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 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 those 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 papilloedema (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), 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 (Zempleni *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 **a**lthough 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; 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).

1.2.13 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 1α, 25(OH) 2D3 is still present at the onset of the deficiency, result in the demineralization of bone, 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 as hormone function

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 extrarenal 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 vitamin D may inhibit the RAS by reducing renin gene expression, increasing 1, 25$(OH)_2$D 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$(OH)_2$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$(OH)_2$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$(OH)_2$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 , 2014).

1.3 Magnesium

1.3.1 Magnesium physiology

Magnesium is the fourth most abundant cation in the body and second most abundant intracellular ion. The average human body (70 kg) contains 1 mole (24 g) of magnesium. Approximately 53% of magnesium in the body is found in bone, 46% in muscle and other organs and soft tissue, and less than 1% is present in serum and red blood cells. Of the magnesium present in serum, about one third is bound to protein, primarily albumin. Of the remaining two thirds, 61% exists in the free or ionized state and about 5% is complexed with

other ions, such as citrate. It is the free ion that is physiologically active in the body (Guyton and Hall, 2006; Bishop *et al.*, 2010).

The role of magnesium in the body is widespread. It is an essential cofactor of more than 300 enzymes, including those important in glycolysis, transcellular ion transport, neuromuscular transmission, synthesis of carbohydrates, proteins, lipids, and nucleic acids, and release of and response to certain hormones (Bishop *et al.*, 2010).

1.3.2 Magnesium regulation

Rich sources of magnesium in the diet include raw nuts, dry cereal, and "hard" drinking water; other sources include vegetables, meats, fish, and fruit. Processed foods, an ever-increasing part of the average diet, have low levels of magnesium that may cause an inadequate intake. This in turn may increase the likelihood of magnesium deficiency. The small intestine may absorb 20%–65% of the dietary magnesium, depending on the need and intake (Bishop *et al.*, 2010).

The overall regulation of body magnesium is controlled largely by the kidney, which can reabsorb magnesium in deficiency states or readily excrete excess magnesium in overload states. Of the nonprotein-bound magnesium that gets filtered by the glomerulus, 25%–30% is reabsorbed by the proximal convoluted tubule (PCT). Henle's loop is the major renal regulatory site, where 50%–60% of filtered magnesium is reabsorbed in the ascending limb. In addition, 2%–5% is reabsorbed in the distal convoluted tubule. The renal threshold for magnesium is approximately 0.60–0.85 mmol/L (1.46–2.07 mg/dL), because this isclose to normal serum concentration, slight excesses of magnesium in serum are rapidly excreted by the kidneys. Normally, only about 6% of filtered magnesium is excreted in the urine per day. Parathyroid hormone (PTH) increases the renal reabsorption of magnesium and enhances the absorption of magnesium in the intestine. Aldosterone and thyroxine apparently have the opposite effect of PTH in the kidney, increasing the renal excretion of magnesium (Guyton and Hall, 2006; Bishop *et al.*, 2010).

1.3.3 Clinical applications

1.3.3.1 Hypomagnesemia

Hypomagnesaemia is most frequently observed in hospitalized individuals in intensive care units or those receiving diuretic therapy or digitalis therapy. There are many causes of hypomagnesaemia; however, it can be grouped into general categories Magnesium-deficient diet as a result of starvation, chronic alcoholism, or magnesium-deficient IV therapy can cause a loss of the ion various gastrointestinal disorders may cause decreased absorption by the intestine, and prolonged vomiting, diarrhea, or laxative use may lead to an magnesium deficiency (Bishop *et al.*, 2010).

Neonatal hypomagnesaemia is a result of various surgical procedures. A primary deficiency has also been reported in infants as a result of a selective malabsorption of the ion. A chronic congenital hypomagnesaemia with secondary hypocalcemia (autosomal recessive disorder) has also been reported; molecular studies have revealed a specific transport protein defect in the intestine (Bishop *et al.*, 2010).

Magnesium loss due to increased excretion by way of the urine can occur as a result of various renal and endocrine disorders or the effects of certain drugs on the kidneys. Renal tubular disorders and other select renal disorders may result in excess amounts of magnesium being lost through the urine because of decreased tubular reabsorption. Several endocrine disorders can cause a loss of magnesium hyperparathyroidism and hypercalcemia may cause increased renal excretion of magnesium as a result of excess calcium ions. Excess serum sodium levels caused by hyperaldosteronism may also cause increased renal excretion of magnesium (Bishop *et al.*, 2010).

A pseudohypomagnesemia may also be the result of hyperaldosteronism caused by increased water reabsorption. Hyperthyroidism may result in an increased renal excretion of magnesium and may also cause an intracellular shift of the ion. In persons with diabetes, excess urinary loss of magnesium is associated with glycosuria. Hypomagnesemia can aggravate the neuromuscular and vascular complications commonly found in this disease

.Excess lactation has been associated with hypomagnesaemia as a result of increased use and loss through milk production, mild deficiencies in pregnancy (Bishop *et al.*, 2010).

Symptoms of hypomagnesaemia: A patient who is hypomagnesaemia may be asymptomatic until serum levels fall below 0.5 mmol/L. A variety of symptoms can occur. The most frequent involve cardiovascular, neuromuscular, psychiatric, and metabolic abnormalities the cardiovascular and neuromuscular symptoms result primarily from the ATPase enzyme's requirement for magnesium. Magnesium loss leads to decreased intracellular K levels because of a faulty sodium potassium pump (ATPase),muscle contraction also requires magnesium and ATPase for normal Magnesium uptake following contraction. Normal nerve and muscle cell stimulation requires Magnesium to assist with the regulation of acetylcholine, a potent neurotransmitter (Bishop *et al.*, 2010).

Hypomagnesaemia can cause a variety of symptoms from weakness to tremors, tetany, paralysis, or coma. The CNS can also be affected, resulting in psychiatric disorders that range from subtle changes to depression or psychosis. Metabolic disorders are associated with hypomagnesaemia. Studies have indicated that approximately 40% of hospitalized patients with hypokalemia are also hypomagnesemic.In addition, 20%–30% of patients with hyponatremia, hypocalcemia,or hypophosphatemia are also hypomagnesaemia. Magnesium deficiency can impair PTH release and target tissue response, resulting in hypocalcemia. Replenishing any of these deficient ions alone, often does not remedy the disorder unless Magnesium therapy is provided. Magnesium therapy alone may restore both ion levels to normal; serum levels of the ions must be monitored during treatment (Bishop *et al.*, 2010).

1.3.3.2 Hypermagnesaemia

Is observed less frequently than hypomagnesaemia, the most common causes for elevated serum magnesium levels is renal failure (GFR, 30 mL/min). The most severe elevations are usually a result of the combined effects of decreased renal function and increased intake of commonly prescribed magnesium-containing medications, such as antacids, enemas, or cathartics. Nursing home patients are at greatest risk for this occurrence (Bishop *et al.*, 2010).

Hypermagnesemia has been associated with several endocrine disorders. Thyroxin and growth hormone cause a decrease in tubular reabsorption of Magnesium, and a deficiency of either hormone may cause a moderate elevation in serum Magnesium. Adrenal insufficiency may cause a mild elevation as a result of decreased renal excretion of Magnesium (Bishop *et al.*, 2010).

Magnesium is a vasodilator, and can decrease uterine hyperactivity in eclampsic states and increase uterine blood flow. This therapy can lead to maternal Hypermagnesemia, as well as neonatal Hypermagnesemia due to the immature kidney of the newborn. Premature infants are at greater risk to develop actual symptoms (Bishop *et al.*, 2010).

Dehydration can cause a pseudohypermagnesemia, which can be corrected with rehydration. Because of increased bone loss, mild serum Magnesium elevations can occur in individuals with multiple myeloma or bone metastases (Bishop *et al.*, 2010).

Symptoms of Hypermagnesemia: Symptoms of Hypermagnesemia typically do not occur until the serum level exceeds 1.5 mmol/L.16 the most frequent symptoms involve cardiovascular, dermatologic, GI, neurologic, neuromuscular, metabolic, and hemostatic abnormalities. Mild to moderate symptoms, such as hypotension, bradycardia, skin flushing, increased skin temperature, nausea, vomiting, and lethargy may occur when serum levels are 1.5–2.5 mmol/L.16 Life-threatening symptoms, such as electrocardiogram changes, heart block, asystole, sedation, coma, respiratory depression or arrest, and paralysis, can occur when serum levels reach 5.0 mmol/L (Bishop *et al.*, 2010).

1.3.4 The biological mechanisms link hypertension with magnesium

Magnesium modulates mechanical, electrical and structural functions of cardiac and vascular cells, and small changes in extracellular magnesium levels and/or intracellular free magnesium concentration may have significant effects on cardiac excitability and on vascular tone, contractility and reactivity. Thus, magnesium may be important in the physiological regulation of blood pressure whereas alterations in cellular magnesium metabolism could contribute to the pathogenesis of blood pressure elevation (Pascal and Rhian, 2000).

A pathological role for magnesium in the etiology and development of hypertension, data from clinical studies have been less convincing. Furthermore, the therapeutic value of magnesium in the management of essential hypertension is unclear roles of magnesium in the regulation of vascular function and blood pressure and introduces novel concepts relating to magnesium as a second messenger in intracellular signaling in cardiovascular cells. A magnesium-rich diet should be encouraged in the prevention of hypertension, particularly in predisposed communities because of the other advantages of such a diet in prevention. The clinical aspect that has demonstrated the greatest therapeutic potential for magnesium in hypertension, is in the treatment of pre-eclampsia and eclampsia (Touyz, 2003).

Magnesium affects blood pressure by modulating vascular tone and reactivity. It acts as a calcium channel antagonist, it stimulates production of vasodilator prostacyclins and nitric oxide and it alters vascular responses to vasoactive agonists (Sontia B and Touyz, 2007).

Magnesium deficiency has been implicated in the pathogenesis of hypertension magnesium also influences glucose and insulin homeostasis, and hypomagnesemia is associated with metabolic syndrome Furthermore, the therapeutic value of magnesium in the management of hypertension is unclear (Sontia B and Touyz, 2007).

The combination of increased intake of magnesium and potassium coupled with reduced sodium intake is more effective in reducing BP than single mineral intake and is often as effective as one antihypertensive drug in treating hypertension. Reducing intracellular sodium and calcium while increasing intracellular magnesium and potassium improves BP response ·Magnesium also increases the effectiveness of all antihypertensive drug classes.Various genetic defects in magnesium transport are associated with hypertension and possibly with cardiovascular disease. Oral magnesium acts as a natural calcium channel blocker, increases nitric oxide, improves endothelial dysfunction, and induces direct and indirect vasodilation (Houston, 2011).

1.4 Rationale

In Sudan hypertension disease is in increase in both sex's 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.

Magnesium plays an important role in hypertension mechanisms it modulates mechanical, electrical and structural functions of cardiac and vascular cells, also increases the effectiveness of all antihypertensive drug classes, Magnesium deficiency has been implicated in the pathogenesis of hypertension.

1.5 General objective

To evaluate magnesium levels among hypertensive vitamin D deficient patients in Khartoum State.

1.6 Specific objectives

1.To estimate vitamin D and magnesium level in study groups.

2. To compare mean concentration of magnesium and vitamin D in patients and control groups.

3. To Correlate between vitamin D levels and study variables (age, BMI, gender and duration).

4. To Correlate between magnesium level and study variables (age, body mass index, gender and duration).

2 Materials and Methods

2.1 Materials

2.1.1 Study Design

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

2.1.2 Study Area

This study was carried out in different hospitals, clinic and centers (East nile model hospital, Khartoum teaching hospital, Alfaroug medical center) 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 (≥20 ng/ml) considered as control, group two deficient vitamin D (10 -19.9 ng/ml) , group three sever deficient vitamin D (up to 9.9 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, 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 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 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 Xylidyl Blue for Magnesium Estimation

2.2.2.1 Principle

Magnesium in sample reacts with xylidyl blue in alkaline medium forming a colored complex that can be measured by spectrophotometry EGTA is included in the reagent to remove calcium interference.

2.2.2.2 Procedure

The absorbance (A) of the standard and samples read at the wavelength 520 nm against the blank.

2.2.2.3 Calculations

The concentration of magnesium is obtained by using the general formula:

(A) sample/ (A) Standard * concentration of standard = concentration of sample.

2.2.3 Statistical Analysis

The data was analyzed using statistical package of social science (SPSS computer program), frequencies, means and Pearson's correlation.

3 Results

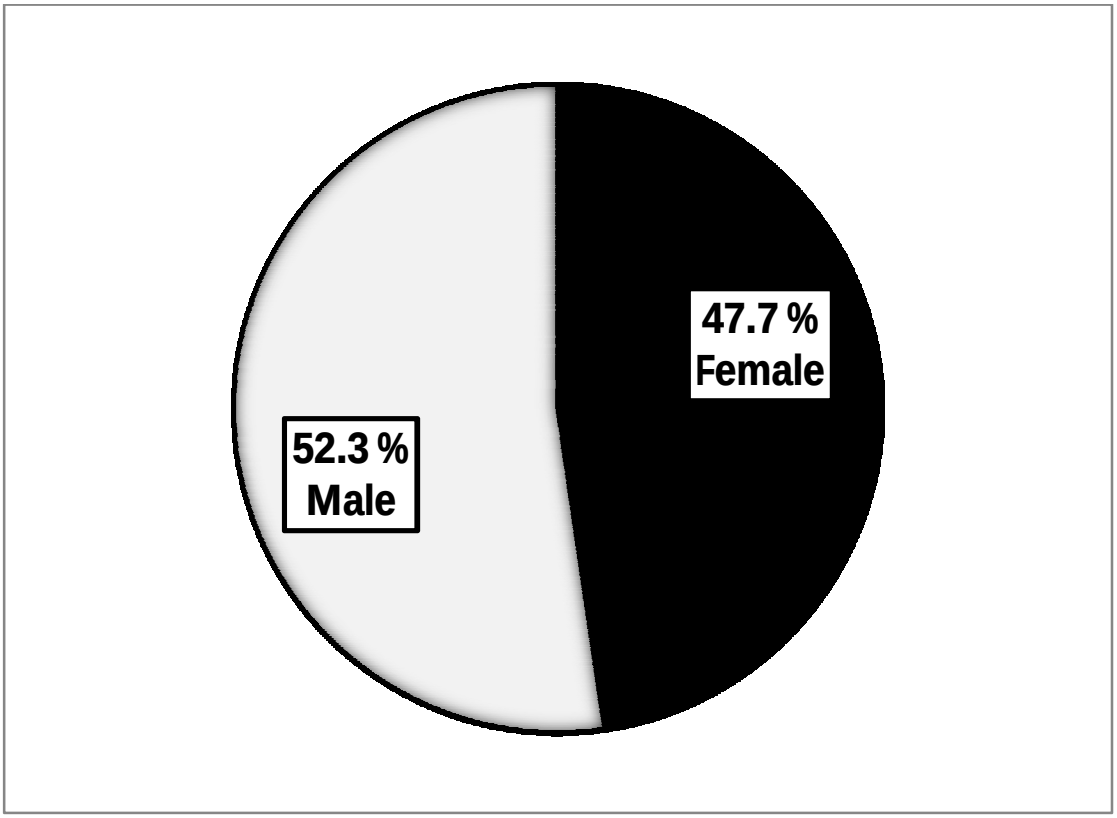

Fig.3.1 Shows frequencies of gender among hypertension patients, results expressed as percentage (%).

Table.3.1 shows frequencies of BMI normal weight (BMI $\leq$ 25 kg/m^2)and over weight (BMI > 25 kg/m^2) in study group classified as male and female, results 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%	19.00%
Deficient vitamin D	37.0%	31.00%
Sever deficient vitamin D	8.60%	50.00%
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	25.3%	35.7%	50.0%	10.7%
Deficient vitamin D	74.7%	43.0%	39.5%	25.0%
Sever deficient vitamin D	00.0%	21.3%	10.5%	64.3%
Total(%)	100%	100%	100%	100%

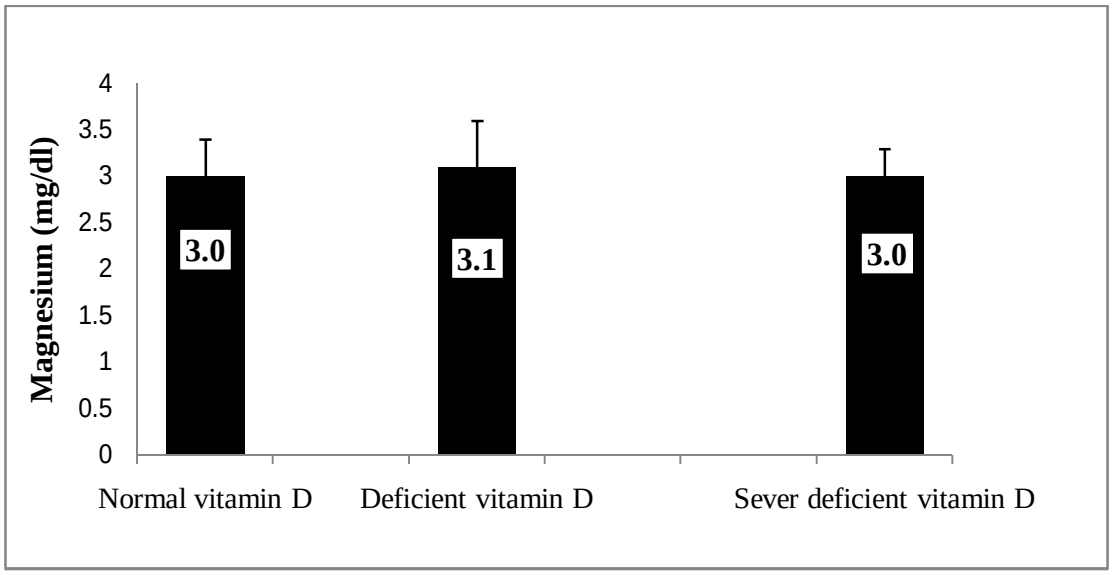

Fig.3.2 Shows mean of magnesium level in study subgroups classified according to vitamin D level, result expressed as (M ± STD), with *P*-value between groups 0.691, with *P*-value within groups:

Normal vitamin D group with deficient vitamin D group, *P*-value 0.524

Normal vitamin D group with sever deficient vitamin D group, *P*-value 0.822

Deficient vitamin D group with sever deficient vitamin D group, *P*-value 0.416

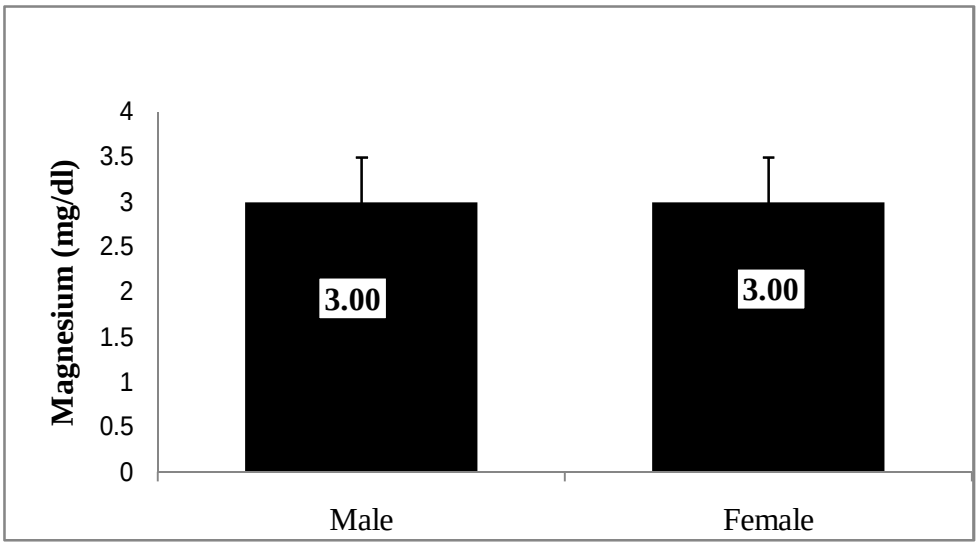

Fig.3.3 Shows mean of magnesium level in study group classified as male and female, result expressed as (M ± STD), with *P*-value 0.665.

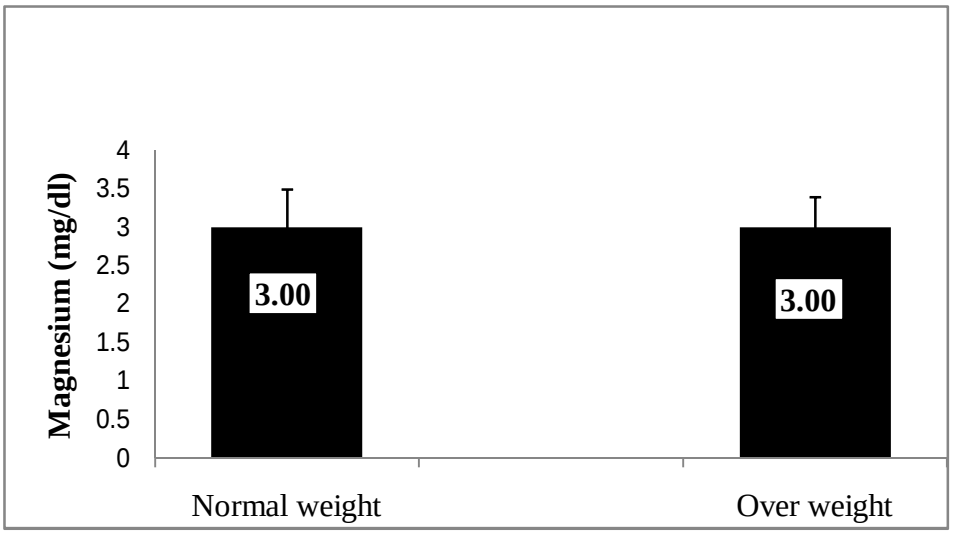

Fig.3.4 Shows mean of magnesium level in study group classified as normal weight (BMI $\leq$ 25 kg/m^2)and over weight (BMI > 25 kg/m^2), result expressed as (M ± STD), with *P*-value 0.828.

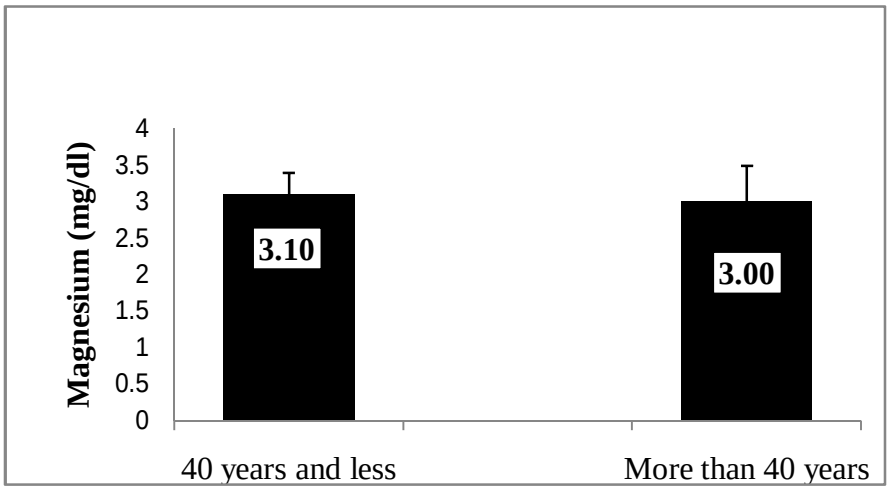

Fig.3.5 Shows mean of magnesium level in study group classified as 40 years and less and more than 40 years, result expressed as (M ± STD),with *P*-value 0.676.

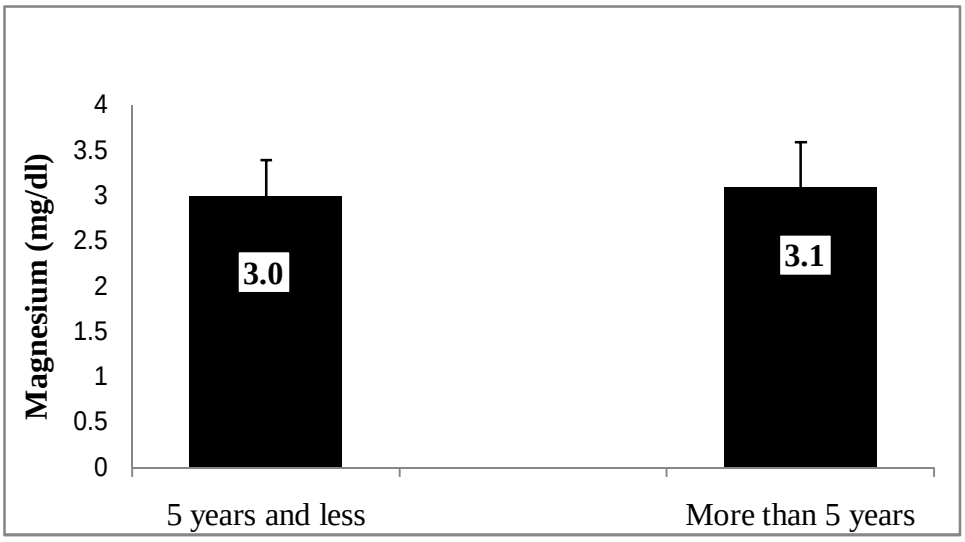

Fig.3.6 Shows mean of magnesium level in study group classified as group with disease for 5 years and less and other with disease for more than 5 years, result expressed as (M ± STD), with *P*-value 0.634.

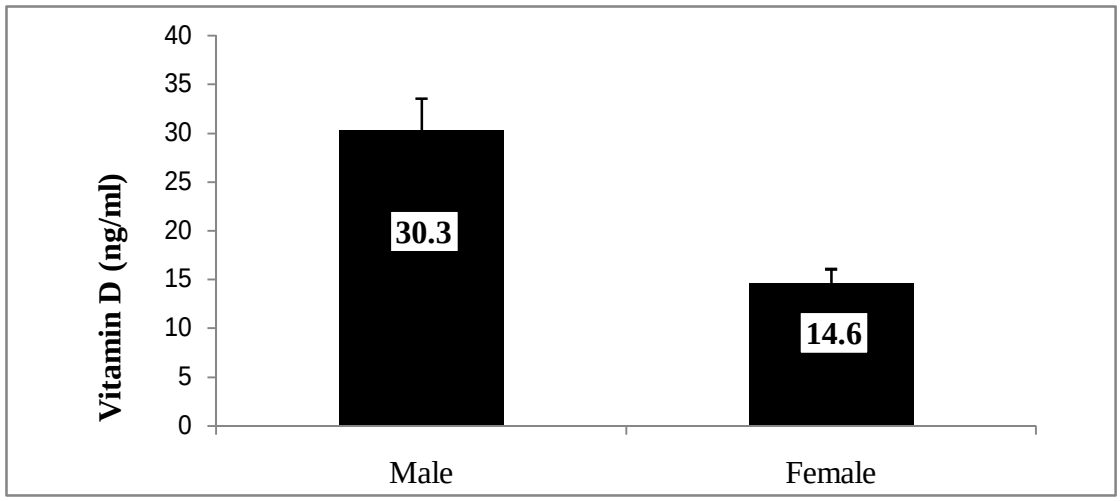

Fig.3.7 Shows mean of vitamin D level in study group classified as male and female, result expressed as (M ± STD), with *P*-value 0.000.

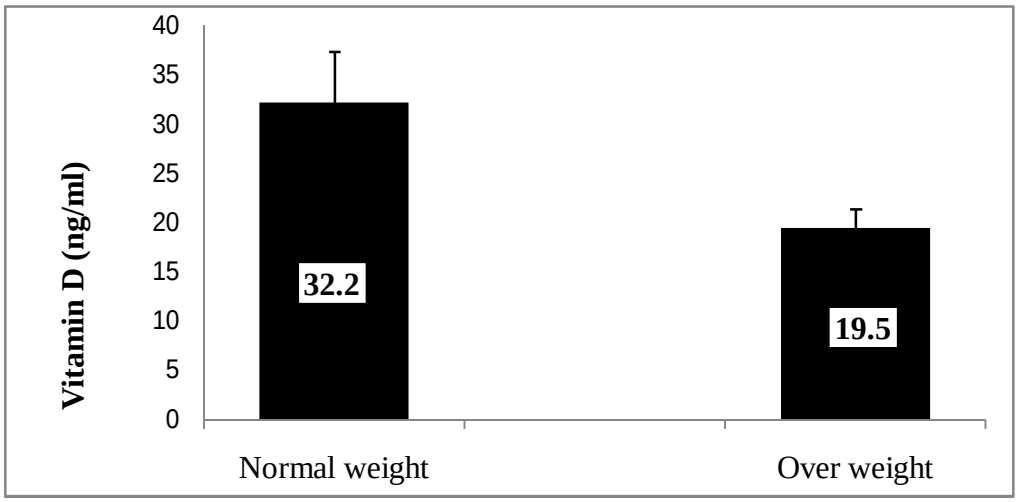

Fig.3.8 Shows mean of Vitamin D level in study group classified as normal weight (BMI ≤ 25 kg/m^2)and over weight (BMI > 25 kg/m^2), result expressed as (M ± STD), with *P*-value 0.033.

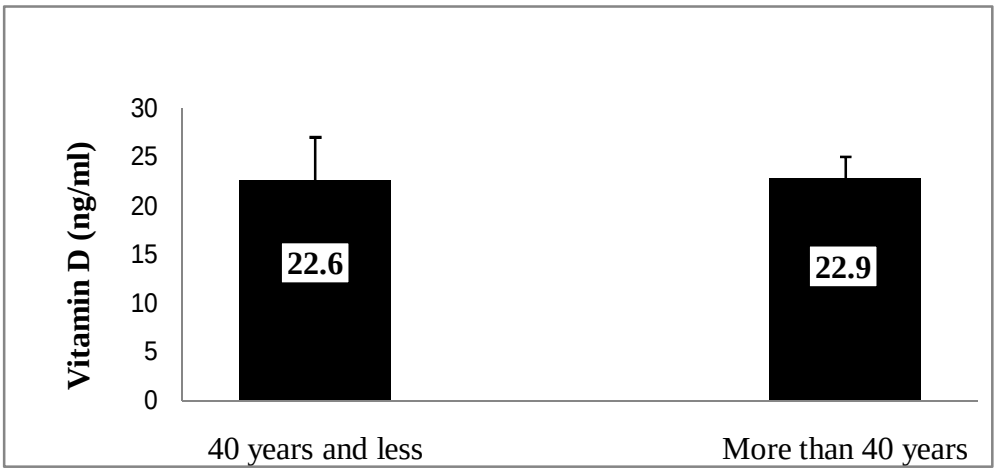

Fig.3.9 Shows mean of Vitamin D level in study group classified as 40 years and less and more than 40 years, result expressed as (M ± STD),with *P*-value 0.959.

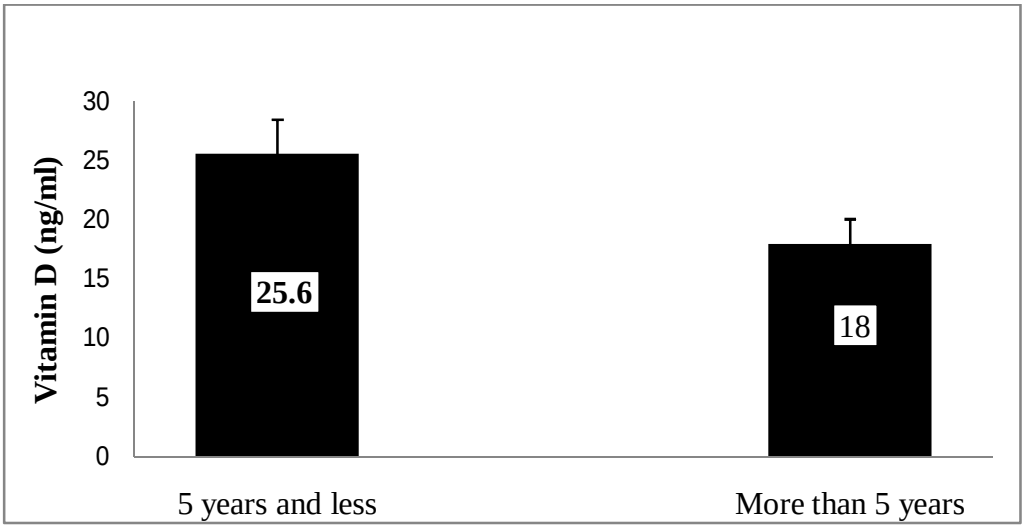

Fig.3.10 Shows mean of Vitamin D level in study group classified as group with disease for 5 years and less and other with disease for more than 5 years, result expressed as (M ± STD), with *P*-value 0.041.

Correlations

Table3.4 Shows Pearson correlation analysis showed the correlation between vitamin D and magnesium, result expressed as (Pearson's r: 0.019, *P*: 0.862).

		Vitamin D
Magnesium	Pearson Correlation (r)	+0.019
	Sig. (2-tailed)	0.862
	N	88

r = Correlation coefficient = +0.019

+ = Positive correlation

Sig= Strength of correlation= 0.862

N = Number of patients

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, researchers reported, magnesium is important in the physiological regulation of blood pressure whereas alterations in cellular magnesium metabolism could contribute to the pathogenesis of blood pressure 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 magnesium levels among vitamin D deficient hypertensive patients in Khartoum State during the period of March to July 2014.

The results of frequencies Showed that the gender variations are equal in hypertension patients approximately 1: 1 fold, this finding similar with previous report that hypertension prevalent in black women to black men, 35.8% and 30.9% respectively, and in white women to white men, 30.2 and 27.7%, respectively **(Kearney *et al.*, 2004)**.

In addition our study showed that hypertensive male were more overweight (80.4%) than female (66.7%), these results indicate that male are more susceptible to complication of obesity in hypertension patients, which confirm the roles of obesity in mechanisms of hypertension, obesity and weight gain have been identified as the most important determinants of hypertension, by which 10% rise in body weight explains a 7 mm Hg rise in systolic blood pressure **(Diaz, 2002)**.Also the current study observed that, vitamin D deficient is more common in female (81%) as (31% deficient and 50% sever deficient) than male whom account (45 %) as (37% vitamin D deficient and 8.6% sever deficient), justifying our finding that in the Sudan male more exposed to the sun light than female lead to trigger the synthesis of vitamin D **(Binkley *et al.*, 2007)**.The present study observed that, vitamin D deficiency was not affected by BMI in the males (74.7%in the normal weight group) as (74% vitamin D deficient and 00.0% Sever deficient vitamin D), (50.0% in the overweight group) as (39.5% vitamin D deficient and 10.5% Sever deficient vitamin D) but was affected by BMI in females (64.3%in the normal weight group) as (43.0% vitamin D deficient and 21.3% Sever deficient vitamin D), (89.3% in the overweight group) as (25.0% vitamin D

deficient and 64.3% Sever deficient vitamin D), this indicate that obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D from cutaneous and dietary sources because of its deposition in body fat compartments, serum vitamin D was also negatively correlated with BMI and body fat mass, different mechanisms relating hypovitaminosis D to obesity occur concurrently, previously reviewed mechanisms include that low vitamin D, may impair insulin action, glucose metabolism and various other metabolic processes in adipose and lean tissue ,vitamin D is affected by BMI, vitamin D status is strongly associated with variation in subcutaneous and especially visceral adiposity **(Worstman*et al*., 2000; Parikh *et al*., 2004; Snijder*et al*., 2005; McGill *et al*., 2008).**

The present study results found that there was insignificant difference in mean magnesium concentration in case groups compared with control group with (*P*-value 0.691 and 0.416) respectively, these finding indicates that magnesium was not affected by vitamin D level, these findings were disagreed with the previous findings, serum vitamin D concentrations are frequently low in patients with magnesium deficiency and may remain low even after 5-13 days of parenteral magnesium administration, magnesium depletion may impair vitamin D metabolism **(Rude, 1985).**

The present study revealed that, insignificant difference between mean of magnesium of in males in comparison with females with (*p*-value 0.665), magnesium is the fourth most abundant cation in the body and second most abundant intracellular ion, in the previous study report that Follow-up was 8 years for new-onset hypertension and 20 years for CVD, there was no association between baseline serum magnesium and the development of hypertension **(Khan *et al.*, 2010).**

Also our results showed that there was insignificant difference between magnesium level and BMI, age of the patients and duration of the disease, with (*p*-valve 0.828, 0.676 and 0.634) respectively, this evidence disagreed with study in which they found that higher serum magnesium level was significantly associated with lower body weight and BMI, serum magnesium was significantly lower in obese children compared with lean children **(Huerta *et al.*,2005; Song *et al,* 2007),** but our study done in adult subjects. Also magnesium measurements in plasma, red blood cells (RBCs), and mononuclear blood cells (MBCs) were made in normal volunteers they compared these parameters with age using analysis of

variance and found no significant differences **(Yang *et al.*, 1990)**. In addition community-based cohort does not support the hypothesis that low serum magnesium is a risk factor for developing hypertension **(Khan *et al.*, 2010)**. Therefore, magnesium may be important in the physiological regulation of blood pressure whereas alterations in cellular magnesium metabolism could contribute to the pathogenesis of blood pressure elevation, magnesium-rich diet should be encouraged in the prevention of hypertension, magnesium increases the effectiveness of all antihypertensive drug classes **(Ma *et al*., 1995; Pascal and Rhian, 2000; Sontia and Touyz, 2007; Houston, 2011).**

The present study revealed that, there is significant increase in the mean of vitamin D level of male compared with female(*P*-value 0.000), which reinforced by mentioned above observation, females were more susceptible to vitamin D deficiency than males, our justification that, Sudanese males expose to sun more than females their work thus exposed to sun light more than female which enhance vitamin D synthesis, the maximal 25(OH)D concentration produced by natural UV exposure **(Binkley *et al*., 2007)**.

The experimental evidence showed by current there was significant increase in mean concentration of vitamin D level in normal weight subject compared with overweight group with (*P*-value0.033), these results were confirmed by previous study found that there is an inverse association between BMI and the serum vitamin D level, therefore researchers noted that, strongly association between vitamin D and variation subcutaneous especially visceral adiposity **(Konradsen *et al*., 2008; Cheng *et al*., 2010; Lagunova *et al*., 2014)**.

Age groups results showed insignificant difference in the mean of vitamin D among age groupswith (*P*-value 0.959), these results agreed with Sherman report, vitamin D level was not affected by the age, in addition to that, no effect of age on the vitamin D concentration and Serum 25-hydroxy- and 1,25-dihydroxyvitamin D did not decline with age in either sex, in the bases of molecular studies **(Sherman *et al*., 1990; Vieth *et al.*, 2008).**

This descriptive cross-sectional study revealed that there was significant decrease in the mean of vitamin D level in patients have < 5years duration of disease compared with >5 years with (*P*-value 0.041), our finding clarify by previous cross-sectional studies have examined the association between plasma vitamin D and either blood pressure or prevalent hypertension. The great majority of these studies demonstrate that lower circulating vitamin

D levels were associated with higher blood pressures or a higher prevalence of hypertension **(Vaidya and Forman, 2010)**.

Finally, the present study showed no correlation between vitamin D and magnesium (*P*-value 0.862, r +0.019), these results noted that magnesium was not affected by vitamin D level, these findings were disagreed with the previous findings, serum vitamin D concentrations are frequently low in patients with magnesium deficiency and may remain low even after 5-13 days of parenteral magnesium administration, magnesium depletion may impair vitamin D metabolism **(Rude, 1985).**

Conclusion

The study concluded that percentage of females is slightly more than males, deficient sever deficient vitamin D group were more frequent than normal one, there is significant difference in the mean of vitamin D level between males, females and normal weight, overweight, age groups results showed insignificant difference in the mean of vitamin D levels, there is a significant difference in the mean of vitamin D with long duration of disease.

There was insignificant difference when compared mean of magnesium in gender groups, BMI groups, age groups, duration of hypertension and vitamin D levels.

Males and females in overweight group were more frequent than males and females in normal weight group and there was no correlation between vitamin D and magnesium level.

Recommendations

1- More research should be performed among large number of patients to determine the correlation between magnesium and study variables (age, BMI, gender and duration), vitamin D and magnesium in hypertensive patients to study the mechanisms and with estimation of more related parameters.

2- More efforts to more routinely estimation of magnesium and vitamin D status in patients with hypertension.

3- Possibly provide supplementation of magnesium and vitamin D to correct depletion in at-risk group (females and overweight) should be evaluated.

4- Magnesium and vitamin D rich diet is a good step for a good health.

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