ORIGINAL RESEARCH PAPER

Biochemistry

KEY WORDS: Anion Gap, Delta Gap, Delta Ratio, High Anion Gap Metabolic Acidosis.

BIOCHEMICAL APPROACH TO THE DIFFERENTIAL DIAGNOSIS OF METABOLIC ACIDOSIS IN CRITICALLY ILL- CHILDREN.

Dr. Aruna Kumari Bandaru M.D (Biochemistry) Associate Professor ,Department of Biochemistry, Government Medical college, Mahabubnagar, Telangana- 509001.

ABSTRACT

Objective: The aim of the study was to simplify the arterial blood gas analysis and facilitate the differential diagnosis of metabolic acidosis (MA) in critically ill- children.

Methods: A descriptive study was conducted on 150 children admitted in the pediatric intensive care unit were analyzed for acid-base status. The Anion Gap (AG) was calculated to identify the causes of MA. Delta gap and Delta ratio used to assess the presence of mixed acid-base disorders.

Results: There was a significant decrease in means of PH, Bicarbonate (HCO_3^-), and Base Excess, while there is increase in AG between the MA and control groups. There was a significant decrease in HCO_3^-, Chloride, and Chloride: Sodium (Cl^-/Na^+) ratio, while there was a significant increase in AG, Delta Gap, and Delta Ratio in high AGMA than Normal AGMA.

Conclusion: Cl^-/Na^+ ratio, Delta Gap and Delta Ratio can be used as indicators in differentiating MA.

INTRODUCTION

Acid-base disorders are among the most common abnormalities seen in critically ill patients, reflect the seriousness of the underlying disease [1-3]. Arterial blood gas analysis plays a vital role in monitoring of postoperative patients and those on intensive support [4]. This simple tool has been underutilized, most frequently due to the difficulty in proper understanding and interpretation [1]. Determination of the anion gap (AG) is very important step in the differential diagnosis of acid-base disorders. Elevated AG usually represents abnormal accumulation of unmeasured anions and indicates a primary disorder metabolic acidosis (MA), regardless of the pH or the serum bicarbonate (HCO_3^-) [3,5,6].

The MA of critical illness is typically multi-factorial (Mixed Metabolic Acid–Base Disturbances), often making a single diagnosis impossible or inappropriate [6]. Therefore, when AG metabolic acidosis is diagnosed, it is imperative to screen for the presence of additional acid-base abnormalities (called mixed metabolic acid-base disorders) [5]. The Delta Ratio or Delta Gap (ΔΔ) can be used to assess the presence and nature of mixed acid-base disorders [5,6].

The chloride to sodium (Cl^-/Na^+) ratio (normal range 0.75–0.79) serves as a simple substitute to quantify the role of hyperchloremia in acid–base disturbances. Patients with MA and high Cl^-/Na^+ ratio may have hyperchloremia as the cause of acidosis. A normal Cl^-/Na^+ and MA indicate a mixed acidosis ie, hyperchloremia and raised AG. A low Cl^-/Na^+ ratio (<0.75) in the MA, suggests a raised AG [2,7].

The aim of the study was (1) to simplify the arterial blood gas analysis for a rapid and easy bedside interpretation, (2) to facilitate the differential diagnosis of metabolic acidosis and (3) to assess the presence and nature of mixed acid-base disorders.

MATERIALS AND METHODS

The present descriptive study was conducted on 150 children admitted to the pediatric intensive care unit (ICU) at Mahabubnagar Government General Hospital with various conditions from age group one day to 5 years over a period of one year were analyzed for acid-base status. No healthy control group was taken as it would have been unethical to procure arterial samples of healthy neonates. However, based on the acid base status were categorized into normal acid base status with normal AG as controls and MA as cases. Among 150 children, 33 children have the PH value of 7.35 -7.45, partial pressure of carbondioxide (PCO_2):35 -45 mmHg, bicarbonate (HCO_3^-):22-28mEq/L were considered to be within normal ranges with normal AG were included in the study as controls. 117 children have PH <7.35, decrease in HCO_3^-(<22mEq/L), normal PCO_2 were defined as MA.

Arterial and venous blood samples were taken simultaneously from all patients for blood analyses including electrolytes estimation. 2 mL of heparinized arterial or venous blood sample was obtained from children aseptically at the time of admission. Samples were carried on ice pack taken in airtight, plastic, disposable syringes, which were flushed with heparin solution prior to sample withdrawal to avoid pH changes. Blood gas and electrolytes were analyzed by using Radiometer ABL 800 Basic analyzer. Appropriate control materials were used to ensure quality control. Blood gas analysis includes pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), Na^+, K^+, Cl^- by respective electrodes. Standard base excess (BE) and HCO_3^- were calculated parameters from PH and PCO_2, which were provided by the analyzer. AG was calculated by using the equation $AG=(Na^+ + K^+) - (Cl^- + HCO_3^-)$ [3], used to further categorize the MA into high anion gap metabolic acidosis (HAGMA) and normal anion gap metabolic acidosis (NAGMA) and helps to identify the causes of MA. A normal AG is 12 ± 4 mEq/L [6,8]. Delta gap and Delta ratio can be used to assess the presence and nature of mixed acid-base disorders [9,10,11].

Delta Ratio=$\Delta AG/\Delta HCO_3^-$ or ΔΔ or Delta Gap=$\Delta AG - \Delta HCO_3^-$
For both formulas: ΔAG =AG (actual)-12(normal) and $\Delta HCO_3^- = 24(\text{normal } HCO_3^-) - HCO_3^-(\text{actual})$ [5,6].
Interpretation of the Delta Gap: [11]

<-6 = Mixed HAGMA and NAGMA (hyperchloremic acidosis)
- -6 to 6 = Only a HAGMA exists
- > 6 = Mixed HAGMA and metabolic alkalosis

Interpretation of the Delta Ratio: [8,10,11]

- <0.4 = pure NAGMA
- 0.4-0.8 = mixed HAGMA and NAGMA.
- 0.8-2.0 = pure HAGMA
- Over 2.0 = HAGMA with pre-existing metabolic alkalosis

Statistical analysis

Statistical analysis was carried out using Sofa Stats software. The significance of the difference between two independent proportions and an unpaired t-test used for "p" values, were calculated by using Medcalc- easy to use statistical software. A p-value of ≤ 0.05 was taken as statistically significant.

OBSERVATIONS AND RESULTS

Among 150 children enrolled in the study, 87(58%) were male and 63 (42%) were female. Based on acid base status children were grouped into controls and MA. Out of total study group, 33 (22%) were controls and 117 (78%) were MA. There was a significant decrease in means of PH (7.22 vs 7.39), HCO_3^-(16.17 vs 23.53mmol/L), and BE (-10.78 vs-1.22) with p<0.0001, while there was a significant increase in AG (18.22 vs 11.18 mmol/L, p<0.0001) between MA and control groups (Table 1).

TABLE 1: Comparison of variables between control and metabolic acidosis

variables	Control Mean ± SD	MA Mean ± SD	p-value	95% CI
PH	7.39 ± 0.02	7.22 ± 0.11	<0.0001*	-0.21 to -0.13
PCO_2(mmHg)	38.67 ± 3.03	40.92 ± 12.1	0.29	-1.9 to 6.4
HCO_3^- (mmol/L)	23.53 ± 1.47	16.17 ± 3.22	<0.0001*	-8.5 to -6.21
BE (mmol/L)	-1.22 ± 2.16	-10.78 ± 10.35	<0.0001*	-13.15 to -5.96
Cl^- (mmol/L)	106.3 ± 2.39	107.5 ± 5.58	0.23	-0.78 to 3.16
Cl^-/ Na^+ ratio	0.78 ± 0.01	0.78 ± 0.04	1	-0.01 to 0.01
AG(mmol/L)	11.18 ± 2.02	18.22 ± 6.97	<0.0001*	4.6 to 9.5

AG: Anion Gap; BE: Base Excess; MA: Metabolic Acidosis; * Significant

Metabolic acidosis was categorized into HAGMA and NAGMA or hyperchloremic MA based on the levels of AG. Among the 117 MA, 68 (58.1%) were HAGMA and 49 (41.9%) were NAGMA. There was a significant decrease in HCO_3^- (15.65 vs 16.89 mmol/L, p=0.04), Cl^- (106.2 vs 109.3 mmol/L, p=0.002), and Cl^-/Na^+ ratio (0.76 vs 0.81, p<0.0001), while there was a significant increase in AG (22.25 vs 12.63 mmol/L), Δ Gap (1.95 vs -6.71 mmol/L), and Δ Ratio (1.36 vs 0.09) with p<0.0001in HAGMA than NAGMA (Table 2).

TABLE 2: Comparison of variables between HAGMA and NAGMA

variables	HAGMA Mean ± SD	NAGMA Mean ± SD	p-value	95% CI
PH	7.2 ± 0.13	7.24 ± 0.09	0.07	-0.00 to 0.08
PCO_2(mmHg)	40.95 ±13.12	40.88 ± 10.66	0.97	-4.6 to 4.4
HCO_3^-(mmol/L)	15.65 ±3.62	16.89 ± 2.4	0.04	0.06 to 2.41
BE (mmol/L)	-11.98 ± 13.22	-9.11 ± 3.19	0.14	-0.95 to 6.69
Cl^- (mmol/L)	106.2 ± 5.07	109.3 ± 5.81	0.002*	1.1 to 5.1
Cl^-/Na^+	0.76 ± 0.04	0.81 ± 0.02	<0.0001*	0.04 to 0.06
AG(mmol/L)	22.25 ± 6.46	12.63± 2.06	<0.0001*	-11 to -8.3
Δ Gap(mmol/L)	1.95 ± 6.26	-6.71 ± 3.51	<0.0001*	-10.6 to -6.69
Δ Ratio	1.36 ± 0.89	0.09 ± 0.42	<0.0001*	-1.54 to -0.99

HAGMA: High anion gap metabolic acidosis; NAGMA: Normal anion gap metabolic acidosis; AG: Anion Gap; BE: Base Excess; * Significant

In metabolic acidosis, the prevalence of Δ Gap <-6 mmol/L was 32.5%, Gap <-6 to >+6 mmol/L was 54.7%, Δ Gap >+6 mmol/L was 12.8%. Among the types of MA, the prevalence of Δ Gap of <-6 mmol/L was significantly more in NAGMA than HAGMA (69.4% vs 5.9%, p<0.0001), where the prevalence of Δ Gap of -6 to +6 mmol/L and >+6 mmol/L were significantly more in HAGMA than NAGMA (73.7% vs 28.6%, p<0.0001 and 20.6% vs 2%, p=0.003). The prevalence of raised Cl-/Na+ was significantly more in NAGMA than HAGMA (73.5% vs 10.3%, p<0.0001) (Table 3).

In Metabolic acidosis, prevalence of Δ Ratio of <0.4 was 35.9%, 0.4 to 0.8 was 17.9%, 0.8 to 2 was 37.6%, >2 was 8.5%. Among the types of MA, the prevalence of Δ Ratio of <0.4 was significantly more in NAGMA than HAGMA (83.7% vs 1.5%, p<0.0001), where the prevalence of Δ Ratio of 0.8 to 2 and >2 were significantly more in HAGMA than NAGMA (63.2% vs 2%, p<0.0001 and 13.2% vs 2%, p=0.03) (Table 3).

TABLE 3: Prevalence of abnormal variables between HAGMA and NAGMA

Variables	Total MA 117	HAGMA No(%)	NAGMA No(%)	p-value	95% CI
Rised Cl^-/Na^+	45(38.5)	7(10.3)	36(73.5)	<0.0001*	46.5 to 74.7
Δ Gap					
<-6 (mmol/L)	38(32.5)	4(5.9)	34(69.4)	<0.0001*	47.3 to 75.2
-6 to +6 (mmol/L)	64(54.7)	50(73.5)	14(28.6)	<0.0001*	26.9 to58.9
>+6 (mmol/L)	15 (12.8)	14 (20.6)	1 (2)	0.003*	6.9 to 29.8
Δ Ratio					
<0.4	42(35.9)	1(1.5)	41(83.7)	<0.0001*	67.9 to 90.1
0.4 to 0.8	21(17.9)	15(22.1)	6(12.2)	0.17	-4.6 to 22.8
0.8 to 2	44(37.6)	43(63.2)	1(2)	<0.0001*	46.5 to 71.8
>2	10(8.5)	9(13.2)	1(2)	0.03*	0.6 to 21.4

HAGMA: High anion gap metabolic acidosis; NAGMA: Normal anion gap metabolic acidosis; AG: Anion Gap; BE: Base Excess; * Significant

The prevalence of increased Cl-/Na+ ratio was significantly high in NAGMA than HAGMA (73.5% vs 13.2%, p<0.0001), the prevalence of normal and decreased Cl^-/Na^+ ratio was significantly more in HAGMA than in NAGMA (60.3% vs 4.5%, p=0.0001 and 26.5% vs 2%, p=0.0004). The prevalence of Δ Gap >+6 mmol/L Δ and Δ Ratio >2 was more in decreased Cl-/Na+ ratio than normal and increased Cl-/Na+ ratio (72.2% vs 2.4% vs 0% and 53% vs 0% vs 0%) (Table 4).

TABLE 4: Comparison of Cl-/Na+ ratio between HAGMA and NAGMA with Δ Gap and Δ Ratio.

Cl^-/Na^+ ratio	HAGMA No (%)	NAGMA No (%)	p-value	Δ Gap (>+6 mmol/L)	Δ Ratio >2
Increased	9(13.2)	36(73.5)	<0.0001*	0(0)	0(0)
Normal	41(60.3)	12(24.5)	0.0001*	1(2.4)	0(0)
Decreased	18(26.5)	1(2)	0.0004*	13(72.2)	9(53)

* Significant

DISCUSSION

Acid-base disorders are currently assessed by the plasma concentration of HCO3- and SBE, and further completed by the use of plasma AG are the most widely methods used to evaluate the metabolic component of acid-base disturbances [1]. In our study the means of PH, HCO3-, BE were significantly decreased and AG was increased with p<0.0001 in MA than in controls. Either of HCO-3or BE can be used to interpret, the metabolic component of the acid–base balance [4]. The BE increases in metabolic alkalosis and decreases in metabolic acidosis, it cannot identify whether an acidosis is due to increased tissue acids, hyperchloremia, or a combination of both [1] . MA is characterized by a low arterial PH, reduced plasma HCO3-, and compensatory respiratory response [3].

Atalan HK etal. 2017 study [2] showed a high Cl/Na+ ratio was detected in 11.5% of the patients with acidosis. In our study 38.5% had raised Cl-/Na+ratio in MA than control. The recognition of hyperchloremia is one of the cause of MA.

In the MA, an elevated AG should be accompanied by an equal decrease in the BE. Similarly, a BE which has changed more than the AG suggests that a non-anion-gap acidosis is also present [10]. In our study AG is increased to 1.63 times and BE is decreased to 8.84 times in MA than in control, suggests that the MA also associate with a non-anion-gap acidosis. The categorization of MA into

NAGMA and HAGMA, primarily used to facilitate the differential diagnosis of MA. However, it also has relevance for predicting the clinical outcome and determining indications for treatment. Although many clinicians presume that acute MA in seriously ill patients will be due to a HAGMA, recent studies indicate that NAGMA or combination of NAGMA and HAGMA might be more frequent [8]. Our study in agreement with Kraut JA etal. 2015 study (8) that MA in seriously ill patients is not only due to a HAGMA (58.1%) but also NAGMA (41.9%). The MA of critical illness is typically multi-factorial (Mixed Metabolic Acid–Base Disturbances), often making a single diagnosis impossible or inappropriate. Δ Gap and Δ Ratio can be used to assess the presence and nature of mixed acid-base disorders.

The mean of HCO_3^-, Cl^-, Cl^-/Na^+ ratio were decreased, while AG, Δ Gap, Δ Ratio were increased in HAGMA than NAGMA. There is no significant rise in BE between HAGMA and NAGMA, and elevated AG is accompanied by almost an equal decrease in the BE. This suggests that BE is used for diagnosis of MA, but not for differential diagnosis.

Based on the Δ Gap assessment, in our study, MA with Δ Gap <-6 mmol/L were 38 (32.5%), among these 34(69.4%) were NAGMA, indicates pure NAGMA (hyperchloremic) and 15 (12.8%) had Δ Gap >+6 mmol/L, among these 14 (20.6%) were HAGMA, indicates that mixed HAGMA associate with metabolic alkalosis. Paulson etal. 1993 study showed 56% of MA group as mixed acid base disturbance [9]. In our study 12.8% of MA associates with mixed acid base disturbance. Based on the Δ Ratio assessment, MA with Δ Ratio <0.4 were 42 (35.9%), among these 41 (83.7%) were NAGMA, indicates pure NAGMA (hyperchloremic), 17.9% had Δ Ratio of 0.4 to 0.8 indicates mixed HAGMA and NAGMA, but no significant difference between HAGMA and NAGMA. 44(37.6%) of MA cases had Δ Ratio of 0.8 to 2, among these 43 (63.2%) were HAGMA, indicates pure HAGMA. Ten (8.5%) of MA cases had Δ Ratio of >2, among these 9 (13.2%) were HAGMA, indicates HAGMA with pre-existing metabolic alkalosis. Thus, 26.4% of MA cases have mixed acid base disturbances. Our study agreed with Rastegar A, 2007 study suggested that mixed disturbances should be considered if the ratio is <0.8 or >2 [9].

An abnormal Cl^-/Na^+ ratio can be the primary reason for a metabolic acid-base disorder [2]. Kurt et al.2015 study [8] indicated the presence of hypochloremic MA in 16% of acid-base disorders. In our study 16.2% had low Cl^-/Na^+ ratio, 45.3% had normal Cl^-/Na^+ ratio, and 38.5% had raised Cl^-/Na^+ ratio with MA.

Our study in consistence with Durward et al. 2001 study [7] showed that raised Cl^-/Na^+ ratio (>0.79) points to hyperchloremia as the cause of acidosis. In our study 36 (73.5%) were NAGMA with increased Cl^-/Na^+ ratio. Thus, raised Cl^-/Na^+ ratio is sensitive indicator of NAGMA (hyperchloremic) for diagnosis and monitoring patients [4, 7]. 18 (26.5%) of HAGMA had decreased Cl^-/Na^+ ratio thus our study in consistence with Durward et al.2001 study that MA associated with a rise in AG should be accompanied by a fall in plasma chloride (Cl^-) relative to sodium (Na^+), while HAGMA associate with normal Cl^-/Na^+ ratio (between 0.75 and 0.79) shows a mixed metabolic acidosis (i.e., mild hyperchloremia associated with a raised AG). In our study, 60.3% of HAGMA had normal Cl^-/Na^+ ratio, indicates presence of mixed MA i.e., mild hyperchloremia associated with a HAGMA. The lower the ratio, the greater is the contribution of the AG. They showed that the Cl^-/Na^+ ratio is a useful marker of the presence of AG with MA and understanding complex acid–base disorders in critically ill pediatric patients [7, 12]. In HAGMA cases, 72.2% of Δ Gap >+6 mmol/L and 53% of Δ Ratio >2 had low Cl^-/Na^+ ratio, indicates HAGMA with pre-existing metabolic alkalosis.

CONCLUSION

Either of HCO_3^-/BE can be used to interpret, the metabolic component of the acid–base balance. It cannot identify the cause of MA. The serum AG has been used to facilitate the differential diagnosis of MA. Δ Gap and Δ Ratio can be used to assess the presence and nature of mixed acid-base disorders. Therefore, when AGMA is diagnosed, it is imperative to screen for the presence of mixed metabolic acid-base disorders. An abnormal $Cl^-/Na+$ ratio can be the primary reason for a metabolic acid-base disorder. Raised Cl^-/Na^+ ratio is sensitive indicator of NAGMA (hyperchloremic) for diagnosis and monitoring patients. HAGMA associate with normal Cl^-/Na^+ ratio shows a mixed metabolic acidosis (i.e. Mild hyperchloremia associated with HAGMA). Low Cl^-/Na^+ ratio is sensitive indicator of pure HAGMA. HAGMA with low Cl^-/Na^+ ratio associate with Δ Gap >+6 mmol/L and Δ Ratio >2, indicates HAGMA with pre-existing metabolic alkalosis. Thus Cl^-/Na^+ ratio, Δ Gap, and Δ Ratio can be used as a bedside tool in differentiating MA, as HAGMA, NAGMA or mixed acid base disturbance in critically ill- children.

ACKNOWLEDGMENT:

I would like to thank professor and HOD of Biochemistry and Pediatric departments, Government general Hospital, Mahabubnagar and the staff for their cooperation during the collection of blood samples.

REFERENCES

1. Mallat J, Barrailler S, Lemyze M, Pepy F, Gasan G, Tronchon L, et al. Use of Sodium-Chloride Difference and Corrected Anion Gap as Surrogates of Stewart Variables in Critically Ill Patients. PLoS ONE 2013; 8(2): e56635.
2. Atalan HK, Gücyetmez B. The effects of the chloride:sodium ratio on acid-base status and mortality in septic patients. Turk J Med Sci 2017 Apr 18;47(2):435-442.
3. Oh YK. Acid-base disorders in ICU patients. Electrolyte Blood Press 2010 Dec;8(2):66-71.
4. Singh V, Khatana S, Gupta P. Blood gas analysis for bedside diagnosis. Natl J Maxillofac Surg 2013 Jul;4(2):136-41.
5. Kraut JA, Nagami GT. The serum anion gap in the evaluation of acid-base disorders: what are its limitations and can its effectiveness be improved? Clin J Am Soc Nephrol 2013 Nov;8(11):2018-24.
6. Fidkowski C, Helstrom J. Diagnosing metabolic acidosis in the critically ill: bridging the anion gap, Stewart, and base excess methods. Can J Anaesth 2009 Mar;56(3):247-56.
7. Durward A, Skellett S, Mayer A, Taylor D, Tibby SM, Murdoch IA. The value of the chloride: sodium ratio in differentiating the aetiology of metabolic acidosis. Intensive Care Med 2001 May;27(5):828-35.
8. Rastegar A. Use of the DeltaAG/DeltaHCO3- ratio in the diagnosis of mixed acid-base disorders. J Am Soc Nephrol 2007 Sep;18(9):2429-31.
9. Kraut JA, Kurtz I. Treatment of acute non-anion gap metabolic acidosis. Clin Kidney J 2015 Feb;8(1):93-9.
10. Tsapenko MV. Modified delta gap equation for quick evaluation of mixed metabolic Acid-base disorders. Oman Med J 2013 Jan;28(1):73-4.
11. Wrenn K1. The delta (delta) gap: an approach to mixed acid-base disorders. Ann Emerg Med 1990 Nov;19(11):1310-3.
12. Kurt A, Ecevit A, Ozkiraz S, Ince DA, Akcan AB, Tarcan A. The use of chloride-sodium ratio inthe evaluation of metabolic acidosis in critically ill neonates. Eur J Pediatr 2012 Jun;171(6):963-9.