Blood Electrolyte Levels in Male and Female Pitbull Dogs

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Authors' contributions

This work was carried out in collaboration among all authors. Author LCC designed the study, wrote the protocol and performed the statistical analysis. Author NCC wrote the first and final draft of the manuscript and managed the analyses of the study. Author EE managed the literature searches, carried out the bench work and collated data. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

The study evaluates blood electrolytes in male and female pit bull dogs to ascertain their level of predominance in extracellular fluid. Blood samples were collected, processed and analyzed for electrolyte levels using standard protocol and absorbance reading taken by spectrophotometric method. Post statistical package for social science (SPSS) analysis indicates that mean serum electrolyte levels of male pit bull dogs were 179.2±66.79 mmol/l for sodium, 3.54±0.55 mmol/l for potassium, 43.72±2.72 mmol/l for bicarbonate, chloride was 116±12.81 mmol/l, phosphorus was 4.26±0.61, calcium and magnesium were 1.74±0.50 and 1.48±0.18 mmol/l respectively. The female pit bulls had a mean serum electrolyte level of 157.4±76.07 mmol/l for sodium, 5.12±1.23 mmol/l for potassium, 75.98±27.98 mmol/l for bicarbonate, chloride and phosphorus are 123.5±17.98 mmol/l and 4.06±0.95 mmol/l, calcium and magnesium were 1.56±0.16 and 1.70±0.39 mmol/l respectively. The result implies that serum concentration of sodium, bicarbonate, chloride, phosphorus and magnesium in both male and female pit bull dogs were significantly ($p<0.05$) higher than normal electrolyte range, while potassium and calcium levels were within range. The electrolyte imbalance could lead to impaired fluid level and electrolyte osmolarity resulting in neurological consequences such as seizure disorders, hypoparathyroidism, metabolic acidosis and alkalosis.

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1. INTRODUCTION

Specific condition resulting in mortality of small dogs and cats population have individually been attributed to disorders in extracellular and intracellular electrolyte concentration, although interactions between electrolyte imbalance and its associated outcome have not been fully explored in large, heterogeneous animal population.

In dogs, most organ systems are physiologically associated with fluid regulation, acid/base balance, electrolyte concentration, kidney function, hypothalamus and most sections of the cardiovascular system [1,2]. Mechanisms geared towards the maintenance of homeostasis ensure that electrolyte concentrations are kept with physiologic range [2]. In critical medical care and veterinary emergency disturbances in electrolyte balance are commonly encountered despite regulatory mechanisms, although they are mostly mild and none life threatening. Aside electrolyte disturbances which are secondary causes of alterations in fluid and electrolyte balance, there are other primary conditions that lead to excessive loss or gain of specific electrolyte as a result of excretion or effusion. Physiologically, marked disturbances in acid-base balance, disruption in enzyme functions, muscle contraction, neuronal excitability and poor membrane potential regulation are consequent on alterations in electrolyte concentration and in most cases could be associated with increased chances of death [3].

Most sodium associated disorders are due to fluid (water) imbalance, which invariably is responsible for sodium related disorders and outcome. Zhang et al. [4] reported that adverse outcomes likened to fluid overload have been recognized in humans and veterinary medicine. Slight alterations in sodium concentrations within reference range irrespective of disease severity in critically ill persons have been associated with high risk of mortality [4].

In veterinary patients, death risks have been known to be associated with potassium disorders, particularly hyperkalemia known to be a major death contributor in kidney injury, obstruction of urinary tract and hypoadrenocorticism patients. With reports of hyperkalemia encounter in veterinary medicine being common, its association to mortality outside specific critical conditions has not been established [5].

Bicarbonate derived from the dissociation of carbonic acid is primarily regulated by the kidney in the maintenance of acid-base balance. An alteration in acid-base balance, leading to loss of bicarbonate is partly necessitated by diarrhoea in humans and animal subjects. This process is achieved by the ability of the kidney to reabsorb filtered bicarbonate and synthesis to maintain a net excretion of acid [6].

According to the report of Durward et al. [7], balance in acid-base and osmolarity maintenance is crucially dependent on chloride regulation. Hence, hyperchloremic acidosis results from decrease in strong ion difference (SID) due to increased plasma chloride concentration, while hypochloremic acidosis results from increased SID due to decreased plasma chloride level. These alterations are clinically useful and evident in increased index suspicion in relation to gastrointestinal obstructions and or determination of un-estimated anions [7]. Acid-base balance can be impacted by disorders in chloride concentration as concerns have in recent times been focused on the potential impact of hyperchloremia (iatrogenic) and its outcome in persons that are critically ill [8]. In human medicine, the extensive use of saline (0.9%) which is likely multi-factorial as a fluid for resuscitation is a putative contributor of acid-base imbalance. According to hypothesis, increased kidney injury in some patients has been related with saline (0.9%) administration and its consequent outcome [9].

Phosphorus, a cation of the extracellular fluid stored primarily in the bone and teeth as hydroxyapatite is metabolically vital as components of metabolic intermediates such as adenosine triphosphate (ATP) and other nucleotides. Calcitonin, calciferol and parathyroid hormone (PTH) regulates its concentration with that of calcium. Phosphorus is readily excreted by the kidney when present in excess. Imbalance in phosphate level results in gastrointestinal disorder and increased excretion.

Increased calcium concentration (hypercalcemia) and its corresponding decrease (hypocalcemia) during emergency and critical care have been prevalently encountered in most animal population [10]. Hypercalcemia in critically ill

animals has likely been associated with kidney disease and tumour. Hypocalcemia in its ionized form has been commonly observed in cats and dogs diagnosed with sepsis and pancreatitis, leading to increased mortality in such patients [11].

Magnesium as the second most abundant intracellular cation is predominant in the bone with a minor quantity found in plasma [12]. Metabolically, aside magnesium function as a cofactor in over 300 enzyme catalyzed reactions essential to optimum body activity, it is important in neuro-muscular transmission, protein and nucleic acid synthesis, carbohydrate and lipid metabolism. It is required in the regulation of intracellular potassium movement and other ion transport.

The evaluation of blood electrolyte concentration of male and female pit bull dog as an indicator for the functional status of the kidney constitutes the aim of the study.

2. METHODOLOGY

2.1 Materials and Reagents

Standard materials, analytical grade reagents and kits were purchased from accredited centers and used for the purpose of this study.

2.2 Sample Collection

The blood sample were collected from twenty four different male and female (12 each) Pit bull dogs breed by NG Pet Universal Koncept, an accredited dog farm located at Eliozu town, Port Harcourt, Rivers State, Nigeria. The blood samples collected via venupuncture were transferred into plain sample bottles, dislodged and centrifuged at 5000rpm for 10 minutes, and the supernatant (serum) carefully transferred into a well labelled cryovials using a pipette. The separated samples were kept in a specimen box before storage at -4°C for safe transportation to the research laboratory.

2.3 Assay Protocol for Electrolyte Analysis

2.3.1 Sodium (Na$^+$) estimation

The principle of the modified method used was on the basis of precipitation of sodium as sodium magnesium uranyl acetate a triple salt, with the excess uranium then reacted with ferrocyanide, producing a chromophore of a varying absorbance which is inversely proportional to the concentration of sodium present in the test sample.

In the procedure, 1.0 ml of filtrate reagent was pipetted into well labelled test tubes, followed by the addition 50 μl of sample. The test tubes containing the samples were vigorously shaken for 3 minutes using a test tube shaker and then centrifuged at 4000rpm for 10 minutes. The supernatant obtained was used for colour development.

For colour development, fresh set of test tubes were labeled according to the corresponding filtrate and then 1.0 ml of acid reagent was pipette into each. Thereafter, 50 μl of supernatant and colour reagent were added to the test tubes and mixed properly. The absorbance reading was taken using a spectrophotometer zeroed with distilled water at 550 nm.

Calculation

$$\frac{\text{Abs. of blank} - \text{Abs. of sample}}{\text{Abs. of blank} - \text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of sodium } (mm/L)$$

Abs. = Absorbance
STD = Standard

2.3.2 Potassium (K$^+$) estimation

The principle used is according to the method of Terri and Sesin [13], where the amount of potassium was determined by the use of sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/L.

In the procedure, 1.0 ml of potassium reagent was pipetted into well labeled test tubes, followed by the addition of 10 μl of sample into respective test tubes. Thereafter, it was properly mixed, allowed to sit at room temperature for 10 minutes and absorbance reading taken using a spectrophotometer zeroed with distilled water at 500 nm.

Calculation

$$\frac{\text{Abs. of unknown}}{\text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of potassium } (mm/L)$$

Abs. = Absorbance
STD = Standard

2.3.3 Chloride (Cl⁻) estimation

The principle is based on the formation of a soluble, non-ionized compound when chloride ions react with mercuric ions, displacing thiocyanate ions from non-ionized mercuric thiocyanate. The displaced thiocyanate ions reacts with ferric ions, forming a coloured complex which absorbs UV-light at 480nm. The colour intensity produced is directly proportional to chloride concentration.

The procedure involves the introduction of 1.5 ml of chloride ion into well labeled test tubes, followed by the addition of 10 µl of calibrator / sample to the respective test tubes, then properly mixed and incubated at room temperature for 10 minutes. The absorbance was read with a spectrophotometer, zeroed with distilled water at 480nm.

Calculation

$$\frac{\text{Abs. of unknown}}{\text{Abs. of calibrator}} \times \text{conc. of calibrator} = \text{conc. of chloride } (mm/L)$$

Abs. = Absorbance

2.3.4 Bicarbonate (HCO₃⁻) estimation

The principle is based on the reaction of phosphoenolpyruvate with carbon dioxide (bicarbonate), catalyzed by phosphoenolpyruvate carboxylase (PEPC) to form oxaloacetate and phosphate ion. The oxaloacetate formed is reduced to malate with simultaneous oxidation of an equimolar amount of NADH to NAD^+ catalyzed by malate dehydrogenase (MDH). This reaction results in a decrease in absorbance at 340nm, and is directly proportional to HCO_3^- concentration in the sample.

In the procedure, 1.0 ml of bicarbonate reagent was pipette into well labeled test tubes and incubated at 37^oC for 3 minutes. Thereafter, 5 µl of water, standard and sample(s) was respectively pipetted into various cuvettes labelled appropriately. The cuvette contents were individually mixed by inversion and incubated for 5 minutes, then absorbance was read at 340 nm.

Calculation

$$\frac{\text{Abs. of blank} - \text{Abs. of sample}}{\text{Abs. of blank} - \text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of bicarbonate } (mm/L)$$

Abs. = Absorbance

STD = Standard

2.3.5 Calcium (Ca²⁺) estimation

The principle of this reaction is based on the formation of a violet complex with o-cresolphthalein complexone in an alkaline solution. In the procedure, then 0.5 ml of calcium reagent was introduced into a set of well labeled test tube, followed by the addition of 25 µ of sample(s), distilled water (blank) and standard to respective tubes. The mixture was properly shaken and allowed to sit at room temperature for 10 minutes, after which absorbance was read using a spectrophotometer zeroed with distilled water as blank at 570nm.

Calculation

$$\frac{\text{Abs. of sample}}{\text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of calcium } (mm/L)$$

Abs. = Absorbance
STD = Standard

2.3.6 Magnesium (Mg²⁺) estimation

The principle is based on the absorption of investigated magnesium by the kidney, since the kidney control magnesium homeostasis via tubular reabsorption. The procedure involved the introduction of 1.0 ml magnesium reagent in a properly labeled test tube, followed by the addition of 10 µl of sample and standard to respective test tubes. The solution was properly mixed and allowed to sit at room temperature for 5 minutes, after which absorbance was read at 540 nm on a spectrophotometer zeroed with distilled water.

Calculation

$$\frac{\text{Abs. of sample}}{\text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of magnesium } (mm/L)$$

Abs. = Absorbance
STD = Standard

2.3.7 Phosphorus (P) estimation

Principally, phosphorus reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex which is measured at 340 nm. The procedure involves the introduction of 1000 µl of phosphorus reagent in a properly labeled test tube, followed by the addition of 10 µl of sample and standard to respective tubes. The solution was well mixed

and allowed to sit at room temperature for 10 minutes. The absorbance was thereafter read at 340 nm on a spectrophotometer zeroed with distilled water.

Calculation

$$\frac{\text{Abs. of sample}}{\text{Abs. of STD}} \times \text{conc. of STD} = \text{conc. of magnesium } (mm/L)$$

Abs. = Absorbance
STD = Standard

3. RESULTS

The estimation of electrolyte concentration in male and female Pitbull dogs indicates an increase in sodium, chloride, bicarbonate and phosphorus levels when compared with respective reference ranges for dogs. The respective standard reference values were obtained from Mitruka and Rawnsley [14]. The concentration of potassium was found to be low in male dogs and high in females compared to the reference ranges as represented in Table 1. However, the difference in sodium, chloride, bicarbonate and phosphorus concentrations were significant (p≤0.05), while that of potassium though different but insignificant (p≥0.05) when compared with their respective reference ranges.

4. DISCUSSION

Electrolytes as ions formed in the body fluid are products of chemical elements or minerals critical for normal cellular function. Importantly, they function in maintaining and regulating blood acidity (pH), muscle activity and nerve function for optimal body processes to occur [15]. Inadequate hydration results in osmotic movement of cellular fluids from intracellular to extracellular space, and such osmotic movement facilitates the loss or imbalance of some electrolytes (e.g. sodium, potassium, calcium and magnesium). Such imbalance in essential electrolyte concentration disrupts normal cellular and body's function.

The mean concentration of sodium in the male and female Pitbull dogs was significantly high compared to the normal experimental range for sodium in dogs. Similar result was obtained in their study conducted by Alexander and Michael [16]. The elevation in sodium concentration was attributed to increased ingestion of diets rich in sodium, as sodium salts e.g. sodium chloride (NaCl) or sodium bicarbonate (NaHCO$_3$). Siew and Davenport [17], also reported excess of aldosterone (hyperaldosteronism) which causes an increase in sodium (Na$^+$) reabsorption by the kidney, resulting in hypernatremia which could lead to seizure disorders as a neurological consequences.

The mean level of potassium estimated for both gender of the pit bull dogs was within the normal experimental reference range for dogs, suggesting a proper neuromuscular cell excitability in the dogs, owing to the fact that potassium is a major determinant of resting membrane potential.

The levels of chloride and bicarbonate were observed to be higher than the normal experimental reference standard for dogs, as an imbalance in chloride and bicarbonate could result to metabolic acidosis or alkalosis, and cellular damage as a consequence.

The calcium concentration in the male and female pit bull dog were observed to be below normal experimental reference range for dogs. The low calcium concentration often referred to as hypocalcaemia is often results to chronic renal failure attributed to decreased synthesis of

Table 1. Mean electrolyte concentration in male and female pitbull dogs

Electrolytes	Concentrations (mmol/l)		
	Male	Female	Reference range
Sodium (Na$^+$)	179.2±66.97[*]	157.4±76.07[*]	137-149
Potassium (K$^+$)	3.54±0.55	5.12±1.23	3.7-5.6
Chloride (Cl$^-$)	116.00±12.81[*]	123.5±17.98[*]	99-110
Bicarbonate (HCO$_3^-$)	43.72±2.72[*]	75.98±27.98[*]	17-24
Calcium (Ca^{2+})	1.74±0.50	1.56±0.16	2.18-2.95
Magnesium (Mg^{2+})	1.48±0.18	1.70±0.39[*]	1.46-2.68
Phosphorus (P)	4.26±0.61[*]	4.06±0.95[*]	0.9-2.45

Results are expressed as Mean±Standard Deviation. Mean values with superscript (*) are statistically different (p≤0.05) compare with reference ranges

1,25-$(OH)_2D_3$ and hyperphosphatemia. As PO^{4-} binds and lowers Ca^{2+}. Hypocalcamia has been reported to be responsible for arrhythmias, a condition associated with irregular heartbeat due to improper electrical signalling function, leading to cardiac dysfunction necessitated by low calcium levels and circulation [3].

Magnesium as a cofactor for over 300 catalytic enzymes, facilitates the normal body function. It is very essential for ion transport, regulating intracellular K^+ movement and also in neuromuscular transmission, carbohydrates, proteins, lipids and nucleic acids synthesis. Excessive levels of magnesium (hyper magnesemia) can lead to neurological consequences e.g. seizure disorders. The magnesium and phosphorus levels of the dogs (male and female) studied were respectively above normal reference range for dogs. This elevation can be attributed to increased dietary intake, gastrointestinal disorders, and excretion by the kidney can lead high phosphorus level (hyperphos phatemia), resulting in hypoparathyroidism and chronic kidney disease. These variations in electrolyte balance (imbalance) evidently affect the normal cellular functions of the dogs, since exercise is vital for the dogs and important in bodily regulation and homeostasis.

5. CONCLUSION

The study revealed the various concentrations of electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phos phorus) in the serum of male and female Pit bull dog specie. The increase in electrolytes found above normal range could result in impaired kidney function, as the kidney is essential for electrolyte regulation and balance. However, maintaining electrolyte balance *in vivo* helps in the maintenance of proper volume of fluid (water) and regulation of biochemical processes during normal cellular and body activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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