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Case Report

Pseudohypobicarbonatemia Associated with Profound Hypertriglyceridemia

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Abstract

A case is presented of falsely low serum bicarbonate levels with normal arterial blood gas bicarbonate levels in a patient with high triglyceride (TG) levels. This false phenomenon is explained by the presence of high triglyceride levels. Most laboratories use an enzymatic/photometric or indirect ion-selective electrode method to measure bicarbonate. Hyperlipidemia interferes with photometric analysis due to its light scattering effect. An ABG analyser employs a direct ion-selective electrode method that is free from the errors of a photometric analyser. Currently, several methods are available to eliminate the interference of lipemia and allow accurate measurement of biological quantities. The clinical laboratory must establish a protocol for handling lipemic samples according to the biological quantity to be tested.

Keywords: Lipemia interference, preanalytical phase, laboratory error, interference testing factitious hypobicarbonatemia, severe hypertriglyceridemia.

INTRODUCTION

Laboratory interference is defined as the process whereby the presence of another substance in the sample causes a change in the measured level of another parameter [1]. Interferences represent a significant source of laboratory errors that can affect the integrity of samples and the reliability of laboratory results. They can also lead to misinterpretation of results, unnecessary investigations and clinician intervention. One of the most common analytical interference in the clinical laboratory is lipaemia [2]. The presence of lipoproteins in a sample can cause analytical errors due to the increased turbidity that results from the high concentration of these particles [3]. Lipoproteins are heterogeneous in size and do not all contribute to turbidity in the same way. Chylomicrons, the largest lipoprotein particles (70 to 1,000 nm), are responsible for the greatest turbidity in the sample. There are three types of very low-density lipoprotein (VLDL) depending on their size: small (27 to 35 nm), medium (35 to 60 nm) and large (60-200 nm). Only large and medium VLDL cause opacity. It is noteworthy that high-density lipoproteins (HDL) (6 to 12.5 nm) and low-density lipoproteins (LDL) (20 to 26 nm) do not cause cloudiness [4].

Hypertriglyceridemia, regardless of its underlying cause, can result in the formation of lipemic aggregates. The primary causes of hypertriglyceridemia are hyperlipidaemia types I, IV and V, as defined by the Fredrickson classification. Secondary causes include diabetes mellitus, insulin resistance, alcoholism, human immunodeficiency virus infection, renal disease, hypothyroidism and parenteral Intralipid administration in hospitalised patients [5]. Furthermore, parenteral nutrition and diluents of poorly water-soluble drugs containing lipid emulsions may also result in the formation of lipaemia [6].

Lipemia can interfere with biochemical results in a number of ways, including the use of spectrophotometry (which is probably the most common method of interference), sample heterogeneity and the volume shift effect.

A case is presented of falsely low serum bicarbonate (CO2) levels with normal arterial blood gas bicarbonate levels in a patient with high triglyceride (TG) levels. It is hypothesised that this may have interfered with accurate serum bicarbonate measurement.

CASE PRESENTATION

A 30-year-old woman with a history of lipoatrophic diabetes, chronic pancreatitis and severe hypertriglyceridemia on insulin and lipid-lowering

therapy for 14 years was admitted to the emergency department with sudden onset of abdominal pain and vomiting. A physical examination revealed a conscious patient with a Glasgow Coma Scale (GCS) score of 15/15, a blood pressure (BP) of 125/60 mmHg, a heart rate of 78 beats per minute (bpm), a respiratory rate of 18/min, and an oxygen saturation of 98% on room air. The physical examination was unremarkable.

The capillary blood glucose level was recorded as 4.2 g/dl on the meter, and the urine dipstick test for ketone bodies (ketonuria) was positive on three occasions. An electrocardiogram was essentially normal.

Biochemical tests were performed using the Abbott ARCHITECT c16000 system.

The initial biological assessment indicated hyperglycaemia at 4.5 g/dl and hyponatraemia at 119 mmol/l. Correction of the sodium concentration for hyperglycemia resulted in a value of 125 mmol/l, accompanied by a kaliemia concentration of 4 mmol/l. The serum chloride concentration was 89 mmol/L, the bicarbonate concentration was 11 mmol/L, and the serum lipase concentration was 76 mg/dL. The β-HCG concentration was negative.

In the absence of clinical signs of acidosis, an arterial blood gas (ABG) was performed, which showed a normal pH of 7.39 and a bicarbonate level of 25 mmol/L, which was incompatible with the results of the serum biochemistry.

Concurrently, the lipid panel demonstrated a markedly elevated total cholesterol level of 14.081 g/L and a markedly elevated triglyceride level of 121.685 g/L. The discrepancy between the bicarbonate levels observed in the biochemical panel and the ABG can be attributed to an erroneous measurement of bicarbonate levels, which occurs in the presence of exceedingly high triglyceride levels. Severe hypertriglyceridemia can interfere with bicarbonate measurement, resulting in the erroneous reporting of pseudohypobicarbonateemia, as observed in this patient.

Following plasmapheresis sessions, there was a gradual improvement in the patient's lipid profile, with a reduction in hypertriglyceridemia to 31.14 g/L. It is noteworthy that the serum bicarbonate level also increased to 18 mmol/L, which provided further confirmation of the diagnosis.

Following a four-day hospital stay, the patient was discharged and started on fenofibrate and statins.

Subsequent consultations with a diabetes and endocrinology specialist were scheduled.

Table: Basic metabolic panel and arterial-blood gas results

Chemistry, serum	Admission	After plasmapheresis	Reference Range
Sodium, mmol/l	119	145	136–145
Potassium, mmol/l	4.37	3.36	3.5–5.1
Chloride, mmol/l	89.47	85.36	98–107
Tco2, mmol/l	11.4	17	22–29
Bun, g/l	impossible to calculate the result	0.23	0,2-0,45
Creatinine, mg/l	impossible to calculate the result	4.05	6 - 12
Glucose, g/l	4.5	2.5	0.7–0.99
Calcium, mg/l	96.47	70.73	84–102
Anion gap			5–17
Arterial blood gases			
Ph, arterial	7,39		7.37–7.47
Pco2, arterial, mmhg	46		35–51
Po2, arterial, mmhg	90		80–100
Hco3– , mmol/l	25		21–26
O2 saturation, %	97		90–98
Lipid panel			
Total cholesterol g/l	14.081	4.42	0-1.99
LDL g/l	6.36	0.44	1-1.59
HDL g/l	0.229	0.14	0.4-0.6
Triglycerides g/l	121.685	31.14	0-1.49
VLDL g/l			

Abbreviations: tCO2 – total serum carbon dioxide, BUN – blood urea nitrogen.

DISCUSSION

Serum bicarbonate concentration is a crucial parameter in the assessment of acid-base balance. Its measurement can be carried out using a variety of methods, each with its own advantages and limitations.

In clinical practice, two main methods are commonly employed in most clinical chemistry laboratories to determine the concentration of tCO2 [7]. tCO2 is quantified directly in serum, either enzymatically or by an indirect ion-selective electrode (ISE) method. Furthermore, bicarbonate levels can be calculated from arterial blood gas (ABG) analysis using the Henderson-Hasselbalch equation, which calculates bicarbonate as a function of pH and partial pressure of carbon dioxide (PCO2) [8].

The Abbott Architect autoanalyser employs phosphoenolpyruvate carboxylase to facilitate the conversion of CO2 and phosphoenolpyruvate to oxaloacetate and phosphate. Malate dehydrogenase then catalyses the reduction of oxaloacetate to malate, accompanied by the oxidation of NADH (reduced nicotinamide adenine dinucleotide). The decrease in light absorbance at 404 nm, as measured using a photometric cell, is proportional to the total CO2 content of the sample. In this type of test, large particles, such as chylomicrons and very low-density lipoproteins, absorb light and cause light scattering interference during analysis. This results in a falsely low TCO2 [9, 10].

Blood gas analysers employ a direct ISE methodology to calculate bicarbonate in the aqueous phase of undiluted blood, utilising directly measured values of blood pH and partial pressure of CO2 (pCO2). This is a non-photometric analysis, which is not subject to the same interference.

The manufacturer's product information indicates that the calculated interference effects, as determined by dose-response methods, exhibit a 99% agreement between the target values and the total CO2 values observed with Intralipidconcentrations of 2000 mg/dL. This phenomenon is not exclusive to the Abbott system. Beckman-Coulter asserts that there is no significant interference up to an Intralipid concentration of 1,000 mg/dL, yet does not guarantee total CO2 agreement above this value. Interference from lipid particles is visible with any autoanalyser that employs the enzymatic reaction and the photometric reaction, regardless of the manufacturer. In vivo, the macromolecular profiles of patient lipids are more complex and varied than those of Intralipid. Consequently, the manufacturer's interference tests may not be comparable with those of patient samples. Different patients with the same triglyceride level may have very different lipid particle populations, with varying degrees of photometric interference [11].

Three mechanisms are involved in the phenomenon of lipemic interference: light scattering, the volume displacement effect and the lack of sample homogenization [12].

The impact of lipemia on laboratory analyses is primarily due to light scattering initiated by lipoproteins, notably chylomicrons and very low-density lipoproteins (VLDL). This scattering phenomenon occurs throughout the visual spectrum (300–700 nm), intensifying as the wavelength decreases. Colorimetric assays, which measure absorbance readings at shorter wavelengths, are particularly susceptible to this interference. The degree of interference in spectrophotometric methods varies based on whether absorbance increases or decreases and the wavelength employed. Consequently, comparing lipemia interference between different analytical methods may not be straightforward. This mechanism influences spectrophotometric, nephelometric, and turbidimetric assays.

The magnitude and nature of light scattering induced by lipoproteins are influenced by their size and composition. Chylomicrons and VLDL exhibit significant heterogeneity in size, resulting in varying degrees of sample turbidity [1, 3].

Furthermore, lipids can also interfere with accurate measurement through the formation of space-occupying lesions by volume displacement, which can lower the aqueous phase of the sample and thus falsely reduce the levels of potassium, chloride, sodium, etc. This results in low and spurious values. This interference can occur with indirect and enzymatic ISE methods [13].

Rifkin *et al*., documented the initial instance of pseudohypobicarbonatemia attributed to severe hyperlipidemia. In their study utilising the Abbott Architect analyser, the patient exhibited a serum bicarbonate level of less than 5 mEq/L, which was in contrast to the normal value observed on arterial blood gas (ABG) analysis. Notably, the patient exhibited a markedly elevated triglyceride level (>7000 mg/dL). It was postulated that the light-scattering phenomenon induced by hyperlipidaemia disrupted the enzymatic method, resulting in falsely low bicarbonate measurements and a diagnosis of high anion gap metabolic acidosis (HAGMA). Subsequent administration of lipid-clearing agents resulted in the resolution of the discrepancy in bicarbonate measurements. Since that time, numerous cases have been documented in which discrepancies have been observed between ABG-derived and auto-analyzer-measured serum bicarbonate levels, particularly in the context of hyperlipidemia [14-16].

Several techniques are available to eliminate lipids from samples, including ultracentrifugation, high-speed centrifugation, lipid extraction using polar solvents, sample dilution, and serum blank preparation. Ultracentrifugation is considered the gold standard method for lipid removal, although it is costly and not accessible in many laboratory settings. In contrast, serum dilution and serum blank preparation are simpler and more routine approaches for mitigating lipid interferences [17].

Centrifugation of serum or plasma represents the primary approach for the removal of lipids. Ultracentrifugation, which involves speeds ranging from 100,000 to 2,000,000×g, is an effective method for eliminating lipids and enhancing the determination of analytes [18, 19]. However, the requisite equipment for ultracentrifugation is not commonly available in most laboratories.

Alternatively, high-speed centrifugation (10,000–15,000×g) can effectively separate large lipoproteins, such as chylomicrons, from serum or plasma [20, 21]. Nevertheless, this method is less efficient for the removal of very low-density lipoproteins (VLDL). Following high-speed centrifugation, a lipid layer forms at the top, and the infranatant, devoid of lipids, is carefully collected to avoid contamination. Alternatively, high-speed centrifugation (10,000–15,000×g) can effectively separate large lipoproteins, such as chylomicrons, from serum or plasma.

Lipids can be extracted using polar solvents such as Lipoclear®, a reagent containing the non-toxic, non-ionic polymer 1,1,2-trichlorotrifluoroethane, which binds lipids [22]. Subsequently, the particles precipitate to the bottom of the tube following centrifugation, and the measurement is performed on the clear supernatant.

The introduction of an exogenous substance may result in interference with the measurement of biochemical parameters. This phenomenon is observed in the case of proteins, calcium, and aspartate aminotransferase when the sample is treated with Lipoclear® [23].

In order to mitigate the effects of an interfering substance, such as lipemia, serum or plasma dilution is often employed, particularly for lipophilic components with significant diffusion in the lipid phase. The dilution of the sample is intended to reduce the level of lipemia to a value below the interference threshold for the relevant analytes, while ensuring that the concentration of the component remains within the quantification limit. A common approach involves the replacement of plasma with an equal volume of an isoosmotic diluent, which effectively reduces the interference in blood samples. It is, however, important to note that this method may yield misleading results due to the potential loss of cells during the replacement process.

CONCLUSION

In conclusion, the turbidity caused by lipemia resulted in a falsely low value for total carbon dioxide, as measured using the enzymatic/photometric method. It is important to consider this artefact when encountering a total carbon dioxide value obtained using this method that is implausibly low or discrepant with a concurrent value obtained using the blood gas method.

REFERENCES

1. Kroll, M. H. (2004). Evaluating interference caused by lipemia. *Clinical chemistry*, *50*(11), 1968-1969.
2. Mainali, S., Davis, S. R., & Krasowski, M. D. (2017). Frequency and causes of lipemia interference of clinical chemistry laboratory tests. *Practical laboratory medicine*, *8*, 1-9.
3. Nikolac, N. (2014). Lipemia: causes, interference mechanisms, detection and management. *Biochemia medica*, *24*(1), 57-67.
4. Soleimani, N., Mohammadzadeh, S., & Asadian, F. (2020). Lipemia interferences in biochemical tests, investigating the efficacy of different removal methods in comparison with ultracentrifugation as the gold standard. *J Anal Methods Chem*, 2020. https://doi.org/10.1155/ 2020/9857636.
5. Nelson, R. H. (2013). Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care: Clinics in Office Practice*, *40*(1), 195-211.
6. Johnson-Arbor, K., Salinger, L., & Luczycki, S. (2015). Prolonged laboratory interference after administration of intravenous lipid emulsion therapy. *Journal of Medical Toxicology*, *11*, 223-226.
7. Smith, A., Barth, J., Xu, K., & Buie, L. W. (2020). Accuracy of Bicarbonate Measurements in the Presence of Hypertriglyceridemia. *Journal of Investigative Medicine High Impact Case Reports*, 8, 1-5.
8. Kraut, J. A., & Madias, N. E. (2007). Serum anion gap: its uses and limitations in clinical medicine. *Clinical journal of the American Society of Nephrology*, *2*(1), 162-174.
9. Rifkin, S. I., & Shaub, B. (2014). Factitious hypobicarbonatemia associated with profound hyperlipidemia. *Renal Failure*, *36*(7), 1155-1157.
10. Stein, H. (2019). Spuriously low serum bicarbonate levels in patients with hyperlipidemia: a report of 4 cases. *American Journal of Kidney Diseases*, *73*(1), 131-133.
11. Stein, H. (2019). Spuriously low serum bicarbonate levels in patients with hyperlipidemia: a report of 4 cases. *American Journal of Kidney Diseases*, *73*(1), 131-133. doi: 10.1053/j.ajkd.2018.04.016
12. Farrell, C. J. L., & Carter, A. C. (2016). Serum indices: managing assay interference. *Annals of clinical biochemistry*, *53*(5), 527-538.
13. Dana, A. P., & Kazory, A. (2020). Spurious low serum bicarbonate level due to severe hypertriglyceridemia: a clinical challenge. *The American Journal of Medicine*, *133*(6), e306-e307.
14. Stein, H. (2019). Spuriously low serum bicarbonate levels in patients with hyperlipidemia: a report of 4 cases. *American Journal of Kidney Diseases*, *73*(1), 131-133.
15. Carag, C., Baxi, P. V., Behara, V., Gashti, C. N., & Rodby, R. (2019). Pseudo-anion gap metabolic acidosis from severe hypertriglyceridemia corrected by plasma exchange. *Clinical Nephrology*, *92*(5), 258-262. doi: 10.5414/CN109627.

16. Echevarria, C., Puscas, M., Abbas, F., Hasoon, M., Kofahl, A., Igwe, M., & Carlson, R. (2014). Spurious bicarbonate from hypertriglyceridemia in diabetic ketoacidosis (DKA). Implications for ICU. *Crit Care Med*, 42, A1647. doi: 10.1097/01.ccm.0000458696.83616.97
17. Lima-Oliveira, G., Volanski, W., Lippi, G., Picheth, G., & Guidi, G. C. (2017). Pre-analytical phase management: a review of the procedures from patient preparation to laboratory analysis. *Scandinavian journal of clinical and laboratory investigation*, *77*(3), 153-163.
18. Calmarza, P., & Cordero, J. (2011). Lipemia interferences in routine clinical biochemical tests. *Biochem Med*, 21, 160–166.
19. Soh, S. X., Loh, T. P., Sethi, S. K., & Ong, L. (2022). Methods to reduce lipemic interference in clinical chemistry tests: a systematic review and recommendations. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *60*(2), 152-161.
20. Dimeski, G., & Jones, B. W. (2011). Lipaemic samples: effective process for lipid reduction using high speed centrifugation compared with ultracentrifugation. *Biochem Med*, 21, 86–92.
21. Castro-Castro, M. J., Candás-Estébanez, B., Esteban-Salán, M., Calmarza, P., Arrobas-Velilla, T., Romero-Román, C., ... & Aguilar-Doreste, J. Á. (2018). Removing lipemia in serum/plasma samples: a multicenter study. *Annals of laboratory medicine*, *38*(6), 518-523.
22. Lipoclear. Available at: http://www.statspin.com/pdfs/64-003957-001D.pdf. Accessed October 27, 2013.
23. Saracevic, A., Nikolac, N., & Simundic, A. M. (2014). The evaluation and comparison of consecutive high speed centrifugation and LipoClear® reagent for lipemia removal. *Clinical biochemistry*, *47*(4-5), 309-314.