ORIGINAL RESEARCH ARTICLE

Investigating Differences in Bicarbonate Levels: Exploring Discrepancies between Venous and Arterial Measurements and Evaluating Stability through Time-point Assessments

Shobha C Ramachandra[1], Akila Prashant[2], Suma MN[3], Swetha NK[4]

Received on: 31 January 2024; *Accepted on:* 20 February 2024; *Published on:* 18 March 2024

Abstract

Introduction: Evaluation of acid-base status is crucial in critical care settings, with bicarbonate serving as a key indicator of electrolyte distribution and anion deficit. This study explored the challenges and uncertainties surrounding the quantification and stability of bicarbonate levels, crucial for accurate clinical assessments.

Methods: The study conducted in the clinical biochemistry laboratory of a tertiary care hospital involves a comparative analysis between serum and arterial bicarbonate levels. We examined serum bicarbonate in 31 patient samples, with concurrently calculated arterial values obtained from blood gas analysis reports of the same patients. Additionally, the stability of serum bicarbonate was assessed at different time intervals.

Results: A significant correlation was observed between serum and arterial bicarbonate ($r = 0.91$), which was reinforced by Bland–Altman analysis. However, the stability assessment revealed a decrease in serum bicarbonate levels at 2 and 4 hours.

Conclusion: This study contributes to the exploration of simplified methods for assessing acid-base status, particularly valuable in less-equipped conditions. The findings underscore the necessity for awareness among healthcare professionals regarding the impact of preanalytical variables, particularly uncapped tube storage, on serum bicarbonate levels.

Keywords: Arterial bicarbonate, Preanalytical variables, Serum bicarbonate, Stability evaluation.

Indian Journal of Medical Biochemistry (2023): 10.5005/jp-journals-10054-0226

Introduction

Evaluating acid-base status is vital in critical care settings, and serum bicarbonate plays a critical role. Bicarbonate ions, under normal physiological conditions, make up approximately 95% of the total serum CO_2 content. However, the stability of the bicarbonate is greatly influenced by the method of estimation, storage of samples, temperature, etc. Bicarbonate is estimated through venous blood or calculated by using the Henderson–Hasselbalch equation in an arterial blood gas (ABG) analyzer and both are used interchangeably. However, there is a lack of consensus regarding the optimal quantification of these parameters.[1]

Bicarbonate levels although calculated through ABG analysis; its practical application is impeded by challenges related to patient acceptance due to associated pain, potential complications, and limitations in the availability, especially in less equipped laboratory settings.[2] Past investigations employing diverse statistical approaches to evaluate the concordance between serum and arterial bicarbonate have yielded conflicting outcomes. Few studies demonstrated strong agreement, while others have reported discordant results.[2–4] This variability underscores the need for further clarification in the assessment of bicarbonate levels in clinical settings.

Clinical laboratory test results serve as crucial tools for clinicians in diagnosing, monitoring, and assessing the prognosis of patients with various diseases.[5] However, the accuracy of test results can be influenced by various preanalytical variables such as sample transportation, storage time, temperature, and also by contact of plasma or serum with cells leading to bias due to on-going metabolism. The stability of analytes is defined by the ability of a product to maintain its stated composition, properties, and performance. The stability of analytes becomes much more crucial for certain critical parameters like bicarbonate, lactate, ammonia, etc. Although it is recommended to analyze freshly drawn serum/plasma for testing, unavoidable delays or the need to reuse samples can introduce challenges, potentially to false test results. Mitigating these factors is crucial for healthcare professionals to make informed decisions based on accurate diagnostic information.[6,7] Bicarbonate stored in unopened containers remains stable for up to 4 hours but its levels decrease upon exposure to air and storage for a longer duration. There is a paucity of studies that have assessed the comparison between serum and arterial bicarbonate levels and also evaluated the stability of serum bicarbonate.

[1–4]Department of Biochemistry, JSS Medical College and Hospital, JSS AHER, Mysuru, Karnataka, India

Corresponding Author: Swetha NK, Department of Biochemistry, JSS Medical College and Hospital, JSS AHER, Mysuru, Karnataka, India, Phone: +91 9886403651, e-mail: swethank@jssuni.edu.in

How to cite this article: Ramachandra SC, Prashant A, MN Suma, *et al*. Investigating Differences in Bicarbonate Levels: Exploring Discrepancies between Venous and Arterial Measurements and Evaluating Stability through Time-point Assessments. Indian J Med Biochem 2023;27(3):50–52.

Source of support: Nil

Conflict of interest: Dr Akila Prashant is associated as Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of the Editor-in-Chief and her research group.

Hence in this study, we intend to conduct a comparative analysis between serum and arterial bicarbonate and also determine the stability of serum bicarbonate levels across various time intervals.

Methodology

The study was conducted in the clinical biochemistry laboratory of a tertiary care hospital, utilizing a total of 31 patient samples received for serum bicarbonate analysis.

Comparison between Arterial Bicarbonate (Calculated) and Serum Bicarbonate

For the comparative analysis, we randomly selected the samples sent to the Clinical biochemistry laboratory for serum bicarbonate estimation. Calculated values for arterial bicarbonate were noted from the blood gas analysis reports of the same patients sent on the same day.

Assessment of Stability of Serum Bicarbonate at Various Time Intervals

The serum bicarbonate was measured at the following three different time points:

- Immediately after the centrifugation (vacutainer cap was closed) and serum separation;
- At 2 hours (two hours after the first analysis); and
- At 4 hours (four hours after the first analysis).

Between the analyses, the samples were stored at room temperature in uncapped vacutainers. We intended to study the stability of bicarbonate in samples stored in uncapped containers at room temperature, taking into consideration the practices in routine clinical laboratory.

Serum bicarbonate was measured using an enzyme-based method on a fully automated Roche Cobas analyzer. Arterial bicarbonate was calculated using the Henderson–Hasselbalch equation on the Radiometer ABG 800 Flex blood gas analyzer.

Statistical Analysis

Statistical analysis was conducted using a Microsoft Excel link. The mean difference in the bicarbonate between the two methods was calculated and analyzed through Pearson correlation, and Bland–Altman plot. The assessment of bicarbonate at various time intervals was performed using one-way analysis of variance (ANOVA). The variables with a *p*-value of 0.05 or less were considered statistically significant.

Results

Comparison between Arterial Bicarbonate (Calculated) and Serum Bicarbonate

In our study, we observed that the mean serum HCO_3– level was 21.4 ± 4.9 mmol/L and arterial HCO_3– was 19.51 ± 4.4 mmol/L. Pearson's correlation analysis revealed a highly significant correlation between serum bicarbonate and arterial bicarbonate ($p < 0.00$; $r = 0.91$). We further evaluated the agreement between the two measurements by Bland–Altman method (Fig. 1). The bias plot demonstrated acceptable agreement for the values of bicarbonate (mmol/L) measured in serum compared to calculated arterial HCO_3– (Table 1).

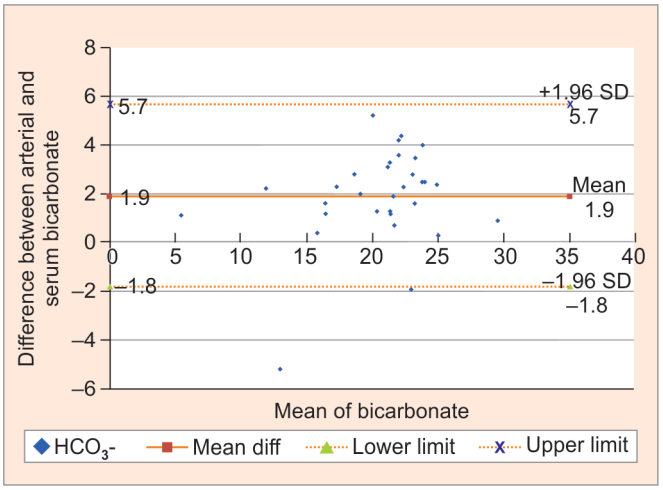

Fig. 1: Bland–Altman plot comparing serum vs arterial bicarbonate

Assessment of Stability of Serum Bicarbonate at Various Time Intervals

The stability comparison at different time intervals (immediate, 2 hours, and 4 hours) demonstrated a notable decrease in serum bicarbonate levels. One-way ANOVA yielded statistically significant results with a *p*-value of below 0.000 (Table 2). This underscores the impact of storage time on the stability of serum bicarbonate, emphasizing the need for careful consideration of preanalytical variables in clinical settings.

Discussion

Evaluating the acid-base status is vital in monitoring the progress of critically ill patients, often achieved through arterial blood gas analysis. Nevertheless, the complications, limited availability of this analysis, and the associated costs underscore the need to explore simpler and more accessible methods for assessing acid-base status using serum samples. The serum bicarbonate serves as a crucial marker for assessing electrolyte distribution and anion deficit. Measuring bicarbonate levels plays a key role in identifying and addressing various acid-base imbalances related to both respiratory and metabolic systems. Consequently, our goal was to investigate the difference between serum and arterial bicarbonate levels and, additionally, assess the stability of serum bicarbonate levels across various time intervals.

In this study, we evaluated the correlation between serum and arterial bicarbonate. The Pearson correlation revealed a significant correlation with $r = 0.91$, $p < 0.0001$. Nevertheless, relying solely on the correlation coefficient to evaluate the agreement between the two methods may not be suitable. Correlation is influenced by the range of values in the samples. The values that appear to exhibit poor agreement may still yield high correlations if the range is sufficiently extensive. Hence, we used Bland–Altman analysis for further confirmation. The Bland-Altman analysis showed a significant degree of agreement between serum versus arterial bicarbonate values. Our study, in line with previous studies, has shown agreement between serum and arterial bicarbonate.[8,9]

Serum bicarbonate measured in venous blood includes plasma bicarbonate, dissolved CO_2, and CO_2 bound to proteins as carbamates. The total CO_2 concentration under resting conditions and normal cardiac output is identified to be 2 mM higher than the

Table 1: Comparison between the serum and arterial blood gas HCO_3– values

Bicarbonate levels in arterial and venous blood sample (n = 31)	*Mean ± SD (mmol/L)*	*Pearson correlation, r (p)*	*Mean difference (mmol/L)*	*Bland–Altman 95% limits of agreement (mmol/L)*
Serum HCO_3–	21.4 ± 4.9	0.91 (<0.0001)	1.91	From −1.8 to 5.72
Arterial HCO_3–	19.51 ± 4.4			

Table 2: Stability comparison between the serum HCO_3– values at different time intervals

Serum HCO_3– Immediate (mmol/L)	*Serum HCO_3– 2 hours (mmol/L)*	*Serum HCO_3– 4 hours (mmol/L)*	*Numbers (n)*	*p-value*
21.4 ± 4.9	17.17 ± 4.5	16.81 ± 4.5	31	**<0.000**

$p < 0.05$ was considered statistically significant

arterial bicarbonate due to CO_2 elimination across the lungs and a gap of 3.2 mM between measured total CO_2 concentration and calculated arterial bicarbonate reflects the difference in bicarbonate concentration (2 mM) and dissolved CO_2 (1.4 mM). The mean difference between serum and arterial bicarbonate in our study was 1.91. Our study further reiterates the fact that serum and arterial bicarbonate can be used interchangeably.[10]

Additionally, we assessed the stability of serum bicarbonate at three distinct time points: immediately, at 2 and 4 hours. A prior stability study of bicarbonate indicated that serum bicarbonate remains stable for 4 hours in closed, uncentrifuged tubes, an additional 2 hours in closed tubes after centrifugation, but becomes unstable within 1 hour in opened tubes.[11] In our study, we used uncapped tubes simulating the routine laboratory practice for the majority of the biochemistry analytes and we tried to assess the extent of variation in the bicarbonate levels at 2 and 4 hours, In our study, we found there was a significant decrease in the serum bicarbonate levels at 2 and 4 hours of storage. This could be mainly attributed to the escape of CO_2 from the uncapped samples. Comparison at different time intervals revealed a significant decrease in serum bicarbonate. Our study reiterates the fact that uncapped serum samples are not suitable for assessing serum bicarbonate levels. However, our study had a few limitations such as a smaller sample size, and other factors such as age, gender, and underlying clinical condition were not considered.

Conclusion

Our study showed that arterial and serum bicarbonate values are comparable and in emergencies, immediate serum bicarbonate may serve as a substitute for arterial bicarbonate. However, for more precise evaluations encompassing additional parameters, arterial blood gas remains necessary. Furthermore, our study emphasizes the need to create awareness among the nursing staff, phlebotomists, and Technicians about the effect of preanalytical variables (uncapped tubes) on serum bicarbonate.

Orcid

Shobha C Ramachandra https://orcid.org/0000-0001-6744-8430
Akila Prashant https://orcid.org/0000-0003-0383-2366
Suma MN https://orcid.org/0000-0002-2614-5377
Swetha NK https://orcid.org/0000-0002-3163-7405

References

1. Kim A, Yamaguchi L, Keswani S, et al. Correlation between serum and arterial blood gas bicarbonate in patients admitted to the intensive care unit. Cureus 2023;15(4):e37703. DOI: 10.7759/CUREUS.37703.
2. Goundan PN, Willard DL, Sahin–Efe A, et al. Comparison of bicarbonate values from venous blood gas and chemistry panels measured at the time of diagnosis and resolution of diabetes ketoacidosis. J Clin Transl Endocrinol 2019;18:100205. DOI: 10.1016/J.JCTE.2019.100205.
3. Ball CM, Featherstone PJ. The introduction of blood gases into clinical practice. Anaesth Intensive Care 2024;52(1):3–5. DOI: 10.1177/0310057X231212299.
4. Chittamma A, Vanavanan S. Comparative study of calculated and measured total carbon dioxide. Clin Chem Lab Med 2008;46(1):15–17. DOI: 10.1515/CCLM.2008.005.
5. Olver P, Bohn MK, Adeli K. Central role of laboratory medicine in public health and patient care. Clin Chem Lab Med 2023;61(4):666–673. DOI: 10.1515/CCLM-2022-1075.
6. Kachhawa K, Kachhawa P, Varma M, et al. Study of the stability of various biochemical analytes in samples stored at different predefined storage conditions at an accredited laboratory of India. J Lab Physicians 2017;9(1):11–15. DOI: 10.4103/0974-2727.187928.
7. Chauhan KP, Patel JD, Prajapati S. Study of specimen stability for biochemical parameters. Int J Clin Biochem Res 2020;5(1):158–163. DOI: 10.18231/2394-6377.2018.0032.
8. de Andrade LGM, Muniz AB, Mondelli AL, et al. Concordance analysis between dosed serum bicarbonate and that calculated by gas analysis in chronic renal patients. J Bras Nefrol 2020;42(4):478. DOI: 10.1590/2175-8239-JBN-2019-0236.
9. Nargis W, Rahman AS, Ahamed BU, et al. Comparison of values of traditionally measured venous bicarbonate with calculated arterial bicarbonate in intensive care unit patients of a hospital in a third-world country. Niger Med J 2014;55(4):285. DOI: 10.4103/0300-1652.137186.
10. Sanghavi SF, Albert TJ, Swenson ER. Will the real bicarbonate please stand up? Ann Am Thorac Soc 2022;19(7):1226–1229. DOI: 10.1513/AnnalsATS.202111-1286CC.
11. Šupak–Smolčić V, Franin L, Horvat M, et al. Serum bicarbonate stability study at room temperature: Influence of time to centrifugation and air exposure on bicarbonate measurement reported according to the CRESS checklist. Clin Chem Lab Med 2023;61(6):1046–1053. DOI: 10.1515/cclm-2022-1182.