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ORIGINAL ARTICLE

COMPARISON OF TWO DIFFERENT MODALITIES OF BICARBONATE FOR DETERMINATION OF ANION GAP IN CRITICALLY ILL PATIENTS

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ABSTRACT

OBJECTIVE: To compare anion gap estimated through measured and calculated bicarbonate modalities to be used interchangeably in critically ill metabolic acidosis patients.

METHODS: This cross-sectional study was conducted at Rehman Medical Institute, Peshawar, Pakistan from September 2019 to March 2020. Out of 390 critically ill patients, 200 cases of metabolic acidosis were selected by non-probability consecutive sampling technique. Measured and calculated bicarbonate values were obtained through Cobas-c 501© (Roche) using enzymatic method and Cobas-b 221© (Roche) blood gas analyzer respectively. Statistical analysis was done by using SPSS-23.

RESULTS: Normal anion gap metabolic acidosis (NAG-MA) and high anion gap metabolic acidosis (HAG-MA) based on calculated bicarbonate levels was observed in 57 (28.5%) and 143 (71.5%) cases as compared to 55 (27.5%) and 145 (72.5%) cases based on measured bicarbonate levels respectively ($p>0.45$). A significant correlation ($r=0.888$ and 0.656, $r^2=0.788$ and 0.431) ($p<.001$) was found between mean values of NAG-MA and HAG-MA respectively, when each was calculated through both modalities of bicarbonate. On applying Bland Altman plot, bias was 1.45±2.89 and -2.14±3.87mmol/L, Upper limit of agreement (LOA) was 7.13 and 5.46 for NAG-MA and HAG-MA, while lower LOA was -4.23 and -9.74 for NAG-MA and HAG-MA respectively. According to the model Bland Altman plot and Story & Postuie criteria, bias and the levels of agreement were not appropriate to conclude that both entities of anion gap could be used interchangeably.

CONCLUSION: Normal and high anion gap metabolic acidosis estimated by measured and calculated bicarbonate cannot be used interchangeably in critically ill patients.

KEY WORDS: Acid-Base Equilibrium (MeSH); Anion Gap (MeSH); Acid-Base Imbalance (MeSH); Acidosis (MeSH); Hydrogen Ion Concentration (MeSH); Blood (MeSH); Body Fluids (MeSH); Fluid Shifts (MeSH); Bicarbonates (MeSH); Buffers (MeSH); Electrolytes (MeSH).

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INTRODUCTION

Acid-base disturbances due to underlying pathology or intensive treatment are frequently encountered in critically ill patients and associated with high morbidity and mortality. The appropriate interpretation of acid-base disorders is important for understanding etiology, making a prompt diagnosis, devising proper therapeutic measures and follow up. The serum anion gap has been in practice to identify discrepancies in the measurement of suspected acid-base disorders and electrolytes imbalances. Anion gap is an important tool in the context that it helps in classifying acid base disturbances into normal and high anion gap metabolic acidosis. Anion gap is not a physiological reality as anions are always equal to cations in normal and abnormal conditions. It is reported because of the presence of unmeasured anions like sulfates, nitrates and proteins etc. which cannot be measured with routine lab methods. Anion gap can be calculated by the following equation: Anion gap = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$

Although sodium, potassium and chloride are components of anion gap yet there is a presumed 1:1 relationship between change in the anion gap and change in the serum bicarbonate levels. As bicarbonate (HCO_3^-) measurement is an important component in the calculation of anion gap, it can play an important role in critically ill patients. Bicarbonate can be a measured or a calculated value. It can be measured in the serum by extracting all the CO_2 present in it by chemistry analyzers or it can be calculated as actual HCO_3^- in the arterial blood by the arterial blood gas (ABG) analyzers. Majority of ABG analyzers use Henderson-Hasselbach equation which can be expressed as; $\mathbf{pH = pKa + Log\ HCO_3 / \alpha\ pCO_2}$ with the pre-requisite of keeping pKa and α (solubility co-efficient) constant, where, pKa is dissociation constant for carbonic acid equivalent to 6.1 in blood at 37°C and α has a value of 0.03 at 37°C. The pK value can be affected by changes in temperature and pH while solubility co-efficient can change due to presence of other substance like lipids, proteins and salts. Therefore, calculated bicarbonate value may lead to anion gap calculation error under some conditions and raising questions on its reliability. Moreover, we know that ABGs analysis is in routine performed for evaluation of acid base status of the patient, but the procedure has some other pitfalls as well. There can be issues of patient compliance because the procedure may be painful, there are chances of developing complications like local hematoma and thrombosis. On the contrary taking blood for measured bicarbonate which is evaluated in venous blood is comparatively safer and easier procedure with lesser chances of complications both to the patient as well as health care worker. Different studies have been conducted to assess whether these two modalities of HCO_3 can be used interchangeably for determining anion gap. Some studies showed good level of agreement while others showed poor level of agreement.

As anion gap measurement is one of the most commonly carried out tests in patients admitted in intensive care units, the present study was conducted to evaluate level of agreement between two entities of anion gap estimation in our local population as no such study has been conducted in Pakistan so far. If we find these two entities comparable with each other, we can recommend using these two interchangeably. The present study was aimed to determine whether anion gap estimated through measured and calculated HCO_3 modalities can be used interchangeably.

METHODS

This cross-sectional study was carried out at Rehman Medical Institute (RMI), Peshawar, Pakistan from September 2019 to March 2020 after obtaining permission from ethical review board.

Sample size was calculated as 186 by using WHO formula (with a prevalence of 14% and margin of error of 5%). All critically ill patients, regardless of gender, admitted in intensive care units (ICU), coronary care units (CCU) and neonatal ICU at RMI were screened for acid base metabolic disorders. Informed consent was taken from patients or their attendants for the study.

Out of 390 critically ill patients selected through non-probability consecutive sampling technique for screening of metabolic disorder, 263 (67.5%) patients had acid base metabolic disorder. Sixty three patients with metabolic disorder other than metabolic acidosis were excluded and 200 patients with metabolic acidosis were finally included in the study.

Venous and arterial blood samples from the patients were taken at the same time and transported in icebox within 15 minutes of sampling. Electrolytes, calculated bicarbonate ($cHCO_3$) and calculated anion gap values were derived from ABG sample using Cobas b 221© (Roche) ABG analyzer which calculates bicarbonate concentration from arterial pH and pCO_2. Measured bicarbonate ($mHCO_3$) and measured anion gap values were estimated by using Cobas c 501© (Roche) by means of enzymatic procedures. This enzymatic procedure uses phosphoenolpyruvate which is based on reaction of bicarbonate with phosphoenolpyruvate in the presence of phosphoenolpyruvate carboxylase. This reaction produces oxaloacetate and phosphate, involving the transfer of a hydrogen ion from reduced nicotinamide adenine dinucleotide (NADH) to oxaloacetate using malate dehydrogenase (MDH). The consumption of NADH causes a decrease in absorbance, which is proportional to the concentration of HCO_3^- in specimen being analyzed. Calculated and measured anion gap were estimated by using calculated and measured HCO_3 respectively with the help of anion gap equation.

Statistical analysis was done by using SPSS 23 version. Demographics like age and gender distribution were calculated and linear regression analysis was used to assess the correlation between both normal and high anion gap estimated through measured and calculated HCO_3. Bland Altman plot was constructed and later on Story and Postuie criteria were applied to it to detect limit of agreement between the two anion gap entities. Model Bland Altman plot was constructed by applying percentage differences against mean of differences to assess agreement in relation to pre-defined total allowable error (TEa). TEa is a quality criterion that establishes an acceptable limit for combined imprecision and bias in one measurement or test result to make sure it is clinically beneficial.

RESULTS

Out of 200 patients with metabolic acidosis, 107(53.5%) were males and 93(46.5%) were females. Twenty (10%) patients were aging less than <15 years, 112 (56%) were ranging in age from 15-65 years and 68 (34%) patients were more than 65 years old. Mean age of patients was 50.13±23.78 years.

On further analysis of metabolic acidosis it was found that 57/200(28.5%) and 55/200 (27.5%) had normal anion gap metabolic acidosis on basis of measured HCO_3 and calculated HCO_3 respectively (Table 1).

On applying linear regression, a significant correlation ($r=0.888$ and 0.656, $r^2=0.788$ and 0.431) was found between mean values of normal anion gap and between mean values of high anion gap respectively

TABLE I: NORMAL AND HIGH ANION GAP ON BASIS OF CALCULATED AND MEASURED BICARBONATE (N=200)

Anion Gap	Metabolic acidosis	
	On basis of calculated bicarbonate	on basis of measured bicarbonate
Normal Anion gap (≤18 mmol/L)	57 (28.5%)	55 (27.5%)
High Anion gap (>18 mmol/L)	143 (71.5%)	145 (72.5%)

(Table II), when each was calculated through both modalities of HCO_3 at 95% confidence interval.

Graphical representation of linear regression analysis is showing that we can have 78.8% and 43.1% of variance for both normal and high anion gap

high anion gap with calculated and measured HCO_3 against the means of both bicarbonate entities respectively. The bias was 1.45 with standard deviation of 2.89 for normal anion gap and 2.14 with standard deviation of 3.87 mmol/L for High anion gap. Bias was actually mean of difference between both the normal and

mmol/L of normal anion gap and 15.2 mmol/L of high anion gap. Out of 55 values for normal anion gap 52(94.5%) and 145 values for high anion gap, 142(97.9%) were within the limit of agreement.

Model Bland Altman plot in terms of percentage difference was constructed

TABLE II: NORMAL AND HIGH ANION GAP ON BASIS OF CALCULATED AND MEASURED BICARBONATE (N=200)

Variable		Mean	Std. Deviation	r value	r^2 value	P value
Normal Anion Gap	With calculated HCO_3	14.42	5.71	0.888	0.788	<0.001
	With measured HCO_3	12.96	6.28			
High Anion Gap	With calculated HCO_3	24.72	4.51	0.656	0.431	<0.001
	With measured HCO_3	26.86	4.82			

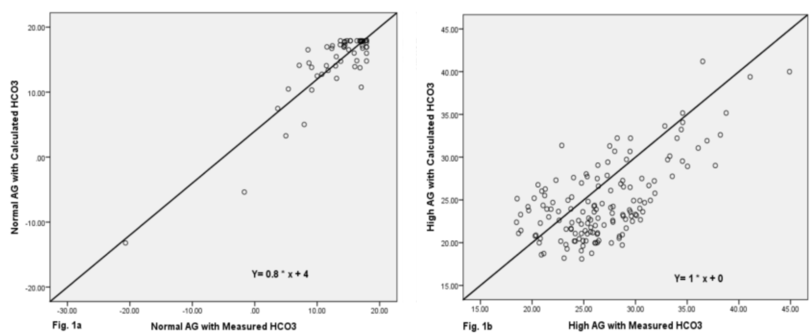

Figure 1a & 1b: Linear regression analysis of normal and high anion gap, estimated by measured and calculated bicarbonate at 95% confidence interval

values and this association is given by the equation Y=0.8*x+4 and Y=1*x+0 showing slope of 0.8 and 1 with intercept of 4 and 0 respectively.

Bland Altman plot was made by plotting differences between normal anion gap with calculated and measured HCO_3 and

high anion gap entities calculated by using one sample t-test. Using the value of bias and SD, the upper limit of agreement (LOA) was 7.13 and 5.46 mmol/L of normal and high anion gap respectively while the lower LOA was -4.23 and-9.74 mmol/L for normal and high anion gap respectively with a total span of 11.36

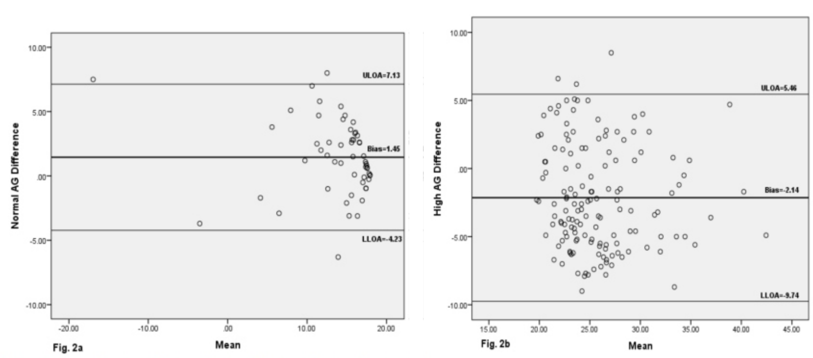

Figure 2a & 2b: Bland altman plot representing levels of agreement between normal and high anion gap entities

against mean of both bicarbonate entities showing upper LOA of 67.27% and lower LOA of -43.85% for normal anion gap and upper LOA of 22.16% and lower LOA of -38.6% for high anion gap at 95% confidence interval. When limits of agreement were compared with predefined total allowable error (TEa) of 10% for 2 instrument comparisons it showed that our limits for both normal and high anion gap exceeded TEa and analytical performance of both instruments for bicarbonate modalities so they were not in agreement and cannot be used interchangeably for calculation of anion gap.

Model Bland Altman plot in terms of percentage difference was constructed against mean of both bicarbonate entities showing upper LOA of 67.27% and lower LOA of -43.85% for normal anion gap and upper LOA of 22.16% and lower LOA of -38.6% for high anion gap at 95% confidence interval. When limits of agreement were compared with predefined total allowable error (TEa) of 10% for 2 instrument comparisons it showed that our limits for both normal and high anion gap exceeded TEa and analytical performance of both instruments for bicarbonate modalities so they were not in agreement and cannot be used interchangeably for calculation of anion gap.

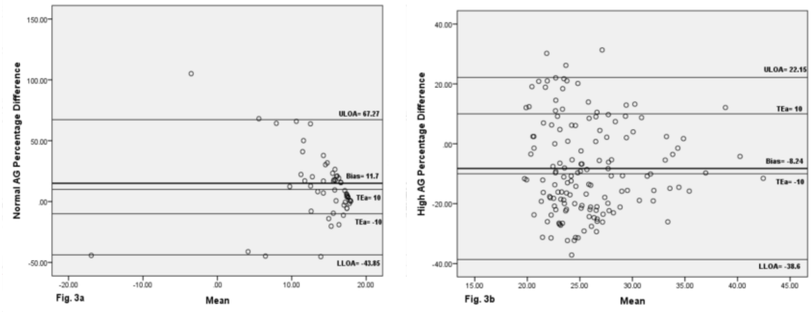

Figure 3a & 3b: Model bland Altman plot showing percentage difference vs mean

DISCUSSION

According to this study, results showed that there was no significant difference between mean of two entities of anion gap. On linear regression analysis "r^2" value for normal and high anion gap was 0.788 and 0.431 respectively and there was a highly significant correlation of <0.01 between the both at a confidence interval of 95%. This was comparable for normal anion gap with the study conducted by Nadzimah et al. which showed a r^2 value of 0.953 and a p value of <0.001. But literature review showed that only establishing correlation is not enough to assess the agreement while comparing two different methods for the measurement of a parameter. Bland Altman plot on other hand is the best way to assess the LOA of normal and high anion gap with calculated and measured HCO_3. On interpretation of Bland Altman plot the bias for normal and high anion gap was 1.45 and -2.14 mmol/L with a SD of 2.89 and 3.87 mmol/L respectively. The LOA for normal anion gap was from 7.13 to -4.23 mmol/L with a span of 11.36 mmol/L while the LOA for high anion gap was from 5.46 to -9.74 mmol/L with a span of 15.2 mmol/L respectively. Apparently with such a big span it seems that there was a good level of agreement between the two anion gap entities with measured and calculated HCO_3 but when Story and Postuie criteria were applied, no agreement was found between them. The Story and Postuie criteria are:

1. The bias between entities should be <±1 mmol/L
2. The Limit of Agreement between the methods should be within a bias of ±2 mmol/L or in a total span of 4 mmol/L in order to be clinically insignificant.

Normal anion gap was fulfilling only the first criterion but high anion gap could not fulfill any one of the above criteria proposed by Story and Postuie, so study was unable to prove any agreement between the two entities of anion gap. This finding was in concordance with conclusion derived by Story et al. In a study Stock et al. illustrated that embodiment of confidence cutoff points and pre-defined error limits grants us luxury of easy visual assessment of method comparison. Stock et al. further concluded that Model Bland Altman plot should display that the level of agreement at 95% C.I. between two methods is equal to predefined TEa for acceptance of analytical performance. Our study showed that neither of our agreement limits of both normal and high anion gap were within TEa of 10%, which means that both of our limits of agreement exceeded the established TEa which further augments the fact that two entities of anion gap cannot be used reciprocally or interchangeably.

Factors like specimen collection, its handling, transportation, imprecision due to analytical techniques and errors of calibration may adversely affect the agreement between calculated and measured bicarbonate. Improper mixing and dilution of ABGs samples with more than required heparin can lower the pH and pCO_2, which in turn can affect bicarbonate values. Therefore, above mentioned factors can alter pKa and α leading to a change in their required constant status.

So, both the anion gap entities cannot be used interchangeably, therefore as suggested by Harold Stein it is preferable to estimate HCO_3 through indirect ion selective electrode method for estimation of anion gap.

CONCLUSION

Normal and high anion gap metabolic acidosis estimated by measured and calculated bicarbonate cannot be used interchangeably in critically ill patients. It apparently showed good level of agreement but according to Story and Postuie criteria, bias and the levels of agreement were not appropriate to conclude the fact that both entities of anion gap could be used interchangeably. This finding was supported by the model Bland Altman plot.

RECOMMENDATIONS

More such studies should be conducted keeping in mind, pre-analytical and analytical factors as mentioned in the discussion and to avoid them as much as possible to get the positive outcomes.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

SO, MH & SS: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

AI & MMD: Conception & study design, critical review, approval of the final version to be published

Je: Analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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