CLINICAL EQUIVALENCE OF HEPARINIZED ARTERIAL PLASMA TO VENOUS SERUM FOR MAGNESIUM AND PHOSPHATE MEASUREMENT AMONG PATIENTS ADMITTED AT THE INTENSIVE CARE UNIT OF MOI TEACHING AND REFERRAL HOSPITAL

BY

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DEPARTMENT OF BIOMEDICAL SCIENCES

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DECLARATION

Declaration by the student

I declare that the work presented is my original work and has not been presented to any other institution of learning for an academic award.

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(Admission number: PG/MSC/00034/2013)

Declaration by supervisors

I confirm that the work presented in this thesis was developed by the candidate under our supervision.

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DEDICATION

To all that have brought out the best of me in this life. God bless you all abundantly.

ABSTRACT

Electrolytes routinely measured in patients in Intensive Care Unit (ICU) are sodium, potassium, chloride, calcium, magnesium and phosphate. Of these; sodium, potassium, chloride and calcium are measured as part of tests done during blood gas analysis (BGA) using arterial blood. However, magnesium and phosphate are measured from a venous serum sample since they cannot be measured during BGA. Both arterial and venous puncture are traumatizing to the patient and trauma increases when both are done numerous times on the same patient in a single day. Plasma obtained from sodium-heparin anticoagulant is yet to be used as a sample of choice for measuring magnesium and phosphate since it is not clear whether there is clinical equivalence between magnesium and phosphate concentrations obtained from venous serum and arterial plasma. It is clinically important that those measurements should give equivalent results and confirm the closeness to the absolute value. The main objective was to assess the equivalence of arterial and venous blood magnesium and phosphate concentrations. The specific objectives were; to determine the mean difference between magnesium concentration in arterial blood plasma and venous blood serum, to determine the mean difference between phosphate concentration in arterial blood plasma and venous blood serum, to determine the correlation between magnesium concentration in arterial blood plasma and venous blood serum and to determine the correlation between phosphate concentration in arterial blood plasma and venous blood serum. This study used a cross-sectional research design and was carried out at Moi Teaching and Referral Hospital (MTRH) where one hundred and fifty-three patients admitted at MTRH ICU underwent arterial and venous puncture to obtain arterial and venous blood which was used for magnesium and phosphate measurement on an automated chemistry laboratory analyzer. Mean difference between magnesium concentration in plasma and serum calculated using paired t-test was 0.03(t=1.23) p=0.22 while mean difference between phosphate concentration in plasma and serum was 0.14(t=1.18) p=0.24. Magnesium correlation between plasma and serum was r=0.98 [p = 0.00 (< 0.05)] and phosphate correlation between plasma and serum was r=0.99 [p = 0.00 (< 0.05)]. The study indicates there is clinical equivalence between magnesium and phosphate concentration in arterial blood plasma and venous blood serum, thus arterial blood plasma samples can be used in place of venous blood serum samples for magnesium and phosphate measurements.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Automated Analyzer
ABG	Arterial Blood Gas
BGA	Blood Gas Analysis
DNA	Deoxyribonucleic Acid
ED	Emergency Department
ICU	Intensive Care Unit
IREC	Institution Review and Ethics Committee
MTRH	Moi Teaching and Referral Hospital
OD	Optical Density
RNA	Ribonucleic Acid
US CLIA	United States Clinical Laboratory Improvement Amendments
mmol/L	Millimole per liter
SD	Standard Deviation

DEFINITION OF TERMS

Arterial puncture	A medical procedure involving insertion of a needle into an artery so as to obtain blood to be used for laboratory analysis.
Blood gas analysis	A test which measures the amounts of oxygen and carbon dioxide in the blood, as well as the acidity (pH) of the blood.
Clinical equivalence	Containing similar or equal value when analyzed in a patient.
Critical patient	Patient with uncertain prognosis, vital signs are unstable or abnormal, there are major complications, and death may be imminent
Electrolyte	A substance that dissociates into ions in solution and acquires the capacity to conduct electricity.
Magnesium	A mineral involved in many processes in the body including nerve signaling, the building of healthy bones, and normal muscle contraction.
Phlebotomy	Opening or puncture of a vein in order to withdraw blood.
Phosphate	An organic compound of phosphoric acid in which the acid group is bound to nitrogen or a carboxyl group in a way that permits useful energy to be released during metabolism.
Plasma	Fluid part obtained from blood collected with an anticoagulant.
Serum	Fluid part obtained from blood collected without an anticoagulant.
Venipuncture	A medical procedure involving insertion of a needle into a vein so as to obtain blood for laboratory analysis.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Disturbances in fluid and electrolytes are among the most common clinical problems encountered in the intensive care unit (ICU) and studies have reported that fluid and electrolyte imbalances are associated with increased morbidity and mortality among critically ill patients (Lee, 2010). In critical patients, electrolyte imbalance can lead to serious and critical events which might adversely affect the outcome of the patient hence quick and accurate assessment of these disturbances demands immediate medical attention (Alanazi, *et al*., 2015). The quickness of that assessment, especially in developing countries, is frequently restricted by the postponement in transporting samples to the laboratory, either because of deficiency of adequate couriers or the nonappearance of quick transport systems (Alanazi, *et al*., 2015). In Moi Teaching and Referral Hospital (MTRH), patient samples for magnesium and phosphate measurement are collected by venipuncture and transported to the central laboratory for analysis separately from samples collected for Blood Gas Analysis (BGA) (Chebii, *et al*., 2017). Thus, the patient undergoes trauma twice. This is done because there is no evidence whether BGA samples can be used for magnesium and phosphate measurement. The same patient will then undergo an arterial puncture to obtain blood for BGA (Chebii, *et al*., 2017).

Magnesium and phosphate are among the electrolytes found within the body and are involved in numerous metabolic processes (Moe, 2008). Almost all metabolic processes are dependent upon or are mediated by electrolytes (Budak, *et al*., 2012). Variation in electrolyte concentrations may be due to a variety of disorders and such disorders must be identified in time to ensure adequate

and timely treatment as electrolyte abnormalities can represent significant risks to life (Budak, *et al*., 2012).

Although frequent blood draws can destroy veins, cause pain, and lead to anemia, ICU patients typically have routine daily blood tests to help detect problems early (Eachempati, 2014).

Under such circumstances it is important to obtain data quickly so as to optimize the therapeutic response interval and allow prompt treatment. In order to decrease the number of phlebotomies in the ICU, and thereby minimize the general discomfort and trauma patients undergo during such activities, it is preferable to measure blood electrolytes on the single sample collected for BGA rather than performing additional venipunctures to collect venous blood in order to check serum levels of magnesium and phosphate as has been routinely done.

Yu *et al.,* (2011) analyzed the concentrations of 163 metabolites in plasma and serum samples collected simultaneously from 377 fasting individuals. Plasma and corresponding serum samples from 83 individuals were re-measured in the same plates and mean correlation coefficients (r) of all metabolites between the duplicates were 0.83 and 0.80 in plasma and serum, respectively, indicating significantly better stability of plasma compared to serum ($p=0.01$). Despite differences in absolute concentration between the two matrices, for most metabolites the overall correlation was high, which reflects a proportional change in concentration. The study showed that reproducibility was good in both plasma and serum.

A study conducted by Jain *et al.,* (2009) in India where 200 paired venous and arterial samples from patients admitted to the ICU were analyzed for electrolytes on arterial blood and venous blood. The mean BGA sodium value was 131.28 (SD 7.33), and the mean venous sodium value was 136.45 (SD 6.50) ($p < 0.001$). The mean BGA potassium value was 3.74 (SD 1.92), and the mean venous potassium value was 3.896 (SD 1.848) ($p = 0.2679$). Based on the analysis, the

authors found no significant difference between the potassium values measured by the blood gas machine and the auto-analyzer. However, the difference between the measured sodium was found to be significant thus concluding that critical decisions can be made by trusting the potassium values obtained from the BGA.

A study was done to determine whether the BGA and venous measurements of potassium, sodium, and hemoglobin levels are equivalent from 200 paired arterial and venous blood samples. The mean BGA and laboratory potassium values were 3.77±0.44 and 4.2±0.55, respectively (P<0.0001). The mean BGA and laboratory sodium values were 137.89±5.44 and 140.93±5.50, respectively (P<0.0001). The mean BGA and laboratory hemoglobin values were 12.28±2.62 and 12.35±2.60, respectively (P = 0.24). Zhang *et al.,* (2015). The results indicate that although there are the statistical difference between BGA and laboratory measured potassium and sodium, the biases do not exceed United States Clinical Laboratory Improvement Amendments (USCLIA)-determined limits. Therefore, all three variables measured by BGA were reliable.

Gupta *et al.,*(2016) did a study to compare the sodium andpotassium results on arterial and venous blood. Data for 112 samples was analyzed and results were the mean sodium level in serum sample was 139.4 ± 8.2 mmol/L compared to 137.8 ± 10.5 mmol/L in ABG (P < 0.05). The mean difference between the results was 1.6 mmol/L. Mean potassium level in serum sample was 3.8 ± 0.9 mmol/L as compared to 3.7 ± 0.9 mmol/L in ABG sample (P < 0.05). The mean difference between the results was 0.14 mmol/L. Statistically significant difference was observed in results of two instruments in low sodium (<135 mmol/L) and normal potassium (3.5-5.2 mmol/L) ranges. The 95% limit of agreement for sodium and potassium on both instruments was 9.9 to −13.2 mmol/L and 0.79 to −1.07 mmol/L respectively.

Put together, these findings suggest that there are variation between different electrolytes when measured/compared between the venous and arterial blood samples. This calls for the need to analyze individual electrolyte in venous and arterial blood samples to determine the clinical equivalence hence improving the patients healthcare system

1.2 Statement of the Problem

Patients in hospital admitted to the ICU routinely undergo arterial puncture to obtain arterial blood to be used in blood gas analysis and measurement of the electrolytes including sodium, potassium, chloride and calcium. This blood sample is usually collected in a sodium heparin anticoagulant pre-treated syringe. The same patient will then undergo venipuncture to obtain venous blood for use in serum biochemical analysis for measurement of other biochemical parameters that will include magnesium and phosphate. Magnesium and phosphate cannot be measured during BGA as the BGA machine does not have a system for measuring the two analytes.

However, both arterial and venipuncture are traumatizing to the patient and trauma increases when both are done numerous times on the same patient in a single day. Also, clinicians after obtaining the first results from the arterial puncture will have to wait for additional results from the second venipuncture before making a conclusive clinical decision regarding the patient's management. Furthermore, an additional venipuncture has cost implications since more consumables are utilized on the patient when performing a second blood draw.

1.3 Justification

Serum from venous blood has been the preferred sample for measurement of magnesium and phosphate due to ease in accessing veins and absence of interfering substances that might be present in anticoagulants which might adversely affect the measurement process and subsequent outcome of results.

However, plasma obtained from sodium heparin anticoagulant is yet to be used as a sample of choice for measuring magnesium and phosphate concentration since no study has been done to establish clinical equivalence between magnesium and phosphate concentrations obtained from venous serum and arterial plasma collected using sodium heparin anticoagulant.

1.4 Study Significance

Clinical equivalence between serum and plasma concentrations of magnesium and phosphate will decrease subsequent trauma to the patient since additional punctures will be reduced and also patient care will improve as clinicians will be able to make decisions quicker due to availability of all results at once. Cost implications on the patient will also be minimized due to decreased use of consumables that would have resulted from a second blood draw.

1.5 Study Objectives

1.5.1 Main objective

To assess whether heparinized arterial magnesium and phosphate concentrations are clinically equivalent to venous magnesium and phosphate concentrations.

1.5.2 Specific objectives

1. To determine the mean difference between magnesium concentration in arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.
2. To determine the mean difference between phosphate concentration in arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.
3. To determine the correlation between magnesium concentration in arterial blood plasma and venous blood serum from patients admitted to the ICU at MTRH.
4. To determine the correlation between phosphate concentration in arterial blood plasma and venous blood serum from patients admitted to the ICU at MTRH.

1.5.3 Research hypotheses

H_0 There is no difference in mean magnesium concentration between arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.

H_0 There is no difference in mean phosphate concentration between arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.

H_0 There is no correlation between magnesium concentration in arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.

H_0 There is no correlation between phosphate concentration in arterial blood plasma and venous blood serum in patients admitted to the ICU at MTRH.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Electrolyte disorders frequently develop in critically ill patients during the course of stay in the ICU Geerse, *et al*., (2010). Therefore, ICU patients are routinely monitored for electrolyte disorders, and it is common practice to correct them (Geerse, *et al*., 2010). Electrolyte abnormalities have been recognized as a preventable cause of cardiac arrest (Sharma, Miclescu, & Wiklund, 2011). Serial measurements of ABG and electrolytes are therefore essential for monitoring ICU patients Srisan *et al.,(*2011). Hazards of frequent blood sampling for ABG, electrolyte and other laboratory investigations include increased infection rate, pain, stress response, patient's discomfort and anemia in newborns (Corwin, 2004). In a multicenter study, anemia was an almost universal finding in ICU patients in the United States (Corwin, 2004). Although anemia in critically ill patients is multifactorial, phlebotomy accounts for greater blood loss than pathologic bleeding and is associated with a higher mortality rate in these patients Von Ahsen, *et al*., (1999). Furthermore, specific conditions like severe underlying disease, patient's critical condition, and frequent previous blood samplings, make repeated venipunctures difficult and uncomfortable (Raghavan & Marik, 2005). In order to decrease the number of phlebotomies, it is preferable to check blood electrolytes on the one single sample collected for ABGs rather than performing additional venipunctures to check serum electrolyte levels Carson, *et al*., (1996).

Although heparin plasma is the preferred sample for many chemistry analytes, with serum and plasma phosphorus results being practically interchangeable, heparin is not acceptable for all analytes Carey, *et al*., (2016). A World Health Organization (WHO) document states that heparin plasma samples are recommended for several analytes Banfi, *et al*., (2002), because the

constituents in plasma are better at reflecting the pathologic situation of a patient than those in serum. Some of the analytes measured in blood plasma include: alkaline phosphatase, aspartate aminotransferase (AST), hemoglobin (plasma), ionized calcium, lactate dehydrogenase, potassium, protein (total), and thyroxine.

The same WHO document also states that heparin plasma samples "can be used without changes of result" for many other analytes on the basis of acceptability of differences observed in comparison of results of testing of plasma samples and serum samples versus criteria on the basis of biologic variation Banfi, *et al*., (2002). Analytes listed include alanine aminotransferase, albumin, amylase, bicarbonate, bilirubin, brain natriuretic peptide, calcitonin, calcium, chloride, cholesterol, cholesterol (HDL), creatinine, creatinine kinase, d-dimer, erythropoietin, ferritin, folate, fructosamine, γ-glutamyl transferase, haptoglobin, homocysteine, insulin, iron, lipase, magnesium, osmolality, sodium, thyroxine, free thyroxine, tri-iodothryonine, triglycerides, urea, uric acid, vitamin B12, and zinc Carey, *et al*., (2016).

2.2 Estimation of Magnesium Levels in the Body

Magnesium is the second most prevalent intracellular cation and has an important role as a cofactor in various enzymatic reactions Rude, *et al*., (2012). Magnesium is an essential, mainly intracellular, cation that has been favorably associated with markers of inflammation and endothelial dysfunction Joosten *et al*., (2013) It also provides energy and regulates various processes in the cell and cell membrane, as well as protein and DNA synthesis, and the regulation of mitochondrial function Rude, *et al*., (2012) Hence, recognition of hypomagnesemia in patients in Medical Intensive Care Unit may be important (Safavi & Honarmand, 2007) as this may be associated with severity of illness or increased mortality and morbidity. The incidence of hypomagnesemia is reported as 50-60% in ICU patients Guerrera, *et al*., (2009). Hypomagnesemia is an important but underdiagnosed electrolyte abnormality in critically ill patients and many studies have been done to find the prevalence of hypomagnesemia and its effects on mortality and morbidity in these patients Limaye *et al.,* (2011). A study done in India found a high prevalence of hypomagnesemia in the critically ill patients and hypomagnesemia was associated with a higher mortality rate in critically ill patients Limaye *et al*., (2011). As a cofactor in numerous enzymatic reactions, magnesium fulfils various intracellular physiological functions. Thus, imbalance in magnesium status—primarily hypomagnesaemia as it is seen more often than hypermagnesaemia—might result in unwanted neuromuscular, cardiac or nervous disorders. Measuring total serum magnesium is a feasible and affordable way to monitor changes in magnesium status, although it does not necessarily reflect total body magnesium content (Jahnen-Dechent & Ketteler, 2012). At present, there is no simple, rapid, and accurate laboratory test to indicate the total body magnesium status (Seo & Park, 2008). The most commonly used method for assessing magnesium status is the serum magnesium concentration (Seo & Park, 2008).

Hypomagnesemia, while typically defined as having serum magnesium concentration below 0.66 mmol/L, with or without accompanying total body depletion, does not lead to clinically significant signs and symptoms until serum levels fall below 0.5 mmol/L. (Swaminathan, 2003) Nonetheless, as magnesium is involved in an array of structural and physiological functions, adverse effects associated with hypomagnesemia may occur in almost every organ system, whether they are clinically acute and overt, or chronic and subtle (Pham, *et al*., 2014). Clinical manifestations of hypomagnesemia that promptly lead to medical attention involve neuromuscular hyper excitability that may range from tremors, fasciculation, tetany, to convulsions, and neuropsychiatric disturbances including apathy, delirium, and even coma Pham, *et al*., (2014). Other potentially life-threatening complications may arise not solely from hypomagnesemia, but also from the associated hypocalcemia and/or hypokalemia, and include atrial and ventricular arrhythmias, torsades de pointe, enhanced sensitivity to digoxin toxicity, and sudden death (Pham, *et al*., 2014). In contrast, long-term adverse complications include altered glucose homeostasis, hypertension, atherosclerosis, osteoporosis, asthma, migraines, and other end-organ damage. Although many paradigms have been explored to minimize the mortality in critical care units, magnesium loss has been scarcely addressed (Zafar, *et al*., 2014); in this respect leading to inconclusive results. Serum magnesium monitoring may have prognostic and perhaps therapeutic implications because critically ill-patients are predisposed to both symptomatic and asymptomatic magnesium deficiency that can lead to some important clinical consequences (such as hypokalemia, cardiac arrhythmias, hypocalcemia, neurotoxicity and psychiatric problems), ultimately increasing the morbidity and mortality (Tong & Rude, 2005). Hyermagnesemia is rarely seen in the absence of renal insufficiency, as normal kidneys can excrete large amounts of magnesium (Seo & Park, 2008). Prevalence of hypermagnesemia varies from 5.7% to 9.3% (Huey, *et al*., 1995). The highest

serum magnesium concentrations reported so far are 18 mmol/L in a 33-week-old premature infant and 13.4 mmol/L in a 78-year-old woman who swallowed water from the Dead Sea Huey, *et al*., (1995), Oren, *et al*., (1987). Severe hypermagnesemia in fact seems to be a feature in patients who drown in the Dead Sea Oren, *et al*., (1987).

2.3 Estimation of Phosphate Levels in the Body

Phosphate is the second most abundant essential mineral in the human body after calcium (Raina, *et al*., 2012). It not only plays a role in numerous biologic processes, including energy metabolism and bone mineralization, but also provides the structural framework for DNA and RNA (Raina, *et al*., 2012). Phosphate is an essential element found in all living cells important for bone structure, energy storage, and gene translation Kemmerly, *et al*., (2014). Normal serum phosphate concentrations range from 0.8–1.45 mmol/L Kemmerly, *et al*., (2014). Critically ill patients have underlying conditions that predispose them to developing hypophosphatemia, including malnutrition and inadequate body phosphorus stores, acute respiratory alkalosis, diabetic ketoacidosis, use of diuretics, alcoholism, and vomiting or gastric losses (Lee, 2010). Hypophosphatemia has been associated with critical conditions such as Gram-negative sepsis and open-heart surgery. The prevalence of hypophosphatemia is high in the ICU, reported to be observed in about 28% in critically ill patients. Hypophosphatemia (plasma phosphate concentration < 2.5 mg/dL or 0.81 mmol/L) may result from decreased intestinal phosphate absorption, increased renal phosphate losses, and a shift of phosphate to intracellular space (Lee, 2010). Approximately 2.4%–100% of critically ill patients are deficient in total body phosphorus for numerous reasons, including impaired absorption, increased renal excretion, or redistribution of inorganic phosphorus within the body Geerse, *et al*., (2010). Phosphate is synthesized through various biochemical pathways such as glycolysis and beta oxidation (Golub, 2011). As a part of signal transduction, phosphate is used in cyclic AMP and products of deoxyribonucleoside diphosphates like dADP, dCDP, dGDP, and dUDP (Golub, 2011). Intensive Care Unit patients are routinely monitored for electrolyte disorders, and it is common practice to correct them Geerse, *et al*., (2010). Hypophosphatemia is one of those frequently encountered electrolyte disorders, for

which many causative factors are present in critically ill patients (Geerse, *et al*., 2010). Hypophosphatemia is one of the frequently encountered electrolyte disorders in critically ill patients, with a prevalence ranging from 20% to 40% (Miller & Slovis, 2000) and even reaching 80% in septic patients Barak, *et al*., (1998). Because the common mechanism in hypophosphatemia-caused complications is impaired energy metabolism, hypophosphatemia has also been described as a metabolic disturbance leading to cellular dysfunction in multiple organ systems Geerse, *et al*., (2010). Three main mechanisms lead to hypophosphatemia: decreased intestinal absorption, increased renal excretion and internal redistribution of inorganic phosphate Yang, *et al*., (2013). The main causes of hypophosphatemia in critically ill patients include severe infection, trauma, postoperative state, malnutrition, respiratory alkalosis and diabetic ketoacidosis Geerse, *et al*., (2010). Zazzo, *et al*., (1995) demonstrated a strong correlation between hypophosphatemia and mortality in surgical intensive care patients. A serum phosphate concentration of more than 1.62mmol/L is considered hyperphosphatemia Raina, *et al*., (2012). In this state, the body induces a physiologically significant down-regulation of serum phosphate by reducing intestinal absorption of dietary phosphates and decreasing re-absorption of phosphate from glomerular filtrate (Raina, *et al*., 2012). Unless there is a significant deterioration of renal function, the phosphate homeostasis is maintained by the action of Parathyroidhormone, Vitamin D3, and phosphatonins Raina, *et al*., (2012). The main underlying causes for hyperphosphatemia are decreased renal function, defective phosphatonin and other phosphaturic factors, a large efflux of intracellular phosphates, and/or a dietary increase in phosphate (Wagner, 2007).

2.4 Serum Versus Plasma as Biological Samples in Clinical Laboratory

Although serum and heparinized plasma specimens are considered equivalent for many assays (Miles, *et al*., 2004), differences in results between these two sample types have been reported for several chemistry analytes. Significant differences between serum and heparinized plasma results have been reported for albumin, alkaline phosphatase, calcium, carbon dioxide, chloride, creatine kinase, glucose, lactate dehydrogenase, inorganic phosphorus, potassium, and total protein (Ladenson, *et al*., 1974). The concentration differences in results for calcium, glucose, inorganic phosphorus, potassium, and total protein between serum and heparinized plasma were felt to be large enough to affect clinical interpretation in certain instances (Miles, *et al*., 2004). For most chemical analytes of interest in testing blood samples, serum was the substrate of choice for many years (Carey, *et al*., 2016). Recently, many hospital and commercial laboratories have converted to heparin plasma samples collected in gel separator tubes for most of these tests (Carey, *et al*., 2016). Heparin plasma samples from anticoagulated patients do not have the clotting issues of serum samples (Carey, *et al*., 2016). Waiting for the right time to centrifuge after the specimen clots is not required with heparin plasma samples (Carey, *et al*., 2016). There is an additional advantage to using heparin plasma samples in that the volume of plasma produced is 15%–20% higher than serum from the same volume of blood; therefore, reduced extraction blood volumes could suffice (Carey, *et al*., 2016). The differences between results obtained from heparin plasma and serum samples have been judged to be acceptably small for most chemical analytes. Pham, *et al.,* (2014), (Tong & Rude, 2005), Zafar, *et al.,* (2014) (Seo & Park, 2008) (Whang & Ryder, 1990) Huey, *et al*., (1995). Miles, *et al*., (2004) did a study on Comparison of Serum and Heparinized Plasma Samples for Measurement of Chemistry Analytes in which twenty apparently healthy volunteers who had been fasting for 12–14 hours had serum and lithium-heparin specimens

collected in that standard draw order during a single venipuncture. The serum samples were analyzed sequentially, followed immediately by sequential analysis of the heparin-plasma samples. Differences in the mean values for the two sample types were compared and were considered clinically significant at 2% for sodium; 5% for calcium, chloride, glucose, and potassium; and 10% for all other analytes tested. Analysis of heparinized plasma samples showed clinically significant decreases relative to serum samples for bile acids (−67%) and potassium (−6.0%). Clinically significant increases were seen for aldolase (+39%), ACE (+22%), and LD (+21%). According to the manufacturers both serum and heparinized plasma samples are acceptable for ACE, aldolase, bile acids, LD, and potassium. Miles, *et al*., (2004). On the Vitros 950 analyzer, similar changes were seen in the concentrations reported for potassium (9.3% decrease) and LD (19% increase) in the plasma samples relative to the serum samples. In addition, mean total bilirubin results were 20% higher in the plasma samples on this analyzer. Both serum and heparinized plasma samples are acceptable for each of these assays according to the manufacturers. Miles, *et al*., (2004). A study was done on the feasibility of using lithium-heparin plasma from a gel separator tube as a substitute for serum in clinical biochemical tests. Three specimen types were labeled as serum with gel separator (S), lithium heparin-plasma (P), and lithium heparin-plasma with gel separator (G). Primarily 120 specimens were centrifuged and analyzed within 2 hours (T_0), 24 hours (T_{24}), and 48 hours (T_{48}). Differences in analyte concentrations between tubes at T_0 and following storage times (T_{24} to T_{48}) were evaluated for statistical significance. Most of the analytes displayed a non-statistical significance for differences between tube types at T_0. However, AST, ALP, LDH, K^+, and phosphorus, which had much higher concentrations in blood cells than plasma, were higher in S than G at T_0. Total protein, which was

composed of fibrinogens and other proteins, was higher in G than S. Glucose, which appeared in blood cells metabolisms, was lower in P than G. Yuan-hua, *et al.,* (2010)

Tze-Kiong, *et al*., (2006) did a study on selected analyte values in serum versus heparinized plasma using the synchron lx pro assay methods/instrument. Blood samples were collected in a clot tube (Vacutainer Tube, Becton Dickinson, Franklin Lakes, NJ) and in a blood collection tube containing lithium heparin anticoagulant (Vacutainer Plasma Separator Tube [PST] II) during a single venipuncture of 100 patients admitted to Emergency Department. All blood samples were centrifuged immediately after collection and aliquots of serum and heparinized plasma samples were assayed singly for glucose (Glu), blood urea nitrogen (BUN), sodium (Na), potassium (K), chloride (Cl), alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase (AMY), lipase (LIP), total bilirubin (TBili), creatine kinase (CK), C-reactive protein (CRP), lactate dehydrogenase (LD), albumin (ALB), and uric acid (UA) using the SYNCHRON LX-20 PRO analyzer (Beckman Coulter, Brea, CA) and reagents from Beckman Coulter. Percent differences between serum versus heparinized plasma samples for all analytes ranged from 0.0% to 10.8%, and were less than ± 4%, except for BUN (–9.4%) and ALB (10.8%). Statistically significant differences between the mean analyte values obtained on serum versus heparinized plasma were observed for only K, CK, LD, and ALB. Compared to mean serum values for K, CK, LD, and ALB, mean plasma values were significantly decreased for K, CK, and LD and increased for ALB.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Moi Teaching and Referral Hospital (MTRH) Intensive Care Unit (ICU). At the time of the study, the ICU had a six-bed patient capacity. Laboratory analysis took place at the Clinical Chemistry Laboratory of MTRH. MTRH is Kenya's second largest national referral facility after Kenyatta National Hospital and it's located in Eldoret Uasin Gishu county. Its geographical coordinates are 0° 30' 44" North, 35° 16' 50" East (figure 3.1). It has a bed capacity of 850 patients and was chosen because of its well-established ICU and clinical chemistry laboratory and its huge catchment areas. MTRH caters for the referral needs of the northern and western parts of Kenya.

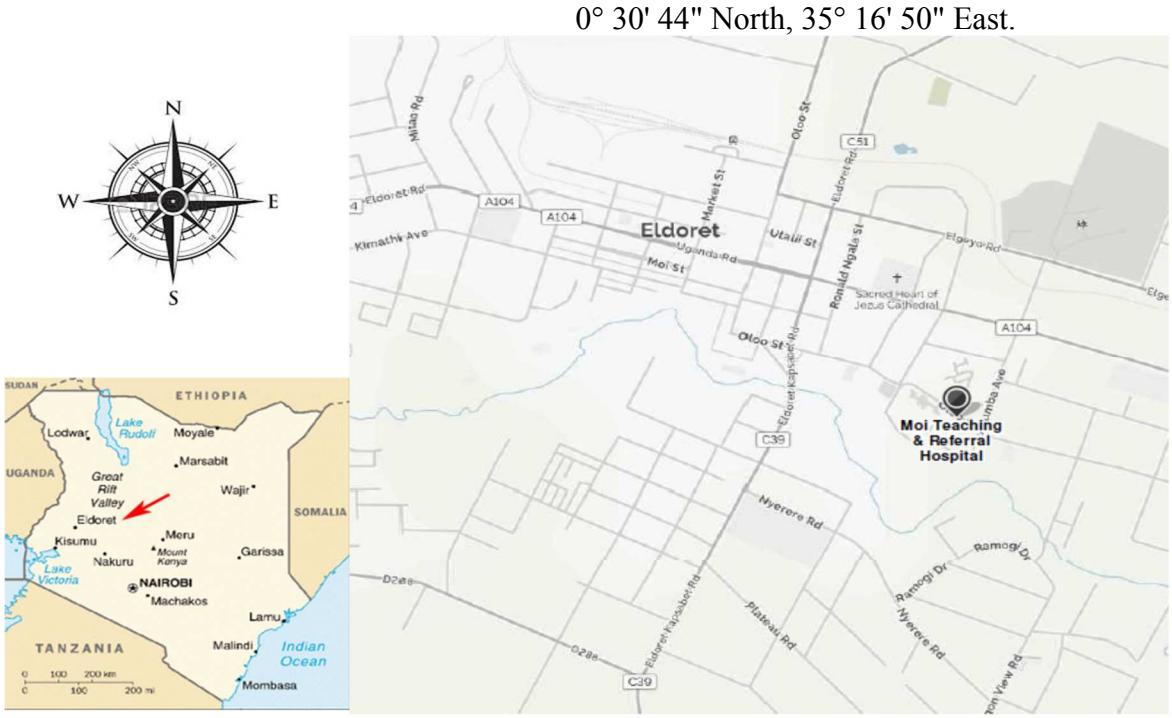


Figure 3.1 Map of Eldoret town.

3.2 Study Design

Cross sectional research design was used in this study.

3.3 Study Population

The study population comprised adult patients of either sex admitted to the MTRH ICU which has a six bed capacity. Within the hospital there are more than six patients requiring ICU admission at any one time due to the 800 bed capacity of the hospital thus the ICU beds are all occupied every other day.

3.4 Eligibility Criteria

3.4.1 Inclusion criteria

All patients admitted to the MTRH ICU with a requisition for both Arterial Blood gas analysis and venous magnesium and phosphate measurement were included in the study.

3.4.2 Exclusion criteria

Patients admitted to the MTRH ICU from whom venous blood could not be obtained due to collapsed veins were not included in the study.

3.5 Sampling Design

Participants were recruited consecutively one after the other for a period of two months till the desired sample size was achieved.

3.6 Sample collection procedure

Arterial puncture and venipuncture was performed by a phlebotomist on patients in ICU for obtaining arterial blood and venous blood. Arterial blood was collected from the radial artery using heparinized syringes containing sodium heparin anticoagulant and subsequently transferred into BD Vacutainer plain tubes for centrifugation at 3000 revolutions per minute (RPM) for 10 minutes.

The resulting supernatant after centrifugation is designated plasma. Following centrifugation, the plasma was transferred into a clean analyzer sample cup for magnesium and phosphate measurement in the automated analyzer COBAS INTEGRA®400 plus (Roche diagnostics Germany) located in the Biochemistry Laboratory of MTRH.

Venous blood was collected from the antecubital vein using non-heparinized syringes and transferred to BD Vacutainer plain tubes. After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature. This took 15-30 minutes. The clot was then removed by centrifugation at 3000 RPM for ten minutes. The resulting supernatant after centrifugation is designated serum. The serum was transferred into a clean analyzer sample cup for magnesium and phosphate measurement in the automated analyzer COBAS INTEGRA®400 plus (Roche diagnostics Germany) located in the Biochemistry Laboratory of MTRH.

3.7 Laboratory Analysis

Plasma and serum samples obtained were utilized for magnesium and phosphate measurement. Magnesium measurement utilizes an arsenazo dye which binds preferentially with magnesium. The absorbance of the arsenazo-magnesium complex was measured at 572 nm. The optical density (OD) obtained was proportional to the concentration of magnesium present in the sample. Phosphate measurement employs a timed-rate method to determine the concentration of phosphate in serum and plasma. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a colored phosphomolybdate complex. The system monitors the change in absorbance at 365 nm at a fixed time interval. This change in absorbance is directly proportional to the concentration of phosphate in the sample.

Values obtained after analysis were entered in a data collection form for subsequent data analysis.

3.8 Sample Size Determination

Using Fishers formula (Israel G D, 1992).

$$n_0 = \frac{z^2 pq}{e^2}$$

Where

z= z value at 95% confidence interval (from the z table this value is equal to = 1.96)

P =estimated proportion (percentage of critical patients in hospital = 10%)

q = 1-p (90%)

e = level of precision (margin of error = 5%)

$$n_0 = \frac{1.96^2 \text{x} 0.1 \text{x} 0.9}{0.05^2} \quad = \quad 138.30 \approx 139 \text{ participants}$$

Adjusting sample size by 10% to cater for any eventuality

$\frac{110 \text{ x } 139}{100} = 152.9 \approx 153$

Therefore 153 participants were recruited in the study.

3.9 Data Analysis

Data was entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 22. Descriptive statistics was used to summarize the data. Paired t-test was used to establish mean differences between magnesium and phosphate concentrations in plasma and serum. Pearson's correlation was used to determine correlation between magnesium and phosphate concentrations in plasma and serum. A p-value of <0.05 was considered statistically significant.

3.10 Ethical Considerations

Ethical approval for the study was sought from Moi Teaching and Referral Hospital and Moi University Institution Review and Ethical Committee (IREC). Consent to collect samples from the

patients was sought from their next of kin who were requested to sign a written consent form authorizing the principal investigator to utilize samples from their loved ones for research purposes. Data obtained from samples analyzed in the study was stored in a password protected file within a password protected computer that only the principal investigator had access to. Coded numbers were used to identify the data generated from each patient sample to ensure confidentiality.

CHAPTER FOUR

RESULTS

4.1 Demographic and Clinical/Laboratory Characteristics of Study Participants

A total of 153 participants were recruited to the study. The age of the participants were between 18 to 85 years with a mean age of 42.58, standard error of mean of 18.69, a median of 39 and a mode of 31. The youngest participant was 18 years old and the eldest was 85 years old. There were 69 females and 84 males, accounting for 45% and 55% of the study populations respectively. The mean age of male participants was 43.7 years while that of female participants was 41.2 years. The youngest participants were both male and female of 18 years while the oldest participant was female aged 85.

4.2 Mean Difference Between Magnesium Concentration in Arterial and Venous Blood

The study matched, cross-tabulated and compared the mean differences between venous (serum) and arterial (plasma) samples for magnesium concentration in mmol/l.

When the concentration absolute values were categorized as either low, normal or high; the results of both plasma and serum samples were similar (Table 4.1).

Table 4.1: Cross-Tabulation of Serum Magnesium versus Plasma Magnesium Categories.

		Plasma Magnesium Scale			
		Low <0.7 mmol/L	Normal 0.7-1 mmol/L	High >1 mmol/L	Total
Serum Magnesium Scale	Low <0.7 mmol/L	23	0	0	23
	Normal 0.7-1 mmol/L	10	87	2	99
	High >1 mmol/L	0	5	26	31
Total		33	92	28	153

Out of 153 samples analyzed, 33 were below the magnesium reference range of 0.70 to 1.05 mmol/l. 92 samples were within range and 28 were above range.

Table 4.2: Mean differences of Serum Magnesium versus Plasma Magnesium.

	Paired Differences					t	Df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Serum Magnesium concentration mmol/l - plasma Magnesium concentration mmol/l	.03013	.05469	.00442	.02140	.03887	6.815	152	.000

The mean difference between serum and plasma magnesium values was found to be 0.0301 ± 0.0547 (95% CI 0.02140 – 0.03887).

4.3 Mean Difference Between Phosphate Concentration In Arterial And Venous Blood

When the concentration absolute values were categorized as either low, normal or high; the results of both plasma and serum samples were similar, Table 4.3.

Table 4.3: Cross-Tabulation of Serum Phosphate versus Plasma Phosphate Categories.

		Plasma Phosphate Scale			
		Low <1 mmol/L	Normal 1.0 -1.5 mmol/L	High >1.5 mmol/L	Total
Serum Phosphate Scale	Low <1 mmol/L	33	0	0	33
	Normal 1.0 -1.5 mmol/L	7	31	0	38
	High >1.5 mmol/L	0	11	71	82
Total		40	42	71	153

Out of 153 samples analyzed, 40 were below the phosphate reference range of 0.85 to 1.45 mmol/l.

42 samples were within range and 71 were above range.

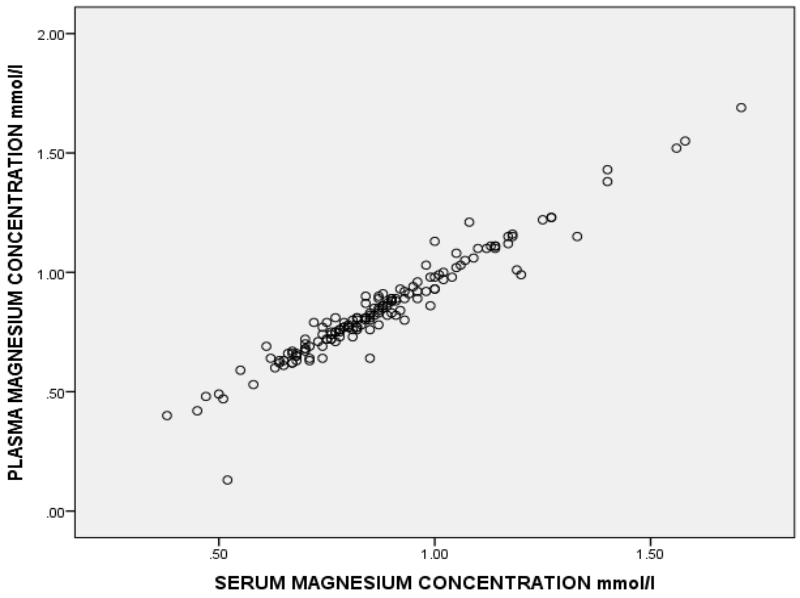
Table 4.4: Mean differences of Serum phosphate versus plasma phosphate.

	Paired Differences							
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Serum Phosphate Concentration Mmol/L - Plasma Phosphate Concentration Mmol/L	.14294	.16326	.01320	.11686	.16902	10.830	152	.000

The mean difference between serum and plasma magnesium values was found to be 0.14294 ± 0.16326 (95% CI 0.11686 – 0.16902).

4.4 Correlation Between Magnesium Concentration in Arterial and Venous Blood

The correlation between magnesium concentration in arterial blood plasma and venous blood serum from 153 patients was done using Pearson correlation and r value obtained was 0.97 [P = 0.00 (< 0.05)]. These results are shown in figure 4.1.



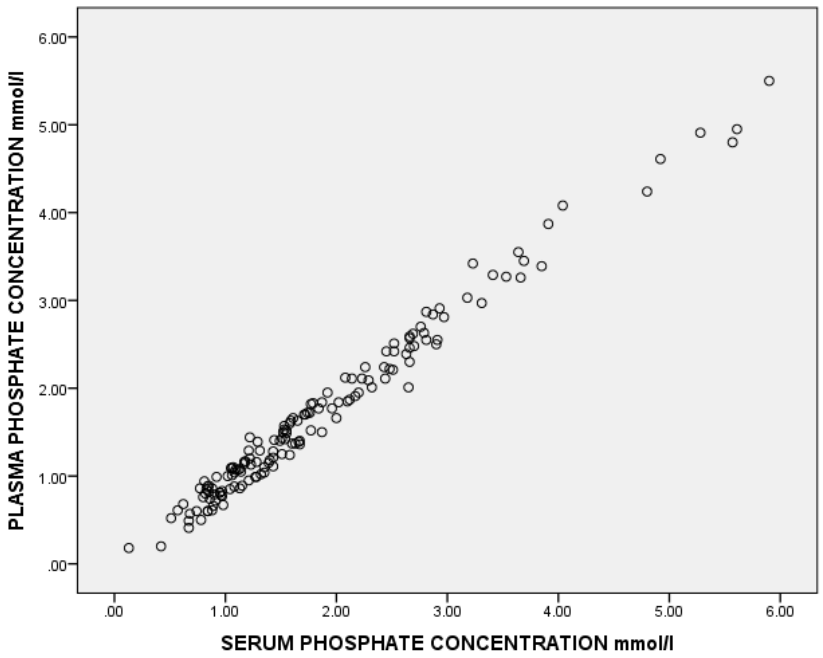
Pearson Correlation = 0.968

P = 0.00 (< 0.05)

Figure 4.1: Correlation between magnesium concentration in arterial and venous blood

4.5 Correlation Between Phosphate Concentration In Arterial And Venous Blood

The correlation between phosphate concentration in arterial blood plasma and venous blood serum from 153 patients was done using Pearson correlation and r value obtained was 0.99 [P = 0.00 (< 0.05)]. These results are shown in figure 4.2.



Pearson Correlation = 0.922

P = 0.00 (< 0.05)

Figure 4.2: Correlation between Phosphate concentration in arterial and venous blood.

CHAPTER FIVE

DISCUSSION

In determining the mean difference between magnesium concentration in arterial blood plasma and venous blood serum, the mean difference obtained indicates that magnesium concentration in the two sample matrices are not significantly different therefore null hypothesis is accepted. This means there is no difference in magnesium concentration between arterial blood plasma and venous blood serum in these patients. These results are comparable to a previous study done by Carey *et al.,*(2016) that found the relationship between simultaneously drawn serum and plasma magnesium in dialysis patients. This suggests that magnesium electrolyte concentration is evenly distributed within serum and plasma components of blood. The current study also demonstrated slightly elevated values of serum magnesium compared to plasma magnesium which might possibly be due to the presence of interfering substances present in anticoagulant used that led to lowering of magnesium concentration within plasma component of blood. Serum magnesium concentrations might have been slightly elevated compared to plasma due to absence of any chemical agent that might have affected its concentration.

The mean difference obtained between phosphate concentration in arterial blood plasma and venous blood serum indicates that phosphate measurement between the two sample matrices is not significantly different. This is possibly due to sample constituent in serum and plasma not having been altered by anticoagulant used in the case of plasma and plain tube used in the case of serum. This is comparable to a study done by Donelly *et al.,* (2009) who used lithium heparin plasma and serum and to evaluate phosphate levels. Carey *et al.,* (2016) found a mean difference that was not significant between simultaneously drawn serum and plasma phosphorus in 101 dialysis patients. The above studies are reflective of the results obtained in the current study indicating that arterial

blood plasma can be used in place of venous blood serum without getting a difference in values. This suggests that alteration in phosphate concentration does not take place in plasma and serum of the same individual.

Correlation between magnesium concentration in arterial blood plasma and venous blood serum showed a positive relationship between magnesium concentration in arterial plasma and venous serum. Thus, as magnesium concentration increases in arterial plasma, the same increase is also observed in venous serum sample. This means there is a correlation between magnesium concentration in arterial blood plasma and magnesium concentration in venous blood serum. Yu *et al*.,(2011) analyzed the concentrations of magnesium in plasma and serum samples and found that despite differences in absolute concentration between the two matrices, the overall correlation was high. Carey *et al*., (2016) did a study to find the relationship between simultaneously drawn serum and plasma magnesium in 76 dialysis patients and found correlation between the two sample matrices. The results of the current study are comparable to these two studies and therefore indicate there is good correlation in magnesium concentration between serum and plasma. This suggests there is no marked alteration that occurs in magnesium concentration in plasma or serum obtained from the same individual and used for analysis.

Correlation between phosphate concentration in arterial blood plasma and venous blood serum also showed agreement in concentrations between the two sample matrices indicating the existence of a positive relationship between phosphate concentration in arterial plasma and venous serum. The correlation suggests that concentration of phosphate in arterial plasma and venous serum is not altered by sample treatment method used prior to analysis. O'Keane *et al.,* (2006) evaluated serum, plasma lithium heparin and serum gel separator for analysis of phosphate and found equivalence among the sample matrices. Carey *et al.,* (2016) did a study to find the relationship

between simultaneously drawn serum and plasma phosphorus in dialysis patients and found correlation between them. Tze-Kiong *et al.,* (2006) analyzed aliquots of serum and heparinized plasma samples for phosphate among other biochemical analytes and percent differences between serum versus heparinized plasma samples were minimal. Results obtained in the current study demonstrated equivalence in magnesium and phosphate concentration between serum and plasma samples suggesting that no alteration in concentration occurs in phosphate concentration between the two sample matrices.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study found no significant difference in magnesium and phosphate concentration in plasma and serum. Magnesium and phosphate concentration in serum was found to correlate with magnesium and phosphate concentration in plasma.

6.2 Conclusions

1. Magnesium concentration in arterial blood plasma and venous blood serum are similar.
2. Phosphate concentration in arterial blood plasma and venous blood serum are similar.
3. There is correlation between magnesium concentration in arterial blood plasma and venous blood serum.
4. There is correlation between phosphate concentration in arterial blood plasma and venous blood serum.

6.3 Recommendations from the Study

1. Heparinized plasma and serum samples could be used interchangeably for magnesium measurement depending on which sample type will be easier to acquire.
2. Heparinized plasma and serum samples could be used interchangeably for phosphate measurement depending on which sample type will be easier to acquire.

6.4 Recommendations/Suggestions for Future Studies

1. Reference ranges be established for heparinized plasma magnesium and phosphate to see how comparable the plasma ranges are to serum reference ranges.
2. Equivalence studies between plasma and serum be done for other biochemical analytes in the human body so as to have additional sample type options for analysis.

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APPENDICES

Appendix I: DATA COLLECTION FORM

sample number	plasma magnesium concentration mmol/l	serum magnesium concentration mmol/l	plasma phosphate concentration mmol/l	serum phosphate concentration mmol/l
1	0.64	0.74	0.94	0.81
2	0.67	0.67	1.77	1.84
3	0.76	0.82	2.09	2.29
4	0.88	0.91	1.39	1.29
5	0.92	0.93	1.43	1.51
6	0.8	0.82	2.91	2.93
7	0.9	0.84	1.4	1.49
8	0.78	0.87	2.11	2.23
9	0.68	0.7	0.79	0.9
10	0.87	0.84	4.08	4.04
11	0.69	0.61	1.44	1.22
12	1.01	1.19	1.16	1.28
13	0.89	0.96	2.81	2.97
14	0.78	0.83	0.2	0.42
15	0.98	1	2.42	2.52
16	0.81	0.77	0.61	0.88
17	0.92	0.98	0.57	0.68
18	0.82	0.91	1.11	1.43
19	0.89	0.93	2.59	2.66
20	0.8	0.93	0.68	0.62
21	0.64	0.71	0.52	0.51
22	0.98	1.04	1.85	2.1
23	0.53	0.58	3.39	3.85
24	1.23	1.27	2.48	2.7
25	1.1	1.14	2.7	2.76
26	1.03	1.06	2.62	2.69
27	0.96	0.96	2.46	2.66
28	0.82	0.86	0.86	0.77
29	0.83	0.9	3.45	3.69
30	0.93	1	2.63	2.79
31	0.93	0.92	1.53	1.53

32	0.81	0.82	1.13	1.23
33	1.03	0.98	3.42	3.23
34	1.16	1.18	2.57	2.66
35	1.02	1.05	3.03	3.18
36	0.76	0.85	3.26	3.66
37	0.73	0.81	0.83	0.97
38	0.83	0.87	1.29	1.21
39	0.7	0.7	0.18	0.13
40	0.82	0.89	4.24	4.8
41	1.15	1.33	4.8	5.57
42	0.81	0.84	0.81	0.95
43	1.69	1.71	4.61	4.92
44	0.85	0.88	0.6	0.84
45	0.77	0.8	0.78	0.96
46	0.89	0.9	2.87	2.81
47	0.92	0.96	1.52	1.77
48	1.22	1.25	4.95	5.61
49	0.63	0.68	1.42	1.54
50	1.11	1.13	3.29	3.41
51	0.77	0.8	0.88	1.08
52	0.59	0.55	0.99	0.92
53	0.75	0.77	0.89	1.15
54	0.74	0.74	1.15	1.39
55	0.68	0.7	1.1	1.35
56	0.47	0.51	1.37	1.6
57	1.43	1.4	1.84	2.02
58	0.82	0.85	1.77	1.96
59	0.77	0.8	1.21	1.43
60	0.89	0.91	0.86	1.13
61	1.11	1.14	2.3	2.66
62	0.76	0.81	1.39	1.66
63	0.93	1	1.6	1.58
64	1.38	1.4	1.66	2
65	0.49	0.5	0.85	1.04
66	1.12	1.17	0.6	0.74
67	0.75	0.77	0.49	0.67
68	0.73	0.78	0.5	0.78
69	0.78	0.8	1.04	1.35

70	0.94	0.95	1.87	2.12
71	0.81	0.85	0.99	1.27
72	0.6	0.63	0.6	0.84
73	0.77	0.74	1.82	1.77
74	0.79	0.72	1.1	1.07
75	1.15	1.18	2.39	2.63
76	0.86	0.88	1.4	1.67
77	0.61	0.65	2.55	2.81
78	0.83	0.85	0.81	0.95
79	0.72	0.75	0.72	0.91
80	0.67	0.7	1.95	1.92
81	0.71	0.77	2.01	2.32
82	0.76	0.78	2.11	2.44
83	1.55	1.58	2.97	3.31
84	1.11	1.14	1.25	1.51
85	0.13	0.52	0.76	0.8
86	1.05	1.07	2.22	2.48
87	0.98	0.99	0.99	1.28
88	0.91	0.88	1.18	1.4
89	0.62	0.67	1.37	1.63
90	0.63	0.64	1.95	2.2
91	1.08	1.05	1.57	1.53
92	1.06	1.09	2.24	2.43
93	0.66	0.68	0.41	0.67
94	0.87	0.89	0.95	1.21
95	0.65	0.68	2.21	2.51
96	0.86	0.88	1.02	1.32
97	0.76	0.78	4.91	5.28
98	1.23	1.27	3.27	3.53
99	0.89	0.87	1.7	1.72
100	0.8	0.84	2.11	2.14
101	0.66	0.66	1.17	1.18
102	0.62	0.64	1.84	1.87
103	0.77	0.82	1.29	1.31
104	0.81	0.84	1.83	1.79
105	1	1.02	3.55	3.64
106	0.42	0.45	1.2	1.22
107	0.79	0.75	1.14	1.17

108	0.69	0.74	1.16	1.17
109	0.4	0.38	0.61	0.57
110	0.85	0.86	1.72	1.74
111	0.72	0.76	1	1.02
112	0.88	0.9	1.08	1.13
113	0.89	0.9	1.66	1.61
114	0.8	0.85	1.72	1.76
115	0.9	0.87	1.52	1.55
116	0.75	0.76	0.8	0.82
117	1.52	1.56	5.5	5.9
118	1.21	1.08	2.12	2.08
119	0.88	0.9	1.41	1.44
120	0.86	0.89	1.04	1.08
121	0.77	0.79	1.07	1.09
122	1.1	1.1	3.87	3.91
123	0.69	0.71	1.09	1.05
124	1.15	1.17	2.5	2.9
125	0.64	0.62	0.85	0.83
126	0.75	0.78	1.7	1.71
127	0.63	0.65	1.01	1.06
128	0.66	0.67	2.24	2.26
129	1.13	1	1.63	1.65
130	0.99	1.01	2.42	2.45
131	0.62	0.67	1.5	1.52
132	1.1	1.12	2.84	2.87
133	0.85	0.87	1.09	1.06
134	0.81	0.82	2.51	2.52
135	0.74	0.76	0.86	0.88
136	0.84	0.87	1.49	1.55
137	0.71	0.73	0.88	0.84
138	0.8	0.81	1.07	1.11
139	0.72	0.75	0.82	0.85
140	0.48	0.47	0.89	0.85
141	0.91	0.94	1.36	1.67
142	0.99	1.2	2.55	2.91
143	0.72	0.76	0.66	0.89
144	0.77	0.79	1.91	2.17
145	0.65	0.68	0.67	0.98

146	0.72	0.7	1.63	1.59
147	0.86	0.99	0.74	0.86
148	0.64	0.85	1.05	1.14
149	0.63	0.71	1.28	1.43
150	0.79	0.79	1.24	1.58
151	0.77	0.82	0.77	0.97
152	0.84	0.92	2.01	2.65
153	0.97	1.02	1.5	1.87
MEAN	**0.84±0.22**	**0.87±0.21**	**1.71±1.02**	**1.85±1.09**

APPENDIX II: CONSENT FORM

My name is **Dominic Stephen Okia Alwala** a Master of Science student in Clinical Chemistry from Maseno University. I am conducting a study on Clinical equivalence of heparinized arterial plasma to venous serum samples for magnesium and phosphate measurement in critical patients in Moi Teaching and Referral Hospital(MTRH) Intensive Care Unit(ICU).

Magnesium and phosphate are electrolytes found in the body and are routinely monitored in ICU patients to aid in assessing their general condition. Arterial samples and venous samples are obtained from these patients to be used in laboratory measurements that include magnesium and phosphate amongst other additional parameters.

Your loved one has been selected to participate in this study because they are currently admitted in ICU and will routinely undergo procedures to obtain blood from the vein and artery. Their participation in this study is entirely voluntary and you may withdraw them from the study at any time if you feel to do so.This study has been approved by the Moi University and MTRH Ethics Review Committee and will involve obtaining 2ml of blood from the artery and 2ml of blood from the vein.

Your participation or declining your loved one to participate in this study will not in any way interfere with the current critical care he/she is receiving in this facility and all information obtained will be treated with confidentiality.

This study is important in finding out whether magnesium and phophate concentrations in plasma and serum are the same so as to eliminate the need of pricking a patient twice for a measurement that can be done on one sample. Findings from this study will be relayed to you and other stakeholders and published in scientific papers.

For any question or concerns about the research, feel free to contact me on mobile phone number 0733252859 or the Chairperson Institution Ethics and Review Committee MTRH building second floor.

DECLARATION

I have read/been read to this consent and all my concerns have been addressed. I therefore agree my loved one to participate in this study

Participant signature and initials:..... Date:.....

Consenters signature :..... Date:.....

Appendix II(b): IDHINI

Jina langu ni **Dominic Stephen Okia Alwala** mwanafunzi wa sayansi katika Hospitali Kemia katika Chuo Kikuu cha Maseno. Ninafanya utafiti juu ya ufananishaji wa kliniki ya plasma ya plastiki ya heparinized kwa sampuli za serum venous kwa kipimo cha magnesiamu na phosphate katika wagonjwa muhimu katika Hospitali ya Mafunzo ya Uhamasishaji na Matibabu (MTRH).

Magnésiamu na phosphate ni electrolytes hupatikana katika mwili na hufuatiliwa mara kwa mara katika wagonjwa wa ICU ili kusaidia katika kutathmini hali yao ya jumla. Sampuli za arterial na sampuli za vimelea zinapatikana kutoka kwa wagonjwa hawa ili kutumika katika vipimo vya maabara ambavyo vinajumuisha magnesiamu na phosphate kati ya vigezo vingine vya ziada.

Mpendwa wako amechaguliwa kushiriki katika utafiti huu kwa sababu sasa wamekubaliwa katika ICU na mara kwa mara wataingia taratibu za kupata damu kutoka kwenye mishipa na mishipa. Ushiriki wao katika utafiti huu ni kikamilifu kwa hiari na unaweza kuwaondoa kwenye utafiti wakati wowote ikiwa unajisikia kufanya hivyo. Utafiti huu umekubaliwa na Chuo Kikuu cha Moi na MTRH Kamati ya Uhakiki wa Maadili na itahusisha kupata 2ml ya damu kutoka kwenye mto Na 2ml ya damu kutoka kwenye mshipa.

Ushiriki wako au kupungua kwa mpendwa wako kushiriki katika utafiti huu kwa njia yoyote haitaingilia huduma ya sasa muhimu anayopokea katika kituo hiki na habari zote zilizopatikana zitatambuliwa kwa siri.

Utafiti huu ni muhimu katika kujua kama magnesiamu na phophate viwango katika plasma na serum ni sawa ili kuondoa haja ya pricking mgonjwa mara mbili kwa kipimo ambacho kinaweza kufanyika kwa sampuli moja. Matokeo kutoka kwa utafiti huu yatapelekwa kwa wewe na wadau wengine na kuchapishwa katika magazeti ya kisayansi.

Kwa swali lolote au wasiwasi juu ya utafiti, jisikie huru kuwasiliana na mimi kwenye nambari ya simu ya simu 0733252859 au Mwenyekiti wa Kamati ya Maadili na Ukaguzi wa Taasisi MTRH kujenga sakafu ya pili.

WASIA

Nimeisoma / nimesoma kwa idhini hii na matatizo yangu yote yameshughulikiwa. Kwa hiyo mimi kukubali mpendwa wangu kushiriki katika utafiti huu

Sahihi ya washiriki na washirika: .... Tarehe:.....

Washauri wa saini: .... Tarehe:.....

Appendix III: SAMPLE COLLECTION

Arterial blood sample collection

1. Assemble all the equipment required for the procedure in the equipment tray
2. Position the patient's arm preferably on a pillow for comfort with the wrist extended (20-30°)
3. Put on gloves and palpate the radial artery on the patient's non-dominant hand (most pulsatile over the lateral anterior aspect of the wrist)
4. Clean the site with an alcohol wipe for 30 seconds and allow to dry before proceeding
5. Attach needle to the ABG syringe and flush the syringe with heparin. Expel the heparin completely and attach fresh needle for sample collection.
6. Palpate the radial artery with your non-dominant hand's index finger around 1cm proximal to the planned puncture site (avoiding directly touching the planned puncture site that you have just cleaned)
7. Warn the patient you are going to insert the needle
8. Holding the ABG syringe like a dart insert the ABG needle through the skin at an angle of 45° over the point of maximal radial artery pulsation (which you identified during palpation)
9. Advance the needle into the radial artery until you observe blood flashback into the ABG syringe
10. The syringe should then begin to self-fill in a pulsatile manner
11. Once the required amount of blood has been collected remove the needle and apply immediate firm pressure over the puncture site with some gauze

12. Remove the ABG needle from the syringe and discard safely into a sharps bin
13. Place a cap onto the ABG syringe and label the sample
14. Yourself or a colleague should continue to apply firm pressure for 3-5 minutes to reduce the risk of haematoma formation
15. Thank the patient
16. Dispose gloves and equipment into an appropriate clinical waste bin
17. Wash hands
18. Take the ABG sample to be analysed as soon as possible after being taken as delays longer than 10 minutes can affect the accuracy of results

Venous blood sample collection

1. Perform hand hygiene; that is wash hands with soap and water, and dry with single-use towels; or if hands are not visibly contaminated, clean with alcohol rub – use 3 ml of alcohol rub on the palm of the hand, and rub it into fingertips, back of hands and all over the hands until dry.
2. After performing hand hygiene, put on well-fitting, non-sterile gloves.
3. Disinfect the entry site with a 70% alcohol swab for 30 seconds and allow to dry completely (30 seconds)
4. Apply firm but gentle pressure. Start from the centre of the venepuncture site and work downward and outwards to cover an area of 2 cm or more.
5. Allow the area to dry. Failure to allow enough contact time increases the risk of contamination.

6. DO NOT touch the cleaned site; in particular, DO NOT place a finger over the vein to guide the shaft of the exposed needle. It the site is touched, repeat the disinfection.
7. Perform venepuncture by anchoring the vein by holding the patient's arm and placing a thumb BELOW the venepuncture site.
8. Enter the vein swiftly at a 30 degree angle or less, and continue to introduce the needle along the vein at the easiest angle of entry.
9. Once sufficient blood has been collected, release the tourniquet BEFORE withdrawing the needle.
10. Withdraw the needle gently and apply gentle pressure to the site with a clean gauze or dry cotton-wool ball.
11. Fill the laboratory sample tube with the collected blood sample
12. Discard used items into the appropriate category of waste
13. Check the insertion site to verify that it is not bleeding, then thank the patient

Appendix IV: ETHICAL APPROVAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2017/54
Approval Number: 0002004

9th January, 2018

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
09 JAN 2018
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dominic Stephen Alwala,
Maseno University,
School of Health Sciences,
P.O. Box 333-40105,
KISUMU-KENYA.

Dear Mr. Alwala,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Clinical Equivalence of Heparinized Arterial Plasma to Venous Serum Samples for Magnesium and Phosphate Measurement in Critical Patients Admitted at Moi Teaching and Referral Hospital Intensive Care Unit".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 2004*** on 9th January, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 8th January, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

DR. S. NYABERA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD

An ISO 9001:2015 Certified Hospital

MOI TEACHING AND REFERRAL HOSPITAL

Telephone:(+254)053-2033471/2/3/4
Mobile: 722-201277/0722-209795/0734-600461/0734-683361
Fax: 053-2061749
Email: ceo@mtrh.go.ke/directorsofficemtrh@gmail.com

Nandi Road
P.O. Box 3 – 30100
ELDORET, KENYA

Ref: ELD/MTRH/R&P/10/2/V.2/2010

15th January, 2018

Dominic Stephen Alwala,
Maseno University,
School of Health Sciences,
P.O. Box 333-40105,
KISUMU-KENYA.

APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Clinical Equivalence of Heparinized Arterial Plasma to Venous Serum Samples for Magnesium and Phosphate Measurement in Critical Patients Admitted at Moi Teaching and Referral Hospital Intensive Care Unit".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

MOI TEACHING AND REFERRAL HOSPITAL
C.E.O
APPROVED
15 JAN 2018
SIGN.....
P.O. Box 3 - 30100, ELDORET

15/01/2018
DR. WILSON K. ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

cc - DCEO, (CS)
- Director of Nursing Services (DNS)
- HOD, HRISM