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(Bishop *et al.*, 2010).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 were 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 hemorrhage and exudates (grade 3), if untreated, it commonly progresses to malignant hypertension, which is characterized by papilledema (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

Blood pressure measurement, sitting pressures is 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), vitamin D does not meet the classical definition of a vitamin. A more accurate description of vitamin D is that it is a prohormone; thus, vitamin D is metabolized to a biologically active form that functions as a steroid hormone (Zemplen*i et al.*, 2007).

1.2.1 Vitamin D structure

Vitamin D refers to a family of structurally related compounds that display antirachitic 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 (Zempleni *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 spinout (Smith *et al.*, 2004; Zempleni *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 1α, 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 1α, 25 (OH) 2D3 steroid hormones (Zempleni *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 (Zempleni *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. 1α, 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 1α,25(OH)2D3. The major controls on the production of 1α, 25(OH) 2D3 are 1α, 25(OH) 2D3 itself, PTH, and the serum concentrations of calcium and phosphate (Bender *et al.*, 2003; Zempleni *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 (Zempleni *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-(OH) 2D, 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 1α, 25(OH) 2D3 were originally postulated to be mediated through the interaction of 1α, 25(OH) 2D3 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 (Zempleni *et al.*, 2007).

1.2.12.3 Vitamin D in non-classical system

Nuclear receptors for 1α, 25(OH) 2D3 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 1α,25(OH)2D3, 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 (1α, 25 (OH) 2D3) and minerals metabolism, the classical target tissues for 1a,25(OH)2D3 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(OH) 2D3. Actions on bone, although the most obvious consequence of vitamin D deficiency is decreased mineralization of bone, 1,25(OH)2D3 apparently does not directly increase bone formation or calcium phosphate deposition in osteoid, actions on kidney, 1, 25(OH) 2D3 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(OH)2D3 and respond to it in a manner that is characteristic of negative feedback. Immunoregulatory roles of 1α, 25(OH) 2D3, 1α, 25(OH) 2D3 has been shown to affect cells of the immune system in a variety of ways.1α, 25(OH) 2D3 reduces the proliferation of HL-60 cells and also induces their differentiation to monocytes and macrophages. The actions of 1α, 25(OH) 2D3 on normal monocytes is controversial but it appears that it may enhance monocyte function. 1α, 25(OH) 2D3 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).

1.2.13Nutritional requirement 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 1α, 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 Hypervitamin 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. Hypervitamin 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 1α, 25-dihydroxyvitamin D_3 [1α, 25(OH) $_2D_3$] 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 1α, 25(OH) $_2D_3$ 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 and salt restriction and inhibition of RAS activity reduce blood pressure. vitamin D as a proximal inhibitor of the RAS vitamin D may inhibit the RAS by reducing renin gene expression, increasing 1, $25(OH)_2D$ concentrations were associated with lower plasma renin activity in hypertension, both 25(OH)D and 1,25(OH)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 $1,25(OH)_2D$. 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, $25(OH)_2D$ as a vascular protective agent it reduces the deleterious effect of advanced glycation end products on the endothelium, reduces inflammatory and atherosclerotic parameters.$1,25(OH)_2D$ 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 Introduction to enzymes

Enzymes are specific biological proteins that catalyze biochemical reaction without alteration the equilibrium point of the reaction or being consumed or undergoing change in composition, enzymes found in all body tissue, some enzyme are specific for plasma such those facilitate coagulation, change in plasma enzyme activities May sometime help to detect localize tissue cells damage or proliferation or to monitor treatment and progress of disease (Bishop *et al*., 2000), with the exception of a few catalytic RNA molecules, or ribosome, the vast majority of enzymes are proteins (Murray *et al*., 2008).

All enzyme molecules possess the primary, secondary and tertiary structural characteristic of protein. In addition most enzyme also exhibit quaternary level of structure (Burits *et al.*, 2008).

Each enzyme usually catalyzes a specific biochemical reaction. The ability of an enzyme to select just one substrate and distinguish this substrate from a group of very similar compounds is referred to as specificity (Lieberman *et al.*, 2013).

1.3.2 Properties of enzymes

Enzyme catalyze many specific physiological reaction, each enzyme contain active site often water-free cavity where substance on which the enzyme act, enzyme may exist in different forms within the some individual, even though the same catalytic function throughout body for enzyme co factor are necessary which may be organic or inorganic but all are non-protein molecules (Bishop *et al*., 2010).

1.3.3 Mechanism of enzymes reaction

Enzymes are highly ordered three dimensional shapes with ridge groove and pockets lined with specific amino acid, the reactant molecule which is called substrate of the enzyme (Fox, 2006).

1.3.4 Possible mechanisms responsible for abnormal levels

I- Increase serum enzyme may due to: 1-in necrosis of cells, due to damage to cells of the tissue the resultant pattern will depend on enzyme content of tissue/organ and on the extent and type of necrosis 2-increase permeability of cell membrane: increase permeability without gross cellular damage such as in early stage of viral hepatitis also in progressive muscular dystrophy. Increase production of enzyme within cells, increase in tissue source of enzyme, these either due to increase rate of production in cells or increase in number of cell (Chatterjea and Chawla, 2010).

II- Impaired excretion: such as in certain renal failure lead to increase level of enzyme (Chatterjea and Chawla, 2010).

III-Decrease serum level, due to decrease formation of the enzyme which may be genetic (Wilson disease with decrease serum ceruloplasmin) or acquired (in hepatitis decrease serum level of pseudo cholinesterase), decrease may also due to enzyme inhibition (as in insect side poisoning decrease serum pseudo cholinesterase) or due to lack of cofactor (as in pregnancy decrease serum glutamate oxaloacetate transferase) (Chatterjea and Chawla, 2010).

1.3.5 Unit of serum enzymes activity

The serum enzyme activity express in international unit (IU) which define as the enzyme which transforms one micromole of substrate per minute under optimal condition (Chatterjea and Chawla 2010).

1.3.6 Value of enzymes assay in clinical practice

Single or serial assay on enzyme activity may provide information on the nature and extent of a disease process. They are many values:

Value in diagnosis: such as creatine kinase (CK) provides good evidence for myocardial information if ECG (electro cardiac graph) doubt full.

Value in differential diagnosis: when the differential diagnosis lies between disease that is because pattern of serum enzyme and on that does not such as differentiating between myocardial infarction and pulmonary embolism (Chatterjea and Chawla, 2010).

Value in ascertaining prognosis, serial enzyme assay is required when a disease regulatory cause serum alteration, value in early detection of such disease (Chatterjea and Chawla, 2010).

1.3.7 Enzymes activity

Enzyme assay usually depend on the catalytic activity of enzyme, since each enzyme molecule can catalyze the reaction of many molecules of substrate, measurement of activity provide greater sensitivity, however condition of assay must be optimized (Marshal and Bangert, 2014).

The catalytic activity of an enzyme molecule depends generally on the integrity of its structure (Burits *et al.,* 2008).

Enzyme activity can be measured as either an increase in product concentration, a decrease in substrate concentration, a decrease in coenzyme concentration, or an increase in concentration of altered coenzyme (Burits *et al.,* 2008).

(1) Endpoint measurements are performed after a reaction proceeds for a designated length of time, then is stopped. Measurement is made of the amount of reaction that has occurred.

(2) Kinetic measurements are multiple measurements of absorbance change made at specific time intervals (Hubbard, 2010).

a. The catalytic mechanism is stated as:

$E + S = ES = E + P$

The transition state for the ES complex has a lower energy of activation than S alone, so the reaction proceeds after the ES complex is formed.

b. Michaelis-Menten constant (Km) expresses the relationship between the velocity of any enzymatic reaction and the substrate concentration Km is the Substrate concentration at which the enzyme yields half the possible maximum velocity of the reaction (Hubbard, 2010).

(1) The Michaelis-Menten hypothesis of the relationship between reaction velocity and substance concentration is **expressed as a formula:**

$$V = \frac{Vmax[S]}{Km+[S]}$$

an international unit (U) of enzyme activity is the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute under specific conditions (e.g., temperature, pH) (Hubbard, 2010).

1.3.8 Enzyme Inhibitors

Competitive inhibitors bind to the active site of the enzyme, causing Km to increase. Noncompetitive inhibitors bind at a place other than the active site, causing Vmax to decrease.

Uncompetitive inhibitors bind to the ES complex; both V max and Km decrease (Hubbard, 2010).

1.3.9 Enzyme nomenclature

Name of some enzyme simply derived by adding the suffix –AES to the name of substrate, name of other enzymes reflect the type of reaction that they catalyze as (dehydrogenase), the common name of an enzyme often tell us a great deal about the function of an enzyme, yet other enzymes have historical names that have no relationship to either the substrate or the reaction that they catalyze such as (tyrosine) (Dennisto*net al*., 2007).

1.3.10 Co factors and co enzymes

are non-protein prosthetic group, co factor may be metal ions, organic compound or organic metallic compound must be bound to the enzyme to maintain the correct configuration of enzyme active site, the term co enzyme specifically refers to an organic group that bind transiently to the enzyme during the reaction it accept or donate chemical groups, often co enzyme contain modified vitamins as a part of their structure (Dennistone*t al*, 2007).

Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), and the metal ions of Co, Cu, Mg, MN, Se, and Zn (Murray *et al.*, 2008).

1.3.11 Factors that influence enzymatic reaction

1.3.11.1 Physiological factors

Age: plasma aspartate transaminase activity is moderately higher during the neonatal period than in adult, also alkaline phosphatase activity of bony origin is higher in children.

Sex: plasma γ-glut amyl transferase activity is higher in men than in women. Physiological condition: plasma alkaline phosphatase activity rise during the last trimester of pregnancy, several such as transaminase and creatin kinase rise moderately in plasma during and immediately after labor or strenuous exercise (Mayne, 1994).

1.3.12 Iso enzymes

Some enzyme exist in more than one forms, these is Iso enzyme may be separated by their different physiological or chemical properties, if they originate in different tissue, such identification will give more information than the measurement of plasma total enzyme activity (Crook, 2006)

1.3.13 Alkaline Phosphatase (ALP)

Alkaline phosphatase (EC.3.1.3.1 Orthophosphoric monoester phosphohydrolase) (Burits *et al.*, 2008)**,** The alkaline phosphatases (ALP) are a group of glycoprotein enzymes that act as phosphotransferases by hydrolyzing various types of monophosphate bond at alkaline pH (Gunatillaka), is a nonspecific enzyme capable of reacting with many different substrates (Bishop *et al.*, 2010).

The protein moieties comprise about 510 amino acid residues, to which is attached various amounts of carbohydrate and sialic acid. Tissue-specific posttranslational

modifications occur to the carbohydrate content, leading to the formation of isoforms, e.g. bone, liver and kidney ALP, each of which contains the same tissue non-specific protein. ALP is found attached to the outer lipid bilayer of cell membranes by a glycosyl phosphatidylinositol group, in the case of liver ALP possibly as a tetramer of identical subunits; if released from cell membranes, ALP is dimeric. Divalent ions such as Mg2, Co 2, Zn 2 are activators of the enzyme, but borate, oxalate, phosphate and cyanide ions are inhibitor of ALP activity (Burits *et al*., 2008)**.**

1.3.13.1 1Tissue Source

Activity of enzyme present on cell surfaces in most human tissue, The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney. In the liver, the enzyme is located on both sinusoidal and bile canaliculi membranes; activity in bone is confined to the osteoblasts, those cells involved in the production of bone matrix. The Specific location of the enzyme within this tissue accounts for the more predominant elevations in certain disorders (Bishop *et al.,* 2010).

Alp exists in multiple forms some of which are true Iso enzyme, encoded at separate genetic loci. The bone, liver and kidney ALP form share common primary structure encoded by same genetic locus but differ in carbohydrate content (Burits, et al, 2008)**.**

Liver ALP is located in the cell membranes of the hepatocyte, and particularly in the outer layer of the cells adjacent to the bile canaliculi and also in the cells lining the sinusoids. Adult intestinal ALP lacks sialic acid and is found in the epithelial cells of the intestinal brush border. The placental enzyme is formed by the syncitiotrophoblast cells lining the microvilli that interface the placental and fetal blood circulations but the placental ALP does not cross into the fetal circulation (Gunatillaka, *et al*)

1.3.13.2 Diagnostic Significance

Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders. In hepatobiliary disorders, elevations are more predominant in obstructive conditions than in hepatocellular disorders; in bone disorders, elevations are observed when there is involvement of osteoblasts. In biliary tract obstruction, ALP levels range from 3 to 10 times ULN. Increases are primarily a result of increased Synthesis of the enzyme induced by cholestasis (Bishop *et al.*, 2010).

In contrast, hepatocellular disorders, such as hepatitis and cirrhosis, show only slight increases. In normal pregnancy, increased ALP activity, averaging approximately 1½ times ULN, can be detected between weeks 16 and 20. ALP activity increases and

persists until the onset of labor. Activity then returns to normal within 3 to 6 days.24 Elevations also may be seen in complications of pregnancy such as hypertension, preeclampsia, and eclampsia, as well as in threatened abortion (Bishop *et al*., 2010).

The response of the liver to any form of biliary tree obstruction is to synthesize more ALP. The main site of new enzyme synthesis is the hepatocytes adjacent to the biliary canaliculi. Some of the newly formed enzyme enters the circulation to raise the enzyme level in serum. The elevation tends to be more marked (more than three-fold) in extra hepatic obstruction (e.g. by stone or by cancer of the head of the pancreas) than in intrahepatic obstruction and is greater the more complete the obstruction. Serum enzyme activities may reach 10 to 12 times the upper limit of normal, returning to normal on surgical removal of the obstruction (Burits *et al*., 2008)**.**

1.3.14 Rationale

In Sudan hypertension disease is in increase in both males and females and occurs in different age groups, it can cause many organ damages and dysfunctions Hypertension is a major risk factor for stroke, ischemic heart disease, peripheral vascular disease, heart failure and chronic kidney disease. Vitamin D is one of the factors that can affect blood pressure. Nowadays, vitamin D has been considered, due to its various effects on health, and numerous studies have been conducted on its various effects on different parts of body and proper functioning of different organs and systems. It is also claimed that vitamin D deficiency leads to many chronic diseases and insufficient intake of vitamin D plays an important role in pathogenesis and progression of hypertension, ALP act as phosphotransferases by hydrolyzing various types of monophosphate bond at alkaline pH use as marker for cholestasis accordingly the present study conducted to evaluate ALP as predictive value for cholestasis in vitamin D deficient hypertensive patients.

1.3.15 General objective

To study Alkaline phosphatase activity among vitamin D deficient hypertensive patients in Khartoum state.

1.3.16 Specific objectives

To estimate vitamin D and ALP activity in study group

To compare between vitamin D level with (BMI, gender, duration of disease and age)

To compare between ALP activity with (vitamin D, BMI, gender, duration of disease and age)

2. Materials and method

2.1. Materials:

2.1.1. Study design:

Descriptive cross section study conducted during period of collection (April to May 2014)

2.1.2. Study area:

This study was conducted in Khartoum town.

2.1.3. Study population:

Case: eighty eight Sudanese hypertensive patients were enrolled for this study then classify according to result of vitamin D level as

Normal vitamin D

Vitamin D deficient

Sever vitamin D deficient

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 were 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 aim of the study

2.2. Method

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 tetramethylebenzidene (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 Limit

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.

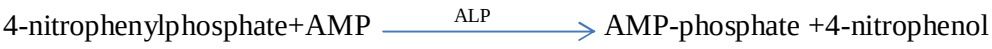
2.2.1.5 Linearity

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 Serum estimation of vitamin and ALP

2.2.2.1 Principle

Alkaline phosphatase(ALP)catalyze in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to2-amino-2-methyl-1-propanol(AMP) liberating 4-nitrophenol, the catalytic concentration is determined from the rate of 4-nitrophenol formation measured at 450nm.



2.2.2.2 Reagent:

ALP reagent provided by bio System Company (appendix Π)

2.2.2.3 Procedure:

All working reagent and instruments brought to reaction temperature

Pipetted into cuvette.Mixed and inserted the cuvette into the spectrophotometer recorded initial absorbance and at 1 minute interval therefor for 3 minute calculated the difference between consecutive absorbance and the average absorbance difference per minute (ΔA/min)

2.2.2.4 Calculation:

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

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times 1 \times Vs} \quad \text{U/L}$$

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

$\Delta A/\text{min} \times 4608 = \text{nKat/L}$

2.2.3. Data analysis:

Appropriate descriptive and analytical statistical procedure were followed using SPSS package (version 14) mean, T test and one way a nova were applied to compare the level of ALP in study groups, and the level of significance was expressed as $P < 0.05$.

3 Results

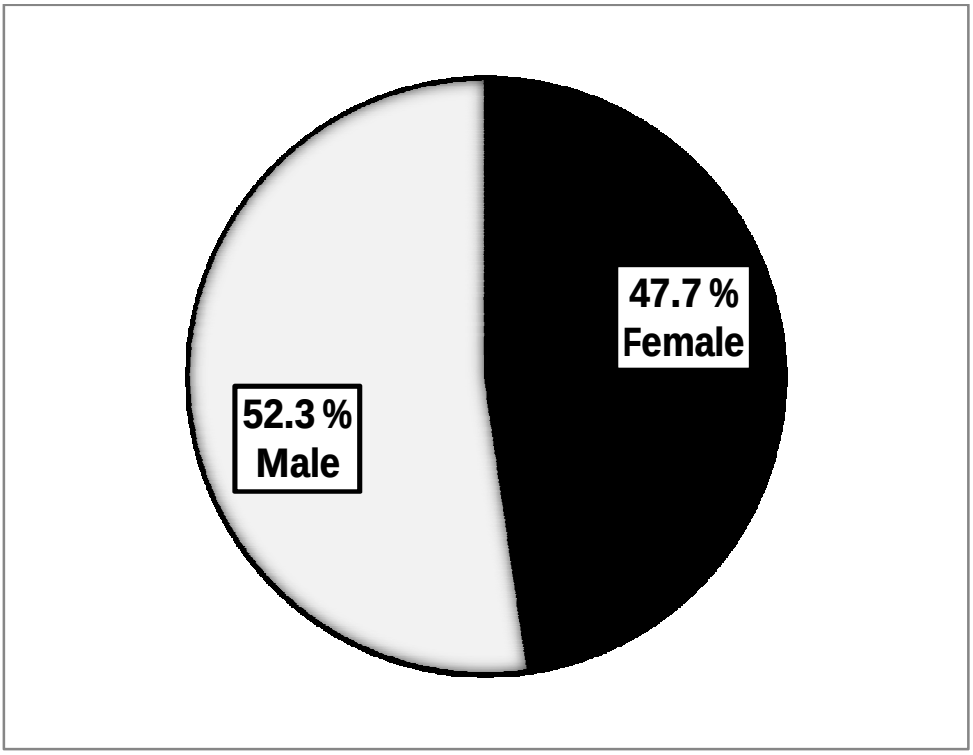


Fig.3.1 Shows frequencies of gender among hypertension patients, results expressed as percentage (%).

Table3.1 Shows frequencies of BMI {(normal weight BMI $\leq$ 25 kg/m^2) and overweight BMI > 25 kg/m^2)} in study group classified as male and female, result expressed as percentage (%).

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

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	54.4 %	19.0 %
Deficient vitamin D	37.0 %	31.09%
Sever deficient vitamin D	8.60 %	50.0 %
Total (%)	100 %	100 %

Table.3.4 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%	10.4%
Deficient vitamin D	22.2%	42.9%	38.9%	27.6%
Sever deficient vitamin D	0.00%	21.4%	11.1%	62.0%
Total (%)	100%	100%	100%	100%

Table.3.5 Shows mean of alkaline phosphatase activity in study group classified as normal deficient and sever deficient vitamin D level result express as (M ± STD) with P.value 0.212

Vitamin D group	Mean of ALP	P.value
Normal	82.5 ±35.1	
Deficient	92.5 ±34.6	0.215
Sever deficient	77.7 ±21.5	0.575

Table.3.6 Shows mean of alkaline phosphatase activity in study group classified as male and female result express as (M ± STD) with P.value 0.841

Gender group	Mean of ALP	P.value
Male	85.2±36.6	0.841
Female	83.8±26.2	

Table.3.7 Shows mean of alkaline phosphatase activity in study group classified as normal and overweight result express as (M ± SD) with P.value 0.041

BMI group	Mean of ALP	P.value
Normal	75.9 ±17.1	0.041
Overweight	87.6 ±35.3	

Table.3.8 Shows mean of alkaline phosphatase activity in study group classified as 40 years and less and more than40 year's result expresses as (M ± STD) with P.value 0.342

Age group	Mean of ALP	P.value
40years and Less than 40	89.6±26.4	0.342
More than 40 years	82.4±33.9	

Table.3.9 Shows mean of alkaline phosphatase activity in study group classified as less than5 years with disease and more than5 years with disease result expresses as (M ± STD) with P.value 0.559

Duration group	Mean of ALP	*P*-value
Less than 5 years	82.9± 29.5	0.559
More than 5 years	87.4±36.0	

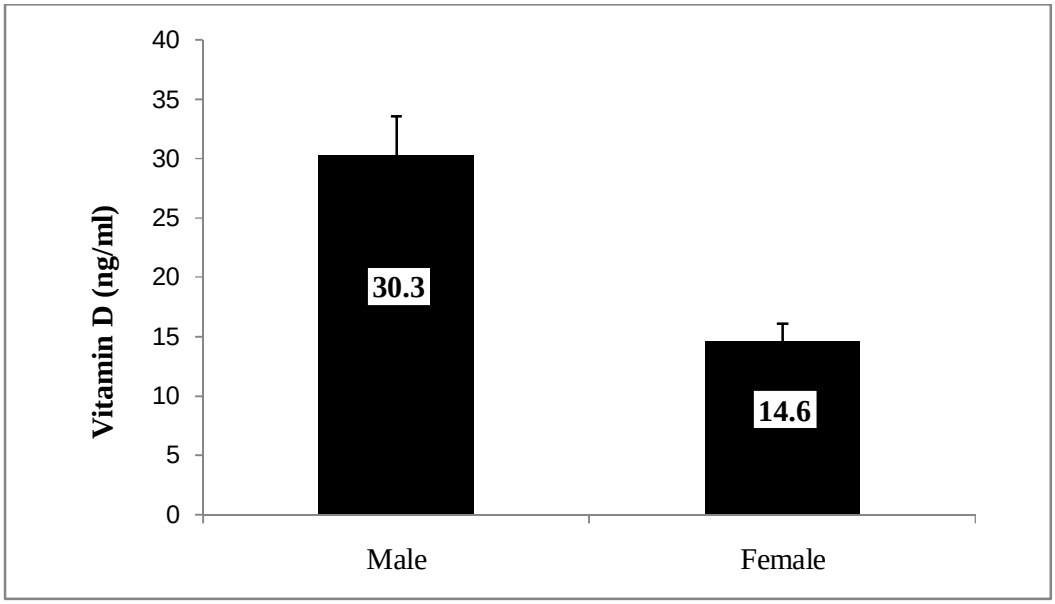


Fig.3.2 Shows mean of vitamin D level in study group classified as male and female, result expressed as (M ± STD) with P-value 0.000.

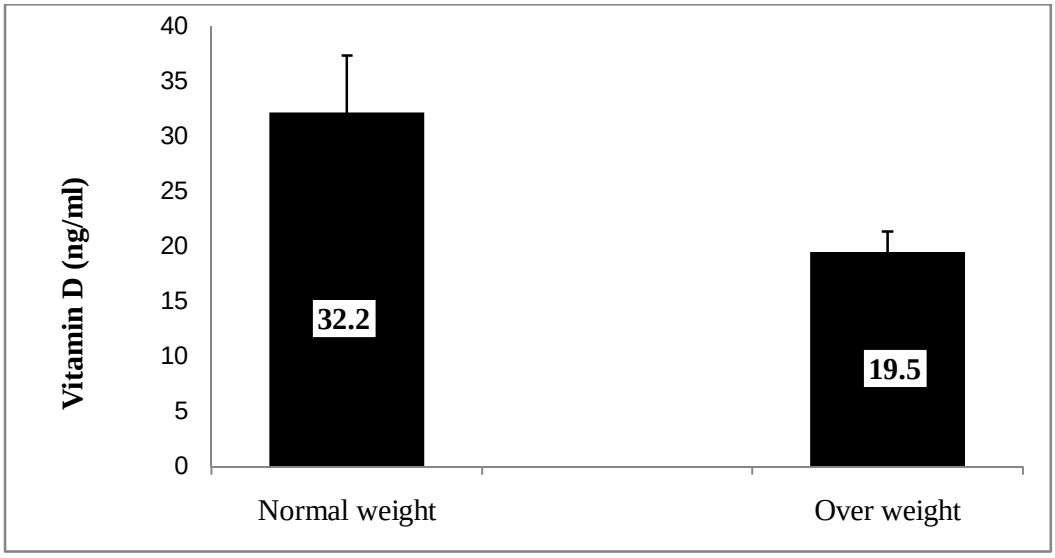


Fig.3.3 Shows mean of Vitamin D level in study group classified as normal weight (BMI $\leq$ 25 kg/m^2) and over weight (BMI > 25 kg/m^2), result expressed as (M ± STD), with P-value 0.033.

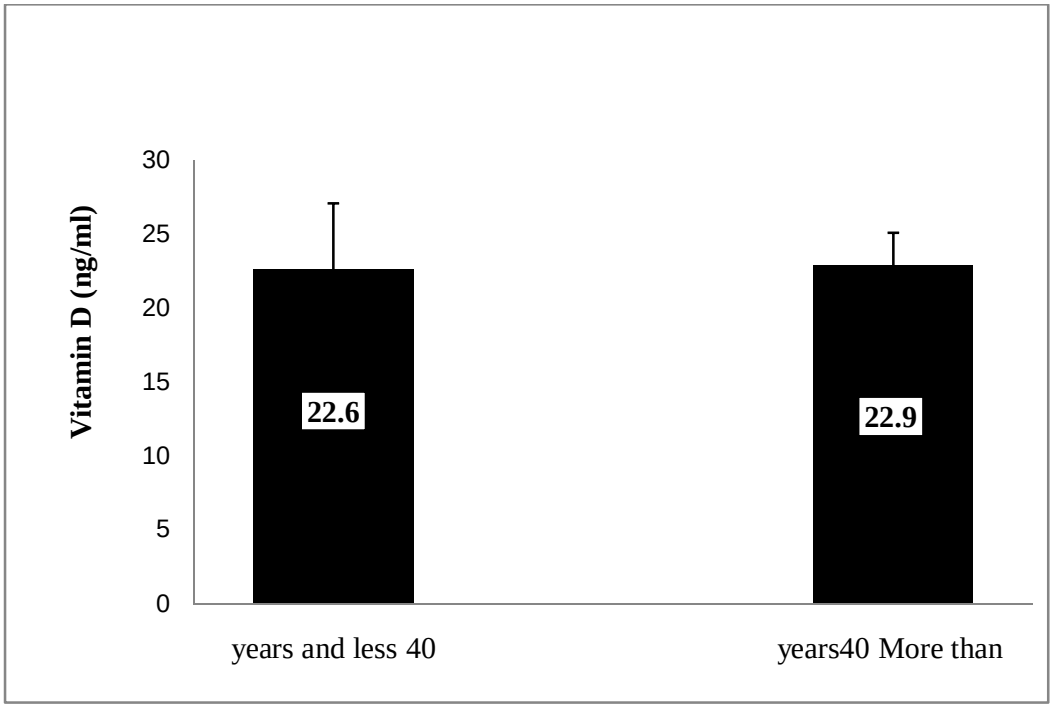

Vitamin D (ng/ml)
30
25
20
15
10
5
0
22.6
22.9
years and less 40
years40 More than

Fig.3.4Shows mean of Vitamin D level in study group classified as 40 years and less and more than 40 years, result expressed as (M ± STD), with P-value 0.959.

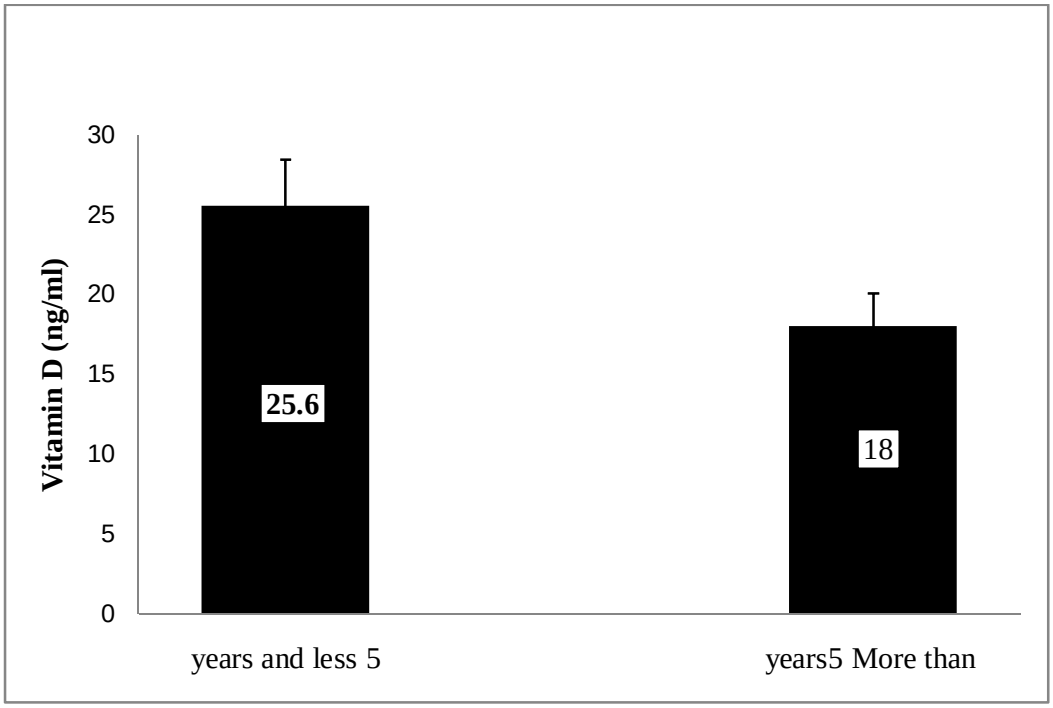


Fig.3.5 Shows mean of Vitamin D level in study group classified as group with disease for 5 years and less and other with disease for more than 5 years, result expressed as (M ± STD), with P-value 0.041.

4 Discussions

Hypertension 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)**,in addition to potential effects on the RAS and regulation of vascular smooth muscle contractility, researcher reported alkaline phosphatase have role in hydrolysis of wide variety of phosphomonoester bound which is an inhibitor of vascular calcification also elevation in vitamin D has direct effects on vascular endothelium and smooth muscle **(Pascal and Rhian, 2000; Vaidya and Forman, 2010)**. Therefore this descriptive cross-sectional study was done to evaluate alkaline phosphatase as predictive value for cholestasis vitamin D deficient hypertensive patients.

The results of frequencies Showed that the genders variations are equal in hypertension patient's approximately 1: 1 male to female. In addition the experimental evidence observed that hypertension patients(male 80.4%, female 66.7%)tend to have gaining weight. these results confirm the roles of obesity in mechanisms of hypertension as it mentioned in **(Diaz, 2002)** study, it was found that a 10% rise in body weight explains a 7 mm Hg rise in systolic blood pressure (SBP) in the population at large, the prevalence of hypertension in the NHANES II was 2.9 times higher in obese than in non-obese adults ,in our study hypertensive female less gaining weight than male may due to nutritional supply or life style.

The results also observed female are more vulnerable to vitamin D deficient than male, which may explained by nature of Sudanese males work lead them more exposed sunlight than females, our findings were agreed with previous report, women had borderline significantly lower vitamin D levels than men, and explained that exposure to sun light is main source for vitamin D synthesis, the maximal vitamin D concentration produced by natural UV exposure **(Binkley *et al.*, 2007; Hagen *et al.*, 2009)**.

The present study revealed that, overweight subjects tend to have vitamin D deficient in both male and female group, this was agreed with **(Worst *et al.*, 2000)** they reported that obesity-associated with vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D_3 from cutaneous and dietary sources because of its deposition in body fat compartments. Serum 25-hydroxy vitamin D was negatively

correlated with BMI and body fat mass. Serum 1, 25-vit D was also negatively correlated with BMI and body fat mass,

Age groups results showed insignificant difference in the mean of vitamin D levels in 40 years and less group in more than 40 years group, these results were confirmed with **(Viethet *al,* 2008)** who reported that no effect of age on the 25-hydroxyvitamin D [25(OH) D] concentration associated with specific vitamin D intakes, there was none of the expected evidence that the young exhibit higher 25(OH) D concentrations than older adults

This descriptive cross-sectional study revealed that there was a significant difference in the mean of vitamin D with long duration of disease; these findings confirm the role of vitamin D in the mechanisms of hypertension **(Forman *et al*, 2010)**, found that, among normotensive individuals, lower 25(OH)D levels were associated with higher circulating Ang II levels, the findings consistent with activation of the RAS in the setting of lower plasma 25(OH)D, these findings may partly explain the higher risk of developing hypertension observed among individuals with vitamin D insufficiency and deficiency.

In this study there was no significant association between serum alkaline phosphatase activity and serum vitamin D_3 level these indicate that alkaline phosphatase are not useful as predictor marker of cholestasis finding agreed with previous study (**Shaheen*et al,* 2012)** which show serum D_3 level may not associate with alkaline phosphatase P.value=0.593 .

The current study revealed that, there was insignificant difference in the mean of alkaline phosphatase activity in age group more than 40 years compare with less than 40 years these findings were agreed with (**Shaheen*et al*., 2012)** which show normal serum ALP level in patients belonging to divers age group, the justification of these as the exact role of ALP at molecular level is still not well defined.

Result of these study show that no significant difference in alkaline phosphatase activity between male and inpregnant female agree with reference **(Meliyanthi, et al)** which show reference value same in male and female

Present study revealed that there was significant increase in ALP activity of overweight group (BMI<26.5) compare with normal group with P.value=0.041 these finding confirm the role of obesity in formation of stones (cholestasis) and ALP use as marker of cholestasis**(Sobia*et al.*, 2012)** show that significant effect of BMI for all enzyme including ALP (P value 0.05).

Conclusion

Study concludes that there was no change in alkaline phosphatase activity in vitamin D deficient hypertensive patients. Female are more susceptible than male for vitamin D deficient. Increase activity of alkaline phosphatase observe in overweight hypertensive patients

Recommendations

Control of hypertension is the best solution for complication avoidance.

further research recommended to study underline mechanism with more parameter related to vitamin D activity and alkaline phosphatase in hypertensive patients (lipid profile, phosphorous, ca^{+2}, vit, D receptor).

Additional research is needed to determine the association between vitamin D and different age groups.

More research should be performed among large number of patients to determine the correlation between alkaline phosphatase and vitamin D.

Explosion to sun light is major source of vitamin D.

Vitamin D need for monitoring and designee for supplement protocol.

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