Evaluation of Plasma Alkaline Phosphatase, Calcium and Phosphorous in Post-menopausal Women with Osteoporosis

A dissertation submitted in partial fulfillment for the requirements of
M.Sc. degree in Medical Laboratory Science – Clinical Chemistry

By

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الآية

قال تعالى:

(اقرأ باسم ربك الذي خلق خلق الإنسان من عقل اقرأ وربك الاكرم الذي علم بالقلم علم الإنسان مالك يعلم).

سورة العلق

صدق الله العظيم
Dedication

To my mother
To my father
To my friends

I dedicate this work
Acknowledgments

Grateful and thanks fullness to ALLAH for giving me strength and ability to complete this work.

I would like to express my sincere thanks and gratitude to my supervisor Dr. Abdelgadir Elmugadam for supervising and support me for completing this work.

Also I would like to thanks the staff of clinical chemistry department especially my respected teachers Dr. Ghada Alfadel and Dr. Mohammed Karrar.

Sincerely thank and gratitude to my family and Emad for continuous encouragement and support.

Finally to every one helped me especially my friends.
Abstract

Osteoporosis is a highly prevalent public health problem with osteoporosis-related fractures that account for high morbidity and mortality.

The study compared the plasma level of Alkaline phosphatase, calcium and phosphorous of 50 women with osteoporosis as test group and 50 apparently healthy women as control group and it is case control study carried out in a period from March to December 2018 in Khartoum state, Sudan.

Venous blood (3ml) was collected from the participants in heparin containers and gently mixed. The plasma level of alkaline phosphatase, calcium and phosphorous measured by spectrophotometer using commercial reagent kits from Biosystem Company.

The obtained data were analyzed by both Student's Independent T test and Pearson correlation test using SPSS version 16 computer program. By analysis of the result, both control and osteoporosis cases had ALP and calcium within normal range, however the difference between the levels was significantly lower in osteoporotic (63.38±20.35) and (7.31±2.28) mg/dl phosphorus was significantly higher in osteoporosis (4.46±1.12) in comparison to the control group (4.00±1.08) yet their values are still within normal range. Also found there is a weak positive correlation between the ALP and the BMI with r = (0.3), but no correlation between the calcium, phosphorus with BMI in case group.

From the result of this study, it is concluded that: Osteoporosis is associated with low plasma level of ALP and calcium, where associate with high level of the phosphorus. Also there is no correlation between the test parameters and age and the BMI of the cases group and there is a weak positive correlation between the ALP and BMI.
.mustakhla al-adrasa

هداثة العظام من أكثر مشاكل الصحة العامة انتشاراً بالإضافة للكسور المصاحبة لهشاشة العظام DELETE

هذه دراسة معاينة تمت في الفترة من مارس إلى ديسمبر 2018 في ولاية الخرطوم / السودان ، حيث تم مقارنة مستوي إنزيم الفوسفاتيز القلوي والكالسيوم والفسفر في بلازما الدم عند 50 من مجموعا من النساء المصابين بهة ذات العظام و50 من مجموعا نساء اصحاء.

تم اخذ 3 مل من الدم الوريدي من الحالات والضوابط وتم وضعها في إباع بحتوي مادة معينة للتجلط تم تقييم إنزيم الفوسفاتيز القلوي والكالسيوم والفسفر بواسطة مقياس الطيف الضوئي باستخدام محالله من شركة الاوزمة الحيوية الألمانية.

تم تحليل النتائج باستخدام الفرق بين المتوسطين غير المعتمدين في برنامج الحزم الاستاذية للعلوم الاجتماعية المحور الإصدار 16.

بعد تحليل النتائج وجد أن هناك انخفاض ذو دلاله إحصائية معنوية في مستوي إنزيم الفوسفاتيز القلوي والكالسيوم ولكنها ضمن معدل الطبيعية لدي النساء المصابات بهة ذات العظام ( 20,35 ± 63,38 ) و ( 2,28 ± 7,31 ) على التوالي بالمقارنة مع النساء الصحية ( 72,38 ± 17,54 ) و ( 9,2 ± 1,19 ).

بينما هناك ارتفاع ذو دلاله إحصائية معنوية في مستوي الفسفر لدى النساء المصابات بهة ذات العظام ( 1,12 ± 4,46 ) مقارنة بمجموعه اصحاء ( 1/1,08 ± 4,00 ) بينما لا زال قيمتها ضمن معدل الطبيعية ووجد أيضاً أن هناك علاقة ضعيفة جداً بين مستوي إنزيم الفوسفاتيز القلوي ومؤشر كتلة الجسم ولكن ليس هناك علاقة ذو دلاله معنوية بين الكالسيوم والفسفر مع مؤشر كتلة الجسم.

ومن نتائج هذه الدراسة نستخلص التالي: هناك علاقة بين مرض هشاشة العظام وانخفاض مستوي إنزيم الفوسفاتيز القلوي والكالسيوم وارتفاع مستوي الفسفر.

بالإضافة إلى أن هناك علاقة ضعيفة جداً بين إنزيم الفوسفاتيز القلوي ومؤشر كتلة الجسم.
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<td>ALP</td>
<td>Alkaline phosphates</td>
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<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BPs</td>
<td>Bisphosphonates</td>
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<td>Ca2+</td>
<td>Calcium</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CBC</td>
<td>Complete blood count</td>
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<tr>
<td>DEXA</td>
<td>Dual-energy X-ray absorbanometry</td>
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<tr>
<td>E</td>
<td>Estrogen</td>
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<td>ECF</td>
<td>Extracellular fluids</td>
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<td>ERT</td>
<td>estrogen replacement therapy</td>
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<td>GH</td>
<td>Growth hormone</td>
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<td>GT</td>
<td>Gastrointestinal tract</td>
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<tr>
<td>Kg</td>
<td>Kilogram</td>
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<tr>
<td>m²</td>
<td>Square meter</td>
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<td>NOF</td>
<td>National Osteoporosis Foundation</td>
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<td>OF</td>
<td>osteoporotic fractures</td>
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<td>OP</td>
<td>Osteoporosis</td>
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<td>OC</td>
<td>Osteoclast</td>
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<td>PHT</td>
<td>parathyroid hormone</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>SLE</td>
<td>Systemic lupus erythromatus</td>
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<td>SPSS</td>
<td>Statistical package for social science</td>
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<td>SERM</td>
<td>selective estrogen receptor modulator</td>
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<td>TIO</td>
<td>Tumor-induced osteomalacia</td>
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<td>TFT</td>
<td>Thyroid function test</td>
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<td>Total alkaline phosphatase</td>
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<td>World Health Organization</td>
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CHAPTER ONE

Introduction, Rational and Objectives
Introduction

1.1 Introduction:

Bone is a metabolically active tissue that experiences continuous remodeling via two reciprocal processes, bone formation and resorption. Respectively, osteoclasts, osteoblasts and osteocytes are responsible for bone resorption, formation and maintenance. (Mohammadi et al., 2014)

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and micro architectural deterioration of bone tissue, leading to bone fragility and increased fracture risk. It is especially prevalent in older postmenopausal women (Meunier et al., 1999), The World Health Organization (WHO) defines osteoporosis as a spinal or hip bone mineral density (BMD) of 2.5 standard deviations (SD) or more below the mean for healthy, young women (T-score of −2.5 or below) as measured by dual energy x-ray absorptiometry (DEXA). (Prentice, 2004)

Osteoporosis is a disease that affects many millions of people around the world. (Prentice, 2004) The exact prevalence of osteoporosis remains unknown, but it may vary among countries and even among ethnic groups in the same country. (Tosun and Press, 2018).

It is characterized by low bone mass and micro architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk. Fragility fractures are most common at the wrist, spinal vertebrae and hip, although they can occur throughout the skeleton. (Prentice, 2004)

The most common cause of osteoporosis is lack of physical stress on the bone because of reduced activity, others include malnutrition to the extent that sufficient protein matrix cannot be formed, lack of vitamin C which is necessary for secretion of intracellular substance by all cells. All the above impair formation of osteoid by the osteoblasts. (Ali, 2018)
White and Asian women are at greater risk for osteoporosis and associated fractures than black women. Other risk factors, such as smoking and lack of exercise, also contribute to the development of osteoporosis, but they are not strong risk factors. In general, the more risk factors a woman has, the higher the likelihood that she has or will develop osteoporosis. However, some risk factors, such as chronic use of high-dose corticosteroids, are sufficiently strong that even in isolation they signal the need for further evaluation, including BMD testing. (Meunier et al., 1999)

Bone mineral density is an indirect measure of the bone calcium content that influences bone strength. Bone density can be measured by a number of techniques; the most commonly used is dual-energy X-ray absorptiometry (DEXA) (Davies et al., 2018).

The mainstay diagnosis of osteoporosis is based on the assessment of BMD at the femoral neck and the anterior–posterior lumbar spine using dual-energy X-ray absorptiometry (DEXA) (Tosun and Press, 2018). A T-score -2.5 standard deviation (SD) is considered osteoporosis (Tosun and Press, 2018).

Menopause is a natural physiological phenomenon resulting from primary ovarian failure secondary to apoptosis or programmed cell death. Ovarian function declines with age. (Ji and Yu, 2015)

The diagnosis and monitoring of treatment for osteoporosis has been confined to clinical assessment, radiography and bone densitometry. However, in recent year’s bio-chemical markers of bone formation and resorption have been developed to quantify bone turnover and remodelling, with possible applications in clinical practice. (Kyd et al., 1998)

Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalase the hydrolysis of phosphate esters at an alkaline pH. 4 the gene for tissue non-
specific ALP encodes the isoenzymes expressed in liver, bone and kidney. In healthy individuals about half the activity of alkaline phosphatase in serum is derived from bone and the remainder from liver. (Kyd et al., 1998)

Serum total alkaline phosphatase (TALP) is the most commonly used marker of bone formation but it lacks sensitivity and specificity. Bone and liver alkaline phosphatase is the same gene product but differ in post-translational action. (Nawawi et al., 2001)

Calcium is an essential nutrient that is involved in most metabolic processes and the phosphate salts of which provide mechanical rigidity to the bones and teeth, where 99% of the body’s calcium resides. The calcium in the skeleton has the additional role of acting as a reserve supply of calcium to meet the body’s metabolic needs in states of calcium deficiency. (Christopher, 1997)

Calcium deficiency is easily induced because of the obligatory losses of calcium via the bowel, kidneys, and skin. In adult animals, calcium deficiency causes mobilization of bone and leads sooner or later to osteoporosis, i.e., a reduction in the “amount of bone in the bone” or apparent bone density. (Christopher, 1997)

Bone mineral consists of calcium phosphate, and phosphorus is as important as calcium in supporting bone augmentation and maintenance. Although typical adult diets contain abundant phosphorus, 10% to 15% of older women have intakes of less than 70% of the recommended daily allowance. When these women take high-dose calcium supplements that consist of the carbonate or citrate salts, all their food phosphorus may be bound and hence unavailable for absorption. Current-generation anabolic agents for treating osteoporosis require positive phosphorus balances of up to 90 mg/d. (Heaney, 2004)
1.2 Rationale:

Osteoporosis is one of the emerging health issues worldwide. It is a silent disease and causes fractures of bones. Due to an increase in longevity of life in Sudan, osteoporotic fractures are becoming a major cause of morbidity and mortality, which is similar to the western part of the world.

With the change in the eating habits the people are not getting adequate nutrition for the wellbeing of the bones and the lack of the activity in the Sudan which increase the prevalence of the disease, but with the advancements in the field of bone mineral density measurement it may now be possible for early detection and rapid treatment which may lead to the better outcome for the patient and may prove less detrimental for the society as well.

The number of patients with osteoporosis is on a rise and it is expected that the number will soon reach 34 million in USA, out of which 80% are expected to be women.

World wide variation in the incidence and prevalence of osteoporosis is difficult to determine because of problems with definition and diagnosis. The most useful way of comparing osteoporosis prevalence between populations is to use fracture rates in older people. However, because osteoporosis is usually not life-threatening, quantitative data from developing countries are scarce. Despite this, the current consensus is that approximately 1.66 million hip fractures occur each year worldwide, that the incidence is set to increase four-fold by 2050 because of the increasing numbers of older people, and that the age-adjusted incidence rates are many times higher in affluent developed countries than in sub-Saharan Africa and Asia.

The purpose of this study was to evaluate the objective of clinical studies on osteoporosis in post-menopausal women in Sudan and compare them with the rest of the world and it is the first research in Sudan about this topic.
1.3 Objectives:

1.3.1 General objective

To evaluation the plasma level of alkaline phosphatase, calcium and phosphorus in post-menopausal woman with osteoporosis.

1.3.2 Specific objectives

1. To measure plasma level of alkaline phosphatase, calcium and phosphorus in comparison of control group.

2. To correlate between alkaline phosphates, calcium and phosphorus in case group regarding to study variables (age, BMI).
CHAPTER TWO

Literature review
2. Literature Review

2.1 Bone:

Bone is living tissue that uses oxygen and gives off waste products in metabolism. Bone requires a blood supply and a source of nutrients to grow and develop. The functions of bone include protection of soft tissue and support for mechanical activity. Bones protect the nervous system, the respiratory system, and the heart. Within the red marrow of bones, hematopoiesis, the formation of blood cells, occurs. (Wendy and Jean, 2007)

2.1.1 The bone remodeling:

Remodelling is the process whereby the skeleton undergoes continual renewal by a phase sequence of bone resorption and formation. In the adult, 95% of bone turnover occurs by remodelling and approximately 10–15% of skeletal surfaces are in the process of being remodelled at any one time. Osteoclasts, the cells responsible for bone resorption, dissolve away a small, discrete portion of the surface. The resulting resorption cavity is refilled by the action of osteoblasts, the bone-forming cells. These lay down bone matrix (osteoid) which gradually becomes mineralized to form new bone. There is a strict chronological sequence of events, with recruitment of osteoblasts occurring sometime after resorption, and with newly-formed bone mineralizing rapidly in the initial stages but more slowly thereafter. As a result, it takes many weeks or months for the entire process to be completed. (Prentice, 2004)

Bone turnover is lower in African-American adolescents compared with Caucasians of the same age, suggesting an influence of remodelling rate on peak bone mass development and later fracture risk, although nothing is known about what an optimal bone turnover rate might be for young people. All these facets need to be considered when considering the role of diet and nutrition of young people in the prevention of osteoporosis. (Prentice, 2004)
2.2 Osteoporosis:

2.2.1 Definition of osteoporosis:

Osteoporosis, a multifactorial systemic skeletal disease, is characterized by low bone mineral density (BMD) and micro-architectural deterioration of bone tissue resulting in bone fragility. BMD measured by dual X-ray absorptiometry is the gold standard to diagnose osteoporosis, according to WHO criteria; osteoporosis is defined as the T-score of less or equal to 2.5 and osteopenia as the T-score between 1.0 and 2.5. The femoral neck and lumbar spine are recommended as the anatomic region of interest, BMD decreases with age, thus primary osteoporosis mainly occurs in women 10-15 years after menopause and elderly men around 75-80 years old.(Ji and Yu, 2015)

With an expanding aging population, osteoporosis and osteoporosis-related fractures are fast becoming important public health issues that are a considerable economic burden on health service resources.(Ji and Yu, 2015)

2.2.2 Clinical presentation of osteoporosis:

Postmenopausal women who require management of osteoporosis may present with >1 of the following: (1) evidence of a vertebral fracture; (2) after a hip fracture; (3) after another type of fracture (e.g., wrist fracture);(4) risk factors for osteoporosis; and (5) concern about the possibility of osteoporosis.

Most patients with osteoporosis are symptomatic, many patients who have experienced >1 fracture will have continuing pain, impaired mobility, and fear of further fractures that may reduce their quality of life. Moreover, such patients have often lost 30% to 50% of their peak bone mass. Although effective therapy can substantially reduce the risk of further fractures, the risk remains appreciable. It is preferable to intervene early in the process of bone loss and thus reduce the risk of the first fracture. This is now achievable, because even in
the absence of previous fracture, the diagnosis of osteoporosis or high risk for developing osteoporosis can be made by measuring BMD. (Meunier et al., 1999)

2.2.2.1 Fraction:

Fracture is the single most important manifestation of postmenopausal osteoporosis, osteoporotic fractures (OF) is usually precipitated by low-energy injuries such as a fall from standing height, Osteoporosis can also be diagnosed in patients with or without fragility fractures. (Binkley et al., 2016)

Fractures due to OP are an important epidemiological as well as a socio-medical problem, after the age of 50 the risk of fractures due to osteoporosis is 40%, which is similar to the risk of coronary heart disease. In Europe, approximately 11.5% of women aged 50-54 years, and 35% of those aged 75-79 years, suffer from at least one vertebral fracture. (Franic and Verdenik, 2018)

A. Vertebral Fracture

Vertebral fractures, may occur during routine daily activities, without a specific fall or injury. (Binkley et al., 2016)

Physicians should be alert for features suggesting vertebral fracture in women aged >65 years, such as kyphosis, height loss, and acute or chronic back pain.

Many vertebral fractures are not associated with acute symptoms; because some degree of kyphosis and height loss may occur in the absence of osteoporosis, it is important to confirm the presence of a vertebral fracture on lateral spine roentgenograms. (Meunier et al., 1999)

B. Hip fracture

Hip fractures are the most serious consequence of osteoporosis; women with hip fracture have an increased mortality of 12 to 20% during the following 2 years. More than 50% of hip fracture survivors are unable to return to independent living; many require long-term nursing homecare. (Binkley et al., 2016)
With the exception of hip fractures caused by severe trauma, most hip fractures occur after a fall and are due to osteoporosis. In addition to having often-severe osteoporosis, patients who have suffered a hip fracture are generally frail and prone to falling, which places them at risk for further fractures. Therefore, patients who have experienced hip fracture should be man-aged actively, with the possible exception of those with a short life expectancy. Contributing causes, such as vitamin D deficiency, protein malnutrition, and other ill-nesses, are common in this population. (Meunier et al., 1999)

C. Other Types of Fracture

Wrist fracture is common; it tends to occur at a somewhat younger age than vertebral or hip fracture and hence often represents the first clinical expression of osteoporosis. Wrist fracture justifies BMD measurement to confirm the presence of osteoporotic disease. Other types of fracture, as of the humerus, rib, or pelvis, are also common in patients with osteoporosis. (Meunier et al., 1999)

2.2.3 Risk factor of osteoporosis:

White and Asian women are at greater risk for osteoporosis and associated fractures than black women. (Meunier et al., 1999)

Low bone mass is only one of several risk factors for osteoporotic fracture and these including both skeletal and non-skeletal factors. Skeletal factors that may act independently of bone mass include skeletal geometry and aspects of bone quality such as turnover, trabecular connectedness, osteocyte viability and osteonal distribution, of those risk factors that are associated with a low bone mass, and may be acting through this pathway, family history and genetic susceptibility are especially important. (Prentice, 2004)

Environmental factors, other than nutritional status and diet, are important determinants of fracture risk. These include cigarette smoking, alcohol abuse, physical inactivity, dependency and use of certain medications. (Prentice,
However, some risk factors such as chronic use of high-dose corticosteroids are sufficiently strong that even in isolation they signal the need for further evaluation, including BMD testing. (Meunier et al., 1999)

The analysis of risk factors for OP clearly shows that besides age and BMD<18, the most important, statistically significant risk factors are decreased height increasing with age, low BMC either in the lumbar spine or in the hip, and previously sustained fractures. All these factors influence the prevalence of OP, which increases with age from 24.9% in the age group 60-64 years to 37.4% in the age group 70-75 years. Nevertheless, low calcium intake, family history of OP, cigarette smoking and corticosteroid use are not statistically prevailing factors for the OP prevalence. (Franic and Verdenik, 2018)

Many women present without specific risk factors for osteoporosis but are concerned that they may have osteoporosis or at risk for developing it. The absence of risk factors does not mean that a woman’s BMD is normal or that a fracture will not occur. (Meunier et al., 1999)

2.2.4 The main type of osteoporosis:

Osteoporosis is traditionally divided into primary and secondary osteoporosis. Both types can occur simultaneously in the same person. The cause of primary osteoporosis is not fully understood. Primary osteoporosis is divided into postmenopausal (type I) and senile (type II) osteoporosis. Secondary osteoporosis is less common and defined as bone loss occurring as a result of other diseases, such as Cushing’s syndrome or malignancy. (Heinz and Ph, 2006)
2.2.4.1 Primary osteoporosis:

Type I: Accelerated or postmenopausal osteoporosis

Osteoporosis is the most prevalent disease in menopausal women, and is strongly associated with low quality of life. (Ji and Yu, 2015)

Primary osteoporosis mainly occurs in women 10-15 years after menopause and elderly men around 75-80 years old. With an expanding aging population, osteoporosis and osteoporosis-related fractures are fast becoming important public health issues that are a considerable economic burden on health service resources. (Ji and Yu, 2015)

Estrogen (E) deficiency causes both the early and late forms of osteoporosis in postmenopausal women and contributes to the development of osteoporosis in elderly men. It is associated with large increases in bone resorption caused by increased osteoclast (OC) numbers (due to enhanced OC formation and reduced OC apoptosis) and by increased OC activity. (Riggs, 2000)

There are two phases of bone loss in women: The first occurs predominantly in trabecular bone and starting at menopause. It results from estrogen deficiency, and leads to a disproportionate increase in bone resorption as compared with formation. This phase could be defined as menopause related bone loss, after 4-8 years, the second phase exhibits a persistent, slower loss of both trabecular and cortical bone, and is mainly attributed to reduced bone formation, this is age related bone loss, which is the only phase that also happens in men. (Ji and Yu, 2015)

Type II Age-related osteoporosis

Age-related osteoporosis occurs in both sexes in women over age 70 and men over age 80. There is very good evidence that the incidence of fractures increases with the lowering of bone mineral density. Type II bone loss typically
results in hip fractures, although fractures occur in other types of bone as well. Elderly men are very susceptible to bone loss, but women get hip fractures about twice as often. It is not clear whether the bone loss is simply an expression of old age which affects some people to a larger extent than others. An underlying disease, a hormonal imbalance or nutrient deficiency may accelerate age-related bone loss.

In general, women have less dense bones than men (usually 30 percent less), and they suffer more bone loss after menopause. This puts women at a disadvantage when age-related bone loss occurs. Women live longer than men, and thus may be more likely to develop fractures. Osteoporosis is rare in young adults and middle-aged men.(Heinz and Ph, 2006)

2.2.4.2 Secondary osteoporosis:

The number of postmenopausal women with osteoporosis from a secondary cause is unknown, but thought to be low, a careful history and physical examination may identify common causes of secondary osteoporosis, if clinical evaluation does not raise suspicion of a secondary cause, and there is currently little evidence to warrant additional testing in postmenopausal women.(Sweet et al., 2009)

Causes of Secondary Osteoporosis:

Chronic medical and systemic diseases(e.g. Amyloidosis, Ankylosing spondylitis, Chronic obstructive pulmonary disease, Human immunodeficiency virus or acquired immunodeficiency syndrome, Inflammatory bowel diseases, Liver disease, Multiple myeloma, Renal insufficiency or renal failure, Rheumatoid arthritis and Systemic lupus erythematosus (SLE))(Sweet et al., 2009)

Endocrine and metabolic disorder(e.g. Cushing syndrome, Diabetes mellitustype 1, Hemochromatosis Hyperadrenocorticism, Hyperparathyroidism
Medication (e.g. Anticonvulsants (e.g., phenobarbital, phenytoin [Dilantin]), Drugs causing hypogonadism (e.g., parenteral progesterone, methotrexate, gonadotropin-releasing hormone agonists). Glucocorticoids Heparin (long term), Immunosuppressant (e.g., cyclosporine [Sandimmune]) (Sweet et al., 2009)

Nutrition (e.g. Thyroid hormone excess, Alcohol (> 2 drinks per day) Anorexia nervosa, Celiac disease, Gastric bypass or gastrostomy, Vitamin D deficiency). (Sweet et al., 2009)

2.2.5 The Epidemiology and prevalence of osteoporosis:

The prevalence of osteoporosis varies between different populations and ethnic groups for example because of the high degree of ethnic variety in China, different studies show variety prevalence of osteoporosis. Considering its high prevalence, the disease imposes a heavy burden on the patients and families as well as the healthcare system. In fact, the numbers of women with osteoporotic fractures are higher than those who experience breast, ovary and uterus cancer. (Mohammadi, Fayyazbakhsh and Barikani, 2018)

The National Osteoporosis Foundation (NOF) estimates that 10.2 million Americans have osteoporosis and that an additional 43.4 million have low bone mass. More than 2 million osteoporosis-related fractures occur annually in the U.S., more than 70% of these occur in women. In the U.S., Medicare currently pays for most of these costs, and as the population ages, the costs of these fractures are estimated to exceed $25 billion. (Binkley et al., 2016)

The aging population is expanding at an unprecedented rate; this explosion in population will lead to a greater number of individuals with osteoporosis. It is estimated that the prevalence of osteoporosis raises from 1/3 in people at
age50e60tomore than50%ofpeople aged over 80 years. By 2050, the global osteoporosis sufferers will reach 6 million (including both males and females),3/4 of who will reside in developing countries.(Ji and Yu, 2015)

In the United States, one in eight men will have an osteoporotic fracture in his lifetime, accounting for 30 percent of hip fractures and 18 percent of the total annual cost of osteoporosis, men have nearly twice the mortality from hip fractures compared with women.(Sweet et al., 2009)

2.2.6 Diagnosis of osteoporosis:

Osteoporosis is diagnosed clinically or radiographically. Osteoporosis may present with low-impact fractures (occurring from a fall at or below standing height) or fragility fractures (occurring spontaneously).Osteoporosis is most commonly diagnosed with a T-score of −2.5 or below as determined by central DEXA scan of the total hip, femoral neck, or lumbar spine. Quantitative computed tomography can be used to assess BMD, but is limited by radiation exposure and cost. Quantitative calcaneal ultrasonography and peripheral DEXA, which measures BMD in the heel, finger, and forearm, are more portable and less costly than central DEXA and can effectively predict fracture risk. Their results, however, do not correlate well enough with central DEXA to be used diagnostically, and they have not been shown to be useful in monitoring treatment over time, Biochemical markers of bone turnover in the serum or urine are not currently recommended for diagnosis.(Sweet et al., 2009)

All postmenopausal women ≥50 years should undergo clinical assessment for osteoporosis and fracture risk, including a detailed history and physical examination.(Binkley et al., 2016)

Laboratory evaluation should include a complete blood count (CBC); comprehensive metabolic panel; 25(OH) D, intact parathyroid hormone (PTH); phosphate; and a 24-hour urine collection for calcium, sodium, and creatinine.
The 24-hour urine calcium collection must occur after the patient is vitamin D replete and has been on a reasonable calcium intake (1,000-1,200 mg/day) for at least 2 weeks. If the patient is receiving thyroid hormone or there is a suspicion for hyperthyroidism, thyroid-stimulating hormone should also be measured. (Binkley et al., 2016)

2.2.7 Treatment of Osteoporosis:

The primary goal of osteoporosis therapy is to reduce the risk of fracture.(Akkawi and Zmerly, 2018)

The North American Menopause Society guidelines suggest that as long as the lowest effective dose of MHT is used, extending treatment for an individual woman's treatment goals are acceptable when the benefits of menopause symptom relief outweigh potential risks and for further prevention of osteoporotic fracture or preservation of bone mass in women with an established reduction in bone mass other therapies are not suitable.(Ji and Yu, 2015)

Pharmacologic and non-pharmacologic treatments for osteoporosis aim to prevent fractures by improving bone strength, preventing falls, and reducing the impact force of falls.(Binkley et al., 2016).Pharmacological agents are classified into two groups: those that decrease bone resorption (antiresorptive agents) and those that increase skeletal formation (anabolic agents)Antiresorptive drugs (bisphosphonates [BPs], denosumab, strontiumranelate, estrogen replacement therapy [ERT], and selective estrogen receptor modulator [SERM]) reduce the rate of bone resorption and the rate of bone formation. The overall changes are associated with increases of BMD, but up to a certain point due to the coupling between bone resorption and formation.7 Anabolic drugs (teriparatide, romosozumab) stimulate bone formation and partially bone resorption.(Akkawi and Zmerly, 2018)
Treatment with teriparatide should always be followed by antiresorptive agents to prevent bone density decline and loss of fracture efficacy. The rationale for using an antiresorptive agent after anabolic therapy is based both on the limited period that anabolic therapy with teriparatide is used and on data showing that, lumbar spine BMD declines if antiresorptive therapy is not initiated after teriparatide therapy. (Binkley et al., 2016)

2.3 Alkaline phosphatase:

Total alkaline phosphatase (ALP) is one of the most frequently and routinely examined biomarkers in clinical laboratory tests. ALP is often considered to be a hepatic marker, but human serum contains a mixture of ALP isoenzymes from bones, the liver, and the intestine. The total ALP concentration represents the sum of the various effects of these isoenzymes. Most of the ALP isoenzymes are derived from the bones and liver, and each account for nearly 50% of the total effect of all isoenzymes in each organ. When serum ALP exceeds normal limits in the absence of obvious liver or bone disease, accurate diagnosis is not possible on the basis of ALP elevation itself, because of its low specificity (Mukaiyama and Kamimura, 2014)

The total ALP is examined by the measurement of the amount of alkaline phosphatase enzyme in the blood stream; the normal levels of ALP with people are various by age, blood type, gender and pregnancy. Unusual concentrations of ALP in blood generally indicate an issue with liver, gall bladder, or bones. The ALP test can be used for diagnosis of bone problems such as rickets, osteomalacia and Paget’s disease. (Kuo and Chen, 2017)

2.4 Calcium:

2.4.1 Introduction:

Calcium is a divalent cation with an atomic weight of 40 and an equivalent weight of 20. In the elementary composition of the human body, calcium ranks
fifth after oxygen, carbon, hydrogen, and nitrogen and it makes up 1.9% of the body by weight. Carcass analyses’ show that calcium constitutes 0.1-0.2% of early fetal fat-free weight rising to about 2% of adult fat-free weight. (Christopher, 1997)

Calcium is one of the main bone-forming minerals and an appropriate supply to bone is essential at all stages of life. (Prentice, 2004)

The tight control of plasma calcium (Ca2+) levels is essential to the performance of many vital physiological functions, Muscle contraction, blood clotting and neuronal excitation all require Calcium. (Bindels et al., 2008)

The extracellular calcium constitutes a pool into which calcium enters from the gut, by absorption, and from bone, by resorption, and from which it leaves via the addition, there are calcium fluxes across all cell membranes. Many neuromuscular and other cellular functions depend on the maintenance of the ionized calcium concentration in the ECF. In fact, the dependence of heart muscle on calcium is such that the ionized calcium concentration can be crudely assayed in a frog heart preparation.” Intracellular calcium has many functions. Calcium fluxes are important mediators of hormonal effects on target organs through the phosphoinositol system and are closely linked with the cyclic AMP systems. The intracellular calcium concentration is kept down by a calcium pump, analogous to the sodium pump, which extrudes from the cells the calcium which flows in by diffusion. The calcification of dead tissue represents the failure of this pump. (Christopher, 1997)

2.4.2 Calcium Intake:

Calcium balance is determined by the relation between calcium intake, on the one hand, and calcium absorption and excretion on the other, dairy products account for about 60% of ingested calcium in Australia, where the mean daily calcium intake is about 20 mmol (800 mg) but the range of intakes is very wide
there is also a wide variation in calcium intake between nations, depending largely on their dairy product consumption. (Christopher, 1997)

2.4.3 Physiology of calcium homeostasis:

Ingested Ca2+ is absorbed by different segments of the small intestine. The active absorption of sodium (Na+) throughout the entire course of the intestine results in a large net water absorption. This mainly occurs in the small intestine, where Ca2+ is concomitantly taken up in a passive, paracellular manner, down their concentration gradient, the active Ca2+ transport takes place largely from the duodenum. (Bindels et al., 2008)

In blood 45% of Ca2+ is present in a free, ionized form, 45% is bound to proteins, and a small fraction, 10%, forms complexes with anions including citrate, sulphate, and phosphate. (Bindels et al., 2008)

2.4.4 Hypercalcemia and hypocalcemia:

Disturbances in both serum and whole body Ca2+ levels can cause severe pathological conditions, the etiology of which is both complex and variable. Hypercalcemia can result from Ca2+ hyper absorption from the gastrointestinal (GI) tract, decreased urinary excretion, or an increased resorption from bone. Elevated serum PTH levels, secondary to hyperparathyroidism or a hypophosphatemic state, will cause increased Ca2+ absorption from the GI tract. Increased Ca2+ loss from bone is caused by elevated PTH and/or 1,25(OH)2D3 levels or skeletal metastasis, while severe dehydration will increase serum Ca2+ concentration without altering the total amount in blood. Symptoms and findings of hypercalcemia include fatigue, electrocardiogram abnormalities, nausea, vomiting, constipation, anorexia, abdominal pain, hypercalciuria, and consequently kidney stone formation. Treatment of hypercalcemia depends on the severity of the abnormality and ranges from dietary adaptation to the administration of calcimimetic compounds that activate
the CaSR in the parathyroid glands, reducing blood PTH levels. Hypocalcemia can result in muscle cramping, depression, psychosis, and seizures. Causes include decreased Ca2+ absorption due to a poor intake, 1,25(OH)2D3 deficiency or resistance, lack of sunlight, decreased bone resorption, a complication of thyroid surgery (i.e. parathyroidectomy), or renal Ca2+ wasting. OralCa2+ and 1,25(OH)2D3 supplementation and ultra-violet light exposure are the current treatments for hypocalcemia. (Bindels et al., 2008)

2.4.5 Calcium as treatment for osteoporosis:

Adequate calcium intake is a fundamental aspect of any osteoporosis prevention or treatment program and part of a lifestyle for healthy bones at any age. For adults aged 50 years and older, the recommended calcium intake including diet, plus calcium supplements, if necessary if dietary intake is insufficient) is 1,200 mg/day. Calcium supplementation has been shown to slightly increase BMD, and a recent meta-analysis from the NOF showed a 15% reduced risk of total and a 30% reduced risk of hip fractures. (Binkley et al., 2016)

The relations between calcium intake and adult BMD have been reported from a large number of cross-sectional and retrospective studies, although there are many other studies where no such association has been observed. Meta-analyses have concluded that calcium intake is a significant determinant of BM but the magnitude of the effect is small, at about 1% of the population variance. Interpretation of this association is difficult, however, because few studies have adjusted adequately for the confounding effects of body size. (Prentice, 2004)

2.5 Phosphorus:

2.5.1 Introduction:

Phosphorus is an essential bone-forming element and, as with calcium, an adequate supply of phosphorus to bone is necessary throughout life. (Prentice, 2004) it is one of the most abundant minerals in the body, and its serum levels
are regulated by a complex set of processes occurring in the intestine, skeleton, and kidneys,(Penido and Alon, 2012).

Maintenance of extracellular and intracellular phosphorus levels within a narrow range is important for many biological processes, including energy metabolism, cell signaling, and regulation of protein synthesis, skeletal development, and bone integrity. The presence of adequate amounts of phosphorus is critical for the process of apoptosis of mature chondrocytes in the growth plate, Without the presence of this mineral in high enough quantities, chondrocytes will not go into apoptosis, and the normal physiological chain of events that includes invasion of blood vessels and the generation of new bone will be blocked, resulting in rickets and delayed growth.(Penido and Alon, 2012)

The pertinent facts about phosphorus are that (1) bone mineral is not just calcium but specifically calcium phosphate; (2) adequate quantities of ingested phosphorus (expressed physiologically as a serum phosphate concentration of 1.5-2.0mmol/L) are essential for bone building during growth4-6; and (3) hypophosphatemia, from whatever cause, limits mineralization at new bone-forming sites at all ages, impairs osteoblast function, and enhances osteoclastic resorption.(Heaney, 2004)

2.5.2. Phosphorus intake:

Phosphorus is a limiting nutrient in the biosphere, Animals get the phosphorus they need by ingesting the protoplasm of other organisms (animal or plant), and plants, by taking up into their roots phosphorus released during decay of other organisms into the superficial soil layers of the biosphere. Phosphorus availability limits bio- mass in both aquatic and terrestrial habitats. As would be expected for a limiting nutrient, phosphorus absorption by the human intestine is relatively efficient, with net absorption typically ranging from 55% to 80%
(depending on concurrent calcium intake and absorption). Moreover, because phosphorus is distributed widely in many foods, it is not likely to function as a limiting nutrient (as is calcium) in animals at the top of the food chain. Nevertheless, circumstances exist in which phosphorus intake may be insufficient to support the bone rebuilding that today may be possible in patients with osteoporosis.(Heaney, 2004)

2.5.3 Physiology of phosphorus homeostasis:

Serum phosphate concentration varies with age, with the highest concentration being in infants [normal range 4.5–8.3 mg/dL (1.50–2.65 mmol/L, conversion factor 0.322], who require more of the mineral for bone growth and soft tissue buildup, and concentrations declining towards adulthood [normal range 2.5–4.5 mg/dL (0.8–1.5 mmol/L). Accordingly, in both the intestinal tract and the kidney, there is an age-related decline in phosphate absorption and reabsorption, respectively, that is correlated with decreased gene and protein expression of sodium–phosphate co-transporters. In human adults, under steady state conditions, a regular Western diet provides between 1000 and 1,600 mg/day (approx. 20 mg/kg/day) of phosphorus. Of this, approximately 16 mg/kg/day is absorbed in the proximal intestine, predominantly in the jejunum. Approximately 3 mg/kg/day is secreted into the intestine via pancreatic, bile, and intestinal secretions, giving a net phosphorus absorption of approximately 13 mg/kg/day, while 7 mg/kg/day appear in the feces. The absorbed phosphorus enters the extracellular fluid pool and moves in and out of bone as needed (approx. 3 mg/kg/day). The rate of bone remodeling is important in determining the concentration of plasma phosphorus, as disproportionate increased bone resorption will lead to a higher plasma phosphorus concentration whereas increased mineralization will lead to a lower one.(Penido and Alon, 2012)
2.5.4 Hypophosphatemia and hypophosphatemia:

Tumor-induced osteomalacia (TIO) is characterized by hypophosphatemia secondary to renal Pi wasting and reduced blood 1,25(OH)2D3 levels. Hypophosphatemia causes decreased bone mineralization and subsequently bone fragility, pain, rickets, and growth retardation. Treatment is aimed at the replacement of Pi and/or 1,25(OH)2D3. Deactivating mutations in FGF23 cause hyperphosphatemic tumoralcalcinosi characterized by hypervitaminosis D and increased intestinal and renal Pi absorption. Consistent with this, FGF23/2 mice exhibit severe hypophosphatemia further substantiating the regulatory role of FGF23 in Pi homeostasis. In humans, renal insufficiency, malignancy, drug abuse, or hypoparathyroidism can lead to hyperphosphatemia. Treatment is with Pi binders or directed at the primary cause. (Bindels et al., 2008)

2.5.5 Phosphorus as treatment of osteoporosis:

Little evidence that, in healthy individuals, the dietary intake of phosphorus influences the risk of osteoporosis, except in the special case of very-low-birth weight infants. Although there is a constant proportion of calcium and phosphorus in bone, the ratio of calcium to phosphorus in the diet can vary over a wide range with no detectable effects on the absorption and retention of either mineral, or on the ability of bone to mineralize appropriately and reduce the risk of osteoporosis. (Prentice, 2004)
CHAPTER THREE

Materials and methods
3. Material and method

3.1 materials:

3.1.1 Study design, area and period

This is an analytical – case control, facility based study conducted in Khartoum State during period from March to December 2018.

3.1.2 Target population and sample size:

Fifty patients women with osteoporosis were enrolled in this study as a test group and fifty apparently healthy women were include as a control group .both the control and test group were matched for age.

3.1.3 Inclusion and Exclusion criteria:

Diagnosed post-menopausal women with osteoporosis were including as test group and healthy subject women as control group .Patient with diabetes, liver disease, renal impairment, and hypertension and bone disorder have been excluded from this study.

3.1.4 Ethical consideration:

 Permission of this study was obtained from local authorities in the area of the study.

 The objectives of the study were explained to all individuals participating in the study.

 An informed consent was obtained from each participant in the study.

 Health education about osteoporosis and its complication was provided to all participants.

3.1.5 Data Collection:

Interview with the test group and the controls were done to obtain the clinical data and provide health education .A questionnaire (see appendix1Page) was specially designed to obtain information which help in either including or excluding certain individual in or from the study.

Blood Sampling

After informed consent and use of local antiseptic for skin (70% ethanol), 3 ml of venous blood was collected from each volunteer in this study, using sterile
disposable plastic syringes, the blood was collected from the cubital vein and centrifuged for 5 minutes at 3000 rpm to obtain plasma and then the obtained plasma was kept at -20 °C till the time of analysis.

**Biochemical measurement:**
The plasma level of alkaline phosphatase, calcium and phosphorous were measured using commercial reagent kits from Biosystem Company.

**Equipment and instrument:**
- Spectrophotometer.
- Centrifuge.
- Sterile heparin containers.
- Disposable syringes.
- 70% alcohol.
- Tourniquets.
- Cotton.
- Micropipettes (automatic pipettes).
- Graduated pipettes.

**3.2 Methodology:**

**3.2.1 Measurement of plasma level of alkaline phosphates:**

**Principle of methods**
Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm. See appendix 2 page.

\[
4 – \text{Nitrophenylphosphate} + \text{AMP}\xrightarrow{\text{ALP}}\text{AMP} – \text{phosphate} + 4 – \text{Nitrophenol}
\]

**Reagent composition and reagent preparation**
See appendix 2 page.

**Procedure of alkaline phosphatase**
- Brought the working reagent and the instrument into reaction temperature.
- Pipetted into a cuvette 1ml of working reagent and 10µl of sample.
- Mixed and inserted the cuvette into the photometer.
- Recorded initial absorbance and at 1min intervals thereafter for 3 minutes.
Calculated the difference between consecutive absorbance, and the average absorbance difference per minute (ΔA/min).

**Calculation**

The ALP catalytic concentration in sample=

\[
\Delta A/\text{min} \times \frac{vt \times 10^6}{\varepsilon \times 1 \times V_s} = \text{U/L}
\]

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 18450, the light path (I) is 1 cm, the total reaction volume (vt) is 1.02, the sample volume (Vs) is 0.02, 1 U/L are 0.0166µkat/L . The following formulas are deduced for the calculation of the catalytic concentration:

\[
\Delta A/\text{min} \times 2764 = \text{U/L}
\]

\[
\Delta A/\text{min} \times 46.08 = \mu\text{Kat/L}
\]

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th>Reaction temperature</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C, up to</td>
<td>75 U/L = 1.25 µKat/L</td>
<td>68 U/L = 1.13 µKat/L</td>
</tr>
<tr>
<td>30°C, up to</td>
<td>87 U/L = 1.45 µKat/L</td>
<td>80 U/L = 1.33 µKat/L</td>
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<tr>
<td>37°C, up to</td>
<td>115 U/L = 1.92 µKat/L</td>
<td>105 U/L = 1.75 µKat/L</td>
</tr>
</tbody>
</table>

**3.2.2 Measurement of plasma calcium:**

**Principle of methods**

Calcium in the sample reacts with methylthymol blue in alkaline medium forming a colored complex that can be measured by spectrophotometry. Hydroxyquinoline is included in the reagent to avoid magnesium interference. (See appendix 3 page)

**Reagent composition and reagent preparation**

See appendix 3 page.
**Procedure of calcium**

Labeled three test tubes as blank, standard and test and pipette 10µl of distilled water, standard and sample in each labeled tube then placed 1000µl of the working reagent in each tube and Mixed thoroughly and let stand the tubes for 2 minutes at room temperature.

Red the absorbance (A) of the Standard and the Sample at 610 nm against the Blank, The color is stable for at least 1 hour.

**Calculation**

The plasma calcium concentration in the sample (mg/dl) =

\[
\text{Calculation} \quad \frac{\text{Å of sample}}{\text{Å of standard}} \times \text{standard concentration (mg/dl)}
\]

**Reference values**

Plasma and serum =8.6 – 10.3 mg/dl =2.15 -2.58 mmol/L

3.2.3**Measurement of plasma phosphorous:**

**Principle of the method**

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry. (See appendix 4 page)

**Reagent composition and reagent preparation**

See appendix 4page.

**Procedure of phosphours**

Labeled four test tubes as reagent blank, sample blank, standard and test and pipetted 10microliters of distilled water in tube labeled reagent blank, and 10µl of sample in tubes labeled sample blank and sample and 10 µl of standard in tube labeled standard then add 1000 µl of the working reagent in reagent blank, sample and standard tubes and 1000 µl of reagent A in tube labeled sample blank, Mixed thoroughly and let stand the tubes for 5minutes at room temperature.

Red the absorbance (A) of the Sample Blanks at 340 nm against distilled water. And Read the absorbance (A) of the Samples and of the Standard at 340 nm against the Regent blank.
Calculation

The plasma level of phosphorus =

\[
\frac{\text{Abs of sample} - \text{Abs Sample Blank}}{\text{Abs of standard}} \times \text{standard concentration (mg/dl)}
\]

Reference values

Adults: 2.5-4.5 mg/dL = 0.81-1.45 mmol/L
Children: 4.0-7.0 mg/dL = 1.29-2.26 mmol/L

3.3 Quality control:

The precision and accuracy of the method used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.4 Statistical analysis:

The data collected in this study were analyzed using Statistical Package for the Social Science program (SPSS program) version 16. 0.5 % was taken as cut off limit for 95 % statistical significance. Frequency and percentage testes were used and then the data were presented in tables. Pearson correlation coefficient and linear regression were used for quantitative variables, and chi-square test for qualitative variables; P value \( \leq 0.05 \) was considered as the level of significance.
CHAPTER FOUR

Results
4. Result

A total of 50 Sudanese women patient with osteoporosis in Khartoum Sudan taken as test group and compared to 50 apparently healthy women were taken as control group.

**Table (4-1)**

Shows the demographic characteristics age (years), weight (kg) height (cm) and BMI (Kg/m²) of cases and control groups.

**Table (4-2)**

Shows mean and standard deviation of ALP, Calcium, phosphorus and BMI, in case and control groups.

**Figure (4.1)**

Shows no correlation between ALP and Calcium with p- value (0.6).

**Figure (4.2)**

Shows no correlation between ALP and phosphorous with p- value (0.7).

**Figure (4.3)**

Shows no correlation between phosphorous and calcium with p- value (0.6).

**Figure (4.4)**

Shows a weak positive correlation between BMI and ALP with p- value (0.02) and r= (0.3).

**Figure (4.5)**

Shows no correlation between BMI and calcium with p- value (0.7).

**Figure (4.6)**

Shows no correlation between BMI and phosphorous with p- value (0.2).
Figure (4.7)
Shows no correlation between Age and ALP with p-value (0.4).

Figure (4.8)
Shows no correlation between Age and calcium with p-value (0.1).

Figure (4.9)
Shows no correlation between Age and phosphorous with p-value (0.4).
Table 4.1: Demographic characteristics of cases and control.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Minimum Cases</th>
<th>Minimum Controls</th>
<th>Maximum Cases</th>
<th>Maximum Controls</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>24</td>
<td>26</td>
<td>90</td>
<td>81</td>
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<tr>
<td>Height (cm)</td>
<td>126</td>
<td>146</td>
<td>178</td>
<td>179</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>42.5</td>
<td>36</td>
<td>93.7</td>
<td>108</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>19.3</td>
<td>15.1</td>
<td>45.3</td>
<td>41.1</td>
</tr>
</tbody>
</table>

The table shows the minimum and maximum of age, weight, height and BMI in case and control group.
Table (4.2): Mean ± standard deviation and P-value of BMI, ALP, Calcium and phosphorus in case and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ±SD of cases</th>
<th>Mean ±SD of control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/l)</td>
<td>63.38±20.35</td>
<td>72.38±17.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.31±2.28</td>
<td>9.02±1.19</td>
<td>0.01</td>
</tr>
<tr>
<td>phosphorus (mg/dl)</td>
<td>4.46±1.12</td>
<td>4.00±1.08</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (Kg /m²)</td>
<td>29.7±5.9</td>
<td>27.08±6.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The table shows the mean ±SD, range in brackets and probability (P).
Independent t test was used for comparison. P-value < 0.05 is considered significant.
**Figure (4-1)** A scatter plot shows correlation between the alkaline phosphates and calcium in test group. $r = (-0.05)$, P-value = (0.6)
Figure (4-2) A scatter plot shows correlation between the alkaline phosphates and phosphorus in test group. $r = -0.07$, $P$-value = (0.7)
**Figure (4-3)** A scatter plot shows correlation between the calcium and phosphorus in test group. $r = (-0.05)$, P-value = (0.6)
Figure (4-4) A scatter plot shows correlation between the BMI and ALP in test group. $r=(0.3)$, P-value = (0.02)
Figure (4-5) A scatter plot shows correlation between the BMI and calcium in test group. \( r = (+0.03), \) P-value = (0.7)
Figure (4-6) A scatter plot shows correlation between the BMI and phosphorus in test group. $r=-0.16$, P-value = (0.2)
Figure (4-7) A scatter plot shows correlation between the Age and ALP in test group. $r= (+0.10)$, P-value = (0.4)
Figure (4-8) A scatter plot shows correlation between the Age and calcium in test group. $r=(+0.20)$, $P$-Value = (0.1)
**Figure (4-9)** A scatter plot shows correlation between the Age and phosphorus in test group. $r= (+0.11)$, $P$-value = (0.4)
CHAPTER FIVE

Discussion, Conclusion and Recommendations
5. Discussion, Conclusion and Recommendations

5.1 Discussion
Osteoporosis is a growing public health problem of the elderly especially in elderly Asians as the people are living longer; it’s qualified by low bone mineral density (BMD), with its associated increased risk of fractures. Ali , (2018)

Osteoporosis is a classical age-related disease that affects women more often than men; its prevalence is higher in post-menopausal women due to many factors that we mentioned above. (Ji and Yu, 2015) Approximately 200 million people suffer from osteoporosis and approximately 8.9 million fractures are caused by osteoporotic fracture, the aim of this study was to evaluate alkaline phosphatase, phosphorus and calcium level in post-menopausal women with osteoporosis. (Akkawi and Zmerly, 2018)

In our study BMI is differ in case group compared to control group which is higher in case group due to high weight and lack of physiological activity of the women in cases group in Sudan which agree with Ali , (2018) showed that BMI significantly higher in osteoporosis compared to the control group in Kirkuk City. On the other hand Sassi et al. (2015) noteworthy that osteopenic and osteoporotic women had the lowest BMI.

plasma alkaline phosphatase was significantly lower in osteoporosis in comparison to the control group yet their values are still within normal range because the treatment lowered the levels of the ALP as they showed in Mukaiyama and Kamimura,( 2014) Bisphosphonate treatment lowered ALP levels, and also Kuo and Chen,(2017) the decrease of total ALP has been demonstrated with the treatment with alendronate from 79.7 U/L to 64.8 U/L but some study shows the increase in level of ALP as in Davies et al., (2018) and in the Ali , (2018) which showed Serum alkaline phosphatase was
significantly higher in osteoporosis (96.89 ± 8.00) in comparison to the control group (81.0 ± 12.4) yet their values are still within normal range.

Both control and osteoporosis cases had phosphorus within normal range, however the difference between the levels was significantly higher in osteoporotic than control groups as they showed in Ali, (2018).

The calcium in our study was decrease in osteoporotic group when compared which control group which disagree with Ali, (2018) which a high level of calcium.

Also we found that a weak positive correlation between the BMI and ALP but.

We observed form our study the BMI in Sudan is higher when compared to other country due to increase the weights and lack of the physiological activity in the post-menopausal women.

5.2 Conclusion

Study concluded that the mean of ALP and calcium level is decrease in post-menopausal women but the mean phosphorus is slightly higher when compared with control group which remain in the normal levels and there is a weak correlation between the BMI with ALP.

5.3 Recommendations

The incidence of postmenopausal osteoporosis is on the rise and one should be careful in the management and suitable pharmacotherapy should be given for every patient.

1. All patients with osteoporosis and fractures should be given advice on lifestyle measures to decrease bone loss. These include eating a balanced diet rich in calcium, moderating tobacco and alcohol consumption, maintaining regular physical activity, and exposure to sunlight.
2. Specific treatment of secondary causes of osteoporosis.
3. The diagnostic evaluation and treatment of patients with osteoporosis is aimed to prevent osteoporotic fractures and, if fractures are present, to initiate effective pharmacological treatment.
4. Weight-bearing aerobic exercises and muscle-strengthening exercises have been shown to be an integral part of osteoporosis prevention, as well as a part of the treatment process.

5. A major role in the prevention of osteoporosis is played by the optimization of intake of calcium (in line with relevant recommendations), protein (1.2 g/kg), potassium (over 3500 mg/day) and magnesium (over 300 mg/day) (B). Appropriate supply of vitamin D reduces the risk of fractures.

6. Hormone therapy (HT), estrogens and progesterone in postmenopausal women and testosterone in men with hypogonadism, having considered the potential risks, prevents bone loss and reduces the risk of fractures.
References


Heinz A. (2006) ‘This report was prepared by Agnes Heinz, Ph.D., a former Director of Nutrition and Biochemistry with the American Council on Science and Health’.


deoxypyridinoline in postmenopausal osteoporosis, 23(2), pp. 79–88.


Appendix
Sudan University of science and technology

Evaluation of Alkaline phosphatase, Calcium and phosphorous in Post-menopausal Women with Osteoporosis

Participant: Questionnaire No. ( )

Name: ...........................................................................................................

Address: ......................................................................................................

Contact phone number: ..............................................................................

Birth date: .................................................................................................

Material Status:

☐ Single ☐ married ☐ divorced ☐ widow

Do you have:

- Menstrual period problem?
- Significant childbirth - related problem?
- Urine loss when you can cough, sneeze or laugh?

Do you now or have you ever had:

- Diabetes
- High blood pressure
- Liver diseases
- Kidney diseases
- Bone diseases

Biochemical findings:

<table>
<thead>
<tr>
<th>Blood investigation</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphates(U/L)</td>
<td></td>
</tr>
<tr>
<td>Calcium(mg/dl)</td>
<td></td>
</tr>
<tr>
<td>phosphorus</td>
<td></td>
</tr>
</tbody>
</table>
PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol.

The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm.

4 - Nitrophenylphosphate + AMP → ALP → AMP + phosphate + 4 - Nitrophenol

CONTENTS

A. Reagent: 2-amino-2-methyl-1-propanol 60 mmol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylidenedimethylacetamide 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.
B. Reagent: 4-Nitrophenylphosphate 60 mmol/L.

STORAGE

Store at 2-8°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:
- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent:
- Cod. 11592 and 11593: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 ml. Reagent A + 1 ml. Reagent B. Stable for 2 months at 2-8°C.
- Cod. 11598: Transfer 25 ml of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 ml. Reagent A + 1 ml. Reagent B. Stable for 2 months at 2-8°C.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer with cuvette holder, thermostable at 25, 30 or 37°C and able to read at 405 nm.
- Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8°C. Heparin may be used as an anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the Instrument to reaction temperature.
2. Pipette into a cuvette (Note 1):

<table>
<thead>
<tr>
<th>Working Reagent Sample</th>
<th>Volume</th>
<th>1.0 ml</th>
<th>20 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 μL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Mix and insert the cuvette into the photometer.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (A/Min).

CALCULATIONS

The ALP catalytic concentration in the sample is calculated using the following general formula:

\[
\text{Activity} = \frac{A_{\text{Min}} \times 10^4}{\text{Volume}}
\]

The motor absorbance (c) of 4-nitrophenol at 405 nm is 1.9450, the lightpath (l) is 1 cm, the total reaction volume (V) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L is 0.0196 μmol/L. The following formulas are deduced for the calculation of the catalytic concentration:

\[
A_{\text{Min}} = \frac{A_{\text{Min}} \times 10^4}{\text{Volume}} \times \frac{1}{x} \times \frac{1}{V_s}
\]

REFERENCE VALUES

<table>
<thead>
<tr>
<th>Reaction temperature</th>
<th>men</th>
<th>women</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C, up to 30°C</td>
<td>76 U/L, 1.25 μmol/L</td>
<td>68 U/L, 1.13 μmol/L</td>
</tr>
<tr>
<td>30°C up to 35°C</td>
<td>87 U/L, 1.45 μmol/L</td>
<td>80 U/L, 1.33 μmol/L</td>
</tr>
<tr>
<td>37°C, up to 42°C</td>
<td>115 U/L, 1.65 μmol/L</td>
<td>105 U/L, 1.75 μmol/L</td>
</tr>
</tbody>
</table>

VALUES AT 37°C

Values at 37°C are obtained from those at 25°C using a conversion factor. Concentrations in growing children are higher and more variable. These ranges are given for orientational only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18009, 18019 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for automatic reagents if controls do not leave within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.0 U/L = 0.017 μmol/L.
- Linearity limit: 1200 U/L = 20 μmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

<table>
<thead>
<tr>
<th>Mean Concentration</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 U/L = 1.02 μmol/L</td>
<td>1.0%</td>
<td>20</td>
</tr>
<tr>
<td>244 U/L = 4.07 μmol/L</td>
<td>0.7%</td>
<td>20</td>
</tr>
</tbody>
</table>

- Reproducibility (run to run):

<table>
<thead>
<tr>
<th>Mean Concentration</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 U/L = 1.02 μmol/L</td>
<td>3.4%</td>
<td>25</td>
</tr>
<tr>
<td>244 U/L = 4.07 μmol/L</td>
<td>1.1%</td>
<td>25</td>
</tr>
</tbody>
</table>

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Lipemia (lipoproteins < 10 g/L) and bilirubin (bilirubin > 20 mg/dL) do not interfere. Hemoglobin (> 2.5 g/L) interferes. Other drugs and substances may interfere.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

DIAGNOSTIC CHARACTERISTICS

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monooesters at alkaline pH.

The enzyme is present in practically all tissues of the body, especially at the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoclastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatocellular damage caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

PRINCIPLE OF THE METHOD
Calcium in the sample reacts with methylthymol blue in alkaline medium forming a coloured complex that can be measured by spectrophotometry. Hydroxychloroquine is included in the reagent to avoid magnesium interference.

CONTENTS

<table>
<thead>
<tr>
<th></th>
<th>COD 11527</th>
<th>COD 11507</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Reagent</td>
<td>2 x 10 mL</td>
<td>1 x 250 mL</td>
</tr>
<tr>
<td>B. Reagent</td>
<td>2 x 10 mL</td>
<td>1 x 250 mL</td>
</tr>
<tr>
<td>G. Standard</td>
<td>1 x 5 mL</td>
<td>1 x 5 mL</td>
</tr>
</tbody>
</table>

COMPOSITION
A. Reagent. Potassium cyanide 7.7 mmol/L, ethanolamine 1.5 mmol/L.
B. Reagent. Methylthymol blue 0.1 mmol/L, hydrochloric acid 10 mmol/L, hydroxychloroquine 17 mmol/L.
C. Calcium/Magnesium Standard. Calcium 10 mg/dL (2.5 mmol/L), magnesium 2 mg/dL.
Aqueous primary standard.

STORAGE
Store at 15-30°C.
Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminated are protected during their use.
Indications of deterioration:
- Reagents: Presence of particulate matter, turbidity, absorbance of the blank over 0.800 at 610 nm.
- Standard: Presence of particulate matter, turbidity.

REAGENT PREPARATION
Standard (S) is provided ready to use.
Working Reagent: Mix equal volumes of Reagent A and Reagent B (Note 1). Mix gently. Stable for 2 days at 2-8°C.

ADDITIONAL EQUIPMENT
- Analyzer, spectrophotometer or photometer able to read at 610 ± 20 nm.

SAMPLES
Serum, heparinized plasma or urine collected by standard procedures.
Calcium in serum or plasma is stable for 10 days at 2-8°C. Anticoagulants other than heparin should not be used.
Collect a 24-hour urine sample in a bottle containing 10 mL of 50 % (v/v) nitric acid. Stable for 10 days at 2-8°C. Centrifuge or filter and dilute 10% with distilled water before testing.

PROCEDURE
1. Pipette into labelled test tubes (Notes 1, 2)

<table>
<thead>
<tr>
<th>Calcium Standard</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Reagent</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

2. Mix thoroughly and let stand the tubes for 2 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 610 nm against the Blank. The colour is stable for at least 1 hour.

CALCULATIONS
The calcium concentration in the sample is calculated using the following general formula:
\[
C_{\text{Sample}} = \frac{C_{\text{Standard}} \times V_{\text{Sample}}}{V_{\text{Standard}}}
\]
where:
- \(C_{\text{Sample}}\) is the calcium concentration in the sample
- \(C_{\text{Standard}}\) is the calcium concentration in the standard
- \(V_{\text{Sample}}\) is the volume of the sample
- \(V_{\text{Standard}}\) is the volume of the standard

If the Calcium Standard provided has been used to calibrate (Note 3):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serum &amp; plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Standard</td>
<td>10 mg/dL calcium</td>
<td>20 mg/dL calcium</td>
</tr>
<tr>
<td>A. Reagent</td>
<td>10 mg/dL calcium</td>
<td>5 mg/dL calcium</td>
</tr>
</tbody>
</table>

REFERENCE VALUES
Serum and plasma: 2.6-10.3 mg/dL = 2.5-8.8 mmol/L
Urine: 100-300 mg/24 h = 2.5-7.5 mmol/24 h
These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL
It is recommended to use the Biochemistry Control Serum level I (cod. 16007, 16009 and 16043) and II (cod. 16007, 16010 and 16034) to verify the performance of the measurement procedure.
Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGY CHARACTERISTICS
- Detection limit: 0.6 mg/dL. Calcium = 0.15 mmol/L.
- Linearity limit: 15 mg/dL. Calcium = 3.75 mmol/L. Calcium. For higher values dilute 1:2 with distilled water and repeat measurement.
- Repeatability (within run):
<table>
<thead>
<tr>
<th>Mean calcium concentration</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 mg/dL = 2.75 mmol/L</td>
<td>0.7 %</td>
<td>20</td>
</tr>
<tr>
<td>13 mg/dL = 3.25 mmol/L</td>
<td>2.6 %</td>
<td>20</td>
</tr>
</tbody>
</table>
- Reproducibility (run to run):
<table>
<thead>
<tr>
<th>Mean calcium concentration</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 mg/dL = 2.75 mmol/L</td>
<td>5.0 %</td>
<td>25</td>
</tr>
<tr>
<td>13 mg/dL = 3.25 mmol/L</td>
<td>4.7 %</td>
<td>25</td>
</tr>
</tbody>
</table>
- Sensitivity: 30 mg/dL = 0.75 mg/L
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.
- Interferences: Hemolysis (hemoglobin < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Lipemia (triglycerides > 1.25 g/L) interfere. Other drugs and substances may interfere.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

DIAGNOSTIC CHARACTERISTICS
Calcium is the most prevalent cation found in the body, distributed in bone (99%), soft tissues and extracellular fluid. Its concentration in plasma is regulated by parathyroid hormone, vitamin D and calcitonin.
Calcium ion is important in the transmission of nerve impulses, in the maintenance of normal muscle contractility, as a cofactor in certain enzyme reactions, and in the coagulation of the blood.
Hypercalcemia can be due to vitamin D intoxication, enhanced renal retention, osteoporosis, sarcoidosis, thyrotoxicosis, hyperparathyroidism, multiple myeloma, idiopathic hypercalcemia of infancy, and carcinoma metastatic to bone.
Elevated calcium concentration in urine is found in nephrolithiasis and metabolic acidosis.
Hypercalcemia may be caused by primary and secondary hyperparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption.
Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES
1. Contamination of glassware with calcium will affect the test. Use acid-washed glassware or plastic tubes.
2. These reagents may be used in several automatic analyzers. Instructions for many of them are available on request.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 16011 and 16044).

BIBLIOGRAPHY
PHOSPHORUS
PHOSPHOMOLYDBDATE/UV

PRINCIPLE OF THE METHOD
Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry.1-4

CONTENTS AND COMPOSITION
A. Reagent: 3 x 40 mL. Sulfuric acid 0.36 mL/L, sodium chloride 154 mmol/L.
DANGER: Causes severe skin burns and eye damage. P262 Wear protective gloves/protective clothing/eye protection/face protection. P301+P330+P331+P332+P333 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
B. Reagent: 1 x 50 mL. Sulfuric acid 0.36 mL/L, sodium chloride 154 mmol/L, ammonium molybdate 35.5 mmol/L. DANGER: Causes severe skin burns and eye damage. P262 Wear protective gloves/protective clothing/eye protection/face protection. P301+P330+P331+P332+P333 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
C. Phosphorus Standard: 1 x 5 mL. Phosphorus 5 mg/L (1.61 mmol/L). Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE
Store at 15-30°C. Reagents and Standards are stable until the expiry date shown on the label when stored tightly closed, and if contamination is prevented during their use.

INDICATIONS OF DECOMPOSITION:
- Reagents: Presence of particulate material, turbidity, absorption of the blank over 0.500 at 340 nm.
- Standard: Presence of particulate material, turbidity.

REAGEN PREPARATION
Standard (G) is provided ready to use. Working Reagent: Mix thoroughly in the proportion: 7 mL Reagent A + 3 mL Reagent B. Stable for 12 months at 15-30°C.

ADDITIONAL EQUIPMENT
- Analyser, spectrophotometer or photometer able to read at 340 ± 20 nm.

SAMPLES
Serum, heparinized plasma or urine collected by standard procedures.

Phosphorus in plasma or serum is stable for 7 days at 2-8°C.

Collected 24-hour urine in a bottle containing 10 mL of 10% (v/v) hydrochloric acid. Stable for 10 days at 2-8°C. Centrifuge or filter the sample and dilute 1:10 with distilled water before measurement.

PROCEDURE
1. Pipette into labelled test tubes: (Note 1)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Diluted Water</th>
<th>Sample Blank</th>
<th>Sample</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
<td>—</td>
</tr>
<tr>
<td>Clarity</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
<td>—</td>
</tr>
<tr>
<td>Clarity</td>
<td>10 mL</td>
<td>10 mL</td>
<td>10 mL</td>
<td>—</td>
</tr>
</tbody>
</table>

2. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.

3. Read the absorbance (A) of the Sample Blanks at 340 nm against diluted water.

4. Read the absorbance (A) of the Samples and of the Standard at 340 nm against the Reagent Blank.

CALCULATIONS
The phosphorus concentration in the sample is calculated using the following general formula:

\[
\text{C sample} = \frac{A_{\text{sample}} - A_{\text{blank sample}}}{A_{\text{standard}} - A_{\text{blank standard}}} \times \frac{C_{\text{standard}}}{C_{\text{sample}}}
\]

If the Phosphorus Standard provided has been used to calibrate (Note 2):

\[
\text{C sample} = \frac{A_{\text{sample}} - A_{\text{blank sample}}}{x + 5 \text{ mg/dL}} \times \frac{25 \text{ mg/dL}}{16} = 1.61 \times x \text{ mmol/L}
\]

REFERENCE VALUES
Serum: Adults: 2.5-4.5 mg/dL (0.61-1.45 mmol/L); Children: 4.0-7.0 mg/dL (1.09-1.89 mmol/L).
Urine: 0.1-1.3 g/24-h = 12.9-42 mmol/24-h

Concentrations in plasma are about 0.25 mg/dL (0.08 mmol/L) lower than in serum. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL
It is recommended to use the Biochemistry Control Serum level I (cod. 18035, 18080 and 18032), level II (cod. 18007, 19101 and 18034) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure. Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS
- Detection limit: 0.13 mg/dL (0.03 mmol/L).
- Linearity limit: 20 mg/dL, phosphorus = 6.40 mmol/L, phosphorus. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run): Mean concentration CV n

<table>
<thead>
<tr>
<th>Mean concentration</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.34 mg/dL = 1.40 mmol/L</td>
<td>1.3%</td>
<td>20</td>
</tr>
<tr>
<td>6.20 mg/dL = 2.16 mmol/L</td>
<td>2.7%</td>
<td>20</td>
</tr>
</tbody>
</table>

- Reproducibility (run to run): Mean concentration CV n

<table>
<thead>
<tr>
<th>Mean concentration</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.34 mg/dL = 1.40 mmol/L</td>
<td>2.9%</td>
<td>25</td>
</tr>
<tr>
<td>6.20 mg/dL = 2.16 mmol/L</td>
<td>2.5%</td>
<td>25</td>
</tr>
</tbody>
</table>

- Sensitivity: 48 mL/L/mg = 146 mL/mmol.
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

- Interferences: hemoglobin (10 g/L), IEP (fibrinogen 10 g/L) and bilirubin (20 mg/dL) do not interfere. Other drugs and substances may interfere.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS
Approximately 80% of the phosphorus in the human body is found in the calcium phosphate salts, which make up the inorganic substance of bone. The remainder is involved in the esterification of carbohydrate metabolism intermediates and is also found as a component of phospholipids, phosphoproteins, nucleic acids and nucleotides. Hypophosphatemia can be caused by shift of phosphate from extracellular to intracellular spaces, increased renal loss (renal tubular defects, hyperparathyroidism) or gastrointestinal loss (diarrhea, vomiting), and decreased intestinal absorption.2-3

Hypophosphatemia is usually secondary to inability of the kidneys to excrete phosphate due to renal failure or hyperparathyroidism.4

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES
1. These reagents may be used in several automatic analyzers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, especially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY