STUDY ON ANION GAP LEVELS IN HYPERGLYCEMIC PATIENTS

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Abstract

To examine the effects of hyperglycemia on some associated metabolic profiles and how they affect the anion gap, we determined the electrolyte profile, glucose, HbA1c, urea, creatinine and pH. Interaction of these metabolic parameters were assessed using spectrophotometric, flame photometric and other biochemical methods. We established a bioregulatory relationship of immense physiological and pathophysiological importance in modulating the anion gap. We observed elevated values of anion gap in hyperglycemic cases resulting in diabetic ketoacidosis as a result of the reduced bicarbonate values which exacerbate further metabolic complications. Minimum values of anion gap for all tasted subject was between 22 mmol/l to 35mmol/l far higher than the reference range (18 mmol/l). A correlation of the anion gap with glucose concentration was strongly positively linear. Similar linearity was also obtained for glucose level versus bicarbonate with reduced pH values. The procedure represent a new and high yielding method for the explanation of the functionality of the metabolites in diabetic condition. We summarise that developing therapies that will inhibit further reduction of bicarbonate will enhance the anion gap to normal and reduce the associated complication in diabetes.

Keywords

Anion, anion gap, hyperglycemic patient.

1. INTRODUCTION

The maintenance of osmotic pressure and water distribution in the various body fluid compartments is primarily a function of the four major electrolytes sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO^-_3). In addition to water homeostasis, these electrolytes play an important role in maintenance of pH, in adequate heart and muscle function, in oxidation, reduction reactions and as cofactors for enzymes **[1]**. Abnormal concentrations of electrolytes may be either the cause or the consequence of a variety of disorders. Electrolytes are classified as either anions, negatively charged ions that move towards the anode, or cations, positively charged ions that move towards a cathode. Physiological electrolytes include Na^+. K^+, Ca^{2+}, $Mg^{2+,}$ Cl^-, HCO^{-3}, H_2PO^{-4}, $H_2PO_4^{2-}$, $SO4^{2-}$, and some organic anions, such as lactate. Although amino acids and proteins in solution also carry an electrical charge, they are usually considered separately from electrolytes. The major electrolytes (Na^+, K^+, Cl^-, HCO^-_3) occur primarily as free ions, whereas significant amounts of Ca^{2+}, Mg^{2+} and trace elements are bound by proteins such as albumin.

Determination of body fluid concentrations of the four major electrolytes (Na^+, K^+, Cl^-, and HCO^-_3) is commonly referred to electrolyte profile. Anion gap (AG) is the difference between the serum sodium concentration and the sum of serum chloride and bicarbonate concentration. AG is high in some form of metabolic acidosis. Metabolic acidosis is a pathological process that leads to the accumulation of acid that lowers the bicarbonate concentration and decrease the pH; also known as primary bicarbonate deficit. Metabolic acidosis is readily detected by decreased plasma bicarbonate, primary perturbation in this acid-base disorder. Bicarbonate is lost in the buffering of excess and may be as a result of: production of organic acids that exceeds the rate of elimination (e.g; the production of acetoacetic acid and B-hydroxybutyric acid in diabetic acidosis and of lactic acid in lactic acidosis. Excessive loss of bicarbonate because of increased renal excretion (decreased tabular reclamation) or excessive loss of duodenal fluid (as in diarrhea). Plasma HCO^-_3 falls; the fall is associated with a rise in the concentration of inorganic anions (mostly chloride) or a concomitant fall in the sodium concentration

When any of these conditions exists, the ratio of d HCO^-_3/CO_2 is decreased because of the primary decrease in bicarbonate. The resulting drop in pH stimulates respiratory compensation via hyperventilation, which lowers PCO_2 and thereby raises the pH[2]. Metabolic acidosis are classified as those associated with either an increased anion gap or a normal anion gap. The concept of the anion gap was originally devised as a quality control rule in which it was noted that if the sum of Cl^- and HCO^-_3 values was subtracted from Na^+ value (Na^+ - [Cl^- + HCO^-_3]), the difference or "gap" averaged 12 mmol/l in healthy subjects [3]. This apparent gap is a result of unmeasured anions (e.g., proteins, SO^{2-}, $H_2PO_4^{2-}$ that are present in plasma. All anion gap metabolic acidosis can be explained by one (or a combination) of eight underlying mechanisms according to the common mnemonic device, MUDPILES: Methanol, uremia of renal failure, diabetes or ketoacidosis, paraldehyde toxicity, ionized iron or ischaemia, lactic acidosis, ethylene glycol and salicylate intoxication [4].

In contrast to high anion gap acidosis, in which bicarbonate is consumed in buffering excess H^+, the cause of acidosis in the presence of a normal anion gap is the loss of bicarbonate rich fluid from either the kidney or gastrointestinal tract. As bicarbonate is lost, more Cl^- ions are reabsorbed with Na^+ or K^+ to maintain electrical neutrality so that hyperchloremia ensues. Normal anion gap acidosis can be divided into hypokalemic, normokalemic acidosis which can be helpful in the differential diagnosis of this type of disorder [5].

In accordance to the principle of electroneutrality the number of millimoles (mmol) per litre of cations in plasma is normally balanced by an equal amount of millimoles per litre of anions. This means that the total amounts in mmol/l of Na^+ + K^+ + Ca^{2+} + Mg^{++} + other cations will be equal to the total amount in mmol/l of Cl^- + HCO^-_3 +HPO_4 + $SO_4^=$ +proteinate. Routinely measured electrolytes are Na^+ + K^+ + Cl^- + HCO^-_3. These four ions in plasma exert the greatest influence on water balance and acid-base relationship. Mg^{++}, Ca^{++} et are considered unmeasured cations. HPO_4^{2-}, proteinate are considered unmeasured anion. Anion gap is the total amount of unmeasured or undetermined anions normally present in the body.

2. MATERIAL AND METHOD

At the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria, blood and urine samples were collected from hyperglycemic patients (n=60) whose fasting blood glucose were $\geq$20mmol/l from both male and female adults aged between 30-45 years. Control subjects (n=40) with fasting blood glucose level ranging between 3.5-6.7 mmol/l and without glucosuria were also used.

Fasting blood sugar was determined enzymatically using glucose oxidase method with Randox laboratory kit (United Kingdom) and determined specifically with unispect 22 D^+

spectrophotometer uniscope, (England). Urine analysis was carried out with Meditest Combi 9 Macherey-Nagel (Germany) to determine presence of ketones and urine glucose. Sodium ((Na^+) and potassium (K^+) were determined by flame photometry using corning. Urea and creatinine were also determined with colorimeter Model No. TIID, Techmel & Techmel, (USA). HCO^-_3 was determined by titration while pH meter was used for determination of pH. Glycated Haemoglobin was determined by ion-exchange high performance liquid chromatography (HPLc-Esi/ms) approach with UV detection.

3. RESULTS

We have shown below two tables representing results obtained from male and female subject and from respective control values.

Table 1: Mean findings for associated metabolic profiles (males) in control and hyperglycemic subjects

Parameters	Control (n=40)	Hyperglycemic (n=60)	p-value 0.05
Glucose	5.6 ± 1.8	23.5 ± 5.3	
Urea	5.0 ± 1.2	19.7 ± 6.4	
Creatinine	105 ± 10.5	550 ± 10.0	
Na^+	137 ± 3.2	149 ± 3.0	
K^+	3.8 ± 0.8	4.8 ± 0.5	
Cl^-	102 ± 0.5	130 ± 3.5	
HCO_3	28 ± 5	68 ± 2.0	
HbA1c	35 ± 4	74 ± 0.05	
pH	7.4 ± 0.01	7.4 ± 0.05	
Urine glucose	Nil	++	
Urine ketone	Nil	++	
Anion Gap	16 ±2.0	24 ± 2	

Comment values are mean ± SEM. Anion gap was calculated as: (Na^+) – (Cl^- + HCO^-_3 mmol/l)

Table 2: Mean finding for associated metabolic profile (female) in control and hyperglycemic subjects

Parameters	Control (n=40)	Hyperglycemic (n=60)	p-value 0.05
Glucose	5.3 ± 1.6	24.4 ± 6.3	
Urea	4.8 ± 1.3	23.2 ± 5.5	
Creatinine	90 ± 10.5	490 ± 5.8	
Na^+	136 ± 3.1	140 ± 2.0	
K^+	3.9 ± 0.6	3.8 ± 3.0	
Cl^-	95 ± 2	125 ± 4	
HCO_3	27.8 ± 5	69.5 ± 2.3	
HbA1c	36 ± 5	67 ± 3.3	
pH	7.4 ± 0.01	7.3 ± 0.01	
Urine glucose	Nil	++	
Urine ketone	Nil	++	
Anion Gap	14 ± 2.0	23 ± 1.0	

Values are mean ± SEM
Anion Gap calculated as: [Na^+] – (Cl^- + HCO^-_3) mmol/l

4. DISCUSSION

In assessing the anion gap in hyperglycemic patients we deployed parameters such as evaluation of concentration of glucose, urea, creatinine, electrolytes pH and glycated haemoglobin. Anion gap was calculated as the difference between the sum of the cations (chloride and bicarbonate) and the anion sodium. The anion gap for all subjects evaluated were higher than normal (reference value 12-18 mmol/l). We observed values higher than this in all subjects which was in tandem with earlier studies [12,19]. A comparison of the anion gap among males and females shows that males had higher values. However this may be due to the severity of the condition. The methods we adopted enhanced an understanding of the biochemical criteria of diabetic ketoacidosis and water and electrolyte deficits in hyperglycemic condition. The biochemical trial of hyperglycemia ketonemia and anion gap metabolic acidosis are strongly interrelated as shown from the results. See table 1 and 2.

Each of these conditions can be caused by other metabolic conditions[5]. To offer an explanation and elucidate the mechanism for this relationship, we aggregated results of previous studies[4,5,6] in rat models which suggested that metabolic acidosis decreases the binding of insulin to its receptors and enhances cortisol production which is complicated in the development of insulin resistance. Recent epidemiological studies have shown an association between clinical markers of metabolic acidosis and greater insulin resistance or prevalence in type 2 diabetes mellitus and concludes that both lower serum bicarbonate and higher anion gap (even with ranges considered normal) were associated with increased insulin resistance among adults. Moreover, higher levels of serum lactate, a small component of anion gap, were associated with higher odds of prevalent type 1 diabetes mellitus in the atherosclerosis risk in communities studies[2]. Insulin deficiency causes the body to metabolize triglycerides and muscle instead of glucose for energy. Serum level of glycerol and free fatty acids (FFAs) rise because of unrestrained lipolysis, as does alanine because of muscle catabolism. Glycerol and alanine provide substrate for hepatic gluconeogenesis, which is stimulated by the excess glucagon that accompanies insulin deficiency. Glucagon also stimulates mictochondrial conversion of free fatty acids with ketones. Insulin normally blocks ketogenesis by inhibiting the transport of FFA derivative into the mitochondrial matrix, but ketogenesis proceeds in the absence of insulin.

The major ketoacids acetoacetate and B-hydroxyl butyric acid, are strong organic acids that create metabolic acidosis. Furthermore hyperglycemia due to insulin deficiency causes an osmotic diuresis that leads to marked losses of water and electrolytes. Urinary excretion of ketones obligates additional loss Na^+ and K^+. Serum Na may fall from natriuresis or rise due to excretion of large volume of free water. K^+ is also lost in large quantities[7]. Despite a significant total body deficit of K^+, initial serum K^+ is typically normal or elevated because of the extracellular migration of K^+ in response acidosis. K^+ levels generally fall further during treatment as insulin therapy drives K^+ into cells. If serum potassium is not monitored and replaced as needed, life threatening hypokalaema may develop.

The lactic acid so produced is transposed to the liver in the coricycle, where it serve as carbon skeleton for gluconeogenesis. Increased levels of glucagon, cathecholamine and cortisol, with, concurrent insulinopemia stimulate gluconeogenic enzymes especially phosphoenolpyruvate carboxykinase (PERCK)[8]. Decreased glucose use is exaggerated further by increased levels of circulating cathecolamines and free fatty acid.

Malonyl COA inhibits carnitine palmitoyl acyltransferase (CPTI), the rate limiting enzyme of ketogenesis. Therefore, reduction in malonyl COA leads to stimulation of CPTI and effective increase in ketogenesis [9], increased production of ketone bodies (acetoacerate and B-hydroxybutyrate [BOHB]) leads to ketonemia. There is also decreased clearance of ketone bodies

in DKA, which contributes to ketonemia[10]. These ketoacids are neutralized by extracellular and cellular buffers, resulting in their loss and subsequent anion gap metabolic acidosis [11]. Fluid and electrolyte abnormalities some occurs as a result of DKA resulting in from development of water and electrolyte balance in DKA resulting hyperglycemia, and hyperketonemia. Osmotic diuresis resulting from hyperglycemia promotes net loss of multiple minerals including sodium, potassium, calcium, Mg^{2+}, Cl^{-} and phosphates[13,14]. Ketoanion excretion, which obligate urinary cation excretion, also contributes to electrolyte derangement although less than hypoglycemia. Insulin deficiency per se also may contribute to renal losses of water and electrolyte because of deficient water and salt resorptive effect of insulin in the renal tubule [15,16].

Intracellular dehydration occur as hyperglycemia and water loss leads to increased plasma toxicity, causing a shift of water out of the cell. This is associated with movement of potassium from the cell to the extracellular component, a phenomenon that is aggravated by acidosis and breakdown of intracelaular protein. Furthermore, the entry of potassium into cell is impaired by lack of effective insulin action [17,18]. Additionally, DKA has to be distinguished from other causes of high anion gap acidosis, including lactic acidosis, advanced chronic renal failure and ingestion of drugs such as salicylate, methanol ethylene glycol.

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REFERENCES

[1] Kirschbaun B, Sica D, Anderson FP (1999). Urine electrolytes and the urine anion and osmolar gaps. The Journal of Laboratory and Clinical Medicine. 133(6): 597-604.
[2] Harris SS, Dawson-Hughes B (2010). No effect of bicarbonate treatment on insulin sensitivity and glucose control in non-diabetic older adults. Endocrine 38:221-6
[3] Gatbow PA, Kaechny WD, Fenessey PV et al (1980). “Diagnositic importance of an increased serum anion gap”. N.Engl J Med (303): 854-858
[4] Maalouf NM, Cameron MA, Moe OW (2007). Low urine pH. A novel feature of the metabolic syndrome. Clin J Am Soc Nephrol 2:883-8
[5] International Expert Committee Report on the role of HbA1c assay in the dispensing of diabetes. Diabetes care. 2009; 32(7): 1327-1334
[6] Andrews RC, Walker BR (1999). Gluco corticoids and insulin resistance: old hormones, new targets. Clin Sci (Lond) 96:513-23
[7] Selvine E, Ning Y, Steffes MW, Glycated hemoglobin and the risk of kidney disease and retinoglobin in adults with and without diabetes. 2011; 60(1): 298-305
[8] Crawford SO, Hougeveen RC, Brancati FL (2010). Association of blood lactate with type 2 diabetes. The Atherosclerosis Risk in communities carotid NRI study. Int J Epidemiol. 39:1647-55
[9] McGarry JD, Woelyje KF, Kuwajima M (1989). Regulation of ketogeneiss and the renaissance of carnitine palmitoyl transferase. Diabetes Metab Rev 5:271-84
[10] Kitabchi AE, Wall B (1995). Diabetic ketoacidosis. Med Clin Noth Am 79(1):10-37
[11] Avogaro A, Crepaldi C, Miola M (1996). High blood ketone body concentration in type 2 non-insulin dependent diabetic patients. J Endocrinol invest 19:99-105
[12] Mehta AN, Emmett JB, Emmett M (2008). Gold Mark: an anion gap mnemonic for the 21st century. The lancet 372 (9642):892
[13] Farawell WR, Taylo EN (2010). Serum anion gap, bicarbonate and biomarkers of inflammation in healthy individuals in a national survey. CMAJ 182:137-41
[14] Kitachi AE, Umpierrez GE, Murphy MB (2001). Management of hyperglycemic crises in patients with diabetes. Diabetes care. 24:131-53

[15] Shulman GI (2004). Unravelling the cellular mechanism of insulin resistance in humans: New insites from magnetic resonance spectroscopy. Physiology. (Bethesd) 19:183-90
[16] Defronzo RA, Bechles AD, (1979). Glucose intolerance following chronic metabolic acidosis in man. Am J Physio, 236 E328-34
[17] Stentz FB, Umpierrez GE, Cuervo, R (2004). Proinflamatory cytokines, markers of cardiovascular risk, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. Diabetes. 53:2079-86
[18] Krant JA, Madia, NE (2006). Serum Anion Gap. Its uses and limitations in clinical medicine. Clinical Journal of the American Society of Neprology 2(1):162-174
[19] Winter SD, Pearson JR, Garbow PA, Schultz AL, Lepoff RB (1990). The fall of the serum anion gap. Archives of internal medicine. 150(2):311-3