بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Medical Laboratory Science

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Determination of plasma sodium and potassium levels using flame photometry and ion selective electrode

تحديد مستويات الصوديوم والبوتاسيوم في البلازما باستخدام الفليم فوتوميتري و الايون سيليكتف اليكترود

A thesis submitted in partial fulfillment of B.Sc (honor) degree in medical laboratory science (Clinical Chemistry)

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الآيه

بسم الله الرحمن الرحيم

قال تعالى :

[إِنَّا عَرَضْنَا الأَمَانَةَ عَلَى السَّمَاوَاتِ وَالأَرْضِ وَالْجِبَالِ فَأَبَيْنَ أَنْ يَحْمِلْنَهَا وَأَشْفَقْنَ مِنْهَا وَحَمَلَهَا الإِنْسَانُ إِنَّهُ كَانَ ظَلُومًا جَهُولا **].**

صدق الله العظيم
سورة الأحزاب
الاية 72

Dedication

To our parents…Who's supported us in all steps in our life and didn't scant anything for us.

To soul of our father Dr- Mohammed AbdAlrahim.

To all our families, friends, teachers, colleague & who have positive impact on our life.

Acknowledgement

Thanks first & last to ALLAH who concede us almightiness to complete this research on requirable face.

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Also we praise all clinical chemistry laboratory staff that help and support us.

Finally we thank students of Sudan University whose give us samples.

Abstract

The study aimed to measure plasma sodium and potassium concentrations using flame photometry and ion selective electrode methods. Fifty (50) apparently healthy students in Sudan University of different age were selected to assess the comparison between sodium and potassium plasma concentration measuring by flame photometer (JENWEY models PFP7 and PFP7/C) and ion selective electrode (EASYLITE), all samples were selected from Sudan University of Science and Technology after agreement of eachindividual at February 2014, three ml from each individuals was taken in a heparin container.

The results showed that significantly difference in sodium plasma concentration average mean (134.9±8.90)mmol/L and (138.4±2.03) mmol/L measured by flame photometer and ion selective electrode respectively (p. value 0.008), no significant difference in concentration of potassium when measured by two methods (p. value 0.17).

The results showed insignificant weak positivecorrelation between flame photometer and ion selective electrode in plasma sodium concentration (p. value .907) (r value .017), but significant weak positive correlation between both methods was observed in plasma potassium concentration (p. value .142) (r value .211).

مستخلص البحث

تهدف هذه الدراسه الى مقارنه مستويات الصوديوم والبوتاسيوم في البلازما بين طريقتي التوهج الضوئي و الايونات المختاره بواسطه القطب الكهربائي ,وقد اجريت هذه الدراسه على أشخاص اصحاء ظاهريا وعددهم 50 في اعمار مختلفه لقياس مستوى الصوديوم والبوتاسيوم في البلازما باستخدام جهازي الفليمفوتوميتريوالايزي لايت , وتم اخذ العينات من طلاب جامعه السودان للعلوم والتكنولوجيا بعد موافقتهم في فبراير 2014 ,اخذت ثلاثه مل في حاويه تحتوي على مانع التجلط (هيبارين) .

اوضحت نتائج هذه الدراسه وجود فرق ذات دلاله احصائيه لمتوسط تركيز الصوديوم (134.9±8.90)م مول / لتر و (138.4±2.03) م مول / لترعند قياسه بطريقتي التوهج الضوئي و الايونات المختاره بواسطه القطب الكهربائي علي التوالي .وعدم وجود فرق ذات دلاله احصائيه لمتوسط تركيز البوتاسيوم (4.065±.7663) م مول / لتر و(3.908±.23) م مول / لتر باستخدام الطريقتين.وايضا اوضحت هذه الدراسه وجود علاقه ايجابيه ضعيفه غير مؤثرة احصائيا لتركيز الصوديوم(p value .907),(r value .017),ووجود علاقه ايجابيه ضعيفه ذات تاثير احصائي لتركيزالبوتاسيوم.(p. Value 0.142) (r. value0.211)عند قياسه بطريقتي التوهج الضوئي و الايونات المختاره بواسطه القطب الكهربائي علي التوالي.

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Introduction & Literature Review

Chapter One
1. Introduction and literature review

1.1 Introduction:

The study aimed to compare between two technical methods flame photometry and ion selective electrode to measured sodium and potassium plasmaelectrolytes, since the accuracy of the result depends mainly on the analytical method used in laboratory, there are different methods used for electrolyte determination, flame photometry is used for the quantitative measurement of sodium and potassium in body fluid based upon measurement of radiant energy emitted. Ion selective electrode is the measurement of the electric potential difference between two electrodes in an electrochemical cell connected by an electrolyte solution, used to select anions or cations for measurement. Sodium ions are major cations of extra cellular fluid. Thus sodium ions are responsible for almost half the osmolality of the plasma and play a central role in maintaining the normal distribution of water and osmotic pressure in the extra cellular fluid compartment, potassium is the major intra cellular cation, is an important part of protein synthesis and maintenance of normal oncotic pressure. (Norbert W,Tiet, 1987)

1.2 Literature review

1.2.1 Electrolyte Composition of body fluid

The body has two main fluid compartments, the intracellular and the extracellular, the extracellular being further divided into blood plasma (vascular compartment) and interstitial fluid. These compartments differ in composition in regard to water and electrolytes. Plasma it consist of large number of organic and inorganic substance dissolved in water, has a volume of 1300-1800 mL/m^2 of body surface and constitutes about 5% of the body volume. Serum the clear liquid that can be separated from clotted plasma, the liquid portion of normal unclotted blood containing the red, white cells and platelets (Norbert W,Tiet, 1987). Maintenance of water homeostasis is paramount to life for all organisms, in mammals, 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 electrolyte play an important role in:

(1) Maintenance of PH.

(2) Proper heart and muscle function.

(3) Oxidation, reduction reaction.

(4) As cofactor for enzymes.

Electrolytes are ions capable of carrying electrical charge, depending on whether they move in an electrical field towards the anode or cathode (Mosby's, 2006).

Abnormal concentration of electrolytes may be either the cause or the consequence of avariety of disorders. Thus determination of concentration of electrolytes is one of the most important functions of clinical laboratory; interpretation of abnormal osmolality and acid base values requires specific knowledge of electrolytes. (Carl A, Burtis, 2002)

Electrolytes are classified as anion or cation, based on the type of charge they carry. Many of the functions listed require electrolyte concentration to be held within narrow ranges, the body has complex system for monitoring and maintaining the concentration of electrolyte.(Carl A, Burtis, 2002).

1.2.1.1 Sodium:

Sodium is the most abundant cation in the extracellular fluid representing 90% of all extracellular cations and largely determined the osmolality of the plasma. Plasma sodium concentration depends greatly on the intake and execration of water and to a lesser degree renal regulation of sodium. Three processes are of primary importance in regulation of sodium concentration:

-The intake of water in response to thirst stimulated or suppressed by plasma osmolality.

- The excretion of water, largely affected by anti diuretic hormone release in response to change in either blood volume or osmolality.

- Blood volume status which affects sodium excretion through aldosterone, angiotensin II and antindiureticprotein. The kidney has the ability to conserve or excrete large amount of sodium depending on sodium contents of the extracellular fluid and the blood volume. Normally 60%-75% of filtrated sodium is reabsorbed in proximal tubule, electro neutrality is maintained by either Cl^-reabsorption or H^+ ion secretion .Some sodium is also reabsorbed in the loop of henle and distal tubules and under control of aldosterone is exchanged for potassium in the connecting segment and cortical collecting tubules.

Table 1.1: Reference range of sodium:

	Sodium concentration:
Daily diet (Nacl) =	130 -260mmol(8-15g)
Body requirement \ day =	1-2 mmol /dl
Urinary sodium excretion* =	40-220 mmol/dl
Cerospinal Fluid	13.8 - 15 mmol/dl

*Urinary sodium excretion varies with dietary sodium intake.

Reference range of sodium in blood:

- Adult / elderly: 136 – 145 mmol / L.
- Child: 136 – 145 mmol / L.
- Infant: 134 – 150 mmol/L.
-Newborn: 134 – 144 mmol/ L.
*Critical value: <120 or >160 mmol/L.

- Clinical applications of sodium

Hypernatremia:

Increase plasma sodium concentration (> 150mmol/L) occurs whenever there is an excess sodium in relation to water. Always reflect hyperosmolarity, with the danger of cellular dehydration .There are numerous causes of hypernatremia. (Nasir S A, Inayatullan,2003)

Hypernatremia is caused by:

- Increased sodium intake.

*Increase dietary intake: if sodium usually in the form of salt it ingested at high quantities without adequate free water, hypernateremia will occur.

*Excessive sodium in intravenous fluids: the normal kidney can excrete about 450-500 mEq of sodium per day if intake of sodium exceeds that amount in apatient without ongoing losses or aprior sodium deficit. Sodium level can be expected to rise.

Decreased sodium loss:

* Cushing syndrome: corticosteroids have an aldosterone like effect.

*Hyperaldosteronism: aldosterone stimulates the kidney to absorb sodium at the level of sodium tubules.

Excessive free body water loss:

*Gastrointestinal loss (without rehydration): if free water is lost residual sodium becomes more concentrated.

*Excessive sweating: Although sweat contains some sodium most is free water, this cause the serum sodium to become more concentrated. If the water loss is replaced without any sodium, the sodium dilution and hypernatremia can occur.

*Diabetes insipidus: deficiency of antidiuretic hormone and the inability of the kidney to respond to antidiuretic hormone cause large free water loss and sodium become concentrated. (Carl A, Burtis)

- In certain stage of pregnancy steroid hormone causes sodium retention as well as water which result in gain in weight. (A C DEB,2008)

In those situations sodium level increases, if however free water is therapeutically provided, sodium levels may become diluted and hypernatremia may occur. (Mosby’s,2006)

Symptoms of hypernatremia:

Symptoms of hypernatremia involve: the CNS due to hyperosmolar state, these symptoms include: altered mental status, lethargy, irritability restlessness, seizures, muscle twitching, hyper reflexes, fever, nausea or vomiting and increased thirst. Serum sodium more than 160 mmol / L is associated with increased mortality by 60%to 75%(Bishop, 2000).

Decreased level of sodium (hyponatremia)

A decreased plasma sodium concentration (<136 mmol/L) can occur when there is a loss of both sodium and water. This happens with some diseases of the liver and kidney, in patient with congestive heart failure, in burn victims (Nasir S A,2003).

Causes of hypornatremia:

-Decreased sodium intake

* Deficient dietary intake: sodium intestinal absorption is highly efficient, deficiency of salt is rare.

- Increased sodium loss:

*Addison's disease: caused by inadequate level of aldosterone and corticosteroid hormones, sodium is not reabsorbed by the kidney and is lost in the urine.

* Diarrhea, vomiting or nasogastric aspiration: sodium in the gastro intestinal content I lost with the fluid, hyponateremia magnified if intravascular fluid replacement does not contain adequate amount of sodium.

* Diuretic administration: many diuretics work by inhibiting sodium reabsorption by the kidney, and level can be diminished or decreased.

* Chronic renal insufficiency: if the kidney losesitreabsorptive capability, large quantities of sodium will be lost with urine.

* Large volume aspiration of pleural or peritoneal fluid: sodium concentration is the same in body fluid, in these fluids, the aspiration of these fluids is compensated by secretion of anti diuretic hormone, which

acts to increase renal reabsorption of free water and sodium will become diluted(Carl A, Burtis,2002).

Increase free body water:

* Excessive oral water intake.

* Hyperglycemia: each 60mg/100ml increase of glucose above normal decreases sodium by 1mEq /L, since osmotic effect of glucose pulls free water from the extracellular space and dilute sodium.

* Excessive intravenous water intake: when intravenous therapy provides less sodium than normal range or volume and ongoing losses, sodium will be diluted.

* Congestive heart failure.

*preipheral edema: the condition are associated with increase free water retention, sodium is diluted.

*Ascitis.

* Pleural effusions.

* Intraluminal bowel loss (Carl A, Burtis,2002).

- Adrenocortical steroids which regulate the metabolism of sodium .In the insufficiency of adrenocortical steroids, the serum sodium level is decreased with an increase in sodium excretion (A C DEB, 2008).

Syndrome of inappropriate or ectopic secretion of antidiuretic hormone, over secretion of antidiuretic hormone stimulates the kidney to reabsorb free water. Sodium is diluted(Mosby’s,2006).

Symptoms of hyponatremia:

Depending on the serum level, between 125mmol/l and 130 mmol/l, symptoms are primarily gastrointestinal; below 125 mmol/l more severe neuropsychiatric symptoms are seen. In general, symptoms include nausea, vomiting, muscular weakness, headach, lathergy and ataxia. More severe symptoms include seizures, coma and respiratory depression (Bishop, 2000).

1.2.1.2 Potassium (K^+):

Potassium is the most major cation within the cell. the intracellular potassium concentration is approximately 150 mmol/L , where the normal serum concentration approximately 4 mmol/L , this ratio is most important determinant in maintaining membrane electrical potential , specifically in neuromuscular tissue , because serum concentration of potassium is so small , minor changes in concentration have significant consequences . Potassium is excreted by kidney, there is no reabsorption of potassium from the kidneys, therefore if potassium is not adequately supplied in the diet or by intravenous administration, in a patient who is unable to eat,serum potassium level can drop rapidly. Potassium is an important part of protein synthesis and maintenance of normal oncotic pressure. It contribute to the metabolic portion of acid -base balance in that the kidneys can shift potassium for hydrogen ions to maintain a physiologic ph. serum potassium concentration depending in many factors:

-Aldosterone (and to a lesser extent glucocorticosteriods) this hormone tends to increase renal loss of potassium.

- acid- base balance:alkalotic states tend to lower serum potassium level by causing a shift of potassium into the cell, acidotic state tends to raise serum potassium levels by reversing that shift.

-Sodium reabsorption: as sodium is reabsorbed, potassium is loss (Carl A, 2002).

Reference range:

#Adult\ elderly: 3.5 – 5.0 mmol/L.

#Child: 3.4 - 4.7 mmol/L.

#Newborn: 3.9- 5.9mmol/L.

Clinical application of potassium

Hyperkalemia:

Increase plasma potassium concentration (>5mmol/l) is a result of redistribution, increase intake, or increased retention. In addition, preanalytical condition such as: hemolysis, thrompocytosis ($>10^6$ /µL) and leuokocytosis ($>10^5$ /µL) (Carl A, 2002).

It is caused by:

- Excessive dietary intake.

- Excessive intravenous intake.

Because the amount of potassium in the serum is so small minimal but significant increase in potassium intake cause elevation in serum level.

-Acute or chronic renal failure: this is the most common cause of hyperkalemia, potassium excretion is diminished and potassium level rise.

- Addison disease.

- Hypoaldosteronism.

- Aldosteron _inhibiting diuretics (spironolactone)

- Crush injury to tissue.

-Hemolysis

-Transfusion of hemolyzed blood.

-Infection: potassium exists in high level in the cell with cellular injury and lyses the potassium within the cell is released into the blood stream.

- Acidosis

-Dehydration: potassium becomes more concentrated in dehydrated patients and serum level appears elevated, when the patient is rehydrated potassium levels may in fact be reduced (Carl A).

Hypokalemia:

Decrease plasma potassium concentration (<3.5mmol/l).

It is caused by

-Deficient dietary intake

- Deficient intravenous intake.

The kidneys cannot reabsorb potassium to compensate for the reduced potassium intake and potassium level declines.

-Gastrointestinal disorders (e.g.: diarrhea, vomiting)

- Diuretics: this medication act to increase renal excretion of potassium.

-Hyperaldesteronism: aldosterone imbalance potassium excretion.

-Cushing's syndrome: glucocorticosteroid have an aldosterone like effect.

- Renal tubule acidosis: renal excretion increased.

- Licorice ingestion: licorice has an aldosterone like effect.

-Alkalosis: to maintain physiological pH during alkalosis, $H^+$are driven out of the cell and into blood (Carl A, 2002).

-Insulin administration: in patient with hyperglycemia, if insulin administrated, glucose and potassium will be driven into the cell, and potassium level drops (Mosby's, 2006).

1.2.2 Type of Techniques used for measurement of sodium and potassium plasma levels:

1.2.2.1 Flame photometry:

Principles:

Flame emission spectrophotometer is based on the characteristic emission of light by atoms of many metallic elements when giving sufficient energy such as that supplied by a hot flame. The wavelength to be used for the measurement of an element depends on the selection of a line of sufficient intensity to provide adequate sensitivity and freedom from the interfering lines at or near the selected wavelength .for example, lithium produces a red color, sodium a yellow color, potassium a violet color, and magnesium a blue color in a flame, these color are characteristic of the metal atom that are present as cations in solution. Under constant and controlled conditions the light intensity ofthecharacteristic wave length produced by each of the atoms is directly proportional to the number of atoms that are emitting energy, which in turn is directly proportional to the concentration of the substance of interest in the sample, although this technique once was widely used for analysis of sodium, potassium and lithium in body fluids, it has been replaced largely by electrochemical technique (Carl A, 2002).

Procedure:

In a flame a solution containing the substance to be determined is passed under carefully controlled conditions as a very fine spray into the air supply of a burner. The solution evaporates and the substance is first converted to the atomic state or it is constituent radicals, in which the electrons in the outer most shell are in their lowest energy state closes to the nucleus the ground state. As the temperature rises the thermal energy of the flame excites these electrons so that they are able to absorb one or more quanta of thermal energy and move into higher energy orbits further from the nucleus. If the energy supplied increases it can become great enough for the electrons to escape from the atom to form an ion. This is the ionization potential at which atomic characteristic are lost. At the temperature used in flame photometer this degree of energy is not reached in doing so the energy previously absorbed is released as quanta of light , the wavelength of which being dependent on the energy levels the electrons can assume , are characteristic of the substance thus giving rise to the emission spectrum(RamnikSood,,2009).

The purpose of the flame photometer is twofold. Chemical bonds are broken to produce atoms, and then atoms absorb energy from the flame and enter an excited electronic state. Then return to the ground state by emitting light energy that is characteristic for the atomic species. The emitted light focused by lenses on mirror, passes through monochromators that are selected to transmit radiant energy from the specific atoms. The monochromator for sodium is set at 589nm, for potassium 767nm, and fop lithium at 671nm. The emitted light is then strikes a photo detector, which generates an electrical signal proportional to the concentration of atom. The basic instrumentation is filter , are used to measure the concentration of solutions that contain fluorescing molecule, the detector placed at right angles to the sample cell and secondary filter that passes the longer wavelength of fluorescent light prevent incident light from striking the photo detector. The fluorescing sample in the cuvate emits radiant energy in all directions. Gas discharge lamps are the most frequently used sources of excitation radiant energy, tungsten lamp are seldom used because little energy is released by them in the ultraviolet region (Bishop M L,2000).

Advantages and dis advantages of flame photometry:

Flame photometry has two advantages: specificity and sensitivity. Fluorometryincreases specificity by selecting the optimal wavelength

For both absorption and fluorescence. Is approximately 1,000 times more sensitive than most spectrophotometric methods. One reason is because emitted radiation is measured directly; it can be increased simply by increasing the intensity of the exciting radiant energy.

The biggest disadvantage is that fluorescence is very sensitive to environmental changes. Changes in pH affect availability of electrons, and temperature changes theProbability of loss of energy by collision rather than fluorescence. Contaminating chemicals or a change of solvents may change the structure. UV light used for excitation canCause photochemical changes. Any decrease in fluorescenceresulting from any of these possibilities is known asQuenching. Because so many factors may change the intensity or spectra of fluorescence, extreme care is mandatoryin analytic technique and instrument maintenance. (Bishop Edward, 2010).

1.2.2.2 Ion Selective Electrode:

An ion selective electrode also known as specific ion electrode (SIE) is atransducer or sensors that convert the activity of a specific ion dissolved in a solution into an electrical potential which can be measured by a voltmeter or pH meter. The voltage is theoretically dependent on the logarithm of the ionic activity, according to the Nernst equation, the sensing part of the electrode is usually made as an ion- specific membrane, along with a reference electrode. ISE is used in biochemical and biophysical researches were measurement of ionic concentrations in an aqueous solution is required (NorbertW,Tietz ,1987).

Principle of ion selective electrode:

The basic of most conventional forms of clinical biochemistry measurement is the production of a coloredcompound. Electrochemistry is different in that themeasurement is based on production of a voltage (potential)or current, and the measuring system is a voltmeter than a photometer. An electrochemical detector, which responds specificallyto a given analyte, is called an ion-selective electrode(ISE). The early electrodes responded to ions, hence the name, but more recently, electrodes have been developedthat respond to metabolites such as glucose and urea, although they are usually included in the same categorybecause they share a common technology. Glucose sensorsused in clinical areas are often ISE devices, and allCritical care analyzers are based on ISE technology. The basis of ISEs is that they produce a potentiometric(voltage change) oramperometric (current change) response to changes in the analyte, i.e. therearetwo types primary electrodes and Secondary electrodesuse other features, such as enzymes, to achieve specificityand release a product that can be detected by an ISE. Electrodes are used in a number of clinical applications (Norbert W,Tietz ,1987).

The most frequently used electrodes are for:

1-Ions: e.g. hydrogen (pH), sodium, potassium, chloride, fluoride, calcium, magnesium, lithium, ammoniumn (NH4).

2-Gases: e.g. oxygen, carbon dioxide, ammonia (NH3).

3- Secondary or complex electrodes:e.g. glucose, urea,Lactate, creatinine.

The advantages of ISEs in clinical applications are that:

- Non-photometric, which means that an optically clear solution is not needed, i.e. whole blood can be used.

- Rapid and direct, allowing measurement of true concentration.

Four elements are required to measure by a potentiometric ISE:

* A measuring half-cell.

* A reference half-cell.

* A salt bridge or equivalent electrical connection.

*A measuring device.

Types of Ion selective electrodes:

There are four main types of ion selective membranes used in ion selective electrode: glass, solid state, liquid based and compound electrode.

glass membrane:

Glass membrane made from an ion exchange type of glass (silicate or chalcogenide) this type of ion elective electrode has a good selectivity but only signal charged cations mainly H^+, Na^+ and Ag^+ . Chalcogenide glass also has selectivity for double charged metal such as $Pb+^2$ and cd^{+2}.

Crystalline membrane:

Made from mono or poly crystallites of a single substance, they have good selectivity because only ions which can introduce themselves into crystal structure which can interfere with electrode response. Selectivity for both cation and anion of membrane forming substance e.g.: fluoride selective electrode, based on LaF_3 crystals.

Ion - exchange resin membrane:

Based on special organic polymer membranes which contain a specific ion exchange substance (resin), is the most wide spread type of ion selective electrode. Usage of specific resins allows preparation of selective electrode for tens of different ions, both single atom and multi atom. e.g.: potassium selective electrode based on ralinomycin as an ion exchange agent (Norbert W,Tietz ,1987).

Applications of ion selective electrode:

In use, the electrode wire is connected to one terminal of a galvanometer or pH meter, the other terminal of which is connected to a reference electrode, and both electrodes are immersed in the solution to be tested. The passage of the ion through the vinyl via the carrier or channel creates an electrical current, which registers on the galvanometer, by calibrating against standard solutions of varying concentration; the ionic concentration in the test solution can be estimated from the galvanometer reading. in practice there are several issues which affecting this measurement leakage between the vinyl and the wall of the capillary , thereby allowing passage of any ion will cause the meter reading to show little , or no change between the various calibration solutions and required that electrode be discarded. The response of the electrode and galvanometer is temperature sensitive and also drifts over time required recalibration to a new reading .so that timing of the reading is critical in order to find the most accurate window after the response has settled, but before it has drift appreciably.

Interference:

The most serious problem limiting use of ion selective electrode is interference from other undesired ions. No ion selective electrode are completely ion specific, all are sensitive to other ions having similar physical properties. Most are these interference are weak enough to be ignored (Norbert W,Tietz ,1987).

1.3.2.4 Advantages and dis advantages of ion selective electrode:

When compared to many other analytical techniques, ISEs are inexpensive and simple to use and,unlike the flame photometer, have a linear response over a wide concentration range. However, they have some disadvantages that require attention if good results are to be obtained:

- First, despite their name, many of them are not entirely ion-specific. For example, sodium electrodeis used also responds to potassium ions**,** although not with thesame sensitivity. This means that Na+ will be overestimated if a high concentration of K+ ispresent. Mathematical techniques have been devised to compensate for this.

-Secondly, they underestimate high concentrations because of "crowding" of the ions at themembrane – some just don't get "seen".

1.3 Rationale:

Laboratory errors become a serious problem in many medical laboratories as increase false interpretation of results well lead to wrong diagnosis. Plasma sodium andpotassium are two important cations and to obtain exact normal value in plasma an accurate and suitable method are required. So, the study aimed to compare between Flame photometry and ion selective electrode important to determine either effective in electrolyte measurement.

1.4 Objective:

General objective:

Measurement of sodium and potassium level using Flame photometry and ion selective electrode methods.

1.4.1 Specific objective:

1-To measure sodium and potassium level using two methods.

2-To find out the suitable method for sodium and potassium separately.

3-To compare between sodium plasma level using two methods.

4-To compare between potassium plasma level using two methods.

Chapter two

Materials & Methods

CHAPTER TWO

2. Materials and Methods

2.1 Study design:

This is an experimental analytical study used to compare between sodium and potassium plasma concentration using ion selective electrode and flame photometry.

2.2 Study population:

This study involved 50 apparently healthy individuals randomly selected from Sudan University of Science and Technology.

2.3 Sample size:

Fifty samples were collected for the study, samples was selected by a simple random sampling method.

2.4 Ethical consideration:

Each participant was informed about the objective of the study, after agreement blood sample are collected.

2.5 Sample collection:

Three ml of venous blood were collected with sterile syringe from healthy individual in heparin container.

2.6 Sample preparation:

Three ml of venous blood collected in heparin container centrifuged at 3000rpm for 5 min, then stored in 18˚C.

2.7 Data analysis:

Data was analyzed to obtain mean, standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer programmed version 16.

2.8 Methods of Measurement of Na+ and K+ levels:

2.8.1 ISE (ion selective electrode):

2.8.1.1 Principle:

An Ion selective electrode consists of a detector electrode and an electrically conductive membrane which separates the sample solution of unknown activity from a solution of fixed ion activity which fills the electrode.A difference in ionic composition of the two solutions causes an electrical potential difference to develop across the membrane, change in potential across the selective membrane are measured with respect to a reference electrode. The potential of which is constant. The change in potential difference between the reference electrode and the ion selective electrode for the sample is proportional with the potential difference for a calibration solution of known composition(Norbert W,Tietz, 1987).

2.8.1.2 Reagents:

Structurally an ISE analyzer is based on an electrochemical cell its basic components as follows: detector electrode and ion selective membrane:

Sodium ISE:

It is made of special glass which is selective of sodium i.e. only sodium ions are free move to any extent.

Potassium ISE:

This usually constructed from polyvinyl chloride (PVC) in which the antibiotic valinomycin is immobilized. The valinomycin selectively transports potassium ions across the membrane.

2.8.1.3 Reference electrode:

This is usually a calomel (Hg\HgCl$_2$) electrode or silver chloride (Ag\AgCl).The electrical potential of the reference electrode must remain constant for long periods.

2.8.1.4 Electrolyte solution:

The circuit of the electrochemical cell is completed by an electrolyte solution. A silver coated wire enables the electric cell to be completed with the reference electrode.

2.8.1.5 Calculation:

The analyzer automatically calculates the analyte concentration voltmeter this measures the potentials developed. Sodium and potassium results were displayed digitally.

Calculation of analyte concentration:

The analyzer automatically calculate the analyte concentration voltmeter this measure the potential developed, sodium and potassium result wereappear in the screen as digital number .

2.8.2 .Flame photometry:

2.8.2.1 Principle:

Flame photometry relies upon the fact that the compound of the alkali and the alkaline earth metals can be thermally dissociated in flame and that some of the atoms produced will be further excited to a higher energy level. When these atoms return to the ground state they emit radiation at a wavelength specific for that element(Bishop M L,2000).

2.8.2.2 Analysis preparation

Calibration of standards:

Sodium:

0.634g was accurately weighed of dry "Analar" quality NaCl dissolved in pure deionized water and washed into a 500ml volumetric flask. The flask filled up to the mark with pure deionized water. To prepare the standard solution for use with the flame photometer, the stock solution was diluted 1 in 50.

Potassium:

0 .477g was accurately weighted of dry "Analar" quality KCL dissolved in pure deionized water and washed into a 500ml volumetric flask. To prepare the standard solution for use with the flame photometer, the stock solution was diluted 1 in 50.

2.8.2.3 Storage of solutions:

Stored solutions were away from direct sun light in a cool place, ideally at temperature below 25C°, Glass containers should not be used for storage as they can affect sodium concentration levels so sample were stored in sealed, plastic vessels.

Quality control:

Accuracy and precision of flame photometer and easylite must be checked using control sera level I and II.

Chapter Three

Results

Chapter Three
3. Results

A group of 50 healthy apparently person was involved in this study to compare between ion selective electrode and flame photometer in measurement of plasma sodium and potassium levels.

Results were analyzed using SPSS.

Table 3.1:Mean difference between plasma sodium concentration Using ion selective electrode and flame photometry.

Table 3.2: Mean difference between plasma potassium using ion selective electrode and flame photometry.

Figure 3.1: Mean sodium by flame photometry and ion selective electrode.

Figure 3.2:Mean potassium by using ion selective electrode and flame photometry

Figure 3.3:Correlation between ion selective electrode and flame photometer in plasma sodium measurement.

Figure 3.4: Correlation between ion selective electrode and flame photometer in plasma potassium measurement.

Table 3.1: Mean difference between plasma sodium concentration Using ion selective electrode and flame photometry

Method	Number	Mean± Std.Deviation	P-value
-Sodium ISE -Sodium flame photometry	50 50	138.4±2.03 134.9±8.90	.008

This table showed that plasma sodium concentration was significantly different between ion selective electrode and flame photometry.

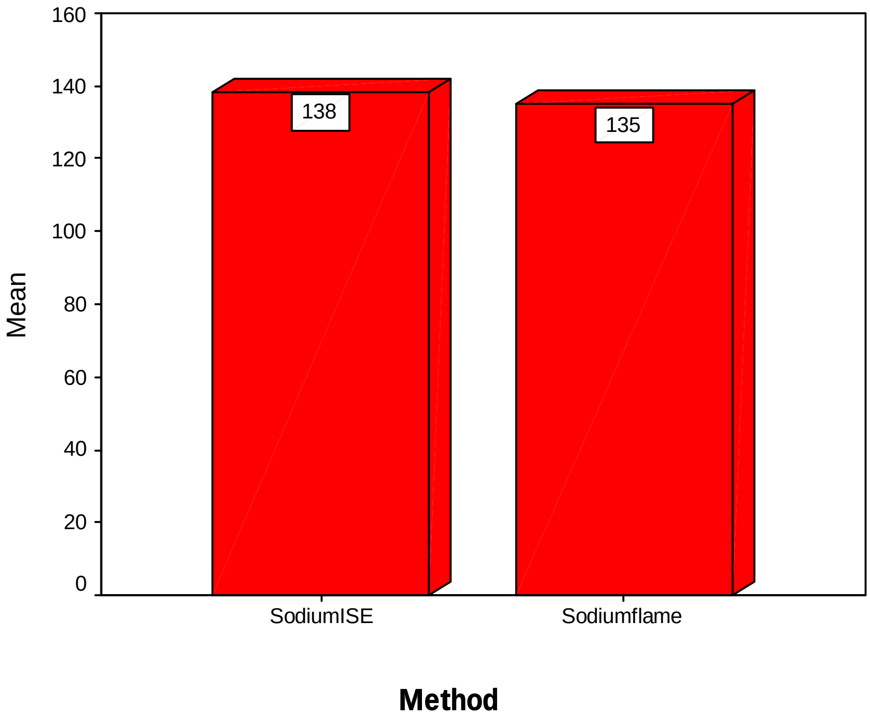

Figure 3.1: Mean of sodium by flame photometry and ion selective electrode

Table 3.2: Mean difference between plasma potassium using ion selective electrode and flame photometry.

Method	Number	Mean± Std.Deviation	P-value
-Potassium ISE	50	3.90± .23	.170
-Potassium flamephotometry	50	4.06± .76	

Plasma potassium concentration was insignificantly difference, no difference between ion selective electrode and flame photometry. (Table 3.2)

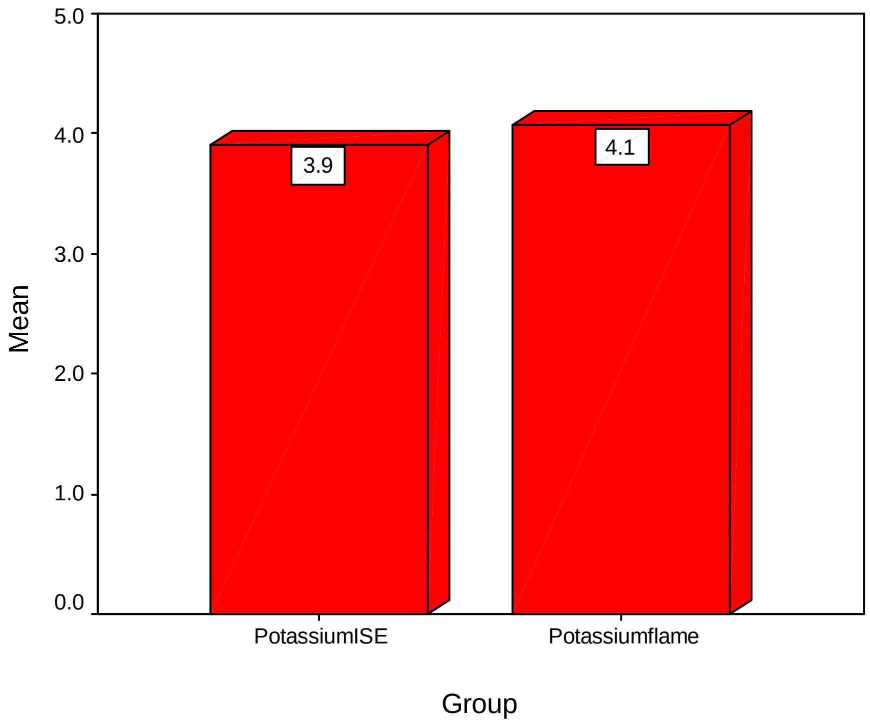

Figure 3.2:Mean potassium by using ion selective electrode and flame photometry

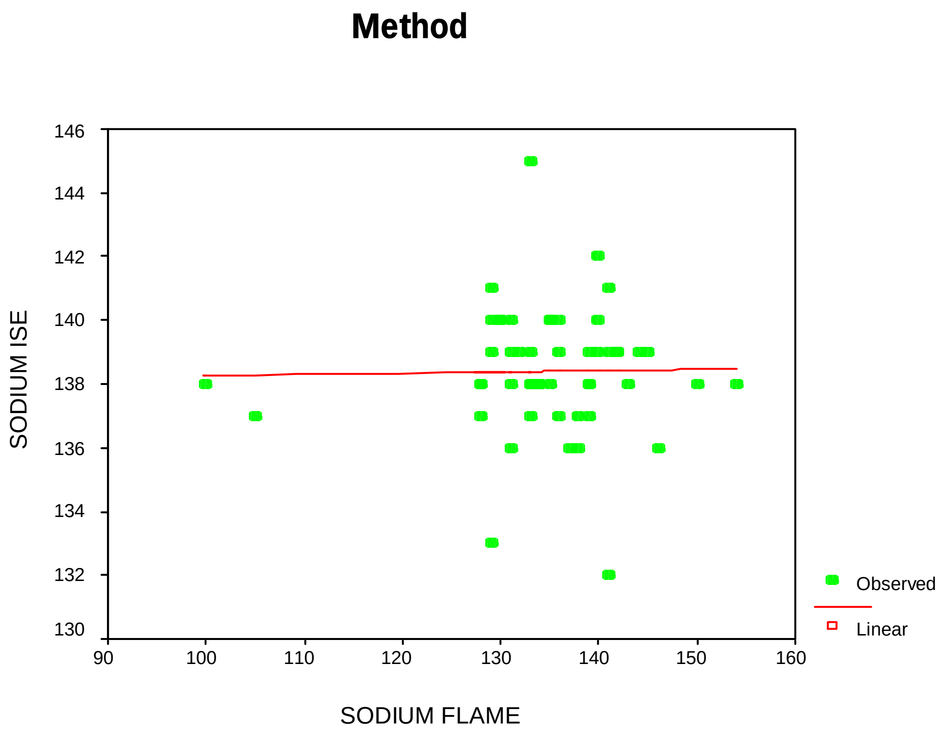

Figure 3.3: showed very weak positive correlation between ion selective electrode and flame photometry in plasma sodium measurement.

r value .017

P value .907

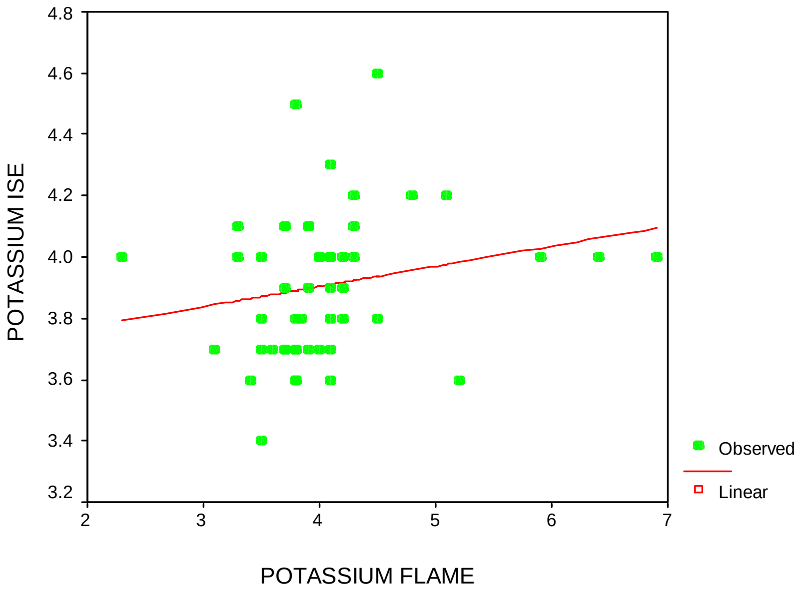

Figure 3.4: plotted showed weak positive correlation between ion selective electrode and flame photometer in plasma potassium measurement .

R value .211

P value .142

Chapter Four

Discussion, Conclusions and Recommendations

CHAPTER FOUR

4. Discussion, Conclusions and Recommendations

4.1 Discussion:

This study aimed to measure sodium and potassium plasma level using flame photometry compared with measurement using ion selective electrode.

The results showed that sodium plasma level was significantly difference when using ion selective electrode compared to flame photometry (p.value .008). Similar finding was reported by worth (1985), who concluded that there was a small but consistent difference between sodium measurements by ion selective electrode and flame photometry. Insignificant difference of plasma sodium level measured by flame photometer and ion selective electrode as reported by Sharma and Sharma (2013), who concluded that there was a good degree of agreement when sodium and potassium measured using convential flame photometer and ion selective electrode.

According to results obtained there was insignificant difference plasma potassium level (p.value.170), when measured by both ion selective electrode and flame photometry.

Also In this study results showed that sodium plasma level measured by ion selective electrode and flame photometry presented insignificant weak correlation. Significant weak positive correlation was obtained for potassium using flame photometry and ion selective electrode.

4.2 Conclusions:

1. There was a significant difference between flame photometry and ion selective electrode when plasma sodium level was measured.
2. There is no difference in plasma potassium measurement using both methods.
3. There were three plasma sodium results compatible by both methods.
4. There were seven compatible plasma potassium results by both methods.

4.3 Recommendations:

To obtain a reliable results control sample should be used to confirm the results of these two analytical methods.

We recommend that to determine lipid profile and protein concentration of sample before electrolytes measurement.

Also hemolytic sample should be avoided.

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