 patients. New Microbes and New Infections, 2020. **37**: p. 100753.
12. Chen, D. and X. Li, *Song q, Hu C, Su F, Dai J.* Hypokalemia and clinical implications in patients with coronavirus disease, 2019.
13. Alemzadeh, E., et al., *The effect of low serum calcium level on the severity and mortality of Covid patients: A systematic review and meta-analysis.* Immunity, Inflammation and Disease, 2021. **9**(4): p. 1219-1228.
14. Basu-Ray, I., A. Adeboye, and M.P. Soos, *Cardiac manifestations of coronavirus (COVID-19)*, in *StatPearls [Internet]*. 2022, StatPearls Publishing.
15. Luo, Y., Y. Li, and J. Dai, *Low blood sodium increases risk and severity of COVID-19: a systematic review, meta-analysis and retrospective cohort study.* medRxiv, 2020: p. 2020.05. 18.20102509.
16. Sterns, R.H., *Disorders of plasma sodium—causes, consequences, and correction.* New England Journal of Medicine, 2015. **372**(1): p. 55-65.
17. Hu, W., et al., *Disorders of sodium balance and its clinical implications in COVID-19 patients: a multicenter retrospective study.* Internal and Emergency Medicine, 2021. **16**: p. 853-862.
18. Ravioli, S., et al., *The syndrome of inappropriate antidiuresis in COVID-19 pneumonia: report of two cases.* Clinical Kidney Journal, 2020. **13**(3): p. 461-462.
19. Yousaf, Z., et al., *COVID-19-associated SIADH: a clue in the times of pandemic!* American Journal of Physiology-Endocrinology and Metabolism, 2020.
20. Yang, C., et al., *Low serum calcium and phosphorus and their clinical performance in detecting COVID-19 patients.* Journal of medical virology, 2021. **93**(3): p. 1639-1651.
21. Frater, J.L., et al., *COVID-19 and the clinical hematology laboratory.* International journal of laboratory hematology, 2020. **42**: p. 11-18.
22. Kelly, A. and M.A. Levine, *Hypocalcemia in the critically ill patient.* Journal of intensive care medicine, 2013. **28**(3): p. 166-177.
23. Nieto-Torres, J.L., et al., *Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome.* Virology, 2015. **485**: p. 330-339.
24. Alfano, G., et al., *Hypokalemia in patients with COVID-19.* Clinical and experimental nephrology, 2021. **25**: p. 401-409.
25. Bowling, C.B., et al., *Hypokalemia and outcomes in patients with chronic heart failure and chronic kidney disease: findings from propensity-matched studies.* Circulation: Heart Failure, 2010. **3**(2): p. 253-260.
26. Li, N., et al., *Side effects of glucocorticosteroids in the management of 1291 patients of SARS.* Beijing da xue xue bao. Yi xue ban= Journal of Peking University. Health sciences, 2004. **36**(5): p. 519-524.
27. Palmer, B.F., *Regulation of potassium homeostasis.* Clinical Journal of the American Society of Nephrology, 2015. **10**(6): p. 1050-1060.
28. Cooper, I.D., et al., *Relationships between hyperinsulinaemia, magnesium, vitamin D, thrombosis and COVID-19: rationale for clinical management.* Open Heart, 2020. **7**(2): p. e001356.
29. Sarvazad, H., et al., *Evaluation of electrolyte status of sodium, potassium and magnesium, and fasting blood sugar at the initial admission of individuals with COVID-19 without underlying disease in Golestan Hospital, Kermanshah.* New microbes and new infections, 2020. **38**: p. 100807.
30. Li, Y., W. Luo, and B. Liang, *Circulating trace elements status in COVID-19 disease: A meta-analysis.* Frontiers in Nutrition, 2022. **9**.
31. Zhao, Y., et al., *effect of hypophosphatemia on the withdrawal of mechanical ventilation in patients with acute exacerbations of chronic obstructive pulmonary disease.* Biomedical reports, 2016. **4**(4): p. 413-416.
32. Lippi, G., A.M. South, and B.M. Henry, *Electrolyte imbalances in patients with severe coronavirus disease 2019 (COVID-19).* Annals of clinical biochemistry, 2020. **57**(3): p. 262-265.

AUTHORS

First Author: Kaleem Maqsood
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0003-2330-3162

Second Author: Shaaf Ahmad
King Edward Medical University/Mayo Hospital,
Hospital Road, Lahore, Punjab 54000, Pakistan
https://orcid.org/0000-0003-0749-187X

Third Author: Azeem Saeed
Allama Iqbal Medical College/Jinnah Hospital,
Lahore Punjab 54000, Pakistan
https://orcid.org/0009-0000-4980-2985

Fourth & Corresponding Author: Prof. Dr. Nabila Roohi
Institute of Zoology, University of the Punjab, Lahore -54590
https://orcid.org/0000-0002-2396-5433