



بسم الله الرحمن الرحيم

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**Calcium and Phosphate Status in Postmenopausal and Premenopausal Women
in SharqElneel Locality.**

مستويات الكالسيوم والفوسفات في النساء السودانيات

بعد وقبل سن اليأس في محلية شرق النيل

Dissertation submitted in partial fulfillment for the Requirement of Master Degree
(M.Sc) in Medical laboratories Science.. Clinical chemistry

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Dedication

I dedicate this research work to my family who inspire me and support me all the time. A special feeling of gratitude to my loving parent who's always being there for me, whose words of encouragement and push for tenacity ring in my ears.

To my supervisor who guide me and help me to get through this process.

Acknowledgment

*First I thank my almighty god for complete of this research
I wish to thank my committee members who were more than
generous with their expertise and precious time, a special
thanks to Dr.AbdelgadirElmugadam my committee chairman
for his countless hours of reflecting, reading, encouraging and
most of all patience throughout the entire process.*

*Word can never help to express my feelings toward everyone
stand beside me to carry this work.*

Abstract

Menopause is a normal, natural transition in the life, there are many changes occur during this period.

This cross-section study, conducted to determine the calcium and phosphate level in Sudanese postmenopausal women compared to premenopausal women in period from March to May 2017.

Fifty samples were collected from postmenopausal women without diseases, and thirty premenopausal women without disease, as control group, were informed about study and verbal consent from participants was obtained. 3ml of venous blood was collected in heparin containers, plasma was separated and investigated for calcium and phosphate levels (using a semi automated selectra analyzer, and biosystem analyzer). And statistical package for social science (SPSS), computer program version 18 was used for data processing.

This study shows that, a significant elevation in calcium and phosphate levels in postmenopausal women compared to premenopausal women. Calcium (mean \pm SD: 2.5 \pm 0.30mmol/L, versus 2.22 \pm 0.0.23mmol/L. p.value=0.00. Phosphate (mean \pm SD: 1.12 \pm 0.23mmol/L, versus 0.93 \pm 0.16mmol/L. P. value =0.00).

Pearson correlation shows that, there is an insignificant positive correlation between the number of birth and level of phosphate (r=0.008 and p. value=0.6). And there is a significant positive correlation between the number of birth and the level of calcium: (r=0.044 and p. value=0.01), in postmenopausal women.

There is an insignificant negative correlation between duration of menstruation and levels of calcium and phosphate in two groups. For postmenopausal women: Calcium (r=0.003 and p.value=0.7). Phosphate (r=0.071 and p.value=0.06).

For premenopausal women: Calcium (r=0.086 and p.value=0.16). Phosphate (r=0.013 and p.value=0.5).

From this study we conclude that, calcium and phosphate levels are affected by menopause, due to many changes that occurring during these years of age.

مستخلص الدراسة

أجريت هذه الدراسة التوقعية لقياس مدى تأثير انقطاع الدورة الشهرية على مستويات الكالسيوم و الفوسفات في الدم في النساء السودانيات في الفترة ما بين مارس حتى مايو 2017.

خمسون عينة دم أخذت من نساء اصحاء لا يعانين من مرض بعد سن اليأس في العمر ما بين 48-80 سنة ، مع ثلاثين عينة دم أخذت من نساء قبل سن اليأس اصحاء لا يعانين من مرض كمجموعة تحكم (مجموعة ضابطة) تم اخبارهم بغرض الدراسة وأخذ موافقتهم .

تم أخذ عينة 3 مل من الدم وتم قياس مستويات كل من الكالسيوم والفوسفات باستخدام كل من (جهاز سيليكترا شبه أوتوماتيكي و جهاز بايوسيسستم للتحليل شله أوتوماتيكي)، وتم استخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية لمعالجة البيانات .

توصلت الدراسة الي ان مستوى الكالسيوم و الفوسفات يزداد في مجموعة النساء بعد سن اليأس مقارنة بالنساء قبل سن اليأس .

"المتوسط \pm الانحراف المعياري عند النساء بعد سن اليأس مقارنة بالنساء قبل سن اليأس"

الكالسيوم (المتوسط \pm الانحراف المعياري) = 0.30 ± 2.5 ممل/لتر، مقارنة ب 0.023 ± 2.22 ممل/لتر، القيمة المعنوية = 0.00). الفوسفات (المتوسط \pm الانحراف المعياري) = 1.12 ± 0.23 ممل/لتر، مقارنة ب 0.16 ± 0.93 ممل/لتر، القيمة المعنوية = 0.00).

تحليل ارتباط بيرسون أظهر انه ليس هناك علاقة ايجابية ذات دلالة احصائية بين عدد الولادات ومستوى الكالسيوم في الدم في المجموعتين، وايضا وجد ان هناك علاقة سلبية ذات دلالة احصائية بين عدد الولادات ومعدل الفوسفات في الدم في مجموعة النساء ما بعد سن اليأس.

لم تثبت الدراسة ان هناك علاقة ذات دلالة احصائية واضحة بين السن ومعدل الكالسيوم والفوسفات في الدم في النساء بعد سن اليأس وقبل سن اليأس .

النساء بعد سن اليأس: الكالسيوم (معامل بيرسون للارتباط = 0.001 ، والقيمة المعنوية = 0.79).

الفوسفات (معامل بيرسون للارتباط = 0.067 ، والقيمة المعنوية 0.07).

النساء قبل سن اليأس : الكالسيوم (معامل بيرسون للارتباط: 0.117 ، والقيمة المعنوية = 0.127).

الفوسفات (معامل بيرسون للارتباط = 0.013 ، القيمة المعنوية = 0.5).

من هذ الدراسة نستنتج أن معدل الكالسيوم والفوسفات في الدم يتأثر بصورة كبيرة عند دخول المرأة في سن اليأس بسبب التغييرات التي تحدث أثناء تلك المرحلة من العمر.

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CHAPTER ONE

Introduction

Objectives

Rational

1. Introduction, Rationale and objectives

1.1 Introduction:

Menopause, also known as the climacteric, is the time in most women's lives when menstrual periods stop permanently, and they are no longer able to bear children (Eunice Kennedy Shriver National Institute of Child Health and Human Development and pubmed, 2013). Menopause typically occurs between 49 and 52 years of age. Medical professionals often define menopause as having occurred when a woman has not had any vaginal bleeding for a year (O'Connor, 2009) It may also be defined by a decrease in hormone production by the ovaries (Sievert and Lynnette, 2006). In those who have had surgery to remove their uterus but they still have ovaries, menopause may be viewed to have occurred at the time of the surgery or when their hormone levels fell (Sievert and Lynnette, 2006). Following the removal of the uterus, symptoms typically occur earlier, at an average of 45 years of age (Nanette, 2002).

Before menopause, a woman's periods typically become irregular, which means that periods may be longer or shorter in duration or be lighter or heavier in the amount of flow. During this time, women often experience hot flashes; these typically last from 30 seconds to ten minutes and may be associated with shivering, sweating, and reddening of the skin (Medlineplus, 2011). Hot flashes often stop occurring after a year or two (Melby, Lock and Kaufert, 2005). Other symptoms may include vaginal dryness, trouble sleeping, and mood changes (Medlineplus, 2011). The severity of symptoms varies between women (Melby, Lock and Kaufert, 2005). While menopause is often thought to be linked to an increase in heart disease, this primarily occurs due to increasing age and does not have a direct relationship with menopause. In some women, problems that were present like endometriosis or painful periods will improve after menopause (Melby, Lock and Kaufert, 2005).

Menopause is usually a natural change. It can occur earlier in those who smoke tobacco (Warren, 2009). Other causes include surgery that removes both ovaries or some types of chemotherapy(O'Connor, 2009). At the physiological level,

menopause happens because of a decrease in the ovaries' production of the hormones estrogen and progesterone. While typically not needed, a diagnosis of f menopause can be confirmed by measuring hormone levels in the blood or urine (MedlinePius, 2012). Menopause is the opposite of menarche, the time when a girl's periods start (Wood and James, 2009). Specific treatment is not usually needed. Some symptoms, however, may be improved with treatment. With respect to hot flashes, avoiding smoking, caffeine, and alcohol is often recommended. Sleeping in a cool room and using a fan may help (MedLineplus, 2010). The following medications may help: menopausal hormone therapy (MHT), clonidine, gabapentin, or selective serotonin reuptake inhibitors(MedLineplus, 2010)(Krause and Nakajima 2015). Exercise may help with sleeping problems. While MHT was once routinely prescribed, it is now only recommended in those with significant symptoms, as there are concerns about side effects (MedLineplus, 2010). High-quality evidence for the effectiveness of alternative medicine has not been found (Melby, Lock and Kaufert, 2005). There is tentative evidence for phytoestrogens (Franco *et al* ,2016).

1.2 Rationale:

Menopause is the normal, natural transition in life that begins between the ages of 35-55. There are many changes occur to the women during this period, which may lead to diseases, important consequences of menopause are osteopenia, a minor reduction in bone mass, and osteoporosis, a severe reduction in bone mass that is associated with a tendency to sustain fractures from minor stresses. This acceleration is associated with decreased production of estrogen and other sex hormones at the onset of menopause(Edward andRobert , 2010).

In Sudan there is no much studies about menopause health, so more researches need to be carried out, to understand the medical condition of menopausal women, and accordingly, there is no published data about calcium and phosphate status in menopausal women in recent years in Sudan.

1.3 Objectives

General objective:

To evaluate calcium and phosphorus status in postmenopausal and premenopausal women in SharqElneel Locality.

Specific objectives:

1-To measure calcium and phosphorus levels in postmenopausal women compared to premenopausal women.

2-To study correlation between menstrual status, number of birth and the blood levels of calcium and phosphorus in postmenopausal women.

3-To determine relationship between age and the blood levels of calcium and phosphorus in the study group.

CHAPTER TWO

Literature review

2. Literature review

2.1 Menopause:

Menopause, also known as the climacteric, is the time in most women's lives when menstrual periods stop permanently, and they are no longer able to bear children (Melby, Lock and Kaufert, 2005). Medical professionals often define menopause as having occurred when a woman has not had any vaginal bleeding for a year (O'Connor, 2009). It may also be defined by a decrease in hormone production by the ovaries (Sievert and Lynnette, 2006). In those who have had surgery to remove their uterus but they still have ovaries, menopause may be viewed to have occurred at the time of the surgery or when their hormone levels fell (Sievert and Lynnette, 2006). Following the removal of the uterus, symptoms typically occur earlier, at an average of 45 years of age (Nanette, 2002).

Before menopause, a woman's periods typically become irregular, which means that periods may be longer or shorter in duration or be lighter or heavier in the amount of flow. During this time, women often experience hot flashes; these typically last from 30 seconds to ten minutes and may be associated with shivering, sweating, and reddening of the skin (Medlineplus, 2011). Hot flashes often stop occurring after a year or two. Other symptoms may include vaginal dryness, trouble sleeping, and mood changes (Medlineplus, 2011). The severity of symptoms varies between women (Melby, Lock and Kaufert, 2005). While menopause is often thought to be linked to an increase in heart disease, this primarily occurs due to increasing age and does not have a direct relationship with menopause. In some women, problems that were present like endometriosis or painful periods will improve after menopause (Melby, Lock and Kaufert, 2005). In younger women, during a normal menstrual cycle the ovaries produce estradiol, testosterone and progesterone in a cyclical pattern under the control of FSH and luteinising hormone (LH) which are both produced by the pituitary gland. During perimenopause (approaching menopause), estradiol levels and patterns of production remain relatively unchanged or may increase compared to young women, but the cycles become frequently shorter or irregular (Prior, 1998). The often observed increase in estrogen is presumed to be in response to elevated

FSH levels that, in turn, is hypothesized to be caused by decreased feedback by inhibin (Burger, 1994). Similarly, decreased inhibin feedback after hysterectomy is hypothesized to contribute to increased ovarian stimulation and earlier menopause (Nehas 2003, Petri 2005).

The menopausal transition is characterized by marked, and often dramatic, variations in FSH and estradiol levels. Because of this, measurements of these hormones are *not* considered to be reliable guides to a woman's exact menopausal status (Burger, 1994).

Menopause occurs because of the sharp decrease of estradiol and progesterone production by the ovaries. After menopause, estrogen continues to be produced mostly by aromatase in fat tissues and is produced in small amounts in many other tissues such as ovaries, bone, blood vessels, and the brain where it acts locally (Simpson and Davis, 2001). The substantial fall in circulating estradiol levels at menopause impacts many tissues, from brain to skin.

In contrast to the sudden fall in estradiol during menopause, the levels of total and free testosterone, as well as dehydroepiandrosterone sulfate (DHEAS) and androstenedione appear to decline more or less steadily with age. An effect of natural menopause on circulating androgen levels has not been observed (Davison *et al*, 2005). Thus specific tissue effects of natural menopause cannot be attributed to loss of androgenic hormone production (Robin *et al*, 2013). Hot flashes and other vasomotor symptoms accompany the menopausal transition. While many sources continue to claim that hot flashes during the menopausal transition are caused by low estrogen levels, in most cases, hot flashes are observed despite elevated estrogen levels. The exact cause of these symptoms is not yet understood, possible factors considered are higher and erratic variation of estradiol level during the cycle, elevated FSH levels which may indicate hypothalamic dysregulation perhaps caused by missing feedback by inhibin. It has been also observed that the vasomotor symptoms differ during early perimenopause and late menopausal transition and it is possible that they are caused by a different mechanism (Prior, 1998).

2.1.1 Causes of menopause:

Age: The typical age of menopause (last period from natural causes) is between 40 and 55 (Minkinet *al*, (1997).

Premature ovarian failure: Premature ovarian failure (POF) is diagnosed or confirmed by high blood levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) on at least three occasions at least four weeks apart. Known causes of premature ovarian failure include autoimmune disorders, thyroid disease, diabetes mellitus, chemotherapy, being a carrier of the fragile X syndrome gene, and radiotherapy (Kalantaridou ,Davis , Nelson,1998).

Surgical menopause: Menopause can be surgically induced by bilateral oophorectomy (removal of ovaries), which is often, but not always, done in conjunction with removal of the Fallopian tubes (salpingo-oophorectomy) and uterus (hysterectomy) (Harlow *et al* ,2012).

2.1.2 Stages of menopause:

2.1.2.1 Premenopause:

Premenopause is a term used to mean the years leading up to the last period, when the levels of reproductive hormones are becoming more variable and lower, and the effects of hormone withdrawal are present (Harlow *et al* , 2012).

2.1.2.2 Perimenopause:

The term "perimenopause", which literally means "around the menopause", refers to the menopause transition years, a time before *and* after the date of the final episode of flow. This transition can last for four to eight years. During perimenopause, estrogen levels average about 20–30% higher than during premenopause, often with wide fluctuations (Prior and Jerilynn, 2013). These fluctuations cause many of the physical changes during perimenopause as well as menopause (Chichester, 2011). Some of these changes are hot flashes, night sweats, difficulty sleeping, vaginal dryness or atrophy, incontinence, osteoporosis, and heart disease. During this period, fertility diminishes but is not considered to reach zero until the official date of menopause. The official date is determined

retroactively, once 12 months have passed after the last appearance of menstrual blood (Prior and Jerilynn, 2013).

The menopause transition typically begins between 40 and 50 years of age (average 47.5) (Hurst and Bradley, 2011), (McNamara, Batur, and DeSapri, 2015). The duration of perimenopause may be for up to eight years (McNamara, Batur, and DeSapri, 2015). Women will often, but not always, start these transitions (perimenopause and menopause) about the same time as their mother did (Kessenich and Cathy, 2013).

2.1.2.3 Postmenopause:

The term "postmenopausal" describes women who have not experienced any menstrual flow for a minimum of 12 months, assuming that they have a uterus and are not pregnant or lactating (Harlow *et al*, 2012).

2.1.3 Management and Treatment:

2.1.3.1 Hormone replacement therapy:

Menopause requires no medical treatment. Instead, treatments focus on relieving your signs and symptoms and preventing or managing chronic conditions that may occur with aging. Treatments may include:

In the context of the menopause, hormone replacement therapy (HRT) is the use of estrogen in women without a uterus and estrogen plus progestin in women who have an intact uterus. HRT may be reasonable for the treatment of menopausal symptoms, such as hot flashes and osteoporosis. Its use appears to increase the risk of strokes the shortest time possible and at the lowest dose possible (Boardman *et al*, 2015). The response to HRT in each postmenopausal woman may not be the same. Genetic polymorphism in estrogen receptors appears to be associated with inter-individual variability in metabolic response to HRT in postmenopausal women (Maryam *et al*, 2011).

It also appears effective for preventing bone loss and osteoporotic fracture (Villiers and Stevenson, 2012) It is often seen as a second line agent for this purpose (Marjoribank *et al*, 2012) There is some concern that this treatment increases the risk of breast cancer (Chlebowski and Anderson, 2015)

Adding testosterone to hormone therapy has a positive effect on sexual function in postmenopausal women, although it may be accompanied by hair growth, acne and a reduction in high-density lipoprotein (HDL) cholesterol (Somboonporn *et al*, 2005). These side effects diverge depending on the doses and methods of using testosterone (Somboonporn *et al*, 2005).

2.1.3.2 Other therapies:

Lack of lubrication is a common problem during and after perimenopause. Vaginal moisturizers can help women with overall dryness, and lubricants can help with lubrication difficulties that may be present during intercourse. It is worth pointing out that moisturizers and lubricants are different products for different issues: some women complain that their genitalia are uncomfortably dry all the time, and they may do better with moisturizers. Those who need only lubricants do well using them only during intercourse.

Low-dose prescription vaginal estrogen products such as estrogen creams are generally a safe way to use estrogen topically, to help vaginal thinning and dryness problems (see vaginal atrophy) while only minimally increasing the levels of estrogen in the bloodstream.

In terms of managing hot flashes, lifestyle measures such as drinking cold liquids, staying in cool rooms, using fans, removing excess clothing, and avoiding hot flash triggers such as hot drinks, spicy foods, etc., may partially supplement (or even obviate) the use of medications for some women.

Individual counseling or support groups can sometimes be helpful to handle sad, depressed, anxious or confused feelings women may be having as they pass through what can be for some a very challenging transition time.

Osteoporosis can be minimized by smoking cessation, adequate vitamin D intake and regular weight-bearing exercise. The bisphosphate drug alendronate may decrease the risk of a fracture, in women that have both bone loss and a previous fracture and less so for those with just osteoporosis (Wells *et al*, 2008).

2.2 Estrogen:

Estrogen (American English) or oestrogen (British English) is the primary female sex hormone as well as a medication. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. *Estrogen* may also refer to any substance, natural or synthetic, that mimics the effects of the natural hormone. The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum level (Files, Ko MG and Pruthi, 2011).

2.2.1 Biological function of estrogen:

The actions of estrogen are mediated by the estrogen receptor (ER), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, estrogen enters passively into the cell where it binds to and activates the estrogen receptor (Lin, 2004). They are:

Structural: Promote formation of female secondary sex characteristics, accelerate metabolism, increase fat store, Stimulate endometrial growth, increase uterine growth, maintenance of vessel and skin, reduce bone resorption, and increase bone formation.

Protein synthesis: Increase hepatic production of binding proteins

Coagulation: Increase circulating level of factors 2, 7, 9, 10, plasminogen, decrease antithrombin III, and increase platelet adhesiveness.

Lipid: Increase HDL, triglyceride, decrease LDL, and fat deposition

Uterus lining: Estrogen together with progesterone promotes and maintains the uterus lining in preparation for implantation of fertilized egg and maintenance of uterus function during gestation period, also upregulates oxytocin receptor in myometrium

Ovulation: Surge in estrogen level induces the release of luteinizing hormone, which then triggers ovulation by releasing the egg from the Graafian follicle in the ovary.

Breast development: Estrogen, in conjunction with growth hormone (GH) and its secretory product insulin-like growth factor 1 (IGF-1), is critical in mediating breast development during puberty, as well as breast maturation during pregnancy in preparation of lactation and breastfeeding. Estrogen is primarily and directly responsible for inducing the ductal component of breast development (Johnson 2003, Norman and Henry 2014, Caod and Dunstall, 2011).

2.3 Osteoporosis:

Is a disease where increased bone weakness increases the risk of a broken bone. It is the most common reason for a broken bone among the elderly. Osteoporosis may be due to lower than normal peak bone mass and greater than normal bone loss. Bone loss increases after menopause due to lower levels of estrogen. Osteoporosis may also occur due to a number of diseases or treatments including alcoholism, anorexia, hyperthyroidism, surgical removal of the ovaries, and kidney disease (WHO, 2014).

The three main mechanisms by which osteoporosis develop are an inadequate peak bone mass (the skeleton develops insufficient mass and strength during growth), excessive bone resorption, and inadequate formation of new bone during remodeling. An interplay of these three mechanisms underlies the development of fragile bone tissue. Hormonal factors strongly determine the rate of bone resorption; lack of estrogen (as a result of menopause for example) increases bone resorption, as well as decreasing the deposition of new bone that normally takes place in weight-bearing bones. The amount of estrogen needed to suppress this process is lower than that normally needed to stimulate the uterus and breast gland. The α -form of the estrogen receptor appears to be the most important in regulating bone turnover (Raisz, 2005). In addition to estrogen, calcium metabolism plays a significant role in bone turnover, and deficiency of

calcium and vitamin D leads to impaired bone deposition; in addition, the parathyroid glands react to low calcium levels by secreting parathyroid hormone (parathyroid hormone, PTH), which increases bone resorption to ensure sufficient calcium in the blood. The role of calcitonin, a hormone generated by the thyroid that increases bone deposition, is less clear and probably not as significant as that of PTH (Raisz, 2005).

2.4 Calcium:

Calcium is the most abundant mineral in the human body. The average adult body contains in total approximately 1 kg, 99% in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22 mmol, of which about 9 mmol is in the plasma (Diem and Lenter, 2013) Approximately 10 mmol of calcium is exchanged between bone and the ECF over a period of twenty-four hours. The concentration of calcium ions inside the cells (in the intracellular fluid) is more than 7,000 times lower than in the blood plasma (i.e. at <0.0002 mmol/L, compared with 1.4 mmol/L in the plasma) (Marshall, 1995).

2.4.1 Function of calcium:

Calcium has several main functions in the body. It readily binds to proteins, particularly those with amino acids whose side chains terminate in carboxyl (-COOH) groups (e.g. glutamate residues). When such binding occurs the electrical charges on the protein chain change, causing the protein's tertiary structure (i.e. 3-dimensional form) to change. Good examples of this are several of the clotting factors in the blood plasma, which are functionless in the absence of calcium ions, but become fully functional on the addition of the correct concentration of calcium salts. The voltage gated sodium ion channels in the cell membranes of nerves and muscle are particularly sensitive to the calcium ion concentration in the plasma (Armstrog and Cota. 1999). Relatively small decreases in the plasma ionized calcium levels (hypocalcemia) cause these channels to leak sodium into the nerve cells or axons, making them hyper-excitabile (positive bathmotropic effect), thus causing spontaneous muscle spasms (tetany) and paraesthesia (the sensation of "pins and needles") of the extremities and round the mouth. When

the plasma ionized calcium rises above normal (hypercalcemia) more calcium is bound to these sodium channels having a negative bathmotropic effect on them, causing lethargy, muscle weakness, anorexia, constipation and labile emotions (Harrison, 2014)

Calcium acts structurally as supporting material in bones as calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$).

Because the intracellular calcium ion concentration is extremely low (see above) the entry of minute quantities of calcium ions from the endoplasmic reticulum or from the extracellular fluids, cause rapid, very marked, and readily reversible changes in the relative concentration of these ions in the cytosol. This can therefore serve as a very effective intracellular signal (or "second messenger") in a variety of circumstances, including muscle contraction, the release of hormones (e.g. insulin from the beta cells in the pancreatic islets) or neurotransmitters (e.g. acetylcholine from pre-synaptic terminals of nerves) and other functions.

In skeletal and heart muscle calcium ions, released from the sarcoplasmic reticulum (the endoplasmic reticulum of striated muscles) binds to the troponin C present on the actin-containing thin filaments of the myofibrils. The troponin's 3D structure changes as a result, causing the tropomyosin to which it is attached to be rolled away from the myosin-binding sites on the actin molecules that form the back-bone of the thin filaments. Myosin can then bind to the exposed myosin-binding sites on the thin filament, to undergo a repeating series of conformational changes called the cross-bridge cycle, for which ATP provides the energy. During the cycle, each myosin protein 'paddles' along the thin actin filament, repeatedly binding to myosin-binding sites along the actin filament, ratcheting and letting go. In effect, the thick filament moves or slides along the thin filament, resulting in muscle contraction. This process is known as the sliding filament model of muscle contraction (Silverthorn and Dee Unglaub 2016, Cooke 2004, Geeves and Michael 2002, Spudich 1989 and Yanagida *et al* 1985).

2.4.2 Normal ranges of calcium:

The plasma total calcium concentration is in the range of 2.2-2.6 mmol/L (9-10.5 mg/dL), and the normal ionized calcium is 1.3-1.5 mmol/L (4.5-5.6 mg/dL). The amount of total calcium in the blood varies with the level of plasma albumin, the most abundant protein in plasma, and therefore the main carrier of protein-bound calcium in the blood. Between 35-50% of the calcium in plasma is protein-bound, and 5-10% is in the form of complexes with organic acids and phosphates. The remainder (50-60%) is ionized (Diem and Lenter, 2013).

2.4.3 Absorption from the intestine:

The normal adult diet contains about 25 mmol of calcium per day. Only about 5 mmol of this is absorbed into the body per day (Barrett, 2016).

Calcium is absorbed across the intestinal epithelial cell's brush border membrane and is immediately bound to calbindin, a vitamin D-dependent calcium-binding protein. Calbindin transfers the calcium directly into the epithelial cell's endoplasmic reticulum, through which the calcium is transferred to the basal membrane on the opposite side of the cell, without entering its cytosol. From there TRPV6 and calcium pumps (PMCA1) actively transport calcium into the body (Balesaria, Sangha and Walters, 2009).

The active absorption of calcium from the gut is regulated by the calcitriol (or 1,25 dihydroxycholecalciferol, or 1,25 dihydroxyvitamin D₃) concentration in the blood. Calcitriol is a cholesterol derivative. Under the influence of ultraviolet light on the skin, cholesterol is converted to previtamin D₃ which spontaneously isomerizes to vitamin D₃ (or cholecalciferol). Under the influence of parathyroid hormone, the kidneys convert cholecalciferol into the active hormone, 1,25 dihydroxycholecalciferol, which acts on the epithelial cells (enterocytes) lining the small intestine to increase the rate of absorption of calcium from the intestinal contents. Low parathyroid hormone levels in the blood (which occur under physiological conditions when the plasma ionized calcium levels are high) inhibit the conversion of cholecalciferol into calcitriol, which in turn inhibits calcium absorption from the gut. The opposite happens when the plasma ionized

calcium levels are low: parathyroid hormone is secreted into the blood and the kidneys convert more cholecalciferol into the active calcitriol, increasing calcium absorption from the gut (Stryer, 2013). Since about 15 mmol of calcium is excreted into the intestine via the bile per day, the total amount of calcium that reaches the duodenum and jejunum each day is about 40 mmol (25 mmol from the diet plus 15 mmol from the bile), of which, on average, 20 mmol is absorbed (back) into the blood. The net result is that about 5 mmol more calcium is absorbed from the gut than is excreted into it via the bile. If there is no active bone building (as in childhood), or increased need for calcium during pregnancy and lactation, the 5 mmol calcium that is absorbed from the gut makes up for urinary losses that are only partially regulated (Barrette *et al*, 2013).

2.4.4 The kidneys:

The kidney filters 250 mmol of calcium ions a day in pro-urine (or glomerular filtrate), and resorbs 245 mmol, leading to a net average loss in the urine of about 5 mmol/d. The quantity of calcium ions excreted in the urine per day is partially under the influence of the plasma parathyroid hormone (PTH) level - high levels of PTH decreasing the rate of calcium ion excretion, and low levels increasing it. The kidney influences the plasma ionized calcium concentration by processing vitamin D₃ into calcitriol, the active form that is most effective in promoting the intestinal absorption of calcium. This conversion of vitamin D₃ into calcitriol is also promoted by high plasma parathyroid hormone levels (Stryer 2013, Tortora and Anagnostakos 2013).

2.4.5 The role of bone:

Although calcium flow to and from the bone is neutral, about 5–10 mmol is turned over a day. Bone serves as an important storage point for calcium, as it contains 99% of the total body calcium. Calcium release from bone is regulated by parathyroid hormone (PTH) in conjunction with calcitriol manufactured in the kidney under the influence of PTH. Calcitonin stimulates incorporation of calcium into bone (Heaney, 2000).

2.4.6 Pathology:

Hypocalcaemia (low blood calcium) and hypercalcaemia (high blood calcium) are both serious medical disorder.

2.4.6.1 Hypocalcaemia:

It is low calcium levels in the blood serum (LeMone, 2015). With levels less than 2.1 mmol/L. Common causes include hypoparathyroidism and vitamin D deficiency (Fong and Khan, 2012). Others causes include kidney failure, pancreatitis, calcium channel blocker overdose, rhabdomyolysis, tumor lysis syndrome, and medications such as bisphosphonates (Soar, 2010).

2.4.6.1.1 Signs and symptoms of hypocalcaemia:

Petechiae which appear as on-off spots, then later become confluent, and appear as purpura (larger bruised areas, usually in dependent regions of the body).

Oral, perioral and acralparesthesias, tingling or 'pins and needles' sensation in and around the mouth and lips, and in the extremities of the hands and feet. This is often the earliest symptom of hypocalcaemia.

Generalized tetany (unrelieved and strong contractions of the hands, and in the large muscles of the rest of the body) are seen.

Latent tetany: Trousseau sign of latent tetany (eliciting carpal spasm by inflating the blood pressure cuff and maintaining the cuff pressure above systolic).

Chvostek's sign (tapping of the inferior portion of the cheekbone will produce facial spasms).

Effects on cardiac output: Negative chronotropic effect, or a decrease in heart rate and negative inotropic effect, or a decrease in contractility (Durlach, 1997).

2.4.6.1.2 Diagnosis and treatment of hypocalcemia:

The calcium deficiency disease is suggested if calcium level is below 8.8 mg/dL. Because a significant portion of calcium is bound to albumin, any alteration in the level of albumin will affect the measured level of calcium. A corrected calcium

level based on the albumin level is: Corrected calcium (mg/dL) = measured total Ca (mg/dL) + 0.8 * (4.0 - serum albumin [g/dL]) (Lippincott and Williams, 2006).

Intravenous calcium gluconate 10% can be administered, or if the hypocalcaemia is severe, calcium chloride is given instead. This is only appropriate if the hypocalcaemia is acute and has occurred over a relatively short time frame. But if the hypocalcaemia has been severe and chronic, then this regimen can be fatal, because there is a degree of acclimatization that occurs (LeMone, 2015)

2.4.6.2 Hypercalcaemia:

It is a high calcium (Ca^{2+}) level in the blood serum. with levels greater than 2.6 mmol/L. Most cases are due to primary hyperparathyroidism or cancer (Minisola *et al* ,2015). Other causes include sarcoidosis, tuberculosis, Paget disease, multiple endocrine neoplasia (MEN), vitamin D toxicity, familial hypocalciurichypercalcaemia, and certain medications such as lithium and hydrochlorothiazide (Minisola *et al* ,2015, Soar *et al*, 2010)

2.4.6.2.1 Sign and Symptoms of hypercalcemia:

Stones (renal or biliary).

Bones (bone pain).

Groans (abdominal pain, nausea and vomiting).

Thrones (polyuria) resulting in dehydration.

Psychiatric overtones (Depression 30–40%, anxiety, cognitive dysfunction, insomnia, coma).

Other symptoms can include fatigue, anorexia, and pancreatitis. Limbus sign seen in eye due to hypercalcemia.

2.4.6.2.2 Diagnosis and Treatment of hypercalcemia:

Abnormal heart rhythms can also result, and ECG findings of a short QT interval suggest hypercalcaemia. Significant hypercalcaemia can cause ECG changes mimicking an acute myocardial infarction (Wesson, Suresh and Parry, 2009). Hypercalcaemia has also been known to cause an ECG finding mimicking hypothermia, known as an Osborn wave (Serafi, Vlieg and Taremi, 2011).

Fluids and diuretics, hydration by increasing salt intake, and forced diuresis. Calcitonin blocks bone resorption and also increases urinary calcium excretion by inhibiting renal calcium reabsorption.

2.5 Phosphate:

Phosphorus is the sixth most abundant element in the human body. A highly reactive substance, it occurs in nature, including in the human body, as phosphate.

2.5.1 Function of phosphate:

Phosphate is critical for a vast array of cellular processes. In addition to providing mineral strength to bone, it is an integral component of the nucleic acids that make up deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The phosphate bonds of adenosine triphosphate (ATP) carry the energy required for all cellular functions.

The addition and deletion of phosphate groups to enzymes and proteins are common mechanisms for the regulation of their activity. Phosphate also functions as a buffer in bone, serum, and urine. In view of the sheer breadth of influence of phosphorus, phosphate homeostasis (as depicted in the image below) is understandably a highly regulated process (Priest *et al*, 2002).

2.5.2 Normal range of phosphate:

Levels are expressed in terms of serum phosphorus mass (mg/dL). One mg/dL of phosphorus is equal to 0.32 mmol of phosphate. The normal adult range for

phosphorus is 2.5-4.5 mg/dL (0.81-1.45 mmol/L). Levels are 50% higher in infants and 30% higher in children, because of growth hormone effects (Priest *et al*, 2002).

2.5.3 Regulation of Phosphate metabolism:

Approximately 60-70% of dietary phosphate, 1000-1500 mg/day, is absorbed in the small intestine. Although vitamin D can enhance the absorption, especially under conditions of dietary phosphate depletion, intestinal phosphate absorption does not require the presence of active vitamin D. Specifically, high serum phosphate and high dietary phosphate intake do not significantly impair intestinal uptake. The movement of phosphate in and out of bone, the reservoir containing most of the total body phosphate, is generally balanced. Renal excretion of excess dietary phosphate intake ensures maintenance of phosphate homeostasis, maintaining serum phosphate at a level of approximately 3-4 mg/dL in the serum (Segawa *et al*, 2009).

2.5.4 Pathology:

2.5.4.1 Hypophosphatemia:

A low level of blood phosphate is defined as a level below 0.8mmol/L. Significant hypophosphatemia (below 0.4mmol/L). True hypophosphatemia can be induced by decreased net intestinal absorption, increased urinary phosphate excretion, or acute movement of extracellular phosphate into the cells (Kerr, Kindt and Daram, 2009).

2.5.4.1.1 Causes of Hypophosphatemia:

Refeeding syndrome (this causes a demand for phosphate in cells due to the action of hexokinase, an enzyme that attaches phosphate to glucose to begin metabolism of glucose), respiratory alkalosis (any alkalemic condition moves phosphate out of the blood into cells), alcohol abuse and malabsorption (O'Brien *et al*, 2003).

2.5.4.1.2 Sign and symptoms of Hypophosphatemia:

Muscle dysfunction and weakness – This occurs in major muscles, but also may manifest as: diplopia, low cardiac output, dysphagia, and respiratory depression due to respiratory muscle weakness.

Mental status changes – This may range from irritability to gross confusion, delirium, and coma.

White blood cell dysfunction, causing worsening of infections. Instability of cell membranes due to low adenosine triphosphate (ATP) levels.

Large pulp chambers in the teeth (O'Brien *et al*, 2003).

2.5.4.1.3 Diagnosis and Treatment:

The diagnosis of hypophosphatemia is often evident from the history. If, however, the diagnosis is not apparent, then measurement of urinary phosphate excretion should be helpful. Phosphate excretion can be measured either from a 24-hour urine collection or by calculation of the fractional excretion of filtered phosphate (FEPO₄) from a random urine specimen.

Standard intravenous preparations of potassium phosphate are available and are routinely used in malnourished patients and alcoholics. Oral supplementation is also useful where no intravenous treatment is available (Shajahan,2015).

2.5.4.2Hyperphosphatemia:

It is abnormally high serum phosphate levels can result from increased phosphate intake, decreased phosphate excretion, or a disorder that shifts intracellular phosphate to extracellular space. However, even severe hyperphosphatemia is for the most part clinically asymptomatic. Morbidity In patients with this condition is more commonly associated with an underlying disease than with increased phosphate values (Priest *et al*, 2002).

2.5.4.2.1 Signs and symptoms of Hyperphosphatemia:

Although most patients with hyperphosphatemia are asymptomatic, they occasionally report hypocalcemic symptoms, such as muscle cramps, tetany, and perioral numbness or tingling. Other symptoms include bone and joint pain, pruritus, and rash. More commonly, patients report symptoms related to the underlying cause of the hyperphosphatemia. These generally are uremic symptoms, such as the following:

Fatigue, shortness of breath, anorexia, nausea, vomiting and Sleep disturbances (Priest *et al*, 2002).

2.5.4.2.2 Diagnosis:

Results from a full chemistry profile can be used as follows in determining the cause of hyperphosphatemia:

Low serum calcium levels along with high phosphate levels: Observed with renal failure, hypoparathyroidism, and pseudohypoparathyroidism.

Blood urea nitrogen (BUN) and creatinine values: Help to determine whether renal failure is the cause of hyperphosphatemia.

Elevated intact parathyroid hormone (PTH) levels: Higher likelihood in patients with renal failure or pseudohypoparathyroidism.

Relatively low levels of intact PTH and normal renal function: Found in patients with primary or acquired hypoparathyroidism.

High serum calcium and phosphate levels: Observed with vitamin D intoxication and milk-alkali syndrome.

Relatively low levels of intact PTH and high 25 and 1,25 vitamin D: Also seen in vitamin D intoxication.

Low levels of PTH and vitamin D: Seen in milk-alkali syndrome (Priest *et al*, 2002).

2.5.4.2.3 Treatment:

Diagnose and treat the cause.

Limit phosphate intake .

Enhance renal excretion.

2.6 Relationship between calcium and phosphate and menopause:

Estrogen is known to act on osteoblasts and osteoclasts through Estrogen receptors ER α and ER β . Estrogen promotes mineralization by stimulating the action of osteoblasts and inhibiting the action of osteoclasts. It also decreases the reactive oxygen species concentration and decreases the degree of demineralization. So it has role in precipitation of calcium and phosphate in the bones, and controlling their level in the blood (Neale and Roberto, 2006). Onset of natural menopause is associated with various endocrinological changes and alteration in bone and mineral metabolism. Estrogen levels decrease significantly after menopause. Decrease estrogen affects the serum and urinary levels of calcium and phosphate indirectly at various levels. Decreased estrogen alters the intestinal absorption, bone resorption and renal re-absorption of calcium and phosphate. All these changes are gradual after natural onset of menopause (Patricia *et al* 2011).

CHAPTER THREE

Marital & Methods

3. Material and Method

3.1 Material:

3.1.1 Study approach: A quantitative methods used to measure plasma calcium and phosphorous level in Sudanese women in SharqElneel, during period from March to April 2017.

3.1.2 Study design: A quantitative, descriptive, analytical cross sectional and Community based study.

3.1.3 Target population and Study area: The test group compares 50 postmenopausal women with 30 premenopausal women as control group, in SharqElneel locality.

3.1.4 Sample size: A total of fifty postmenopausal women as test group were enrolled in this study in addition to thirty premenopausal women were involved as control group.

3.1.5 Inclusion criteria: Sudanese postmenopausal women and healthy volunteer were included.

3.1.6 Exclusion criteria: Postmenopausal women with hypertension, thyroid, muscle diseases were excluded, and premenopausal women who take contraceptive agent were excluded.

3.1.7 Ethical consideration: The aim and benefits of this study were explained to participants and verbal consent was obtained from each participant.

3.1.8 Data collection:

Interview a questionnaire: was used for each participant in this study to obtain the clinical data.

3.1.9 Blood sample collection:

Local antiseptic 70% ethanol was used to clean the skin. venous blood (2ml) were taken from each participant by standard procedure in heparin anticoagulant container, and then centrifuged at 3000 rpm for 3 minutes and plasma obtained for calcium and phosphate, the plasma was separated in Eppendorf tubes and kept in refrigerator(20°C) until used.

3.2 Methods:

3.2.1 Measurement of blood calcium:

3.2.1.1 Biochemical measurement and instruments used:

Biosystem-semi automated was used for estimation of calcium.

3.2.1.2 Reagent composition: preparation of working reagent and assay condition for measurement of plasma calcium.

3.2.1.3 Procedure: The reagents were first brought to room temperature, then following amounts were pipette according to the table:

	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Calcium STD(10mg\dl)	–	0.010ml	-
Sample	–	–	0.010ml
Distilled water	0.010ml	–	–

Mixed well thoroughly and the tubes incubated for 10 minutes at room temperature.

The absorbance (A) of standard and sample was recorded at 600nm against the blank.

3.2.1.4 Calculation:The calcium concentration in sample was calculated using formula:

$$\text{Calcium(mmol/L)}: = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc of standard}$$

3.2.2 Measurement of plasma phosphate:

3.2.2.1 Biochemical measurement and instruments used:

Selectra-semi automated was used for estimation of phosphate.

(For principle and procedure see appendix).

3.2.3 Quality control:The precision and accuracy of all methods in this study were checked each time batch was analyzed by including commercially prepared sera.

3.2.4 Statistical analysis: Statistical package social science (SPSS version 18) software was used for data analysis. The mean and standard deviation of plasma level of calcium and phosphate were calculated, and Independent t-test was used for comparison (significant level was set at $p < 0.05$). Linear regression analysis was used to assess correlation between the age and number of births and the plasma level of calcium and phosphate, and the result were presented in the form of tables and figures.

CHAPTER FOUR

Results

4.Results

The blood premenopausal levels of calcium and phosphate were estimated in two groups of women after and before menopause, age, number of birth and duration of menstruation were taken in consideration; to find out if they effect on the levels of calcium and phosphate.

Table (4.1) shows the demographic data of postmenopausal women, and women, age number of birth and duration of menstruation (mean± SD).

The level of biochemical parameter of plasma Calcium and phosphate in postmenopausal women and also compared with premenopausal women, the results were presented as follow:

Table (4.2) represents the mean of the plasma levels of calcium and phosphate (mmol\L) in postmenopausal women and control subjects, there are significant increase in postmenopausal women compared to premenopausal women.

Calcium (mean±SD:2.5 ±0.30mmol\L, versus2.22±0.0.23mmol\L. p.value=0.00. Phosphate (mean±SD: 1.12± 0.23mmol\L, versus 0.93±0.16mmol\L. P. value =0.000).

Figure (4.1) show the correlation between calcium level (mmol\L) and age among postmenopausal women. Scatter plot shows: $r= 0.001$ and $p. value=0.79$ (insignificant positive correlation).

Figure (4.2) shows correlation between calcium level (mmol\L) and numbers of birth among postmenopausal women. Scatter shows: $r=0.051$ and $p. value = 0.1$ (insignificant positive correlation).

Figure (4.3) show correlation between calcium level (mmol\L) and duration of duration of menstruation among postmenopausal women. Scatter plot shows: $r= 0.003$ and $p.value = 0.7$ (insignificant positivecorrelation).

Figure (4.4) shows the correlation between phosphate level (mmol\L) and age among postmenopausal women. Scatter plot shows: $r=0.067$ and $p. value =0.070$ (insignificant positivecorrelation).

Figure (4.5) show the correlation between phosphate level (mmol\L) and number of birth among postmenopausal women. Scatter plot shows: $r=0.044$ and $p. value=0.14$ (insignificant positive correlation).

Figure (4.6) show the correlation between phosphate level (mmol/L) and duration of menstruation among postmenopausal women. Scatter plot shows: $r=0.07$ and $p.value = 0.06$ (insignificant positive correlation).

Table (4.3): represents the correlation between age, number of birth and the plasma levels of calcium and phosphate (mmol/L) in premenopausal women.

Table (4.1): Demographic Data of two groups:

	Postmenopausal Women	Premenopausal women
Age in years (mean \pm SD)	62.78 \pm 9.8	29.17 \pm 8
Number of birth (mean \pm SD)	5.34 \pm 2.8	1.57 \pm 2.03
Durationof menstruation in years (mean \pm SD)	48.4 \pm 10.3	14.93 \pm 7.8

*Age, number of birth and duration of menstruation given in mean \pm SD.

Table (4.2): The mean of calcium and phosphate levels (mmol/L) in Sudanese postmenopausal and premenopausal women:

Study group	Mean \pm SD	P. value
Calcium (mmol/L)		
Postmenopausal (50)	2.5 \pm 0.30	0.00
Premenopausal (30)	2.22 \pm 0.23	
Phosphate (mmol/L)		
Postmenopausal (50)	1.12 \pm 0.23	0.00
Premenopausal (30)	0.93 \pm 0.16	

*Results given in mean \pm SD, P. value \leq 0.05 considered significant.

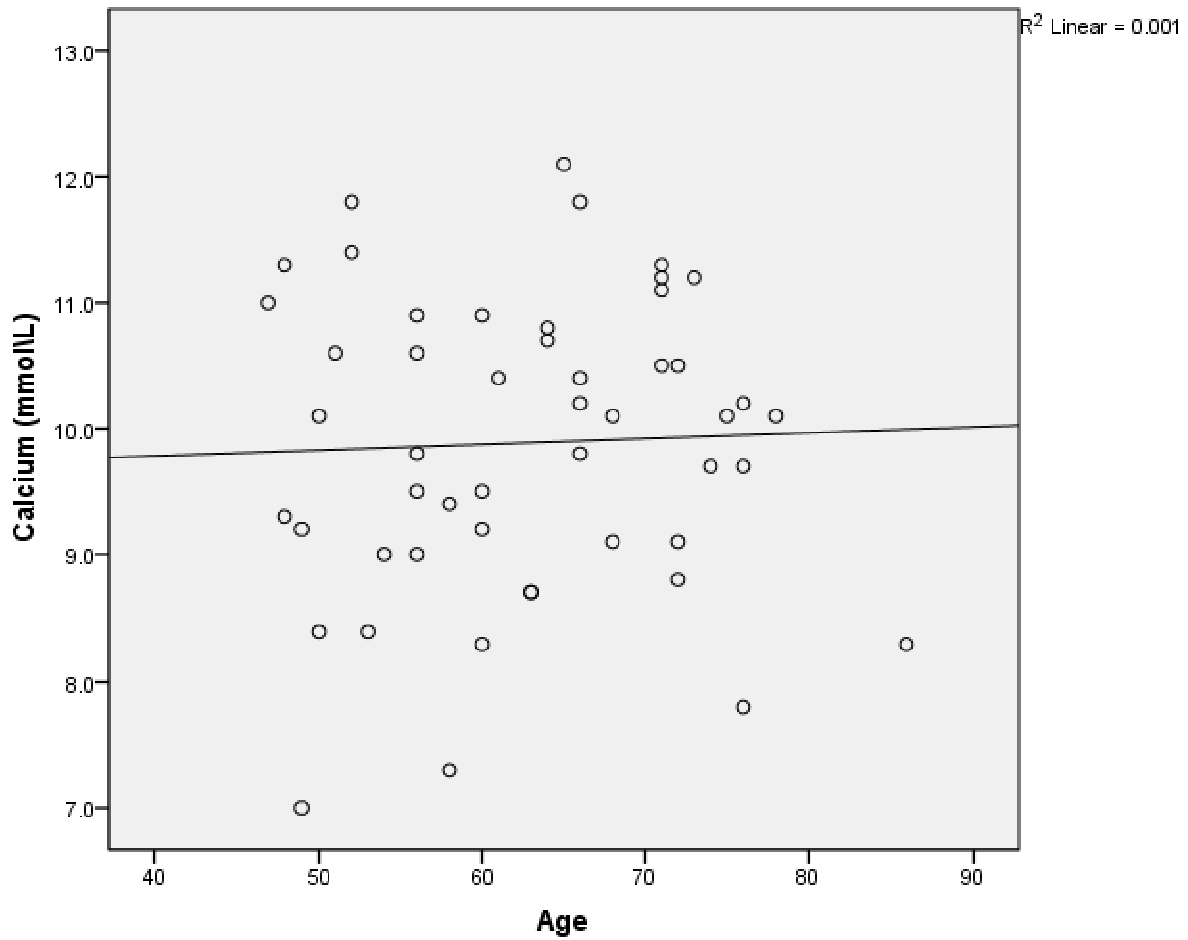


Figure (4.1): scatter plot of calcium level and age among postmenopausal women.

P.value = 0.79

$r = 0.001$

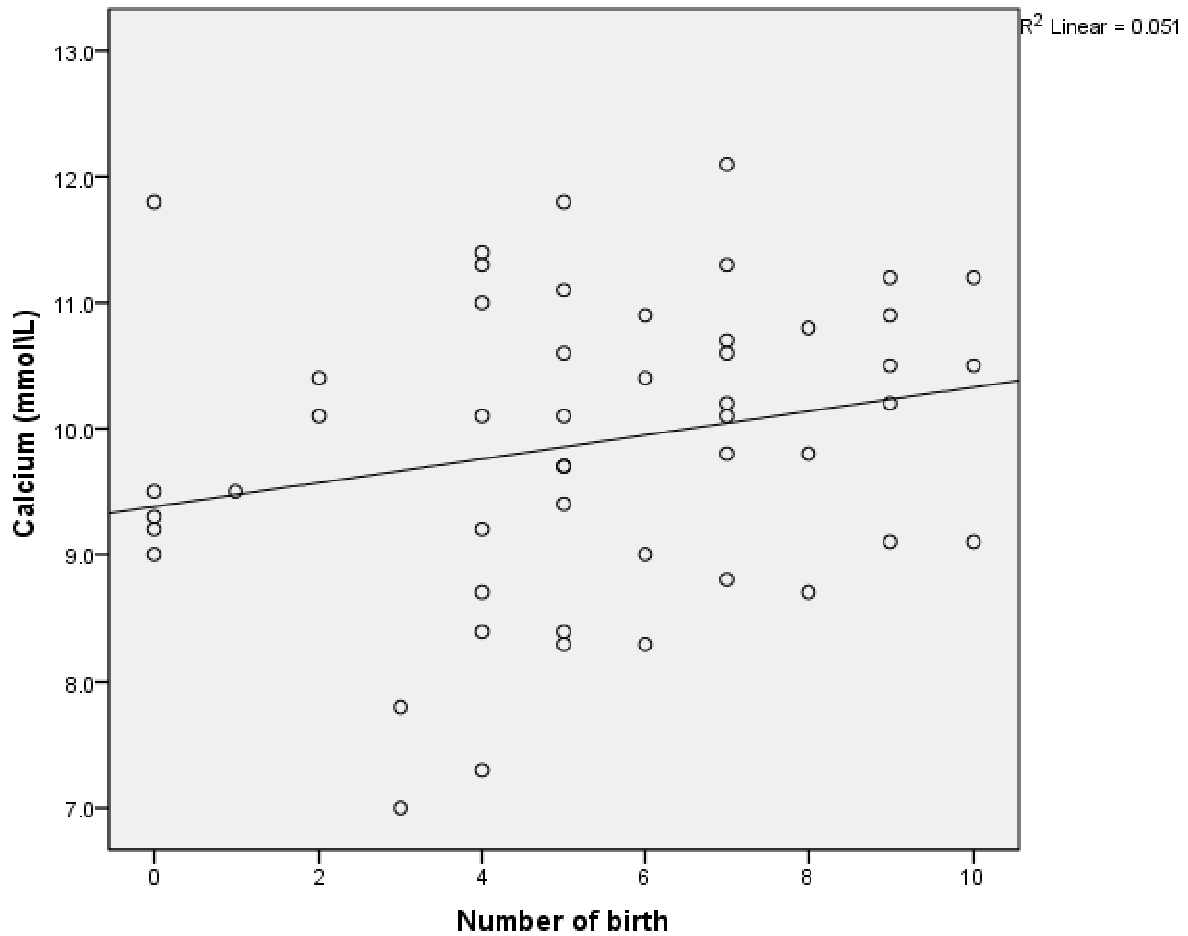


Figure (4.2): Scatter plot of calcium level and number of birth in postmenopausal women.

P.value = 0.115

r = 0.051

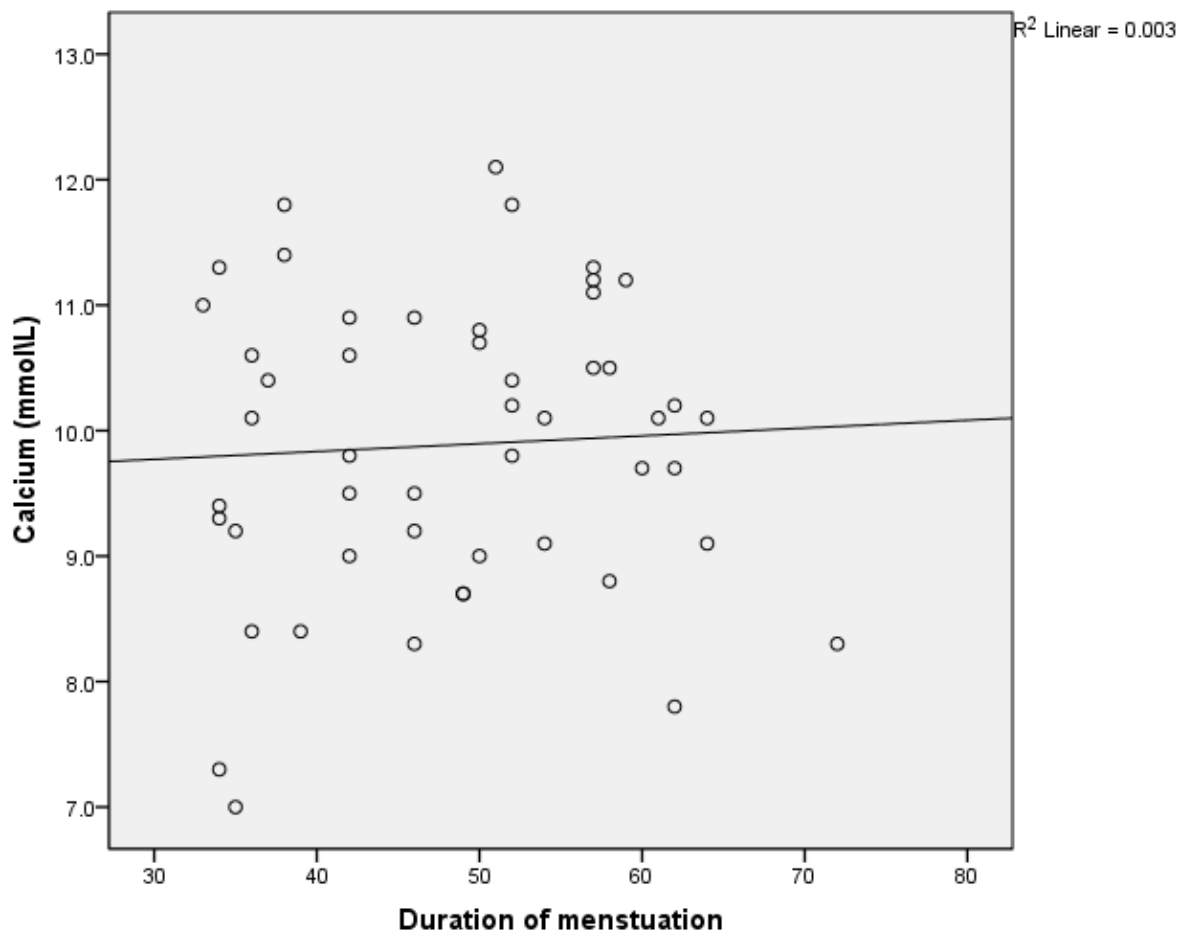


Figure (4.3): Scatter plot of calcium and duration of menstruation postmenopausal women. P.value = 0.71 r = 0.003

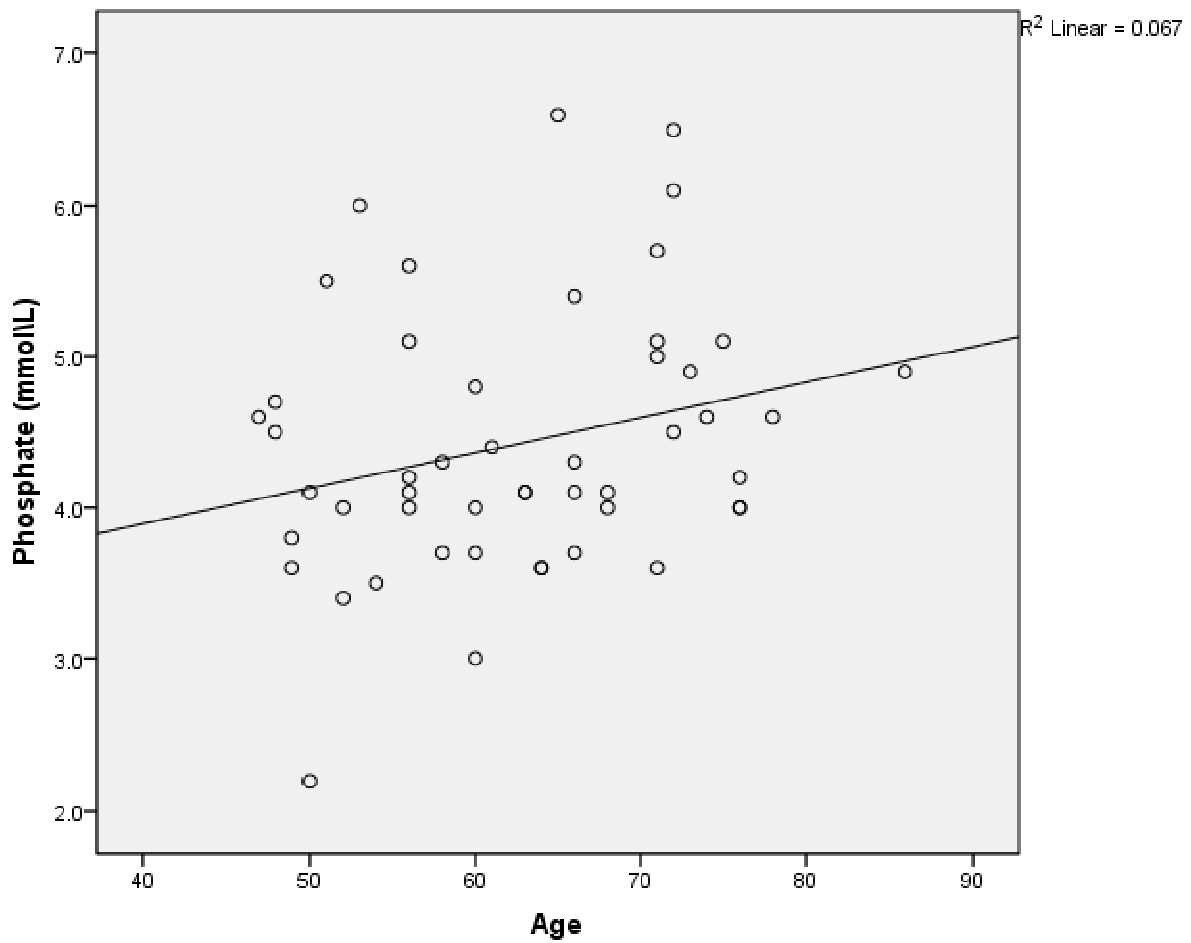


Figure (4.4): Scatter plot of Phosphate and age in postmenopausal women.

P.value = 0.070

r = 0.067

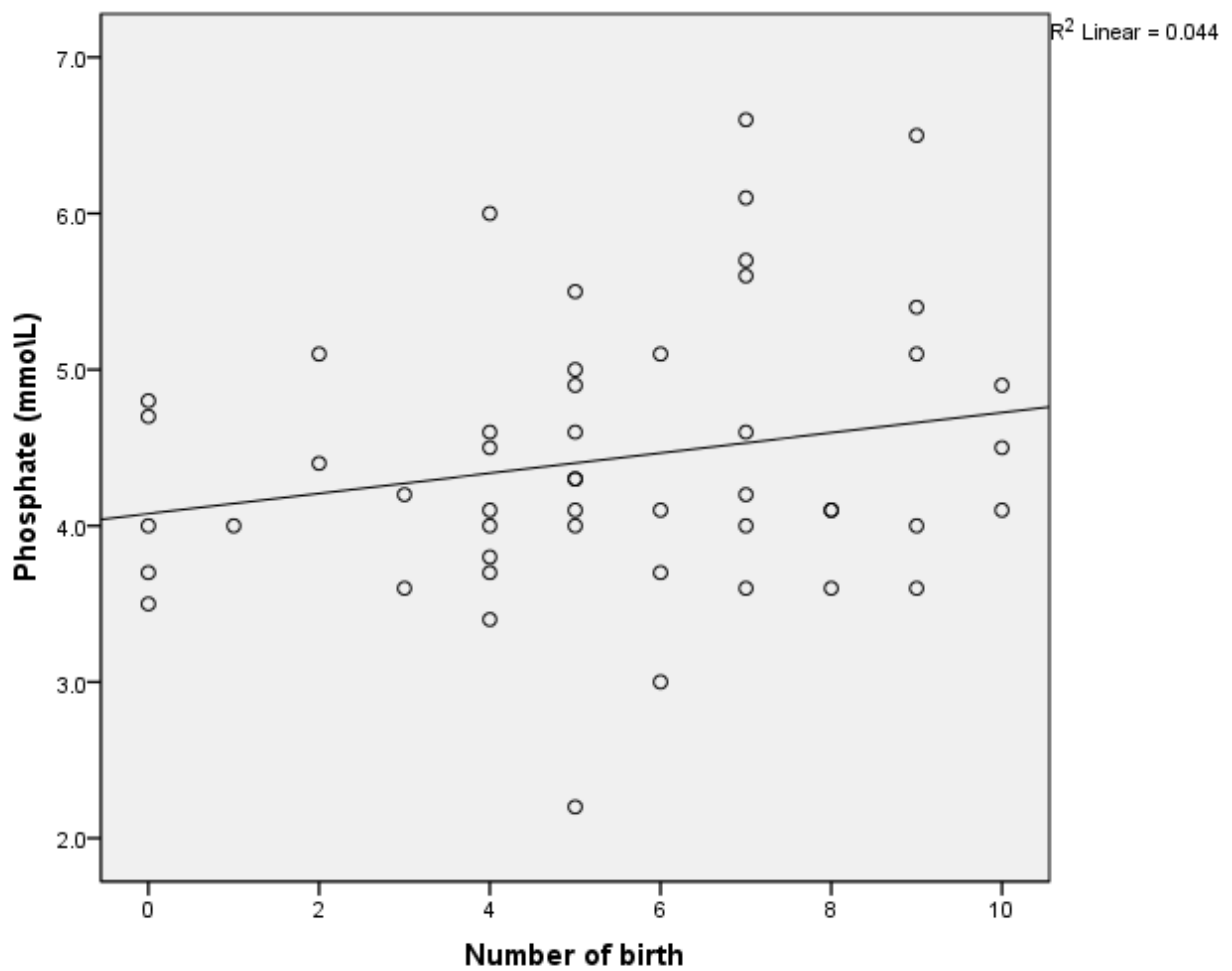


Figure (4.5): Scatter plot of phosphate level and number of birth in postmenopausal women. P.value = 0.146 r = 0.004

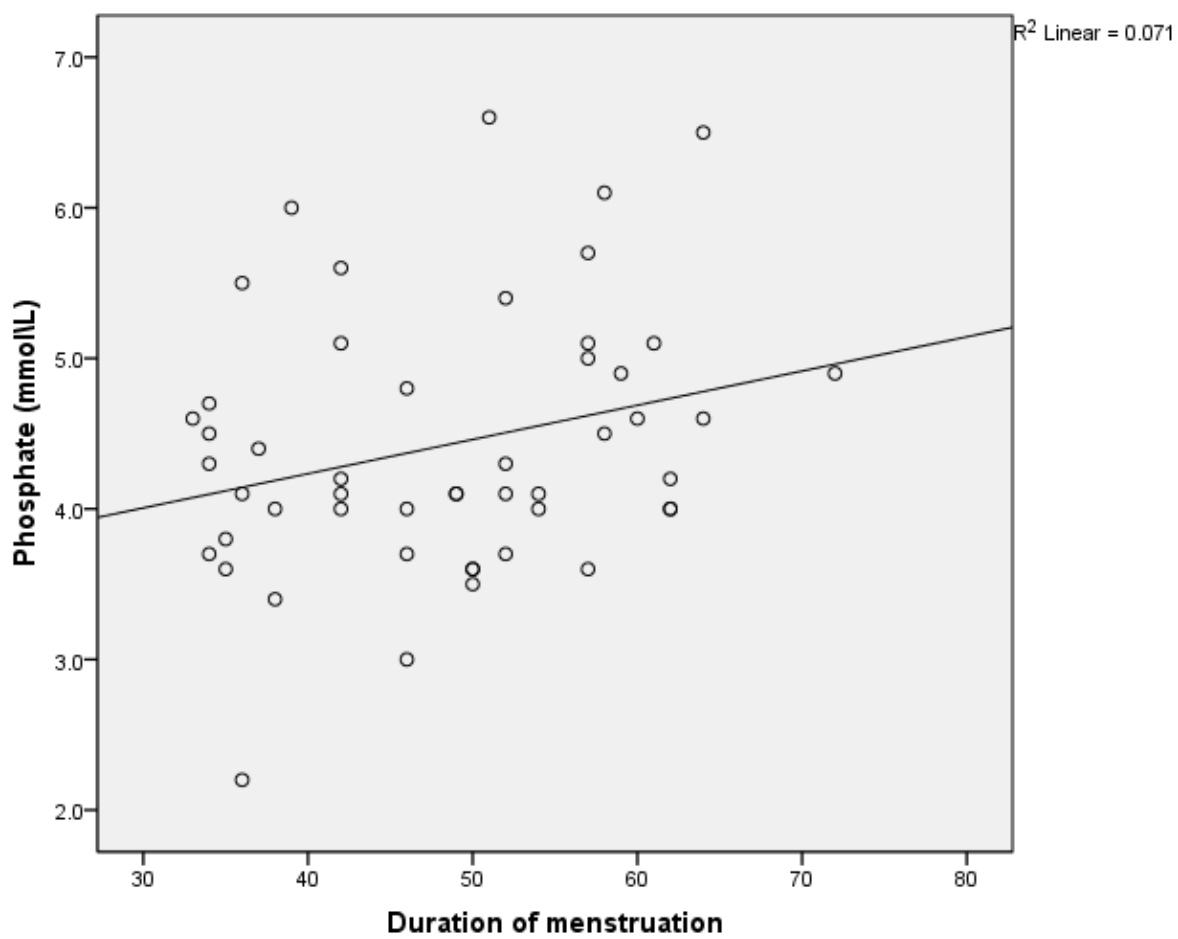


Figure (4.6): Scatter plot of phosphate and duration of menstruation postmenopausal women.

P.value = 0.06

r = 0.071

Table (4.3): Correlations between variables in premenopausal women:

Correlation between variables in premenopausal women.	P. value	R.value
Age \ Ca	0.0117	0.127
Number of birth \ Ca	0.053	0.127
Menstrual duration \ Ca	0.16	0.086
Age \ Phosphate	0.55	0.013
Number of birth \ Phosphate	0.6	0.008
Menstrual duration \ Phosphate	0.5	0.013

CHAPTER FIVE

Discussion, Conclusion & Recommendation

5. Discussion, Conclusion and Recommendation

5.1 Discussion:

Onset of natural menopause is associated with various endocrinological changes and alteration in bone and mineral metabolism (Patricia *et al.*, 2011). Estrogen level decrease significantly after menopause. Decrease estrogen affects the serum levels of calcium and phosphate indirectly at various levels. Decreased estrogen alters the intestinal absorption, bone resorption and renal re-absorption of calcium, magnesium and phosphate (Arthur, 2012).

Calcium and phosphate status were evaluated in postmenopausal and premenopausal women in the present study. The results obtained from this study indicate that, there are significant increases in calcium and phosphate levels in the blood of postmenopausal women, when they are compared to premenopausal women.

This study result agrees with result carried in India by (Sonuet *al*, 2015), Which found that the phosphate level increased in postmenopausal women, but the calcium is decreased. Observed higher serum phosphate levels could be due to increased demineralization observed in postmenopausal women secondary to decreased estrogen.

This result is in agreement with finding done by (Christopher, 1990) in Australia, which showed there was significantly increasing in the level of plasma calcium and phosphate of postmenopausal women compared to premenopausal women. The cause of these results is due to a rise in the ultrafiltrable fraction, which in turn was accounted for by rises in the ionized and complexed fractions, of which the complexed fraction was the most significant and proportionately the largest. The rise in the complexed fraction was accounted for by the increase in plasma bicarbonate.

The result obtained agrees with another result, which found that, the decline in estrogen secretion result in increasing of the calcium and phosphate levels, the study indicate that the decline in estrogen secretion results in hypercalcemia and hyperphosphatemia (Nihon, 1975).

The result agreed with previous study carried out in India, which said that, the phosphate level was significantly increased in postmenopausal women when it is compared to premenopausal women (Sonuet *al.*, 2015).

The result of this study disagrees with result of (Qureshiet *al.*, 2010), which found that Serum calcium levels were significantly lower in postmenopausal women than in pre-menopausal women, this may be due to that postmenopausal women have increased bone turnover as indicated by increased serum parathyroid hormone levels.

It also disagrees with (Dhananjay and Hina 2014) in which Serum calcium was significantly decreased in postmenopausal women as compared to that in premenopausal women, this may be due to estrogen deficiency which may induce calcium loss due to decreased intestinal calcium absorption and decreased renal calcium conservation.

The finding of this study disagrees with result obtained by the same authors in the same year (Sonuet *al.*, 2015), in which the calcium level is significantly decreased.

The result also agrees with the finding with another study which found that the calcium level is increased after menopause (Lori and Bess, 1989).

The result obtained from this study indicates that, there are insignificant positive correlation between age, number of birth and duration of menstruation and calcium and phosphate levels in postmenopausal women.

In group of premenopausal women there is only significant positive correlation between age and calcium level, and between duration of menstruation and phosphate level, while there is insignificant negative correlation between age and number of birth and phosphate level.

5.2 Conclusion:

Result and finding of this study, concluded that:

- 1 Plasma calcium and phosphate levels are significantly increased in postmenopausal women.
- 2 Plasma calcium and phosphate levels are not affected by age, and by duration of menstruation.
- 3 Plasma calcium is affected by the number of birth, while Plasma phosphate level is not affected by the number of birth.

5.3 Recommendation:

- 1 Calcium and phosphate level should be regularly monitored in the blood of postmenopausal women.
- 2 Health education for the community to understand that the menopause is not a medical condition, but a life transition or a symbol of aging.
- 3 More extensive investigation should be done to monitor the bone diseases in postmenopausal women such as Bone density test.

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Appendix:

1. Questionnaire:

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Sudan University for Science and Technology

College of Graduate Studies

**Questionnaires on premenopausal and
postmenopausal women**

Name:.....

Telephone NO:.....

Age:.....Years

Tribe :.....

Use of contraceptive agent : Yes () No ()

Do you have a : answer with **Yes** or **No** :

Kidney disease:

Hypertension:

Malignancy:

Thyroid diseases:

Bone disease :

Fractures:

Result :

Calcium level :mmol/L	Phosphorus level :..... mmol/L
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Appendix 2:

MR.PHOSPHORUS DIAGNOSTIC KIT FOR DETERMINATION OF INORGANIC PHOSPHORUS CONCENTRATION

Kit name Kit size Cat. No

MR.Phosphorus mini 2 x 25 ml GB22MR

MR.Phosphorus 100 2 x 50 ml GB23MR

REAGENTS

Package

MR.PhosphorusMR.Phosphorus mini 100

R1-Phosphorus 2 x 25 ml 2 x 50 ml

R2-STANDARD 1 vial 1 vial

R2-STANDARD is phosphorus ions standard solution: Refer standard value mentioned in the vial.

Working reagent preparation and stability

The reagent is ready to use.

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light, avoid contamination!

Concentrations in the test

0.4 mmol/l

100 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- Reagent 1-MG is classified as an irritant!

MR.Phosphorus page 1

MR.Phosphorus page 1

METHOD PRINCIPLE

Direct phosphomolybdate reaction without deproteinization.
Phosphate ions form with molybdate ions in acid solution
proportional amounts of unreduced phosphomolybdate complex. The
concentration of the complex formed is determined by measuring its
absorbance $\lambda=340$ nm

ammonium molybdate

sulphuric acid

hydrochloric acid 100 mmol/l

Contaminated glassware is the greatest source of error.

Disposable plastic ware is recommended for the test.

The reagent is usable when its absorbance is less than 0.350

(read against distilled water, wavelength $\lambda=340$ nm, cuvette $l=1$
cm, at temp. 25°C).

Reagent 1-PHOSPHORUS is classified as an irritant!

Ingredients: sulphuric acid;

Xi – Irritant.

R 36/38: Irritating to eyes and skin.

S 26-28-30-45: In case of contact with eyes, rinse
immediately with plenty of water and seek medical advice. After
contact with skin, wash immediately with plenty of water. Never add
water to this product. In case of accident or if you feel unwell, seek
medical advice immediately (show the label where possible).

ADDITIONAL EQUIPMENT

automatic analyzer or photometer able to read at 340 nm (Hg 365 nm,
334 nm);

thermostat at 37°C ;

general laboratory equipment;

SPECIMEN

Serum, heparinized plasma (recommended: heparine lithium, sodium
or ammonium salt) free from hemolysis, 24-hours urine.

Serum is the preferred specimen! Level of inorganic phosphate in
heparinized plasma is about 0.2 to 0.3 mg/dl (0.06 – 0.10 mmol/l)
lower than in serum.

Serum should be separated from red blood cells as soon as possible after blood collection, because erythrocytes contain several times higher phosphate concentration than normal serum.

Urine preparation: to prevent phosphate precipitation in urine, specimens should be collected in HCl, 20-30 ml of 6 mol/l for 24-h specimen. Then dilute 1 part of acidified urine with 10 parts of distilled water. Multiply the result by the dilution factor.

Serum and plasma can be stored up to 7 days at 2-8°C. For longer storage samples should be frozen at -20°C.

24-hours urine samples can be stored up to 7 days at 2-8°C.

Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength 340 nm (Hg 365 nm, 334 nm)

temperature 20-25°C / 37°C

cuvette 1 cm

Mix well, incubate for 5 min. at the determination temperature. Read the absorbance of test A(T) and standard A(S) against blank(B). The absorbance is stable within 60 minutes.

Calculation

phosphorus concentration = $A(T) / A(S) \times \text{standard concentration}$

0.87 – 1.45

2.3 – 3.7 0.74 – 1.20

2.8 – 4.1 0.90 – 1.32

0.4 – 1.3 12.9 – 42.0

Pipette into the cuvette:

blank test standard

(B) (T) (S)

R1-Phosphorus 1000 µl 1000 µl 1000 µl

Bring up to the temperature of determination. Then add:

standard - - 10 μ l

sample - 10 μ l -

distilled water 10 μ l - -

REFERENCE VALUES

serum / plasma mg/dl mmol/l

Age 0 - 10 days 4.5 - 9.0 1.45 - 2.91

10 d - 24 months 4.5 - 6.7 1.45 - 2.16

24 mon - 2 years 4.5 – 5.5 1.45 - 1.78

12 -20 years 2.7 – 4.5

> 60 years male

> 60 years female

24-hours urine: mg/24h mmol/24h

It is recommended for each laboratory to establish its own reference ranges for local population.

Corporate Office :

No13, Dhandapani Street, Radha Nagar,

Chrompet Chennai - 600 044.

PH:044 - 4557 4989,

Works : No.87, Gandhi Salai,

Alapakkam, Chennai - 600 063.

e-mail:genuinebiosystem@gmail.com

website;www.genuinebiosystem.com

SYSTEM PARAMETERS

Method : Endpoint

Wavelength : 340 nm

Zero Setting : Reagent Blank

Temperature Setting : 25°C/ 37°C

Incubation Temperature : 37°C

Incubation Time : 5 mins

Delay time : ----

Read time : ----

No. of Reading : ----

Interval time : ----

Sample Volume : 0.01 ml (10 ul)

Reagent Volume : 1.0 ml (1000 ul)

Standard Concentration : Refer Standard vial

Units : mg/dl

Factor : ----

Reaction slope : Increasing

Linearity : 15 mg/dl

QUALITY CONTROL

Sensitivity / Limit of Quantitation: 0.25 mg/dl (0.08 mmol/l).

Linearity: up to 15 mg/dl (4.85 mmol/l). For higher concentration of phosphorus dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

Specificity / Interferences

Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

For Fully Automated analyzers by using multicalibrators or phosphorus standard, the calibration curve can plot and the same should be prepared every 6 weeks or with change of reagent lot number.