

Relationship between Serum Anion Gap and Diabetes Mellitus

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Abstract

Diabetes mellitus is the most common metabolic disorder occasioned by derangement in glucose equilibration between the ECF and ICF. The derangement is known to affect the appropriate balance of electrolytes that serves as a buffer in the body. In this study anion gap was evaluated among outpatient diabetics as compared to non-diabetics control group. The categorization into the study or control groups was done by serum glucose estimation using glucose oxidase method. The study group mean age was 51 ± 14 as against control group of 47 ± 10 . One hundred and fifty subjects were divided in two groups based on serum glucose concentration. Group A (control group) consisted of 50 subjects with mean serum glucose concentration 4.3 ± 1.7 mmol/l and anion gap 13.8 ± 2.6 , group B (diabetics) consisted of 100 subjects that had serum glucose concentration 15.0 ± 3.9 and anion gap 18.4 ± 2.5 . The glucose was estimation by glucose oxidase method, whereas the anion gap was calculated by subtracting the concentrations of sodium and potassium from the concentrations of chloride and bicarbonate. The concentrations of the electrolytes were assayed using ion selective electrodes (ISE). A statistical significant difference $P < 0.05$ was observed between group A and B glucose concentrations and the anion gap. The abnormal anion gap was created by the insufficiency of bicarbonate used for the buffering of the electrolytes variability occasioned by derangement in glucose metabolism and distorted hormonal secretion. Hence metabolic acidosis is strongly linked with diabetics as a result of distorted anion gap. Healthcare providers and takers should ensure that anion gap estimation is factored into investigations for the management of diabetics. Also, patients with deranged anion gap should be placed as an emergency case for proper management. Clinicians should ensure that patient's anion gap is within the reference anion gap range so as to prevent development into metabolic acidosis and subsequent ketoacidosis.

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Keywords

Anion Gap, Diabetes Mellitus, Serum Anion, Serum Cation, Serum Buffers

1. Introduction

The anion gap is the difference in the measured cations (positively charged ions) and the measured anions (negatively charged ions) in serum, plasma, or urine [1]. The magnitude of this difference (*i.e.* “gap”) in the serum is often calculated in medicine when attempting to identify the cause of metabolic acidosis, lower than normal pH in the blood. It is most commonly used in the differential diagnosis of acid-base disorders; it also has been used to assess quality control in the chemical laboratory [2] and to diagnose paraproteinemias [3] [4] and intoxications with lithium [5] bromide [6] or iodide [7]. The concentrations are expressed in units of milliequivalents/liter (mEq/L) or in millimoles/litre (mmol/l). Anion gap is an “artificial” and calculated measure that is representative of the unmeasured ions in plasma or serum (serum levels are used more often in clinical practice).

There are two major ways of calculating anion gap. It is calculated either by including potassium in the formula or by excluding potassium in the formula. The reason for the later way has become widely accepted, as potassium concentrations, being very low, usually have little effect on the calculated gap. However, this work will utilize the formal way of calculation which includes potassium. With potassium, it is calculated by subtracting the serum concentrations of chloride and bicarbonate (anions) from the concentrations of sodium and potassium (cations):

$$= ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

Without potassium, this leaves the following equation:

$$= [\text{Na}^+] - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

Commonly measured cations include sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}) and Magnesium (Mg^{2+}). Cations that are generally considered “unmeasured” include a few normally occurring serum proteins, and some pathological proteins (e.g., paraproteins found in multiple myeloma). Likewise, commonly “measured” anions include chloride (Cl^-), bicarbonate (HCO_3^-) and phosphate (PO_4^{3-}), while commonly “unmeasured” anions include sulfates and a number of serum proteins.

In normal health there are more measurable cations compared to measurable anions in the serum; therefore, the anion gap is usually positive. Because we know that plasma is electro-neutral (uncharged), we can conclude that the anion gap calculation represents the concentration of unmeasured anions. The anion gap varies in response to changes in the concentrations of the above-mentioned serum components that contribute to the acid-base balance. Calculating the anion gap is clinically useful, as it helps in the differential diagnosis of a number of disease states.

The normal reference values ranged from 8 to 15 mEq/L plasma when not including $[\text{K}^+]$ and from 10 to 18 mEq/L plasma when including $[\text{K}^+]$. Some specific sources use 8 - 16 mEq/L [8]. This research pegged its reference range on the work of [8]. Anion gap can be classified as either high, normal or, in rare cases, low.

High anion gap: Anion gap is affected by changes in unmeasured ions. A high anion gap indicates acidosis. E.g. In uncontrolled diabetes, there is an increase in ketoacids due to metabolism of ketones. Ketoacids are unmeasured anions, so there is a resulting increase in the anion gap. In these conditions, bicarbonate concentrations decrease, in response to the need to buffer the increased presence of acids (as a result of the underlying condition). The bicarbonate is consumed by the unmeasured cation (H^+) (via its action as a buffer) resulting in a high anion gap. Renal failure causes high anion gap acidosis by decreased acid excretion and decreased HCO_3^- reabsorption. Accumulation of sulfates, phosphates, urate, and hippurate accounts for the high anion gap.

Normal anion gap: Normal gap does not necessary mean that all is well with the body system. Proportionality of the electrolytes that constitute the anion gap calculation is what determines differential diagnosis of pathological normal anion gap from physiological anion gap. In patients with a normal anion gap the drop in HCO_3^- is primarily pathological and deleterious to the body system. This distortion must be compensated urgently by the body system to avoid further deterioration. This is achieved by increase in Cl^- to buffer the imbalance, since the only other major buffering anion is chloride. This compensation mechanism leads to hyperchloremic acidosis. The loss HCO_3^- which is replaced by a chloride anion results in a normal anion gap.

Low anion gap: A low anion gap is frequently caused by hypoalbuminemia. Albumin is a negatively charged protein and its loss from the serum results in the retention of other negatively charged ions such as chloride and bicarbonate. As bicarbonate and chloride anions are used to calculate the anion gap, there is a subsequent decrease in the gap. In hypoalbuminaemia the anion gap is decreased from 2.5 to 3 mmol/l per 1 g/dL in serum albumin [9]. Common conditions that reduce serum albumin in the clinical setting are hemorrhage, nephrotic syndrome, intestinal obstruction and liver cirrhosis. The anion gap is sometimes reduced in multiple myeloma, where there is an increase in plasma IgG (paraproteinaemia) [10].

The first systemic description of Diabetes Mellitus was written by the Arelaeus of Cappadosis in Asia Minor, probably in the first century AD, the disease as “a melting down of flesh into the urine”. The discovery by Van Mering and Minikowaski in 1889 that pancreatotomy causes a metabolic disorder called Diabetes Mellitus is the result of insulin deficiency.

The World Health Organization (WHO) defined Diabetes Mellitus on the basis of laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion, even if the fasting concentration is normal. The definition stands if only the laboratory investigation carried out more than twice still give diabetic range values. The major characteristic of diabetes is the body's inability to regulate the level of sugar or glucose in the blood. This is based on insufficiency in insulin secretion by the beta cells of the islet cells of the langerhans or insensitivity of glucose absorptive cell membranes. There are two basic types of diabetes; insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). However, Diabetes Mellitus is a group of metabolic disorders characterized by an increased blood glucose level resulting from defects in insulin secretion, in insulin action or both. It is based on the underutilization of glucose by the body, producing hyperglycaemia-elevated glucose level. The symptoms and signs of diabetes are based on the excessive occupation of glucose in the blood streams rather than the cells where metabolic activities that drive the body systems occur.

Diabetes Mellitus is a worldwide diseased condition that cut across the world. Nigeria is currently estimated to have 6.9 million people with diabetes and this will double in 15 years if proper measures are not adopted. People mostly affected are between 45 and 64 years. Also 20 and 44 years were also being diagnosed with diabetes. Diabetes mellitus is common disease in man. A predisposition to the disease is probably inherited as an autosomal recessive trait. About 25% of relatives of diabetics show abnormal glucose tolerance as compared to 1% in general population. Common symptoms of diabetes are lethargy, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections. Severe hyperglycaemia may lead to ketoacidosis and subsequently diabetic coma. Chronic hyperglycaemia causes a long-term damage, dysfunction and failures of various cells, tissues and organs. Long-term complications of diabetes are: Macroangiopathy: Ischaemic heart disease (IHD), stroke, peripheral vascular disease (PVD). Microangiopathy: Retinopathy, nephropathy. Neuropathy: Peripheral neuropathy, autonomic neuropathy. Cataract. Diabetic foot. Diabetic heart.

A lot of research had linked the usefulness of anion gap calculation to acid resulting diseases. This mainly based on the derangement of the buffering system of the body as a result of correcting the system disturbance. Anion gap is a very useful tool in the diagnosis and management of diabetes ketoacidosis. Diagnose by an arterial pH < 7.30, with an anion gap > 12 and serum ketones in the presence of hyperglycemia. It had been shown that lower bicarbonate and higher anion gap are independently associated with insulin resistance [11]. A study carried out had shown the usefulness of anion gap as a screen for mixed acid-base disorders with diabetic acidosis [12]. This work is saddled with the responsibility of critically studying the relationship between anion gap and diabetes mellitus. It will further design an anion gap template in the diagnosis of diabetes mellitus.

1.1. Statement of the Problem

A trend has been observed whereby an increasing number of patients attending the diabetes clinic at the Niger Delta University Teaching Hospital Okolobiri, Yenagoa, Bayelsa State, Nigeria for diabetes management monitoring still encounter diabetes complication despite the stringency to the prescribed drugs. It is well established that effective glucose metabolism is anchored on good electrolytes buffering system. Any derangement in electrolytes buffering affect glucose metabolism and diabetes complication is ensuing. Anion gap is a useful tool in diabetes ketoacidosis diagnosis management as it accounts for the unmeasured anion in the body that are also of critical importance in the effective drive of the body mechanisms. This work is design to study the anion gap trend in diabetic patients as a management tool to prevent diabetes complications.

1.2. Aim and Objectives

1) Aim

To evaluate the true pattern of Anion Gap amongst diabetics.

2) Objectives

The objectives of this study are three folds:

- 1) To evaluate if a correlation exists between anion gap and diabetes mellitus exists.
- 2) To utilize anion gap in early diagnosis of diabetes complication and its management.
- 3) To establish an anion gap range in diabetes mellitus that is tolerable by the body.

2. Materials and Methods

2.1. Study Location

This study was conducted at the departments of Chemical Pathology of the Niger Delta University Teaching Hospital, Yenagoa, Bayelsa State. Bayelsa state is located within Latitude 4° 15' North and Latitude 5° and 23' South. It is also within longitude 5° 22' West and 6° 45' East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people.

2.2. Study Subjects

One hundred (150) subjects were utilized in the studies. The subjects were divided into two groups. The group A was made up of 50 non-diabetic subjects (control) that are apparently healthy. Group B constituted 100 diabetes patients as study subjects. The group B is defined based on WHO standard on diabetes mellitus. Diabetes mellitus patients that have developed into ketoacidosis were excluded from the study.

2.3. Ethical Clearance

Ethical approval was granted by the department involved in the study. Participation was voluntary and informed consent was obtained as verbal or written depending on the literacy level of individual participants.

2.4. Sample Collection

In total, the 150 blood samples were collected from 150 subjects by venepuncture. The blood was immediately introduced into plain containers and separated after coagulation. Samples were assayed immediately after separation. Sample use was serum.

2.5. Analysis

Ion selective electrode (ISE) was used for the estimation of the electrolytes, whereas glucose oxidase for glucose estimation. The anion gap was calculated from the electrolytes data obtained using ISE, using the formulas:

$$([\text{Na}] + [\text{K}]) - ([\text{Cl}] + [\text{HCO}_3])$$

2.6. Statistical Analysis

Data were analyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 15) and expressed as mean \pm SE. Student *t*-test was used for comparing values of the diabetic group and the control group. Percentages and pictorial expression were also used for data presentation.

3. Results

Table 1 shows a comparison of the Mean \pm SD of non-diabetic subjects (control) (group A) and diabetic subjects (group B). The result shows that there were significant differences ($P < 0.05$) in mean values for the anion gap and glucose estimation. The anion gap of group B is significantly elevated as compared to higher group A.

Table 2 shows a comparison of Mean \pm SD of electrolytes measured between non-diabetic and diabetic subjects. The result shows that there were significant differences ($P < 0.05$) in mean values for serum bicarbonate

Table 1. A comparison of mean \pm SD of the anion gap and glucose estimation measured between diabetic and non-diabetic subjects.

Parameters Measured	Reference Range	Control Mean \pm SD	Diabetics Mean \pm SD	P-Value	Comment
		Group A (n = 50)	Group B (n = 105)		
Age (yr)		47 \pm 10	51 \pm 14	$P > 0.05$	NS
Anion gap (mmol/l)	08 - 15	13.8 \pm 2.6	18.4 \pm 2.5	$P < 0.05$	S
Glucose (mmol/l)	2.5 - 5.6	4.3 \pm 1.7	15.0 \pm 3.9	$P < 0.05$	S

Ns: Non Significant; S: Significant.

Table 2. A Comparison of mean \pm SD of electrolytes measured between non-diabetic and diabetic subjects.

Parameters Measured	Reference Range	Control Mean \pm SD	Diabetic subjects Mean \pm SD	P-Value	Comment
		(n = 50)	(n = 105)		
Sodium (mmol/l)	135 - 145	143 \pm 10	142 \pm 8	$P > 0.05$	NS
Potassium (mmol/l)	3.0 - 5.0	3.9 \pm 1.4	5.9 \pm 1.9	$P > 0.05$	NS
Chloride (mmol/l)	95 - 110	108 \pm 11	110 \pm 15	$P > 0.05$	NS
Bicarbonate (mmol/l)	20 - 30	25 \pm 5	17 \pm 4	$P < 0.05$	S

concentration as compared between group A and B. Other assayed electrolytes did not show statistical significance ($P > 0.05$).

4. Discussion

The research carried out showed a statistical significant elevation of serum anion gap and glucose concentration in group B as compared to group A. This agreed with a work that exhibited lower bicarbonate and higher anion gap which independently associated with insulin resistance [11]. Diabetes mellitus is a metabolic syndrome characterized by electrolytes variability as a result of the high concentration of glucose in the extracellular fluid and low concentration in the intracellular fluid. This has a preponderance of distorting the fine equilibrium of electrolytes balance between the intracellular and the extracellular. This research work brought to bear a statistical difference between anion gap of diabetic subjects and that of non-diabetic subjects. This could be ascribed to the disjunction of the equilibration of glucose between the extracellular and the intracellular environments. The increased concentration of glucose in the extracellular creates inappropriate concentration gradients that tend to move fluid from the intracellular to the extracellular. The increase of water concentration in the extracellular distorts the fine electrolytes' balance, hence leading to polydipsia and polyuria. This cascade of malfunction further instigates the rennin-angiotensin pathway and the hypothalamus to balance the derangement. All these further distort the fine ion balance because membrane transportation is involved and both utilize the same membrane for metabolic activities. The elevation was created by a significant decrease in serum bicarbonate. **Figures 1-3** clearly showed the deficit of bicarbonate resulting from its massive utilization for buffering. Bicarbonate is a useful buffer that is spent in maintaining equilibrium in the production of hydrogen ions. As bicarbonate decreases in diabetics, there are preponderances of an increase in anion gap. The mean anion gap observed in the control group as showed in tables above was 13.8 as against 18.4 observed amongst diabetics. The increase is due to the decreased concentration of bicarbonate. Hence, 18.4 value of anion gap amongst diabetics should be treated as an emergency. Anion gap is complication of any diseases that distorts ions' balance in the body. Increasing anion gap detection is useful in the diagnosis of acid-base disorders, assessment of quality control in the chemical laboratory, and detection of such disorders as multiple myeloma, bromide intoxication, and lithium intoxication [13] [14]. Diabetes mellitus is also associated with acid-base distortion. Hence, based on the data generated, diabetes mellitus is a clear disease of ions distortion cascaded by bicarbonate utilization without equal production.

MEAN ANION GAP COMPARISON BETWEEN NON DIABETIC AND DIABETIC SUBJECTS

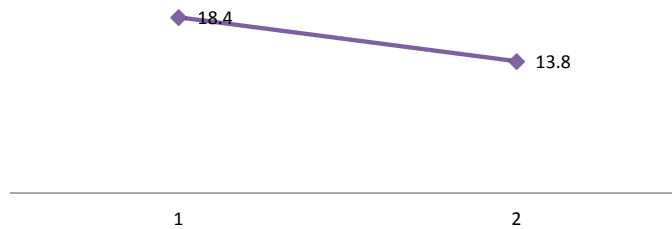


Figure 1. Mean anion gap comparison between non-diabetic and diabetic subjects.

ANION GAP CONCENTRATION COMPARISON BETWEEN NON DIABETIC AND DIABETIC SUBJECTS

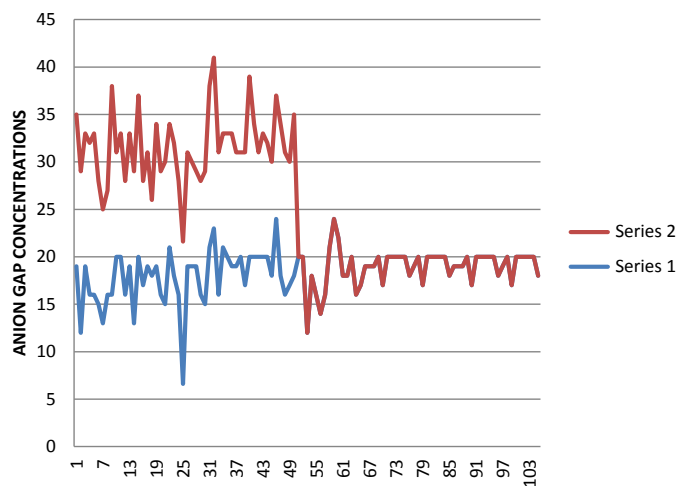


Figure 2. Anion gap concentrations comparison between non-diabetic and diabetic subjects.

A COMPARISON OF MEAN \pm SD OF ELECTROLYTES MEASURED BETWEEN DIABETIC AND NON DIABETIC SUBJECTS

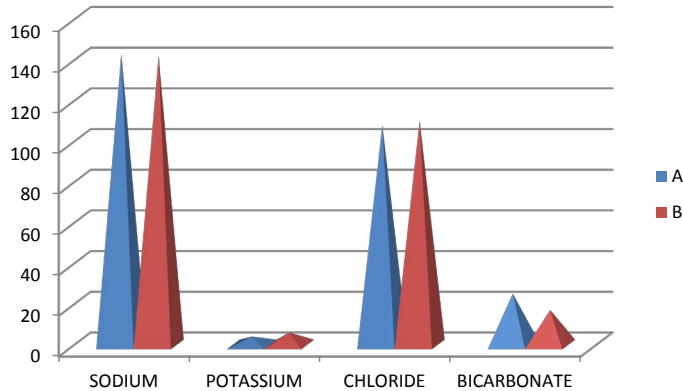


Figure 3. A comparison of mean \pm SD of electrolytes measured between non-diabetic and diabetic subjects.

Hence, from the research carried out, it is explicit to state that diabetic subjects are more prone to metabolic acidosis created by distorted anion gap, occasioned by the inappropriate concentration of glucose in the ECF. The various complications of diabetes had also been linked to the distortion of the fine balance of electrolytes

between the intracellular and the extracellular. Hence the stringency in containing the anion gap within the normal range will reduce the preponderance of diabetes complications. Healthcare givers of diabetes mellitus patients should ensure that the anion gap is within the normal range.

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