Original Article

Effect of High Atmospheric Carbon Dioxide Concentrations on the Serum Total Carbon Dioxide Measurement

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Background: Serum total carbon dioxide (TCO_2) measurements are easily affected by numerous factors. Whether an irregularly high atmospheric CO_2 concentration affects the TCO_2 measurement remains unclear.

Materials and Methods: In Somdech Phra Debaratana Medical Center laboratory (SDMC) and the main building laboratory (Building 1) located within Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, the repeated TCO_2 measurements using an enzymatic assay in three levels of human based control material were performed every two hours, over a one-day period. TCO_2 in a total of 150 patient sera were measured. Simultaneously, atmospheric CO_2 levels were determined.

Results: Atmospheric CO_2 levels in SDMC and Building 1, ranged from 763 to 1,560 ppm and 602 to 787 ppm, respectively. Repeated TCO_2 measurements for SDMC, the measured TCO_2 concentrations of all control materials clearly increased between 10:00 a.m. and 4:00 p.m., with the peak at 2:00 p.m., which was related to an increase in the atmospheric CO_2 concentration. By contrast, in Building 1, the measurements were considerably stable. Moreover, considering patient data (n=12,042), the estimate median TCO_2 concentration in SDMC was likely to increase between 10:00 a.m. to 4:00 p.m. as well. The association between the bias (y), difference TCO_2 concentration obtained between the SDMC and the Building 1, and the increasing atmospheric CO_2 (x) was $y = 0.0038x - 0.016$, $R^2=0.6813$. Using regression equations, TCO_2 level increased by approximately 0.4 mmol/L for every 100 ppm of CO_2 increase in atmosphere.

Conclusion: High atmospheric CO_2 concentrations can result in falsely high TCO_2 values, which may lead to markedly wrong interpretations, especially in patients with a tendency to have low TCO_2 concentrations.

Keywords: Serum total carbon dioxide, Atmospheric carbon dioxide, Acid-base disorder

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In clinical practice, serum total carbon dioxide (TCO_2) is used as a marker to detect respiratory acidosis, respiratory alkalosis, metabolic acidosis, and metabolic alkalosis, especially, the acid-base derangement in chronic kidney disease[1,2]. Serum TCO_2 measurements are easily affected by numerous factors, including instrument, reagents, and environment variables[3]. Typically, falsely low levels of TCO_2 can be found in routine analysis when a sample is exposed to air, because air causes the loss of CO_2. Exposure to air can result in a loss of TCO_2 of up to 4 to 6 mmol/L in an hour[4,5].

Because of human activities, the atmospheric concentration of CO_2, a greenhouse gas, have been rising extensively since the Industrial Revolution[6]. Although living things emit CO_2 when they breathe, CO_2 is widely considered to be a pollutant when associated with cars, planes, power plants, and other human activities that involve the burning of fossil fuels, such as gasoline and natural gas. However, little is known regarding whether an irregularly high CO_2 concentration in the atmosphere can affect the TCO_2 measurement.

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Materials and Methods

Core laboratories in university hospital

In the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, there are two main laboratories, Somdech Phra Debaratana Medical Center (SDMC) laboratory and the main building laboratory (Building 1), which are approximately 600 meters apart. To assure the same quality, the testing process used in the SDMC was the same as that used in Building 1, including the use of instrumentation, reagents and supplies, middleware, and quality management system. Both laboratories comply with the regulatory requirements of the International Organization for Standardization (ISO) 15189. Eventually, in 2016 to 2017, both laboratories implemented the Six Sigma technique for evaluating the quality of laboratory results by participating in the Westgard Sigma Verification of Performance Program[7].

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (MURA2018/969).

Determination of CO_2

In both Building 1 and SDMC laboratories, TCO_2 was measured using an enzymatic assay (Abbott Laboratories, IL, USA). Atmospheric CO_2 was measured using a TSI Quest™ EVM Environmental Monitor (TSI Incorporated, Minnesota USA). The within-laboratory imprecision for TCO_2 determination was determined according to the Clinical and Laboratory Standards Institute EP05A3: Evaluation of Precision of Quantitative Measurement Procedures[8].

The present study was performed between February 2019 and April 2019. The researchers used two types of samples to study the effects of atmospheric CO_2 concentration on the TCO_2 measurement, the quality control materials from human serum (Technopath Clinical Diagnostics, Tipperary, Ireland) and the individual patient specimens. One hundred fifty patients' sera (TCO_2 ranging from 12.8 to 32.0 mmol/L) with a usual mix of diseases and without any consideration to diagnosis were collected from the SDMC laboratory. Each aliquoted sample was transferred directly to Building 1 laboratory via the Tempus 600 tube transport system (TimedicoA/S, Bording, Denmark), which sent sample tubes to the Building 1 laboratory (600 meters from the SDMC laboratory) within 60 seconds. Immediately after the sample arrived to Building 1 laboratory, the TCO_2 concentration and the atmospheric CO_2 level were measured in parallel between both laboratories.

Statistical analysis

Correlation between the difference of TCO_2 concentration and the atmospheric CO_2 concentration was analyzed using simple linear regression analysis. Outcome was considered statistically significant when the p-value was less than 0.05. Statistical analysis was carried out using the SPSS Statistics for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Atmospheric CO_2 concentrations in the SDMC and the Building 1 laboratories ranged from 763 to 1,560 ppm and 602 to 787 ppm, respectively. The coefficient of variation for within-laboratory imprecision of TCO_2 determination in the SDMC (ranging from 5.33% to 5.70%) was higher than that in Building 1 (ranging from 3.14% to 4.35%).

To investigate the effect of atmospheric CO_2 on the TCO_2 measurement, the researchers conducted repeated measurements of TCO_2, with five replicates per run, every two hours over a one-day period, in three different levels of quality control materials from human serum. Simultaneously, in both the SDMC and Building 1, the levels of atmospheric CO_2 were determined. Figure 1 shows the observed TCO_2 concentration over a one-day period in the SDMC (Figure 1A) and in Building 1 (Figure 1B). In the SDMC, the measured TCO_2 concentrations of all quality control materials clearly increased between 10:00 a.m. and 4:00 p.m., with the peak at 2:00 p.m., which was related to an increase in the atmospheric CO_2 concentration. The researchers observed that the overall effect of atmospheric CO_2 on TCO_2 was not dependent on the analyte concentration. By contrast, in Building 1, not only the measured TCO_2 concentration of each control material but also the atmospheric CO_2 concentration at any time was considerably stable.

In addition, the researchers assessed the actual patient results of TCO_2 measured by SDMC (n=12,042) during March 2019 as tabulated in Table 1. It was found that a shift in median of TCO2 concentrations also occurred between 10 a.m. and 6 p.m.

By using patient sera (n=150), the bias (difference TCO_2 concentration obtained between the SDMC and the Building 1) versus the increasing atmospheric CO_2 concentration is shown in Figure 2. The association between the bias of the TCO_2 concentration (y) and the increasing atmospheric CO_2 (x) was $y = 0.0038x - 0.016$, $R^2=0.6813$, $p<0.001$. Using regression equations, the researchers estimated the TCO_2

Table 1. The actual patient results of TCO_2 obtained from SDMC laboratory during March 2019 (n=12,042)

Time interval	Number of samples	Serum total carbon dioxide (mmol/L)	
		Range	Median
2:00 a.m. to 6:00 p.m.	510	10.7 to 33.2	22.0
6:00 a.m. to 10:00 a.m.	6,678	10.2 to 33.9	24.2
10:00 a.m. to 2:00 p.m.	3,473	11.1 to 36.8	25.8
2:00 p.m. to 6:00 p.m.	1,069	11.5 to 35.5	25.7
6:00 p.m. to 10:00 p.m.	244	12.5 to 32.8	23.7
10:00 p.m. to 2:00 a.m.	68	11.3 to 31.2	21.3

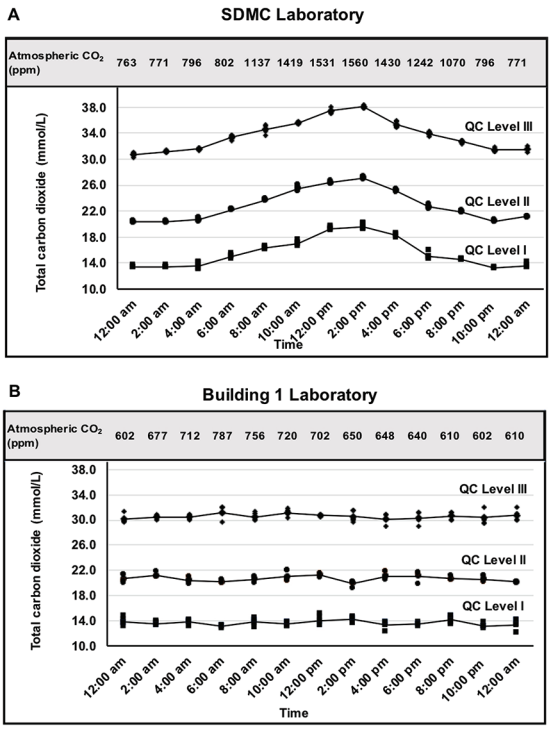

Figure 1. Measuring TCO_2 concentrations in samples and atmospheric CO_2 throughout the day from SDMC (A) and Building 1 (B) laboratories.

concentration increased by approximately 0.4 mmol/L for every 100 ppm of CO_2 increase in the atmosphere.

Discussion

TCO_2 measurements are easily affected by numerous factors. The exposure of a sample to air causes loss of CO_2, thus, the measurement procedure must be rapidly and carefully performed. However, the present study results indicated that an irregular high atmospheric CO_2 concentration can result in falsely high values of TCO_2 in patient samples. Therefore, atmospheric CO_2 is an important environmental

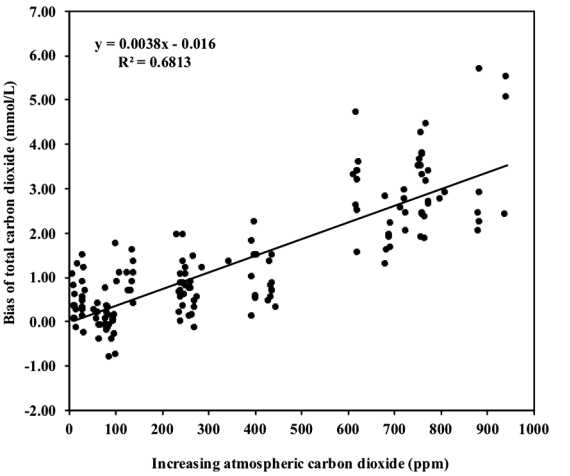

Figure 2. Bias plot of TCO_2 concentration obtained between the SDMC and the Building 1 versus the increasing atmospheric CO_2 concentration.

factor resulting in variability in the measurement of total amount of CO_2 in serum. The present study result was in line with the researchers' previously study[9], which demonstrated that the irregular high atmospheric CO_2, especially in daytime, was not only altering the systematic error or bias of the TCO_2 measurement procedure, but also altering the random error or imprecision of the measurement procedure.

The CO_2 in blood has three main components, bicarbonate anion (HCO_3-), gaseous dissolved carbon dioxide (dCO_2), and carbonic acid (H_2CO_3). It comprises of bicarbonate 95%, followed by 5% of dCO_2 and H_2CO_3. At equilibrium, the amount of dCO_2 can be estimated by multiplying the partial pressure of CO_2 (pCO_2) by the solubility coefficient of CO_2 ($dCO_2 = pCO_2 \times CO_2$ solubility coefficient)[10]. Since arterial pCO_2 and CO_2 solubility coefficient at pH of 7.4 and body temperature are approximately 40 mmHg and 0.0308 mmol/L/mmHg, respectively, the concentration of dCO_2 is around 1.2 mmol/L. This

corresponds to 20-fold lower than the concentration of HCO_3-, where the normal range in arterial blood as 21 to 27 mmol/L[10]. Because the ambient air contains less CO_2 than blood, there is a tendency for dCO_2 to be lost from the specimen into the atmosphere.

The atmospheric pressure is 760 mmHg, which is simply the sum of the partial pressures of each constituent gas. The air comprises approximately 0.03% of CO_2, therefore, is the pCO_2 is (0.03×760)/100=0.23 mmHg. In the SDMC, atmospheric CO_2 increased almost to 1,600 ppm (0.15%) in the afternoon, thereby, the pCO_2 in the air was (0.15×760)/100=1.22 mmHg, which increases 5-times, approximately. Based on the limited information available in the literature, the underling mechanisms to explain the irregular atmosphere CO_2 in the SDMC, which is more diffusion and solubility in the plasma or serum samples, is not clear. However, the researchers believe that it may be related to the pCO_2 between air and sample and the dCO_2 level in the sample as well as the environmental factors. Based on several sources[10], the pCO_2 in venous blood ranging from 40 to 50 mmHg is higher than in the arterial blood (35 to 45 mmHg), but this pressure in plasma or serum fraction is not known. In a pre-analytical phase, the samples are not handled anaerobically during processing of the centrifuged and aliquoted plasma or serum fraction. Consequently, dissolved dCO_2 is lost to the atmosphere, which would result in a decrease of dCO_2 in the samples by a half compared to whole blood (0.76 mmol/L vs. 1.4 mmol/L)[11]. As known, the solubility coefficient of CO_2 is not constant and increases as the temperature falls[12]. This coefficient at room temperature would increase when compared to that at body temperature. Due to the decrease in dCO_2 and the increase in CO_2 solubility coefficient, one can imply that the plasma or serum pCO_2 would be much lower. In an analytical process, preservation of anaerobic condition is not practical when the time plasma is placed on an instrument and the time it takes to sample. Taking together, it is possible that the irregular atmosphere CO_2 in the SDMC may be more diffusion and solubility in the plasma or serum samples.

The CO_2 continues to pile up in the atmosphere, which is a global environmental problem. According to the data from the National Oceanic and Atmospheric Administration (Earth System Research Laboratory, US Department of Commerce), the atmospheric CO_2 concentration globally averaged over the marine surface sites was 395 ppm in 2015 and will be 410 ppm in 2019[13]. Bangkok, Thailand is one of the largest and dense cities in the world. Traffic is heavy throughout the day and usually heavier around a few roads and intersections. The SDMC building is located at a busy intersection, which is one of the areas with heavy traffic. Moreover, many patients, approximately 5,000 outpatients per day, are present in the building, resulting in the release of more CO_2 into the building's atmosphere. Any irregularly high CO_2 in the atmosphere may dissolve in the sample, leading to falsely high values. The present study results demonstrated that the TCO_2 concentration increases by approximately 0.4 mmol/L for every 100 ppm of CO_2 increase in the atmosphere. Falsely elevating serum bicarbonate and producing a spurious reduction in serum anion gap may lead to wrong interpretations, especially in patients with a tendency to have low TCO_2 concentrations, such as those with kidney disease[14], diabetes ketoacidosis[15], respiratory alkalosis[16], and metabolic acidosis[17]. Because pollutants in the air are not always visible and the source of pollutants can be different[6], laboratory buildings near busy roads in large and dense cities worldwide should be concerned about the effect of unexpected high atmospheric CO_2 concentrations on test measurements.

The environment of a large building structure cannot be easily corrected. An irregularly high atmospheric CO_2 concentration, especially in daytime, can alter both systematic and random errors of the measurement procedure. To prevent erroneous results from being reported, a more stringent quality control strategy should be applied to this measurement procedure. Thus, the more likely the result can cause severe harm to the patient, the more frequent the quality control events should be scheduled[7,18]. Because of the considerable effect of atmospheric CO_2 on the bias of the measurement procedure, standardization curves should be frequently checked.

Conclusion

A high atmospheric CO_2 concentration can result in falsely high TCO_2 values. The TCO_2 concentration increases by approximately 0.4 mmol/L for every 100 ppm of CO2 increase in the atmosphere. The falsely high TCO_2 values lead to markedly wrong interpretations, especially in patients with a tendency to have low TCO_2 concentrations. Because CO_2 continues to build-up in the atmosphere and the air pollutants are not always visible, laboratory buildings in large and dense cities worldwide should be concerned about the effect of unexpected high atmospheric CO_2 concentrations on test

measurements.

What is already known on this topic?

Serum TCO_2 measurements are easily affected by numerous factors such as environment variables. Greenhouse gases have been rising extensively since the Industrial Revolution. Little is known regarding whether an irregularly high atmospheric CO_2 concentration can affect the TCO_2 measurement.

What this study adds?

Any irregularly high CO_2 in the atmosphere may dissolve in the blood sample, leading to falsely high levels of TCO_2. For every 100 ppm of CO_2 increase in the atmosphere, the TCO_2 concentration increases by approximately 0.4 mmol/L, which may lead to wrong interpretations, especially in patients with a tendency to have low TCO_2 values, such as those with kidney disease, diabetes ketoacidosis, respiratory alkalosis, and metabolic acidosis.

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Conflicts of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Fencl V, Jabor A, Kazda A, Figge J. Diagnosis of metabolic acid-base disturbances in critically ill patients. Am J Respir Crit Care Med 2000;162:2246-51.
2. Kraut JA, Madias NE. Metabolic acidosis of CKD: An update. Am J Kidney Dis 2016;67:307-17.
3. International Federation of Clinical Chemistry and Laboratory Medicine. IFCC Scientific Division, Working Group on Selective Electrodes. IFCC reference measurement procedure for substance concentration determination of total carbon dioxide in blood, plasma or serum. Clin Chem Lab Med 2001;39:283-8.
4. Scott MG, LeGrys VA, Hood JL. Electrolytes and blood gasses. In: Burtis CA, Ashwood ER, Burns DE, editors. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. St. Louis, MO: Elsevier Saunders; 2012. p. 807-35.
5. Bray SH, Tung RL, Jones ER. The magnitude of metabolic acidosis is dependent on differences in bicarbonate assays. Am J Kidney Dis 1996;28:700-3.
6. Harris JM, Roach B. Global climate change: Science and economics. In: Harris JM, Roach B, editors. Environmental and natural resource economics: A contemporary approach. 4th ed. New York: Taylor and Francis; 2016. p. 306-34.
7. Westgard JO. Six sigma quality design & control: Desirable precision and requisite qc for laboratory measurement processes. 2nd ed. Madison, WI: Westgard QC; 2006.
8. Clinical and Laboratory Standards Institute. CLSI document EP05-A: Evaluation of precision of quantitative measurement procedures; Approved guideline. 3rd ed. Wayne, PA: CLSI; 2014.
9. Kumproa N, Vanavanan S, Promnuch S, Pornpen Srisawasdi P. Sigma metric as an essential tool for quality management: Case study from serum total carbon-dioxide. J Med Tech Assoc Thai 2019;47:7016-26.
10. Adrogué HJ, Madias NE. Normal acid-base values. In: Gennari FJ, Adrogue HJ, Galla JH, Madias NE, editors. Acid-base disorders and their treatment. Boca Raton, FL: Taylor and Francis; 2005. p 789-99.
11. Arthurs GJ, Sudhakar M. Carbon dioxide transport. Continuing Education in Anaesthesia, Critical Care & Pain 2005;5:207-10.
12. Christmas KM, Bassingthwaighte JB. Equations for O2 and CO2 solubilities in saline and plasma: combining temperature and density dependences. J Appl Physiol (1985) 2017;122:1313-20.
13. Global Monitoring Division, National Oceanic and Atmospheric Administration. Trends in atmospheric carbon dioxide [Internet]. 2019 [cited 2019 May 15]. Available from: https://www.esrl.noaa.gov/gmd/ccgg/trends/global.html.
14. Wallia R, Greenberg A, Piraino B, Mitro R, Puschett JB. Serum electrolyte patterns in end-stage renal disease. Am J Kidney Dis 1986;8:98-104.
15. Adrogué HJ, Wilson H, Boyd AE 3rd, Suki WN, Eknoyan G. Plasma acid-base patterns in diabetic ketoacidosis. N Engl J Med 1982;307:1603-10.
16. Krapf R, Beeler I, Hertner D, Hulter HN. Chronic respiratory alkalosis. The effect of sustained hyperventilation on renal regulation of acid-base equilibrium. N Engl J Med 1991;324:1394-401.
17. Adeva-Andany MM, Fernández-Fernández C, Mouriño-Bayolo D, Castro-Quintela E, Domínguez-Montero A. Sodium bicarbonate therapy in patients with metabolic acidosis. ScientificWorldJournal 2014;2014:627673.
18. Westgard JO, Westgard SA. Six sigma quality management system and design of risk-based statistical quality control. Clin Lab Med 2017;37:85-96.