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**Evaluation of Calcium and Phosphate Levels among
Hypertensive Vitamin (D) Deficient Patients In Khartoum State**

تقويم مستويات الكالسيوم و الفوسفات بين مرضى إرتفاع ضغط الدم الذين
يعانون من نقص فايتمين (د) في ولاية الخرطوم

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قال تعالى :

وَقُلْ اَعْمَلُوا فَسَيَرَى اللّٰهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ
اِلٰى عَالَمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ

صدق الله العظيم

سورة التوبة الآية 105

Dedication

To my Parents ..

To my brothers and sisters ..

To my teachers and friends ..

Who are always in my heart

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Firstly of all we must be regard our thanks to God for helping us all the way till the completion of this work.

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Abstract

Descriptive cross-sectional hospital based study was conducted in the Nile-east model and Khartoum teaching hospital, Khartoum state during the period from march 2014 to august 2014 . In order to estimate vitamin D, calcium and phosphate levels in Sudanese patients with hypertension. Eighty eight subjects were enrolled in this study, and then classified based on vitamin D results into three groups (group normal vitamin D, group deficient vitamin D and group sever deficient vitamin D). The group of normal vitamin D consider as control group.

Vitamin D was estimated using Euroimmun 25-oH vitamin D competitive ELISA kits, calcium was estimated by spectrophotometric method and phosphate was estimated using phosphomolybdic acid method, and results data obtained were analyzed using (SPSS version 16).

The results showed that female hypertensive patients are more susceptible to vitamin D deficient (81%) than male hypertensive patients (45.7%). There was no significant (p -value >0.05) change in the mean of serum calcium and phosphate level in hypertensive vitamin D deficient patients classified according to gender, age and body mass index. Based on these results, monitoring of serum vitamin D should be useful and recommended measure in hypertensive patients regularly.

Concluded, more studies are needed to evaluate the possible effect of vitamin D, calcium and phosphate levels with hypertension.

المستخلص

أجريت هذه الدراسة بمستشفى الخرطوم ومستشفى شرق النيل بولاية الخرطوم خلال الفترة من مارس الى اغسطس 2014 وذلك بهدف قياس مستوى فيتامين د والكالسيوم و الفوسفات فى مصل مرضى ارتفاع ضغط الدم . تم جمع ثمانية و ثمانين عينة دم من المرضى و قسمت الى ثلاثة مجموعات على حسب نتائج فيتامين د (مجموعة ذات تركيز طبيعي من فيتامين د و مجموعة ذات نقص من فيتامين د و مجموعة ذات نقص حاد من فيتامين د) المجموعة التي تتضمن تركيز طبيعي من فيتامين د أعتبرت كمجموعة ضابطة . قيس معدل فيتامين د باستخدام الطريقة المناعية الانزيمية بينما قيس الكالسيوم بطريقة الطيف الضوئي و قيس معدل الفوسفات باستخدام طريقة الموليبدات الفوسفاتية.

تم جمع النتائج وحللت البيانات احصائيا عن طريق (spss اصدار 16) حيث وجدت الدراسة ان النساء المصابات بارتفاع ضغط الدم تنقص نسبة تركيز فيتامين د بنسبة 81% بصورة أكبر مقارنة بالرجال المصابين بارتفاع ضغط الدم بنسبة تبلغ 45.7% .

لم تكن هناك اي فروق معنوية في متوسط تركيز الكالسيوم و الفوسفات لدى المرضى مقارنة بوزنهم وعمرهم و نوعهم.

استنادا على هذه النتائج أوصت الدراسة بالفحص و المتابعة الدورية لقياس فيتامين د لمرضى ارتفاع ضغط الدم كما أوصت بمزيد من الدراسات فيما يتعلق بفيتامين د و الكالسيوم و الفوسفات وعلاقتهم بارتفاع ضغط الدم .

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Chapter One

Introduction and Literature Review

1 Introduction and Literature Review

1.1 Hypertension

Hypertension (HTN) or high blood pressure is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease (EL-Guindy, 2005). Hypertension is defined as persistent systolic blood pressure (BP) of at least 140 mm Hg and/or diastolic pressure of at least 90 mm Hg, or BP that is controlled to guideline recommended levels using antihypertensive medication (Sobh 2000; Rosendorf, 2005; Bishop *et al.*, 2010).

1.1.1 Epidemiology

Hypertension is an important public health challenge worldwide because of its high prevalence and concomitant increase in risk of disease. In 2005, approximately 75 million people had high BP: 34 million males and 39 million females. Data have established that death from ischemic heart disease and stroke increases progressively and linearly so that for every 20 mm Hg systolic or 10 mm Hg diastolic increase in BP, there is a doubling of mortality from ischemic heart disease and stroke (Bishop *et al.*, 2010). Hypertension was more prevalent in black women than in black men, 35.8 and 30.9% respectively, and in white women than in white men, 30.2 and 27.7%, respectively (Kearney *et al.*, 2004). Earlier studies of hypertension prevalence in the Sudan was estimated at 7.5% (Elzubier *et al.*, 2000).

1.1.2 Classification of hypertension

The classification is based on the mean of two or more properly measured seated blood pressure readings on two or more office visits. Normal blood pressure is defined as levels <120/80 mmHg. Systolic blood pressure of 120–139 mmHg or diastolic blood pressure 80–89 mmHg is classified as prehypertension and these patients are at increased risk for progression to hypertension. Hypertension is divided into two stages. First stage includes patients with systolic blood pressure 140–159 mmHg or diastolic blood pressure 90–99 mmHg, second stage includes patients with systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg (El-Guindy, 2005).

Isolated systolic hypertension is defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure < 90 mmHg. Accelerated hypertension is characterized by markedly elevated blood pressure (diastolic blood pressure usually > 120 mmHg) associated with retinal haemorrhage and exudates (grade 3), if untreated, it commonly progresses to malignant hypertension, which is characterized by papilla edema (grade 4) (El-Guindy, 2005).

1.1.2.1 Essential hypertension

Is systemic hypertension of unknown cause that results from dysregulation of normal homeostatic control mechanisms of blood pressure in the absence of detectable known secondary causes over 95% of all cases of hypertension are in this category? In the mechanisms and theories of essential hypertension primary hypertension tends to cluster in families, but a specific genotype has not been identified. A number of associations have been suggested, but none has been confirmed (Rosendorf, 2005).

1.1.2.2 Secondary hypertension

Secondary hypertension is secondary to many diseases as renal diseases, endocrine diseases, neurological causes and pregnancy induced HTN and other diseases (Chiong et al., 2008). Secondary hypertension symptoms are according to the secondary disease as sleep apnea, cushing's, hyperthyroidism, renal artery stenosis, polycystic kidney disease, adrenal tumors (Hui., 2011).

1.1.3 Complications and target organ damages of hypertension

Vascular Hypertrophy, left Ventricular Hypertrophy, heart Attack and Brain Attack, hypertensive Encephalopathy, hypertension Related Renal Damage, hypertensive Retinopathy, hypertensive emergencies and urgencies (Rosendorf, 2005).

1.1.4 Diagnosis of hypertension

1.1.4.1 Blood pressure measurement

Sitting pressures are usually adequate for routine measurement of blood pressure. Patients should sit quietly with back supported for 5 minutes, with arm bared and supported at the level of the heart in patients aged ≥ 65 years. Ambulatory blood pressure is usually several mmHg lower than office blood pressure (El-Guindy., 2005).

1.1.4.2 Laboratory investigations

Laboratory investigations should be directed at providing evidence of additional risk factors, searching for secondary hypertension and assessing presence or absence of target organ

damage. They include routine tests, recommended tests and specific tests for extended evaluation of hypertensive complications and causes of secondary hypertension (El-Guindy, 2005).

1.1.5 Treatment of hypertension

lifestyle modifications are often the only therapy indicated for patients with relatively mild hypertension and little overall cardiovascular risk, and they are always indicated along with drug therapy for the remainder. Drug therapy should begin if blood pressure remains above the goal of therapy after assiduous application of lifestyle modifications or if the patient starts with a blood pressure so high or cardiovascular risk (Rosendorf, 2005).

1.1.6 Prevention of hypertension

Prevention include, weight control, increased physical activity, limiting dietary sodium to ≤ 2.4 per day (equivalent to 6 g of sodium chloride), Abstention from alcohol and increased dietary potassium (El-Guindy, 2005).

1.2 Vitamin D

The generic term vitamin D designates a group of chemically related compounds that possess antirachitic activity. The two most prominent members of this group are vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) and vitamin D does not meet the classical definition of a vitamin. A more accurate description of vitamin D is that it is a prohormone and thus, vitamin D is metabolized to a biologically active form that functions as a steroid hormone (Zemleni *et al.*, 2007).

1.2.1 Vitamin D structure

Vitamin D refers to a family of structurally related compounds that display anti rachitic activity. Members of the D-family are derived from the cyclopentanoperhydrophenanthrene ring system, which is common to other steroids, such as cholesterol, vitamin D has only three intact rings; the B ring has undergone fission of the 9, 10-carbon bond resulting in the conjugated triene system that is present in all the vitamins (Zemleni *et al.*, 2007).

1.2.2 Vitamin D nomenclature

Vitamin D is named according to the new revised rules of the International Union of Pure and Applied Chemists (IUPAC). Vitamin D is designated seco because its B ring has undergone fission. Asymmetric centers are named using R, S notation and Cahn's rules of priority. The configuration of the double bonds is notated E, Z; E for Trans, Z for cis. The

formal name for vitamin D3 is 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene- 3b-ol and for vitamin D2 it is 9,10-seco (5Z,7E)-5,7,10(19), 21-ergostatetraene-3b-ol (Zempleni *et al.*, 2007).

1.2.3 Chemical properties

Vitamin D3 (C₂₇H₄₄O) Three double bonds; melting point, 848C 858C; Ultra violet (UV) absorption maximum at 264–265 nm with a molar extinction coefficient of 18,300 in alcohol or hexane, insoluble in H₂O; soluble in benzene, chloroform, ethanol, and acetone; unstable in light; will undergo oxidation if exposed to air at 248C for 72 h; best stored at 08C. Vitamin D2 (C₂₈H₄₄O) Four double bonds; melting point, 1218C; UV absorption maximum at 265 nm with a molar extinction coefficient of 19,400 in alcohol or hexane, same solubility and stability properties as D3 (Zempleni *et al.*, 2007).

1.2.4 Isolation of vitamin D metabolites

Since vitamin D is a steroid, it is isolated from tissue by methods that extract total lipids, the technique most frequently used for this extraction is the method of Bligh and Dyer, over the years a wide variety of chromatographic techniques have been used to separate vitamin D and its metabolites. These include paper, thin-layer, column, and gas chromatographic methods (Zempleni *et al.*, 2007).

1.2.5 Physiology of vitamin D

Vitamin D functions through its vitamin D endocrine system, vitamin D3 must be sequentially hydroxylated at the C-25 position and then the C-1 position to generate the steroid hormone, 1a, 25(OH) 2D3, before it can produce any biological effects. The activation of vitamin D2 occurs via the same metabolic pathway as that of vitamin D3, vitamin D2 has only 25%–30% of the biological activity of vitamin D3 (Zempleni *et al.*, 2007).

1.2.6 Absorption of vitamin D

Vitamin D can be obtained from the diet, in which case it is absorbed in the small intestine with the aid of bile salts, the specific mode of vitamin D absorption is via the lymphatic system and its associated chylomicrons, only about 50% of a dose of vitamin D is absorbed. However, considering that sufficient amounts of vitamin D can be produced daily by exposure to sunlight, it is not surprising that the body has not evolved a more efficient mechanism for vitamin D absorption from the diet (Zempleni *et al.*, 2007).

1.2.7 Synthesis of vitamin D

Chemical Synthesis of vitamin D is that vitamin D is derived from cholesterol, the first synthesis of vitamin D resulted from the first chemical synthesis of cholesterol, as a consequence of a hydrogen shift the top panel depicts the dynamic changes occurring within the seco-B conjugated triene framework of the hormone (C5, 6, 7, 8, 9, 10, 19). Photochemical Production of Vitamin D₃ although the body can obtain vitamin D from the diet, the major source of this prohormone can be its production in the skin from 7-dehydrocholesterol. The highest concentrations of 7-dehydrocholesterol are found in the stratum basale and the stratum spinosum (Smith *et al.*, 2004; Zemleni *et al.*, 2007; Nowson *et al.*, 2012).

1.2.8 Transport by vitamin D binding proteins (vitamin DBP)

Vitamin DBP, referred to group-specific component of serum or Gc-globulin, vitamin DBP is the serum protein that serves as the transporter and reservoir for the principal vitamin D metabolites throughout the vitamin D endocrine system, these include 25(OH) D₃, the major circulating metabolite, and the steroid hormone 1 α , 25(OH) 2D₃. DBP binds 88% of the total serum 25(OH) D₃ and 85% of serum 1, 25(OH) 2D₃, yet only 5% of the total circulating DBP actually carries vitamin D metabolites, the concentration of the free hormone may be important in determining the biological activity of the 1 α , 25 (OH) 2D₃ steroid hormones (Zemleni *et al.*, 2007).

1.2.9 Storage of vitamin D

Following intestinal absorption, vitamin D is rapidly taken up by the liver thus blood has the highest concentration of vitamin D when compared with other tissues (Zemleni *et al.*, 2007).

1.2.10 Metabolism of vitamin D

Before vitamin D can exhibit any biological activity, it must first be metabolized to its active forms. 1 α , 25(OH) 2D₃ is the most active metabolite known, but there is evidence that 24, 25(OH) 2D₃ is required for some of the biological responses attributed to vitamin D, vitamin D undergoes its initial transformation with the addition of a hydroxyl group to the 25-carbon to form 25(OH)D₃, the major circulating form of vitamin D, the production of 25(OH) D₃ is catalyzed by the cytochrome P450 enzyme, vitamin D₃ 25-hydroxylase, the kidney is

considered the primary source of circulating $1\alpha,25(\text{OH})_2\text{D}_3$. The major controls on the production of $1\alpha, 25(\text{OH})_2\text{D}_3$ are $1\alpha, 25(\text{OH})_2\text{D}_3$ itself, PTH, and the serum concentrations of calcium and phosphate (Bender *et al.*, 2003; Zemleni *et al.*, 2007).

1.2.11 Catabolism and excretion of vitamin D

The catabolic pathway for vitamin D is obscure, but it is known that the excretion of vitamin D and its metabolites occurs primarily in the feces with the aid of bile salts, very little appears in the urine (Zemleni *et al.*, 2007).

1.2.12 Physiological action of vitamin D

1.2.12.1 Action of vitamin D in endocrine system

The most clearly established effects of vitamin D are to maintain calcium and phosphate homeostasis, and to optimize bone health and muscle function. The hormonal form, $1, 25-(\text{OH})_2\text{D}$, increases active intestinal calcium (and phosphate) absorption, when calcium concentrations decrease below normal, even slightly, coupled to a G protein system, stimulate the secretion of parathyroid hormone. Parathyroid hormone then proceeds to the osteoblasts and to the proximal convoluted tubule cells within seconds. Most importantly, in the convoluted tubule cells that serve as the endocrine gland for the vitamin D hormone, 1 -hydroxylase concentrations are markedly elevated. This signals the vitamin D hormone, which by itself stimulates intestinal absorption of calcium or together with parathyroid hormone, at higher concentrations, stimulates mobilization of bone calcium and renal reabsorption of calcium, the increase in serum calcium concentrations exceeds the set point of the calcium sensing system, shutting down the parathyroid gland-induced cascade of events (Norman, 2008; Katsilambros *et al.*, 2010; Harvey and Ferrier., 2011).

1.2.12.2 Non genomic action of vitamin D

The rapid or non genomic responses mediated by $1\alpha, 25(\text{OH})_2\text{D}_3$ were originally postulated to be mediated through the interaction of $1\alpha, 25(\text{OH})_2\text{D}_3$ with a novel protein receptor located on the external membrane of the cell, this membrane receptor has now been shown to be the classic VDR (heretofore largely found in the nucleus and cytosol) associated with caveolae present in the plasma membrane of a variety of cells (Zemleni *et al.*, 2007).

1.2.12.3 Vitamin D in non-classical system

Nuclear receptors for $1\alpha, 25(\text{OH})_2\text{D}_3$ are found in a variety of tissues and cells not directly involved in calcium homeostasis, thus, the role of the vitamin D endocrine system has

expanded to include a broader range of effects on cell regulation and differentiation, the expression of more than 100 proteins is known to be regulated by $1\alpha,25(\text{OH})_2\text{D}_3$, including several oncogenes by far extending the classical limits of vitamin D actions on calcium homeostasis, the presence of muscle weakness or myopathy during metabolic bone diseases related to vitamin D deficiency (Zempleni *et al.*, 2007).

1.2.12.4 Specific functions of active vitamin D

Active vitamin D ($1\alpha, 25 (\text{OH})_2\text{D}_3$) and minerals metabolism, the classical target tissues for $1\alpha,25(\text{OH})_2\text{D}_3$ are those that are directly involved in the regulation of mineral homeostasis, serum calcium and phosphorous, actions on Intestine, deficiency of vitamin D severely impairs intestinal transport of both calcium and phosphorus, although calcium uptake is usually accompanied by phosphate uptake, the two ions are transported by independent mechanisms, both of which are stimulated by $1, 25(\text{OH})_2\text{D}_3$. Actions on bone, although the most obvious consequence of vitamin D deficiency is decreased mineralization of bone, $1,25(\text{OH})_2\text{D}_3$ apparently does not directly increase bone formation or calcium phosphate deposition in osteoid, actions on kidney, $1, 25(\text{OH})_2\text{D}_3$ increases reabsorption of both calcium and phosphate. PTH secretion is increased in vitamin D deficiency, and hence tubular reabsorption of phosphate is restricted. actions on the parathyroid glands, the chief cells of the parathyroid glands are physiological targets for $1,25(\text{OH})_2\text{D}_3$ and respond to it in a manner that is characteristic of negative feedback. Immunoregulatory Roles of $1\alpha, 25(\text{OH})_2\text{D}_3$, $1\alpha, 25(\text{OH})_2\text{D}_3$ has been shown to affect cells of the immune system in a variety of ways. $1\alpha, 25(\text{OH})_2\text{D}_3$ reduces the proliferation of HL-60 cells and also induces their differentiation to monocytes and macrophages. The actions of $1\alpha, 25(\text{OH})_2\text{D}_3$ on normal monocytes is controversial but it appears that it may enhance monocyte function. $1\alpha, 25(\text{OH})_2\text{D}_3$ appears to reduce levels of HLA-DR and CD4 class II antigens on monocytes or macrophages with no effect on the expression of class I antigens (Zempleni *et al.*, 2007; Harvey and Ferrier., 2011).

1.2.13 Nutritional requirements and recommended dietary allowance of vitamin D

The vitamin D₃ requirement of healthy adults has never been precisely defined. Since vitamin D₃ is produced in the skin on exposure to sunlight and can be retained in vertebrate tissues, humans may not have a requirement for vitamin D when sufficient sunlight is available. The international unit (IU) of vitamin D₃ is defined as “the vitamin D activity of

0.025 mg of the international standard preparation of crystalline vitamin D₃. Thus, 1.0 IU of vitamin D₃ is 0.025 mg (Zempleni *et al.*, 2007).

The adequate intake allowance of vitamin D is 200 IU=day (5 mg=day) for infants, children, adult males, and females (including during pregnancy and lactation) up to age 51. For males and females ages 51–70 or more than 70, the adequate indicated level is set at 400 IU=day (10 mg=day) or 600 IU=day (15 mg=day), respectively (Goodman, 2002; Zempleni *et al.*, 2007).

1.2.14 Food sources of vitamin D

For the most part, vitamin D is present in unfortified foods in only very small and variable quantities. The vitamin D that occurs naturally in unfortified foods is generally derived from animal products. Salt-water fish such as herring, salmon, and sardines contain substantial amounts of vitamin D, and fish-liver oils are extremely rich sources. However, eggs, veal, beef, unfortified milk, and butter supply only small quantities of the vitamin. Plants are extremely poor sources of vitamin D; fruits and nuts contain no vitamin D; and vegetable oils contain only negligible amounts of the provitamin (Zempleni *et al.*, 2007).

1.2.15 Vitamin D deficiency

A deficiency of vitamin D results in inadequate intestinal absorption and renal reabsorption of calcium and phosphate, as a consequence, serum calcium and phosphate levels fall and serum alkaline phosphatase activity increases, in response to these low serum calcium levels, hyperparathyroidism occurs. Increased levels of PTH, along with whatever 1 α , 25(OH) 2D₃ is still present at the onset of the deficiency, result in the demineralization of bone and this ultimately leads to rickets in children and osteomalacia in adults (Zempleni *et al.*, 2007).

1.2.16 Hypervitaminosis D

Excessive amounts of vitamin D are not available from natural sources. However, vitamin D intoxication is a concern in those patients treated with vitamin D or vitamin D analogs for hypoparathyroidism, vitamin D-resistant rickets, renal osteodystrophy, osteoporosis, psoriasis, some cancers, or in those who are taking supplemental vitamins. Hypervitaminosis D is a serious problem as it can result in irreversible calcification of the heart, lungs, kidneys, and other soft tissues (Bender *et al.*, 2003; Zempleni *et al.*, 2007).

1.2.17 Vitamin D and hormone D

The steroid hormone $1\alpha, 25\text{-dihydroxyvitamin D}_3$ [$1\alpha, 25(\text{OH})_2\text{D}_3$] and its receptor, the vitamin D receptor (VDR), has resulted in significant contributions to good bone health in addition to the kidney's endocrine production of circulating $1\alpha, 25(\text{OH})_2\text{D}_3$ a paracrine production of this steroid hormone in extra renal organs. This article identifies the fundamentals of the vitamin D endocrine system, including its potential for contributions to good health (DeLuca, 2004).

1.2.18 Biological mechanisms relating vitamin D with hypertension

1.2.18.1 Vitamin D and the Renin-Angiotensin System (RAS)

Dietary sodium and increased activity of the RAS are known to contribute to hypertension; salt restriction and inhibition of RAS activity reduce blood pressure. Vitamin D as a proximal inhibitor of the RAS may inhibit the RAS by reducing renin gene expression, increasing $1, 25(\text{OH})_2\text{D}$ concentrations were associated with lower plasma renin activity in hypertension, both $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$ were inversely associated with plasma renin and angiotensin II concentrations (Wang, 2009; Vaidya and Forman, 2010).

1.2.18.2 Vitamin D and intracellular calcium homeostasis

Calcium homeostasis has long been linked to blood pressure regulation; however, this concept evolved with the demonstrations that intracellular calcium concentrations were positively associated with blood pressure and that the flux of calcium into vascular smooth muscle cells may be facilitated by $1,25(\text{OH})_2\text{D}$. This suggests that vitamin D may play a role in regulating vascular tone by influencing the concentration of calcium in vascular smooth muscle cells (Vaidya and Forman, 2010).

1.2.18.3 Vitamin D and other vascular mechanisms

In addition to potential effects on the RAS and regulation of vascular smooth muscle contractility, the link between vitamin D and hypertension has also been hypothesized to be mediated by other direct effects on vascular endothelium and smooth muscle. $1, 25(\text{OH})_2\text{D}$ as a vascular protective agent it reduces the deleterious effect of advanced glycation end products on the endothelium, reduces inflammatory and atherosclerotic parameters. $1,25(\text{OH})_2\text{D}$ has been implicated in the growth of vascular myocytes and has been shown to enhance prostacyclin production (possibly via the cyclooxygenase pathway) in cultured vascular smooth muscle cells (Vaidya and Forman., 2010).

1.2.18.4 Secondary hyperparathyroidism

There are also other mechanisms involved in the relationship between blood pressure and vitamin D. Secondary hyperparathyroidism, commonly seen in vitamin D deficiency, could be the reason for hypertension. The mechanism is not completely clear, but it is a well known association that high PTH levels affect vascular smooth muscle cells and increase vascular stiffness and promotes hypertension (Jafari and Paknahad, 2012).

1.3 Calcium

Calcium is the fifth most abundant element in the human body. It is an essential element that is only available to the body through dietary sources (Munro, 2010). The main sources of calcium are: milk, cheese, eggs, fish, green vegetables and fruits (Kamal, 2007). Current dietary calcium recommendations range from 1000 to 1500 mg/d, depending on age. In some individuals, particularly the elderly, calcium supplements may be needed to achieve the recommended dietary calcium intake (Munro, 2010).

1.3.1 Calcium Physiology

Calcium Physiology In 1883, Ringer showed that Ca^{2+} was essential for myocardial contraction. While attempting to study how bound and free forms of Ca^{2+} affected frog heart contraction, McLean and Hastings showed that the ionized/free Ca^{2+} concentration was proportional to the amplitude of frog heart contraction, whereas protein-bound and citrate-bound Ca^{2+} had no effect, from this observation, they developed the first assay for ionized/free Ca^{2+} using isolated frog hearts. Although the method had poor precision by today's standards, the investigators were able to show that blood-ionized Ca^{2+} was closely regulated and had a mean concentration in humans of about (1.18 mmol/L). Because decreased ionized Ca^{2+} impairs myocardial function, it is important to maintain an ionized Ca^{2+} at a near normal concentration during surgery and in critically ill patients. Decreased ionized Ca^{2+} concentrations in blood can cause neuromuscular irritability, which may become clinically apparent as irregular muscle spasms, called tetany (Bishop *et al.*, 2010).

1.3.2 Calcium Regulation

Three hormones, PTH, vitamin D, and calcitonin, are known to regulate serum Ca^{2+} by altering their secretion rate in response to changes in ionized Ca^{2+} . PTH secretion in blood is stimulated by a decrease ionized Ca^{2+} and, conversely, PTH secretion is stopped by an increase in ionized Ca^{2+} . PTH exerts three major effects on both bone and kidney. In the bone, PTH activates a process known as bone resorption, in which activated osteoclasts break down bone and subsequently release Ca^{2+} into the ECF. In the kidneys PTH conserves Ca^{2+} by increasing tubular reabsorption of Ca^{2+} ions, PTH also stimulates renal production of active vitamin D. Vitamin D3, a cholecalciferol, is obtained from the diet or exposure of skin to sunlight. Vitamin D3 is then converted in the liver to 25-hydroxycholecalciferol (25-OH-D3), still an inactive form of vitamin D. In the kidney, 25-OH-D3 is specifically hydroxylated to form 1, 25-dihydroxycholecalciferol (1,25-[OH]₂-D3), the biologically active form. This active form of vitamin D increases Ca^{2+} absorption in the intestine and enhances the effect of PTH on bone resorption. Calcitonin, which originates in the medullary cells of the thyroid gland, is secreted when the concentration of Ca^{2+} in blood increases. Calcitonin exerts its Ca^{2+} -lowering effect by inhibiting the actions of both PTH and vitamin D. Although calcitonin is apparently not secreted during normal regulation of the ionized Ca^{2+} concentration in blood, it is secreted in response to a hypercalcemic stimulus (Bishop *et al.*, 2010).

1.3.3 Calcium Distribution

About 99% of calcium in the body is part of bone. The remaining 1% is mostly in the blood and other ECF. Little is in the cytosol of most cells. In fact, the concentration of ionized calcium in blood is 5,000 to 10,000 times higher than in the cytosol of cardiac or smooth muscle cells. Maintenance of this large gradient is vital to maintain the essential rapid inward flux of calcium (Bishop *et al.*, 2010).

Calcium in blood is distributed among several forms. About 45% circulates as free calcium ions (referred to as ionized calcium), 40% is bound to protein, mostly albumin, and 15% is bound to anions, such as HCO_3 , citrate, PO_4 , and lactate. Clearly, this distribution can change in disease. It is noteworthy that concentrations of citrate, HCO_3 , lactate, PO_4 , and albumin can change dramatically during surgery or critical care. This is why ionized calcium

cannot be reliably calculated from total calcium measurements, especially in acutely ill individuals (Bishop *et al.*, 2010).

1.3.4 Clinical Applications

1.3.4.1 Hypocalcemia and Hypercalcemia

Hypocalcemia and hypercalcemia are terms used clinically to refer to abnormally low and high serum calcium concentrations. It should be noted that, because about one half of serum calcium is protein bound, abnormal serum calcium, as measured by total serum calcium, may occur secondary to disorders of serum proteins rather than as a consequence of changes in ionized calcium (Munro., 2010).

1.3.4.2 Causes of Hypocalcemia

Primary hypoparathyroidism glandular aplasia, destruction, or removal, Hypomagnesemia, Hypermagnesemia, Hypoalbuminemia (total calcium only, ionized not affected by) chronic liver disease, nephritic, syndrome, malnutrition, Acute pancreatitis, Vitamin D deficiency, Renal disease, Rhabdomyolysis and Pseudohypoparathyroidism (Bishop *et al.*, 2010).

1.3.4.3 Causes of Hypercalcemia

Primary hyperparathyroidism adenoma or glandular,hyperplasia,Hyperthyroidism,Benign, Familial hypocalciuria Malignancy, Multiple myeloma, Increased and Prolonged vitamin D, immobilization Thiazide diuretics (Bishop *et al.*, 2010).

1.3.5 Biological mechanisms relating Calcium with hypertension

The free intracellular calcium concentration determines the tension in vascular smooth muscle cells, thereby resulting in peripheral vascular resistance. Calcium has direct effect on peripheral vascular tone. Alternations in intracellular calcium are thought to be involved in the common pathway mediating the secretion and action of many hormones, including the presser action of catecholamines and angiotensin II (Sudhakar,2004) Ionized serum Ca^{2+} is reported to be lower in low-renin hypertensive patients and higher in high-renin hypertensive patients than in normal-renin hypertensives or in normotensives. Plasma renin activity in essential hypertension has a continuous negative correlation with serum Mg and a positive correlation with serum ionized Ca^{2+} . Hence, plasma renin in hypertension may reflect (or contribute to) Ca^{2+} and Mg flux changes across cell membranes (Resnick,1983)Besides these, there are many other factors which are, directly or indirectly, implicated in the pathogenesis of essential hypertension and are influenced by serum calcium level. Endothelial cell dysfunction is one of

them which is accompanied by a decrease in the production and/or the release of nitric oxide and the increase of contracting factors with resultant increase in peripheral vascular resistance (Ramon,1999). Another notable factor which is indirectly involved in the pathogenesis of essential hypertension is altered lipid metabolism in the situation of low serum calcium level or decreased dietary calcium intake. Low calcium diet or low serum calcium stimulates increased production of 1,25-dihydroxyvitamin D which in turn, stimulates adipocyte Ca^{2+} influx and, as a consequence, stimulates lipogenesis, suppresses lipolysis, and increases lipid accumulation; whereas increasing dietary calcium inhibits these effects and markedly accelerates fat loss (Zemel *et al.*, 2004). Many researchers even recommend a regular consumption of the recommended daily levels of dietary calcium to combat with hypertensive disorders (David *et al.*, 1999).found that calcium supplementation reduced blood pressure in hypertensive individuals during chronic nitric oxide synthase inhibition and abrogated the associated impairments in endothelium-dependent and endothelium-independent arterial relaxation. High-calcium diet had been found to enhance the vaso-relaxation in nitric oxide-deficient hypertension (Jolma *et al.*, 2000).

1.4 Phosphate

1.4.1 Phosphate Physiology

Phosphate compounds participate in many of the most important biochemical processes. The genetic materials deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are complex phosphodiester, most coenzymes are esters of phosphoric or pyrophosphoric acid. The most important reservoirs of biochemical energy are ATP, creatine phosphate, and phosphoenolpyruvate. Phosphate deficiency can lead to ATP depletion, which is ultimately responsible for many of the clinical symptoms observed in hypophosphatemia. Alterations in the concentration of 2,3-bisphospho-glycerate (2,3-BPG) in red blood cells affect the affinity of hemoglobin for oxygen, with an increase facilitating the release of oxygen in tissue and a decrease making oxygen bound to hemoglobin less available. By affecting the formation of 2,3-BPG, the concentration of inorganic phosphate indirectly affects the release of oxygen from hemoglobin (Bishop *et al.*, 2010).

1.4.2 Regulation of Phosphate

Phosphate in blood may be absorbed in the intestine from dietary sources, released from cells into blood, and lost from bone. In healthy individuals, all these processes are relatively constant and easily regulated by renal excretion or reabsorption of phosphate. Disturbances to any of these processes can alter phosphate concentrations in the blood; however, the loss of regulation by the kidneys will have the most profound effect. Although other factors, such as vitamin D, calcitonin, growth hormone, and acid-base status, can affect renal regulation of phosphate, the most important factor is PTH, which overall lowers blood concentrations by increasing renal excretion. Vitamin D acts to increase phosphate in the blood. Vitamin D increases phosphate absorption in the intestine and phosphate reabsorption in the kidney. Growth hormone, which helps regulate skeletal growth, can affect circulating concentrations of phosphate. In cases of excessive secretion or administration of growth hormone, phosphate concentrations in the blood may increase because of decreased renal excretion of phosphate (Bishop *et al.*, 2010).

1.4.3 Distribution of Phosphate

Although the concentration of all phosphate compounds in blood is about 12 mg/dL (3.9 mmol/L), most of that is organic phosphate and only about 3 to 4 mg/dL is inorganic phosphate. Phosphate is the predominant intracellular anion, with intracellular concentrations varying, depending on the type of cell. About 80% of the total body pool of phosphate is contained in bone, 20% in soft tissues, and less than 1% is active in the serum/plasma (Bishop *et al.*, 2010).

1.4.4 Clinical Applications

1.4.4.1 Hypophosphatemia

It occurs in about 1% to 5% of hospitalized patients. The incidence of hypophosphatemia increases to 20% to 40% in patients with the following disorders: diabetic ketoacidosis, chronic obstructive pulmonary disease (COPD), asthma, malignancy, long-term treatment with total parenteral nutrition (TPN) (Bishop *et al.*, 2010), inflammatory bowel disease, anorexia nervosa, and alcoholism. The incidence increases to 60% to 80% in ICU patients with sepsis. In addition, hypophosphatemia can also be caused by increased renal excretion, as with hyperparathyroidism, and decreased intestinal absorption, as with vitamin D

deficiency or antacid uses, although most cases are moderate and seldom cause problems and severe hypophosphatemia (1.0 g/dL or 0.3 mmol/L) requires monitoring and possible replacement therapy. There is a 30% mortality rate in those who are severely hypophosphatemic versus a 15% rate in those with normal or mild hypophosphatemia (Bishop *et al.*, 2010).

1.4.4.2 Hyperphosphatemia

Patients at greatest risk for Hyperphosphatemia are those with acute or chronic renal failure. An increased intake of phosphate or increased release of cellular phosphate may also cause Hyperphosphatemia. Because they may not yet have developed mature PTH and vitamin D metabolism, neonates are especially susceptible to Hyperphosphatemia caused by increased intake, such as from cow's milk or laxatives. Increased breakdown of cells can sometimes lead to Hyperphosphatemia, as with severe infections, intensive exercise, neoplastic disorders, or intravascular hemolysis. Because immature lymphoblasts have about four times the phosphate content of mature lymphocytes, patients with lymphoblastic leukemia are especially susceptible to Hyperphosphatemia (Bishop *et al.*, 2010).

Rationale

Hypertension is one of the most serious problems worldwide, and there is strong association between vitamin D, calcium and Phosphate level and Hypertension, which is give a reason to be a target for researchers to find out a new ways for Prognosis and follow up, in the Sudan this is first study to evaluate the effect of Calcium and Phosphate level in Hypertensive vitamin D deficient Patients.

Objectives**General objective**

To evaluate calcium and Phosphate levels among Hypertensive Vitamin D deficient Patients in Khartoum State.

Specific objectives

- 1- To estimate serum Vitamin D, calcium and Phosphate levels in the study group (Hypertensive Vitamin D deficient Patients).
- 2- To compare mean Vitamin D, calcium and Phosphate levels in serum of Hypertensive Patients with risk factors (BMI, age and gender).
- 3- To compare between serum calcium and Phosphate levels and vitamin D status (Deficient, sever deficient and normal vitamin D).

Chapter Two

Materials and Methods

2 Materials and Methods

2.1.1 Study Design

Descriptive cross-sectional study, conducted during the period of March to August 2014.

2.1.2 Study Area

This study was carried out in different hospitals, (Nile east model hospital, and Khartoum teaching hospital) in Khartoum state.

2.1.3 Study Population

Eighty eight hypertensive patients were enrolled in this study, and then classified based on vitamin D results into three groups, group normal vitamin D (>30 ng/ml) considered as control, group two deficient vitamin D ($20 -30$ ng/ml) , group three sever deficient vitamin D (< 20 ng/ml).

2.1.4 Inclusion Criteria

Specimens were collected from hypertensive patients, serum specimens collected from these patients when they were fasting.

2.1.5 Exclusion Criteria

Other diseases like diabetes mellitus, renal diseases and patients under vitamin D supplement are excluded.

2.1.6 Collection of Samples

samples were collected by using dry, plastic syringes, tourniquet was used to make the veins more prominent, blood samples (5ml) was collected in plane containers from each volunteer under septic condition. All blood samples were allowed to clot at room temperature and then they were centrifuged at 4000 rpm to obtain the serum samples, and stored in -20° until the analysis.

2.1.7 Ethical Considerations

Study was approved from ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained and all patients were informed by aims of the study.

2.2 Methods

2.2.1 Vitamin D Estimation

2.2.1.1 Principle

The ELISA kit is designed for the in vitro determination of 25-OH Vitamin D in human serum or plasma samples. In the first analysis step, the calibrators and patient samples are diluted with biotin labeled 25-OH Vitamin D and added to micro plate wells coated with monoclonal anti-25-OH Vitamin D antibodies. During the incubation an unknown amount of 25-OH Vitamin D and known amount of biotin labeled 25-OH Vitamin D compete for the antibody binding sites in micro plate wells plate. Unbound 25-OH Vitamin D is removed by washing. For the detection of bound biotin labeled 25-OH Vitamin D, a second incubation is performed using peroxidase labeled streptavidin. N a third incubation using the peroxidase substrate tetramethylebenzidine (TMB) the bound peroxidase promote the color reaction. The color intensity is inversely proportional to the 25-OH Vitamin D concentration in the sample. Results of the samples calculated directly using a standard curve.

2.2.1.2 Procedure

Prior to use in the assay, reagents and samples were stand at room temperature, samples (200µl) were pipette in biotin/sample buffer for dilution, in each micro plate wells, and then plate incubated for 2 hours at room temperature, the wells were emptied and subsequently washed three times using 300 µl of working strength wash buffer for each wash, enzyme conjugate streptavidin/peroxidase (100µl) were pipette into each of the micro plate wells and Incubated for 30 minutes at room temperature, wells were emptied and washed as step 3. Chromogen substrate solution (100µl) was pipette into each of the micro plate wells and Incubated for 15 minutes at room temperature. Stop solution (100µl) was pipette into each of the micro plate wells in the same speed and the same order as chromogen substrate solution was introduced. Photometric measurement of the color intensity was made at a wavelength 450 nm and a reference wavelength 620 nm and 650 within 30 minutes of adding stop solution. Prior to measuring the micro plate was shacked slightly to ensure homogenous distribution of the solution.

2.2.1.3 Calculation of Results

The standard curve from which the 25-OH vitamin D in the serum samples can be taken was obtained by point-to-point plotting of the extinction values measured for six calibration sera

against the corresponding units. Use “4-PL” or “cubic-spline” plotting for calculation of the standard curve by computer.

2.2.1.4 Detection Limits

The lower detection limit is defined as the mean value of an analyte-free sample minus three times the standard deviation and is the smallest detectable 25-OH vitamin D concentration. The detection limit of 25-OH vitamin D ELISA is 1.6 ng/ml.

2.2.1.5 Linearity

The linearity of the test was investigated by diluting three samples with calibrator one and determining the concordance. The average concordance amounted to 98%.

2.2.2 Calcium Estimation

2.2.2.1 Principle

The measurement of calcium in the sample is based on formation of color complex between calcium and O-Cresolphthaleine in alkaline medium.



The intensity of the color formed is proportional to calcium concentration in the sample.

2.2.2.2 Procedure

Briefly according to manufactured all conditions were adjusted and all reagents brought at room temperature, 2ml from R1 (Ethanalamine) were pipette into blank, standard, and sample tube, and 1drop from R2 (O-Cresolphthaleine and 8-hydroxyquinolein) was added to each tube. 20µl from standard were pipette to standard tube, and 20µl from sample to sample tube. The tubes were mixed, and incubated for 5 minutes absorbance of sample and calibrator were measured, against the blank solution, using Biosystem BTS-310 spectrophotometer.

2.2.2.3 Calculation

Serum and plasma: (A) sample/ (A) standard × 10 (standard Conc.) in mg/dl

2.2.3 Phosphate Estimation

2.2.3.1 Principle

Inorganic Phosphate reacts with molybdic acid forming phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum color measured photometrically at wavelength 710 nm.

The intensity of the color formed is directly proportional to the inorganic phosphate concentration in the sample.

2.2.3.2 Procedure

Prior to use in the assay all reagents and samples were allowed to reach room temperature (15-30 °C), then Three test tubes were labeled as blank standard and test, 1.5ml of phosphomolybdate reagent was pipetted to each of the labeled tubes, 0.05ml of standard phosphate concentration 5 mg/dl was pipette into tube labeled standard, 0.05ml of sample was pipetted into tube labeled sample, content of each tube were mixed and incubated for 10 minutes at 37 °C or 30 minutes at room temperature then read the absorbance of calibrator and sample against the blank the color was stable for 2 hours.

2.2.3.5 Calculation of results

(The absorbance of sample/the absorbance of calibrator) X calibrator concentration (5mg/dl).

2.2.3 Statistical Analysis

The data was analyzed using statistical package of social science (SPSS computer program) tests were applied to compare the mean of serum calcium and phosphate level with hypertensive vitamin D deficient patients by using one way ANOVA test and one sample T-test. Statistics were calculated using 95% confidence intervals, also Microsoft excel 2010 was used in order to draw chart for data presentation.

Chapter Three

Results

Results

3.1 Percentage of gender

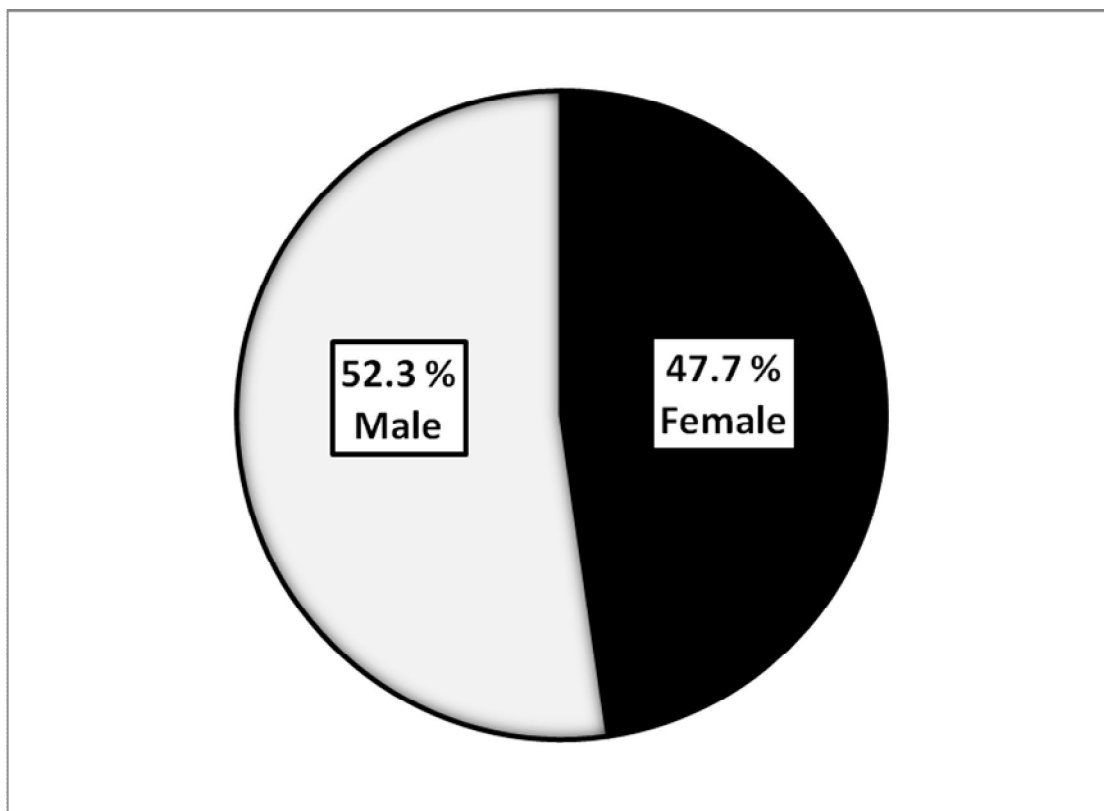


Fig.3.1 Shows frequencies of gender among hypertensive patients, results expressed as percentage (%).

3.2 Frequency of gender and BMI

Table.3.1 The results of frequency (BMI in kg/m^2 versus gender) shows percentages of normal weight (BMI <26.5) and over weight (>26.5) in male (19.6% normal weight and 80.4% overweight) versus female which account for (33.3% normal weight and 66.7% overweight).

BMI	Gender	
	Male	Female
Normal weight	19.6 %	33.3 %
Over weight	80.4 %	66.7 %
Total (%)	100 %	100%

3.3 Frequency of gender and vitamin D status

Table.3.2 shows frequencies of gender (male and female) in study subgroups classified according to vitamin D level, result expressed as percentage (%).

Vitamin D groups	Gender	
	Male	Female
Normal vitamin D	54.3 %	19.0 %
Deficient vitamin D	37.0 %	31.0 %
Sever deficient vitamin D	8.70%	50.0 %
Total (%)	100 %	100 %

3.4 Frequency of BMI gender and vitamin D status

Table.3.3 Shows frequencies of vitamin D level in study group classified as gender that have normal weight and other who have over weight, result expressed as percentage (%).

Vitamin D groups	BMI			
	Normal weight		Over weight	
	Gender			
	Male	Female	Male	Female
Normal vitamin D	77.8 %	35.7 %	50.0 %	10.3 %
Deficient vitamin D	22.2 %	42.9 %	38.9 %	27.9 %
Sever deficient vitamin D	0.00 %	21.4 %	11.1 %	62.1 %
Total (%)	100 %	100 %	100 %	100 %

3.5 Mean concentration of vitamin D level among gender

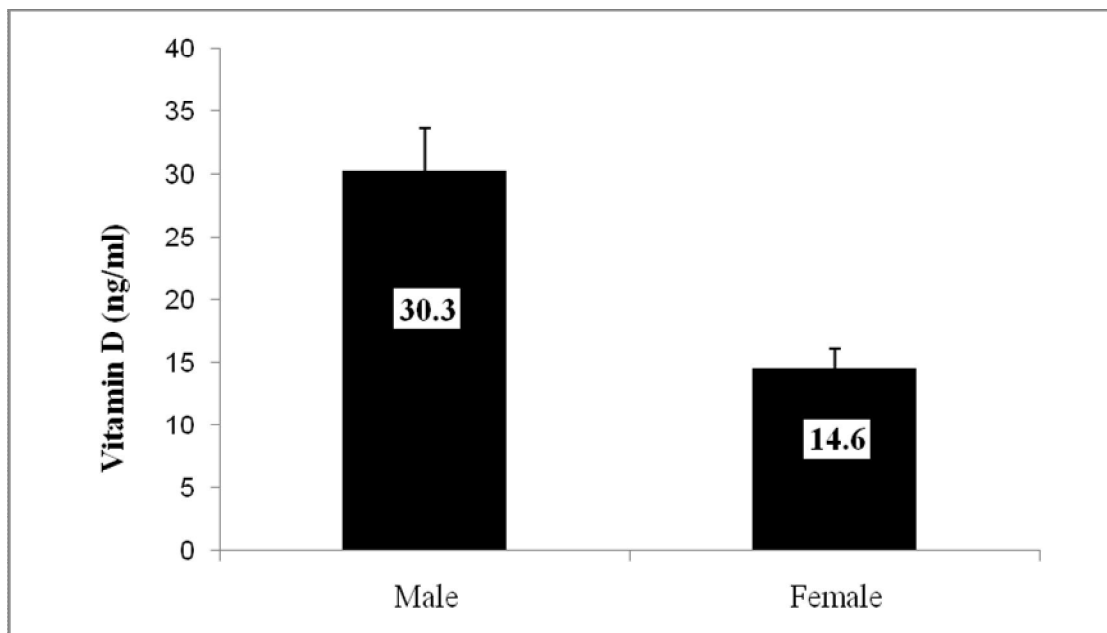


Fig.3.2 Shows mean of vitamin D level in study group classified as male and female, result expressed as ($M \pm SD$) with P-value 0.000.

3.6 Mean concentration of vitamin D level among patient with normal and over weight.

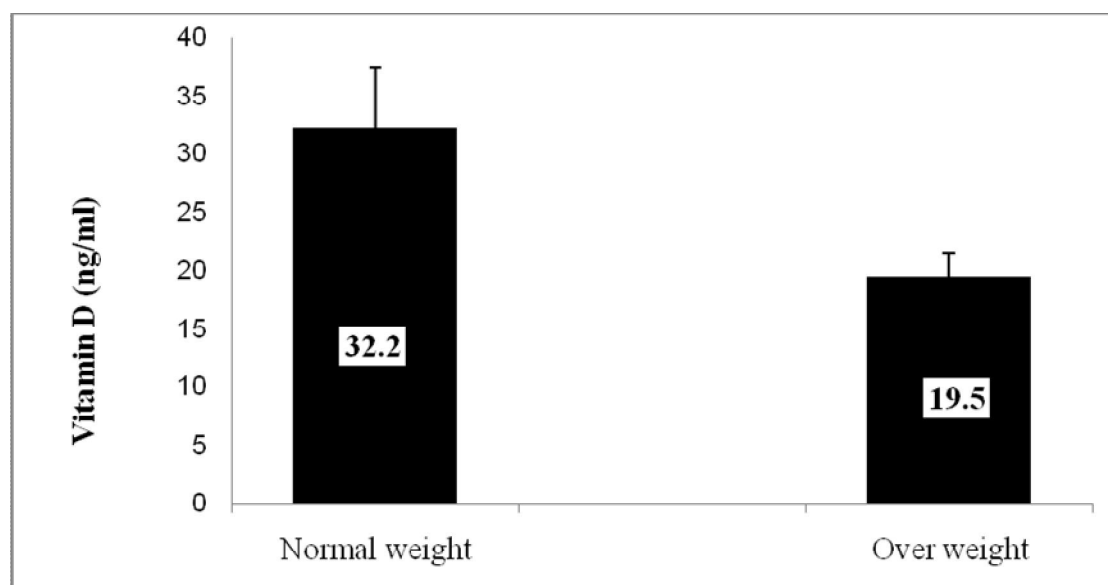


Fig.3.3 Shows mean of Vitamin D level in study group classified as normal weight ($\text{BMI} \leq 25 \text{ kg/m}^2$) and over weight ($\text{BMI} > 25 \text{ kg/m}^2$), result expressed as ($M \pm \text{STD}$), with P-value 0.033.

3.7 Mean concentration of calcium and phosphate level among patient with normal, deficient and sever deficient vitamin D.

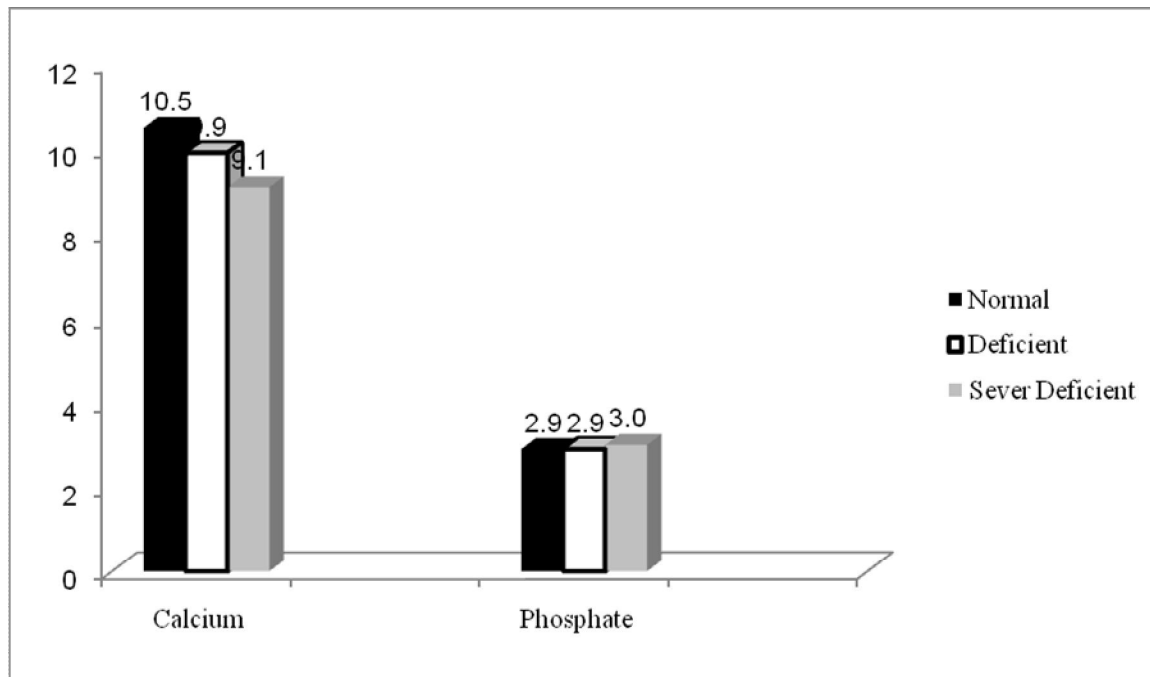


Fig.3.4 Shows mean of calcium and phosphate level in study group classified as normal, deficient and sever deficient vitamin D group, result expressed as ($M \pm STD$) with P-value (0.148, 0.959) respectively.

3.8 Mean concentration of calcium and phosphate level among gender.

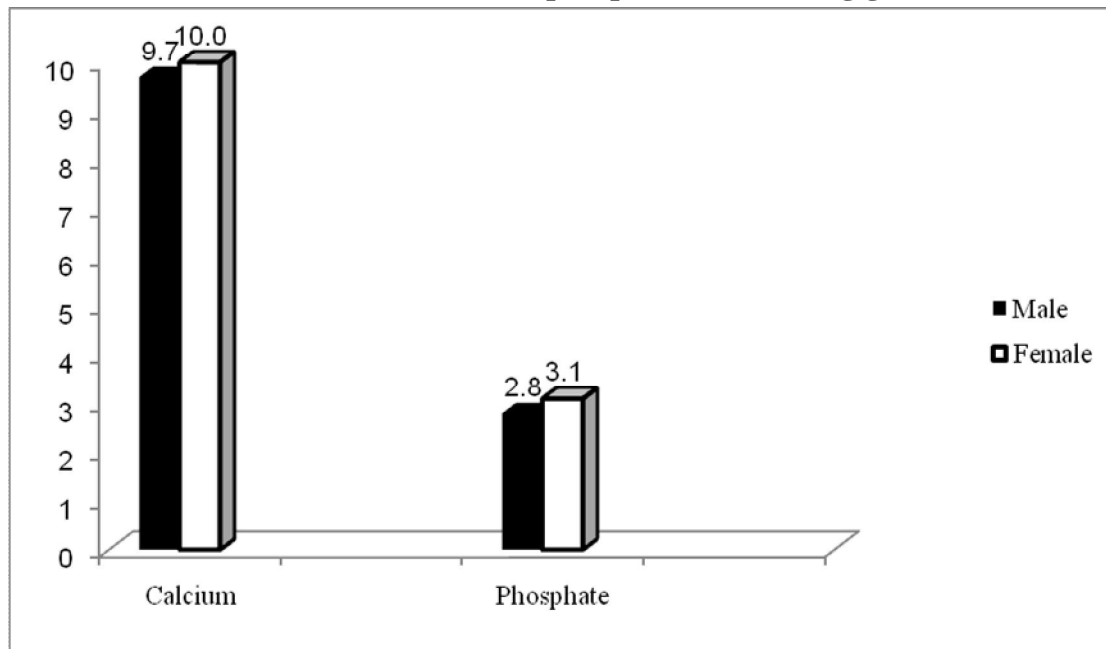


Fig.3.5 Shows mean of calcium and phosphate level in study group classified as male and female, result expressed as ($M \pm STD$) with P-value (0.633, 0.086) respectively.

3.9 Mean concentration of calcium and phosphate level among patient classified by age.

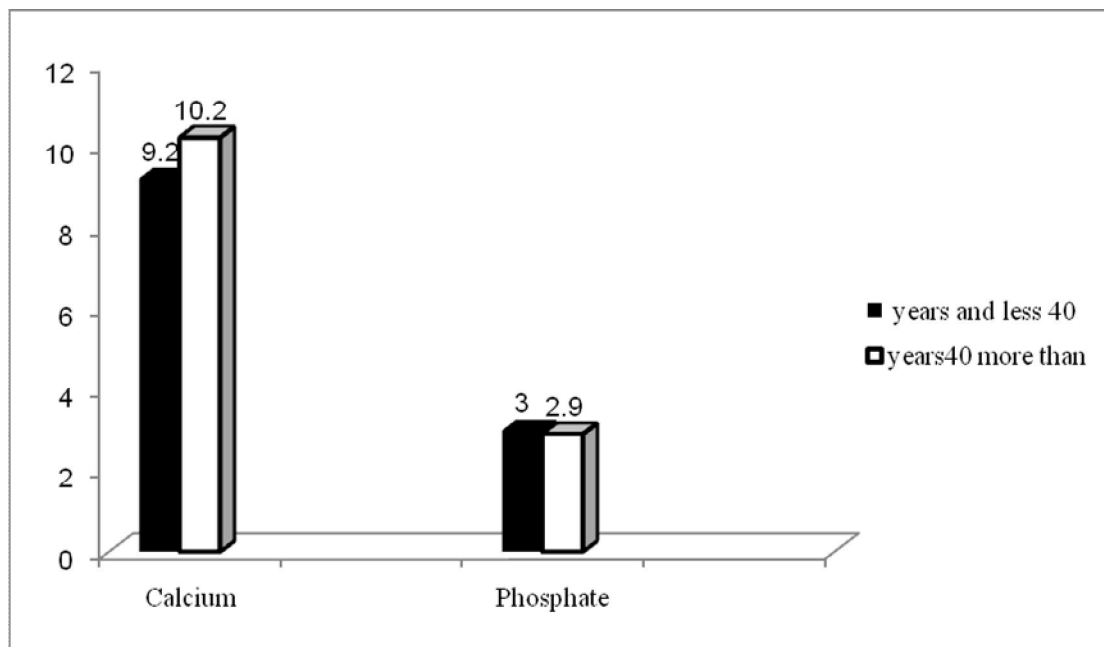


Fig.3.6 Shows mean of calcium and phosphate level in study group classified by age as 40 years and less and more than 40 years, result expressed as ($M \pm STD$), with P-value (0.107, 0.776) respectively.

3.10 Mean concentration of calcium and phosphate level among patient with normal and over weight.

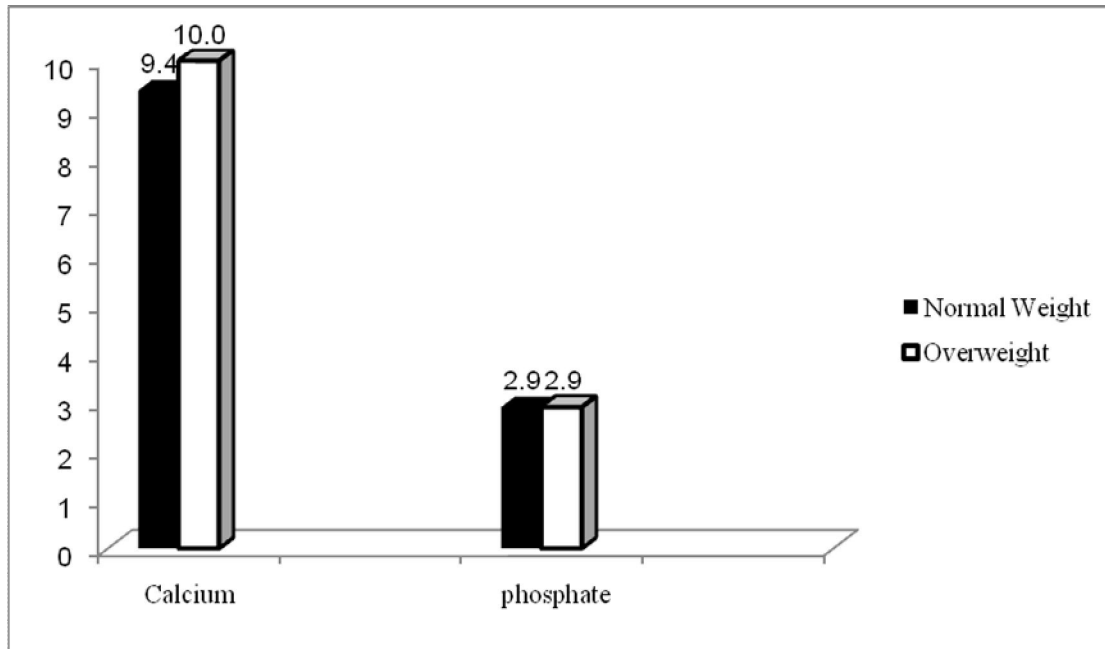


Fig.3.7 Shows mean of calcium and phosphate level in study group classified as normal weight ($BMI \leq 26.5 \text{ kg/m}^2$) and over weight ($BMI > 26.5 \text{ kg/m}^2$), result expressed as ($M \pm STD$), with P-value (0.333, 0.946) respectively.

Chapter Four

Discussion

4 Discussion, Conclusion and Recommendations

4.1 Discussions:

This study is descriptive cross sectional hospital based study was conducted in order to evaluate serum calcium and phosphate levels among hypertensive vitamin D deficient patients, in addition to compare serum calcium and phosphate levels with hypertensive vitamin D deficient risk factors (age, gender and BMI).

Results of frequency showed, gender variations are approximately equal in hypertensive patients (52.3%) male and (47.7%) female. In addition study observed that female are more susceptible to vitamin D deficient than male (81%) female and (45.7%) male with vitamin D deficient, it is possible that due to life style changes such as working indoors, occlusive clothing, and increase use of sunscreen creams, so female expose less to the sun less often than male, resulting in reduced synthesis of vitamin D.

There is a consistent association in the published literature between increasing BMI and lower serum vitamin D concentrations. In our study over weight patients are more frequent in both sexes male and female (80.4%, 66.7%), but study observed percentage of male have increased overweight are more than female, and also serum vitamin D levels inversely correlated with BMI, as fact noted that body fat act as a reservoir for storage of the fat soluble vitamin D, reducing its bioavailability, therefore same time the release of vitamin D from the fat is extremely slow and proportional to the concentration of the vitamin D in the adipose tissue. However, excess body fat results in its increased sequestration and low availability and, as a consequence, low serum vitamin D levels (Zoya *et al.*, 2009; Vanlint, 2013; Bischof *et al.*, 2006). This study showed that, there was no significant difference between mean serum calcium and phosphate levels in both cases groups (vitamin D deficient and sever deficient) in comparison with control with (p -value 0.148 and 0.959) respectively, previous study conducted by (Katharina *et al.*, 2013) reported that, epidemiological studies have largely, but not consistently confirmed a positive correlation of PTH levels and blood pressure, since PTH regulate calcium level, therefore our study assume that calcium and phosphate are not affected by vitamin D status.

Notably, there was no significant difference in mean of calcium and phosphate level of hypertensive male in comparison with female with (p - value 0.633 and 0.086) respectively. In

contrast our study disagreed with previous report stated that, there was strong association between, calcium and phosphate level in vitamin d deficient individuals (Fu *et al.*, 1998).

The study observed that, there was no significant difference in mean calcium and phosphate levels in the age groups (<40 and >40 years) with (*p*-value 0.107 and 0.776) respectively. This results Disagreed with (Gregory *et al*, 2010; Sudharkar *et al.*, 2004), who state that, significant difference in serum calcium and phosphate with hypertension, also the study showed that, there was no significant difference in calcium and phosphate level when compared between normal and overweight hypertensive vitamin d deficient patients with(*p*- value 0.333and 0.946) respectively, this result disagreed with (daniele *et al* ., 2014), who state that significant difference in calcium and phosphate level among hypertensive vitamin D deficient.

Finally, the interpretation of contrast these results may be due to our study did not take the calcium and phosphate supplementation in the consideration or may possibly due to the relatively small sample size.

4.2 Conclusion

Hypertensive patient tend to have vitamin d deficient, and female are more susceptible to vitamin D deficient than male. There no significant difference in Serum calcium and phosphate of hypertensive vitamin D deficient and sever deficient compared with those hypertensive patients with normal vitamin D level.

3.2 Recommendations

1. Monitoring of serum vitamin D should be useful and recommended measure in hypertensive patients.
2. The present study recommended for vitamin D supplementation trails.
3. More studies recommended evaluating the underline mechanisms of association between hypertension, calcium, phosphate, and vitamin D, with estimation of some valuable parameters (PTH, vitamin D receptors).

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Appendices

بسم الله الرحمن الرحيم
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Evaluation of Calcium and Phosphate Levels among Hypertensive Vitamin (D) Deficient Patients In Khartoum State

Questionnaire NO:

Date:

Name:

Age:Sex:

Body weight (kg):

Body height (cm):

Body mass index:.....

Systolic:Diastolic:mmHg

Duration of Disease:

Investigations:

Vitamin D:ng/ml

Calcium:mg/dl

Phosphate:mg/dl