

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, 84-85°C; 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 24°C for 72 h; best stored at 0-8°C. Vitamin D2 (C₂₈H₄₄O) Four double bonds; melting point, 121-128°C; 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, 1 α , 25(OH) 2D₃, before it can produce any biological effects. The activation of vitamin D₂ occurs via the same metabolic pathway as that of vitamin D₃, vitamin D₂ has only 25%–30% of the biological activity of vitamin D₃ (Zempleni *et al.*, 2007).

1.2.6 Absorption of vitamin D

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

1.2.7 Synthesis of vitamin D

Chemical Synthesis of vitamin D is that vitamin D is derived from cholesterol, the first synthesis of vitamin D resulted from the first chemical synthesis of cholesterol, as a consequence of a hydrogen shift the top panel depicts the dynamic changes occurring within the seco-B conjugated triene framework of the hormone (C5, 6, 7, 8, 9, 10, 19). Photochemical Production of Vitamin D₃ although the body can obtain vitamin D from the diet, the major source of this prohormone can be its production in the skin from 7-dehydrocholesterol. The highest concentrations of 7-dehydrocholesterol are found in the stratum basale and the stratum spinosum (Smith *et al.*, 2004; 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 1 α , 25(OH) 2D₃. DBP binds 88% of the total serum 25(OH) D₃ and 85% of serum 1, 25(OH) 2D₃, yet only 5% of the total circulating DBP actually carries vitamin D metabolites, the concentration of the free hormone may be important in determining the biological activity of the 1 α , 25 (OH) 2D₃ steroid hormones (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. 1 α , 25(OH) 2D₃ is the most active metabolite known, but there is evidence that 24, 25(OH) 2D₃ is required for some of the biological responses attributed to vitamin D, vitamin D undergoes its initial transformation with the addition of a hydroxyl group to the 25-carbon to form 25(OH)D₃, the major circulating form of vitamin D, the production of 25(OH) D₃ is catalyzed by the cytochrome P450 enzyme, vitamin D₃ 25-hydroxylase, the kidney is considered the primary source of circulating 1 α ,25(OH)2D₃. The major controls on the production of 1 α , 25(OH) 2D₃ are 1 α , 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 (PTH). Parathyroid hormone then proceeds to the osteoblasts and to the proximal convoluted tubule cells within seconds. Most importantly, in the convoluted tubule cells that serve as the endocrine gland for the vitamin D hormone, 1-hydroxylase concentrations are markedly elevated. This signals the vitamin D hormone, which by itself stimulates intestinal absorption of calcium or together with parathyroid hormone, at higher concentrations, stimulates mobilization of bone calcium and renal reabsorption of calcium, the increase in serum calcium concentrations exceeds the set point of the calcium sensing system, shutting down the parathyroid gland-induced cascade of events (Norman, 2008; Katsilambros *et al.*, 2010; Harvey and Ferrier, 2011).

1.2.12.2 Non genomic action of vitamin D

The rapid or non-genomic responses mediated by 1 α , 25(OH) 2D₃ were originally postulated to be mediated through the interaction of 1 α , 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 1 α , 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 $1\alpha,25(\text{OH})_2\text{D}_3$, including several oncogenes by far extending the classical limits of vitamin D actions on calcium homeostasis, the presence of muscle weakness or myopathy during metabolic bone diseases related to vitamin D deficiency (Zempleni *et al.*, 2007).

1.2.12.4 Specific functions of active vitamin D

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

effect on the expression of class I antigens (Zempleni *et al.*, 2007; Harvey and Ferrier, 2011).

1.2.13 Nutritional requirements and recommended dietary allowance of vitamin D

The vitamin D₃ requirement of healthy adults has never been precisely defined. Since vitamin D₃ is produced in the skin on exposure to sunlight and can be retained in vertebrate tissues, humans may not have a requirement for vitamin D when sufficient sunlight is available. The international unit (IU) of vitamin D₃ is defined as “the vitamin D activity of 0.025 mg of the international standard preparation of crystalline vitamin D₃. Thus, 1.0 IU of vitamin D₃ is 0.025 mg (Zempleni *et al.*, 2007).

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

1.2.14 Food sources of vitamin D

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

1.2.15 Vitamin D deficiency

A deficiency of vitamin D results in inadequate intestinal absorption and renal reabsorption of calcium and phosphate, as a consequence, serum calcium and phosphate levels fall and serum alkaline phosphatase activity increases, in response to these low serum calcium levels,

hyperparathyroidism occurs. Increased levels of PTH, along with whatever $1\alpha, 25(\text{OH})_2\text{D}_3$ 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 $1\alpha, 25$ -dihydroxyvitamin D_3 [$1\alpha, 25(\text{OH})_2\text{D}_3$] and its receptor, the vitamin D receptor (VDR), has resulted in significant contributions to good bone health in addition to the kidney's endocrine production of circulating $1\alpha, 25(\text{OH})_2\text{D}_3$ a paracrine production of this steroid hormone in 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 $1, 25(\text{OH})_2\text{D}$ concentrations were associated with lower plasma renin activity in hypertension, both $25(\text{OH})\text{D}$ and $1,25(\text{OH})\text{D}$ were inversely associated with plasma renin and angiotensin II concentrations (Wang, 2009; Vaidya and Forman, 2010).

1.2.18.2 Vitamin D and intracellular calcium homeostasis

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

1.2.18.3 Vitamin D and other vascular mechanisms

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

1.2.18.4 Secondary hyperparathyroidism

There are also other mechanisms involved in the relationship between blood pressure and vitamin D. Secondary hyperparathyroidism, commonly seen in vitamin D deficiency, could be the reason for hypertension. The mechanism is not completely clear, but it is a well-known association that high PTH levels affect vascular smooth muscle cells and increase vascular stiffness and promotes hypertension (Jafari and Paknahad, 2014).

1.3 C-reactive protein

1.3.1 Definition

C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation, it is an acute phase protein. Its physiological role is to bind to phosphocholine expressed on the surface

of dead or dying cells and some type of bacteria, in order to activate the complement system the C1Q complex (Thompson *et al.*, 1999). CRP is synthesized by the liver in response to factors released by fat cells (adipocyte). It is a member of the pentraxins family of proteins. It is not related to C-peptide or protein C. C-reactive protein was the first pattern recognition receptor (PRR) to be identified (Mantovani *et al.*, 2008).

1.3.2 History and nomenclature of CRP

CRP was so named because it was first discovered as a substance in the serum of patient with acute inflammation that reacted with the C- (capsular) polysaccharide of pneumococcus. Discovered by Tillet and Francis in 1930, it was initially thought that CRP might be a pathogenic secretion as it was elevated in people with a variety of illness including cancer; however discovery of hepatic synthesis demonstrated that it is a native protein (Faraj and Salem, 2012).

1.3.3 Genetic and biochemistry of CRP

The CRP gene is located on the first chromosome (1q21-q23). CRP is a 224-residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape and a member of the small pentraxins family (Faraj and Salem, 2012).

1.3.4 Function of CRP

The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial, viral, or fungal infections; rheumatoid and other inflammatory disease; malignancy; and tissue injuries and necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. During the acute phase response, levels of CRP rapidly increase within two hours of acute insult, reaching a peak at 48 hours. With resolution of the acute phase response, CRP declines with a relatively short half-life of 18 hours. Measuring CRP level is screen for infectious and inflammatory diseases. Rapid and marked increases in CRP occur in inflammation, infection, trauma, tissue necrosis, malignances and autoimmune disorders. Because there are a large number of disparate conditions that can increase CRP production, and an elevated CRP

production, an elevated CRP level does not diagnose specific disease. An elevated CRP level can provide support for the presence of an inflammatory disease, such as rheumatoid arthritis, polymyalgia rheumatica or giant cell arthritis (Pepys and Hirschfield, 2003). The physiological role of CRP is to bind to phosphocholine expressed on the surface of dead or dying cells, in order to activate the complement system. CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Thus, CRP participates in the clearance of necrotic and apoptotic cells (Pepys and Hirschfield, 2003). CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory process occurring in the body. This increment is due to arise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocyte. CRP assists complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limit within six hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production. And hence severity of precipitating cause. Serum amyloid A is a related acute-phase marker that responds rapidly in similar circumstances (Pepys and Hirschfield, 2003).

1.3.5 Clinical significance of CRP

Scleroderma, polymyositis, and dermatomyositis often elicit little or no CRP response. CRP levels also tend not to be elevated in SLE unless serositis or synovitis is present. Elevation of CRP in the absence of clinically significant inflammation can occur in renal failure. CRP level is an independent risk factor for atherosclerotic disease. Patients with high CRP concentrations are more likely to develop stroke, myocardial infarction, and severe peripheral vascular disease (Danesh *et al.*, 2004).

1.3.6 Role of CRP in cardiovascular disease

Recent research suggest that patients with elevated basal levels of CRP are at increased risk of diabetes, hypertension and cardiovascular disease (Pradhan *et al.*, 2001). Although one group of researchers indicate that CRP may be only a moderate risk factor for cardiovascular diseases (Danesh *et al.*, 2004), this study known as Reykjavik study was found to have some problems for this type of analysis related to the characteristics of the population studied, and there was an extremely long follow-up time which may have attenuated the association between CRP and future outcomes, others have shown that CRP can exacerbate ischemic necrosis in a complement-dependent fashion and that CRP inhibition can be a safe and effective therapy for myocardial and cerebral infarcts; so far, this has been demonstrated in animal models only (Pepys *et al.*, 2006). It has been hypothesized that a high CRP levels might reflect a large benefit from statins. This is based on the JUPITER trial that found elevated CRP levels without hyperlipidemia benefited. Statins were selected because they have been proven to reduce levels of CRP (Ridker *et al.*, 2008). A subsequent trial however failed to find that CRP was useful for determining statin benefit (Emberson *et al.*, 2011). To clarify whether CRP is a bystander or active participant in atherogenesis, a 2008 study compared people with various genetic CRP variants. Those with a high CRP due to genetic variation had no increase risk of cardiovascular disease compare to those with a normal or low CRP (Zacho *et al.*, 2008).

1.3.7 Diagnostic use of CRP

There are two different tests for CRP. The standard test measures a much wider range of CRP levels but is less sensitive in the lower ranges. The high-sensitivity CRP (hs-CRP) test can more accurately detect lower concentrations of the protein (it is more sensitive), which makes it more useful than the CRP test in predicting a healthy person's risk for cardiovascular disease (Faraj and Salem, 2012). CRP is used mainly as a marker of inflammation, Apart from liver failure; there are few known factors that interfere with CRP production (Pepys and Hirschfield, 2003).

Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. Blood, usually collected in a serum-separating tube, is analyzed in a medical laboratory or at the point of care. Various analytical methods are available for CRP determination, such as ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination. A high sensitive CRP (hs-CRP) test measures low levels of CRP using laser nephelometry, the test gives results in 25 minutes with sensitivity down to 0.04 mg/L, immunoturbidimetry (Immunoturbidimetric Method) this reagent is intended for the in vitro quantitative determination of CRP concentration in serum or plasma on automated clinical chemistry analyzers. Normal concentration in healthy human serum is usually lower than 10mg/L, slightly increasing with aging. Higher levels are found in late pregnant women, mild inflammation, and viral infections (10- 40) mg/L, active inflammation, bacterial infection (40 – 200mg/L), severe bacterial infection and burns (more than 200mg/L) (Clyne and Olshaker, 1999). CRP is more sensitive and accurate reflection of the acute phase response than the ESR. The half-life of CRP is constant. Therefore CRP levels are mainly determined by the rate of production. In the first 24hours, ESR may be normal and CRP elevated. CRP returns to normal more quickly than ESR in response to therapy (Clyne and Olshaker, 1999).

1.3.8 Cardiology diagnostic test of CRP

Arterial damage results from white blood cell invasion and inflammation within the wall. CRP is a general marker for inflammation and infection, so it can be used as a very rough proxy for heart disease risk. Since many things can cause elevated CRP, this is not a very specific prognostic indicator. Nevertheless, a level above 2.4mg/L has been associated with a doubled risk of a coronary event compared to levels below 1mg/L (Lloyd-Jones *et al.*, 2006).

1.3.9 CRP and hypertension

1.3.9.1 Pathophysiologic Implications

Some cross-sectional studies reported greater plasma CRP concentrations in treated or untreated patients with hypertension than in normotensive individuals. However, the effects of current or previous antihypertensive treatment and of hypertension duration could not be excluded in those studies. Moreover, in some of these studies, greater plasma CRP concentrations among patients with hypertension might be explained by a clustering of common positive CRP covariates (i.e. age, female sex, increased body mass index and lipid concentrations) among hypertensive patients. The association between elevated CRP levels and high blood pressure may have three different pathophysiologic explanations. First, causation may be involved, and indeed several studies hypothesise that CRP may induce a decrease in endothelium dependent relaxation, a potential risk factor for hypertension. Reverse causation might also be implicated, whereby high blood pressure may induce inflammation and raise CRP levels. On the other hand, the association could be explained by confounding, because CRP and high blood pressure share several risk factors such as lower sociodemographic position, lack of physical activity, smoking, and abdominal obesity (Ingle and Patel, 2011).

1.3.9.1.1 Causative connection

Several studies suggest that; CRP may have negative effects on vascular function and structure, and recent data have clearly documented that CRP is produced not only by the liver, but also in human atheroma by vascular smooth muscle cells and endothelial cells. Higher levels of CRP may increase blood pressure through a variety of biological effects in endothelial cells, which ultimately result in vasoconstriction and increased production of endothelin-1. The putative proatherogenic and hypertensive effects of CRP might also be mediated by upregulation of angiotensin type 1 receptor expression. Despite the documented effects of CRP on the vessel wall, its role in vascular biology remains elusive. Indeed, some data suggest that CRP might inhibit neutrophil adhesion and platelet aggregation. These contradictory data can be in part reconciled when

considering that CRP could dissociate into individual subunits during inflammation (Ingle and Patel, 2011).

1.3.9.1.2 Reverse causality

Hypertension may accelerate the atherosclerotic process through inflammatory mechanisms mediated by the vasoactive peptides angiotensin II and endothelin-1. A number of mechanisms may account for the proinflammatory effects of angiotensin II. Angiotensin II is primarily involved in the inflammatory process by modulating proinflammatory transcription factors such as NF- κ B, and by inducing the release of cytokines. Angiotensin II is also responsible for the activation of nicotinamide adeninedinucleotide (phosphate) (NADPH) oxidase in endothelial cells and in vascular smooth muscle cells. NADPH oxidase is the major source of vascular reactive oxygen species, which lead to endothelial dysfunction, endothelial growth, inflammation, and upregulation of endothelin-1. The renal protection afforded by inhibitors of the renineangiotensinealdosterone system appears to be at least in part due to inhibition of tissue macrophage infiltration. Moreover, pharmacological inhibition of the renineangiotensinealdosterone system results in reduced endothelial dysfunction, oxidative stress, inflammation, and plasminogen activator inhibitor-1 concentration. Endothelin- 1 is an important mediator of vascular inflammation in its own, via activation of NF- κ B and NAD(P)H⁺ oxidase. Cyclic strain has been shown to increase adhesion molecule expression by endothelial cells, to upregulate endothelial cell secretion of proinflammatory cytokines, which result in enhanced monocyte adhesion to the endothelium, and to increase oxidative stress in endothelial Cells (Ingle and Patel, 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.

Several studies hypothesise that CRP may induce a decrease in endothelium dependent relaxation, a potential risk factor for hypertension. Reverse causation might also be implicated, whereby high blood pressure may induce inflammation and raise CRP levels, several workers have reported elevated levels of CRP in hypertensive individuals. In the Sudan little is known about the association between vitamin D, CRP and cardiovascular disease in vitamin D deficient hypertensive patients, accordingly present research conducted to study CRP as predictor markers for cardiovascular disease in hypertensive patients with vitamin D deficient.

1.5 General objective

To study CRP as a predictor marker for cardiovascular disease among hypertensive vitamin D deficient patients in Khartoum State.

1.6 Specific objectives

- 1- To estimate vitamin D and CRP levels in study group.
- 2- To compare between CRP level in cases (vitamin D deficient and severe deficient) and control group (normal vitamin D).
- 3- To correlate between vitamin D levels and study variables (gender, BMI, age and duration).
- 4- To correlate between CRP level and study variables (gender, BMI, age 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, normal vitamin D group (≥ 20 ng/ml) considered as control, deficient vitamin D group (10-19.9 ng/ml) , sever deficient vitamin D group (up to 9.9 ng/ml).

2.1.4 Inclusion criteria

Overnight fasting specimens were collected from essential hypertensive patients.

2.1.5 Exclusion criteria

Patients with chronic inflammatory diseases like rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythematosus (SLE), autoimmune diseases, tuberculosis, diabetes, stroke, any hepatic or renal diseases and malignancies were excluded from this study.

Other diseases like diabetes mellitus 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 plain containers from each volunteer under aseptic condition. All blood samples were allowed to clot at room temperature, and then they were centrifuged at 4000 rpm to obtain the serum samples, and stored in -20° until the analysis.

2.1.7 Ethical Considerations

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

2.2 Methods

2.2.1 Vitamin D Estimation

2.2.1.1 Principle

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

Briefly according to manufactured, reagents and samples were brought 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 shaken slightly to ensure homogenous distribution of the solution. Absorbance was measured using (SUNRISE Techan) ELISA reader.

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 CRP Estimation

2.2.2.1 Principle

Particle enhanced immunoturbidimetric assay.

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

2.2.2.2 Procedure

Dispensing and all processes done automatically by using Cobas C 311 automated chemistry analyzer.

2.2.2.3 Calculations

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: $\text{mg/L} \times 9.52 = \text{nmol/L}$

$\text{mg/L} \times 0.1 = \text{mg/dL}$

2.2.3 Statistical Analysis

The data was analyzed using statistical package of social science (SPSS) computer program using frequencies, t-test and ANOVA, result was expressed as percentage (%) and (mean \pm SD), and significance difference was considered as (P -value <0.05).

3 Results

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

Table.3.1 Shows percentages of BMI, classified as normal weight ($\text{BMI} \leq 26.5 \text{ kg/m}^2$) and over weight ($\text{BMI} > 26.5 \text{ kg/m}^2$) among gender (male and female).

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

Table.3.2 Presenting the percentages of vitamin D status (normal, deficient and sever deficient) among gender (male and female).

Vitamin D groups	Gender	
	Male	Female
Normal vitamin D	54.4%	19.0%
Deficient vitamin D	37.0%	31.0%
Sever deficient vitamin D	08.6%	50.0%
Total (%)	100%	100%

Table.3.3 Shows percentages (%) of vitamin D status (normal, deficient and sever deficient) among both gender classified based on body weight, normal weight ($BMI \leq 26.5 \text{ kg/m}^2$) and over weight ($BMI > 26.5 \text{ kg/m}^2$).

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 Presenting the mean of hs CRP level in study group classified according to vitamin D status (normal, deficient and sever deficient), result expressed as ($M \pm STD$), with P -value between groups 0.203, and with P -value within groups:

Normal vitamin D group with deficient vitamin D group, P -value 0.795

Normal vitamin D group with sever deficient vitamin D group, P -value 0.049

Deficient vitamin D group with sever deficient vitamin D group, P -value 0.156

Fig. 3.3 Shows mean of hs CRP level in study group classified based on gender (male and female), result expressed as ($M \pm STD$), with P -value 0.374.

Fig. 3.4 Shows mean of hs CRP level in study group classified based on body weight, normal weight ($\text{BMI} \leq 26.5 \text{ kg/m}^2$) and over weight ($\text{BMI} > 26.5 \text{ kg/m}^2$), result expressed as ($M \pm \text{STD}$), with P -value 0.014.

Fig. 3.5 Presenting mean of hs CRP level in study group, classified as 40 years and less and more than 40 years, result expressed as ($M \pm \text{STD}$), with P -value 0.223.

Fig. 3.6 Shows mean of hs CRP level in study group based on duration of disease, as group with disease for 5 years and less and other with disease for more than 5 years, result expressed as ($M \pm \text{STD}$), with P -value 0.330

Fig. 3.7 Shows mean of vitamin D level in study group classified based on gender (male and female), result expressed as ($M \pm STD$), with P -value 0.000.

Fig. 3.8 Shows mean of Vitamin D level in study group classified based on body weight, normal weight ($\text{BMI} \leq 26.5 \text{ kg/m}^2$) and over weight ($\text{BMI} > 26.5 \text{ kg/m}^2$), result expressed as ($M \pm \text{STD}$), 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 ($M \pm \text{STD}$), with P -value 0.959.

Fig. 3.10 Shows mean of Vitamin D level in study group based on duration of disease, as group with disease for 5 years and less and other with disease for more than 5 years, result expressed as ($M \pm STD$), with P-value 0.041.

Correlations

Table.3.4 Shows Pearson correlation analysis showed the correlation between vitamin D and hs CRP, result expressed as (Pearson's r : 0.137, P : 0.205)

		Vitamin D
hs CRP	Pearson Correlation	
	(r)	-0.137
	Sig. (2-tailed)	0.205
	N	88

r = Correlation coefficient = -0.137 (negative correlation)

- = Negative correlation

Sig= Strength of correlation= 0.205 (weak correlation)

N = Number of patients

4 Discussions

Nowadays, hypertension is one of the most important causes of death all over the world because of its adverse effects on cardiovascular system and vitamin D is one of the important factors that may influence blood pressure. Many studies have shown the modulatory effect of this vitamin on rennin-angiotensin system as well as its inhibitory effect on vascular

smooth muscle hypertrophy (**Jafari and Paknahad, 2014**). Hypertension is one of the major risk factors for cardiovascular diseases, and it has been reported that hypertension is in part an inflammatory disorder and several workers have reported elevated levels of CRP in hypertensive individuals (**Dar et al., 2010**). Therefore this descriptive cross-sectional study was carried out to study hs CRP levels as predictor marker for cardiovascular disease among vitamin D deficient hypertensive patients in Khartoum State during the period of March to July 2014, in addition to correlate between vitamin D levels, hs CRP levels and study variables (gender, BMI, age and duration).

Concerning to gender, the results of frequencies showed that the gender variation are approximately equal 1: 1 fold (52.3% male and 47.7% female), with slightly increase in male percentage, this result was similar to previous reports in Saudi Arabia, Sudan, Kuwait and Egypt as (28.6%, 73.6%, 85.7%, and 61.76%) for males, and (23.9%, 26.4%, 14.3%, and 38.24%) for females, respectively (**Al-Nozha et al., 2007; Sherif et al., 2008; Almajed and Sadek, 2012; Abd El-Mohsen et al., 2013**), and in conflict with others study which stated that, female are more affected by hypertension than males, as reported in Sudan and Turkey as (76.3% and 31.1%) for female, and (23.7% and 14.1 %) for male respectively (**Elzubier et al., 2000; Dogan et al., 2012**). The possible explanation for our results that men are at risk of hypertension that, significant evidence that androgens, such as testosterone, play an important role in gender-associated differences in blood pressure regulation (**Reckelhoff, 2001**).

According to estimated BMI, our study revealed that hypertensive male were more overweight (80.4%) than female (66.7%), these results indicate that hypertensive male are more susceptible to complication of obesity, which confirm the roles of obesity in mechanisms of hypertension as reported by **Dustan**; that obesity and hypertension are closely linked,

obesity is a potent risk factor for hypertension and hypertension may predispose to obesity (**Dustan HP, 1991**). Our possible explanation for this result is that women are more likely to consume healthier food than men.

Analysis of frequency revealed that, vitamin D deficient are more common in female (81%) than male (45.6%), and the result of independent t-test showed significant increase in mean concentration of vitamin D in male compared to female with (P -value 0.000), thus females were more susceptible to vitamin D deficiency than males, the possible justification for our result is that males spend more time outdoors and women wear sun protective clothing and avoid sun exposure which may affect vitamin D synthesis. Our finding was agreed with previous study performed in Bahrain, that vitamin D deficiency was significantly higher in females (67.6%) than males (31.2%), in addition to study in Jordan that, the prevalence of low vitamin D status was (37.3%) in females compared to (5.1%) in males. Also agreed with study performed in Malaysia that, median vitamin D concentration of rural women was significantly higher [69.5 (59.0-79.1) nmol/L] compared to urban women [31.9 (26.1-45.5) nmol/L] ($p < 0.001$), since rural women spent more time in the sun compared to urban women (**Nurbazlin et al., 2013; Batieha et al., 2011; Golbahar et al., 2013**), and in conflict with others study which stated that there was a low vitamin D status despite abundant sun exposure (**Binkley et al., 2007**).

The present study results showed significant decrease in mean vitamin D level of overweight group in comparison with normal weight group with (P -value 0.033), in addition vitamin D inversely correlate with body weight in female in contrast there was no correlation between body weight and vitamin D in male. This observation was agreed with previous studies which revealed that obese subjects had significantly lower basal

25-hydroxyvitamin D concentrations than did age-matched control subjects, the prevalence of vitamin D deficiency was highest in individuals with BMI ≥ 40 , being as high as 32% among women and 46% among men, this indicates 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, this apparent decrease in vitamin D bioavailability with increased adiposity has been hypothesized to be due to the increased sequestration of vitamin D in fat, because vitamin D is fat soluble and is readily stored in adipose tissue, it could be sequestered in the larger body pool of fat of obese individuals **(Worstman *et al.*, 2000; Parikh *et al.*, 2004 ; Lagunova *et al.*, 2009; Tsiaras and Weinstock, 2011).**

To our knowledge the current research is the first study link between hypertension, vitamin D and CRP, thus results of present study provide experimental evidence that, there was significant increase in mean CRP level in severe deficient compared with control group with (P -value 0.049), followed by insignificant difference when compared vitamin D deficient with control group with (P -value 0.156), though the CRP a marker for cardiovascular disease our results indicates that CRP is useful predictor marker for cardiovascular disease in severe deficient hypertensive patients. Since both vitamin D deficiency and inflammation have been linked to cardiovascular disease, these differences improve the association between vitamin D deficient and increasing incidence of vascular inflammation, vitamin D is now recognised to have broader functions, including effects on immune regulation. Insufficient vitamin D status may lead to impaired immune function, with an increased vulnerability to proinflammatory state. In vitro studies have shown that vitamin D can decrease concentrations of inflammatory cytokines like TNF α and IL-6, while increasing secretion of the anti-inflammatory cytokine IL-10, other studies indicated that vitamin D shifts the immune

response from an inflammatory (T-helper cell 1: TH-1) to an anti-inflammatory (TH-2) profile, but human studies have reported conflicting results. A similar result was reported that hypertensive elderly patients showed significant negative associations between vitamin D and the pro-inflammatory markers IL-6 and CRP. Similar observation was made that, vitamin D deficiency is independently associated with elevated hsCRP, but this observation was made on children and young adults with lupus erythematosus **(Robinson *et al.*, 2014; Laird *et al.*, 2014)**.

The present study revealed that there was insignificant difference between mean of hs CRP in males in comparison with females with (P -value 0.374.). This finding was agreed with study performed in Nigeria that, among the hypertensive subjects, there was no significant difference in the level of C-reactive protein between the males and the females, also agreed with study performed in India that, within the patient group, the male hypertensive subjects does not show any significant difference in hs-CRP levels as compared to female hypertensive subjects **(Dar *et al.*, 2010; Idemudia and Idogun, 2012)**. It has been reported that hypertension is in part an inflammatory disorder and several workers have reported elevated levels of CRP in hypertensive individuals when compared to normotensive healthy individuals which confirm the role of CRP in hypertension or vice versa, several studies hypothesise that CRP may induce a decrease in endothelium dependent relaxation; a potential risk factor for hypertension, reverse causation might also be implicated, whereby high blood pressure may induce inflammation and raise CRP levels **(Dar *et al.*, 2010; Ingle and Patel, 2011; Idemudia and Idogun, 2012; Yanchun *et al.*, 2012)**. In conflict to earlier facts, study results performed in Korea showed that the level of hsCRP was not a risk factor for hypertension among adults aged over 50 years, living in a rural area **(Lee *et al.*, 2005)**.

Our study showed significant increase in mean CRP level of overweight group in comparison with normal weight group with (P -value 0.014), this finding confirms the association of hs CRP levels with body fat mass. The production of CRP is regulated by cytokines, principally interleukin-6 (IL-6), and serum CRP levels reflect IL-6 activity in humans. It was demonstrated that IL-6 is released in vivo by subcutaneous adipose tissue and is thereby able to have systemic effects, particularly in obese subjects, thus, adipose tissue may play a role in the regulation of serum CRP concentrations via IL-6 production (**Bastard *et al.*, 1999**). Our results were confirmed by previous studies found that hs-CRP level is high in obese patients and there was close relationship between BMI and hs-CRP serum levels, thus serum CRP concentrations were significantly correlated with BMI (**Bastard *et al.*, 1999 ; Gokalp *et al.*, 2007**).

Also our results showed that there was insignificant difference between CRP level and age of the patients, with (P -value 0.223), this evidence agreed with study performed in India in which they found that the levels of hs-CRP obtained in the different age group of the male and female were not differ significantly (**John and Srinivasan, 2014**).

The present study revealed that there was insignificant difference between hs CRP level and duration of hypertension, with (P -value 0.330), our finding was disagreed with previous study performed in India which reported that, the difference in the elevation levels of hs-CRP was found to be depend on duration, patients with shorter duration of hypertensive history were found to have significantly elevated levels of hs-CRP compared to those with longer duration of hypertensive history (**Dar *et al.*, 2010**).

Concerning age of the study group, our results showed insignificant difference in the mean of vitamin D among the age groups with (P -value 0.959), these results agreed with previous study revealed that, serum

25-hydroxy- and 1,25-dihydroxyvitamin D did not decline with age in either sex, in conflict another study revealed that older adults are often considered at increased risk of vitamin D deficiency due to limited sun exposure, decreased capacity for cutaneous vitamin D synthesis and reduced intake of dietary vitamin D (**Sherman *et al.*, 1990; Tsiaras and Weinstock, 2011**).

Also our study revealed that there was significant decrease in the mean of vitamin D level in patients have < 5years duration of hypertension compared with >5 years with (*P*-value 0.041). The majority of observational data suggest that lower levels of vitamin D may be associated with a higher blood pressure and a higher risk of developing hypertension, although conflicting studies exist. Experimental studies in animals, as well as some observational and experimental data in humans, suggest that vitamin D and its metabolites are integrally related to blood pressure and the RAS. Nevertheless, randomized, controlled trials have thus far failed to confirm that vitamin D supplementation lowers blood pressure (**Vaidya and Forman, 2010**). In a series of cohort and interventional studies a relationship was seen between serum vitamin D and blood pressure levels, while in some other no correlation was observed. The existence of polymorphism in vitamin D receptor gene can be a reason for these contradictory results. Among interventional studies, there are a studies supporting the idea that vitamin D supplementation reduces blood pressure. In contrast, there are studies claiming that vitamin D is not correlated with blood pressure. It seems that difference in dose and duration of vitamin D supplementation are the reason for this contradiction (**Jafari and Paknahad, 2014**).

Finally, the present study showed negative correlation between vitamin D and hs CRP (*P*-value 0.205, *r* -0.137). A similar observation was made by previous study as mentioned earlier that hypertensive elderly patients

showed significant negative associations between vitamin D and the pro-inflammatory markers IL-6 and CRP (**Laird *et al.*, 2014**). In contrast, there was no clear evidence that vitamin D supplements may reduce the CRP level, as reported that there was no statistically significant difference in hs-CRP after correction of hypovitaminosis D (**Carlson *et al.*, 2013**).

Conclusion

In conclusion, CRP as a useful predictor marker for cardiovascular disease in vitamin D deficient hypertensive patient, since they tend to have higher CRP level compared with control group. The study concluded that percentage of males is slightly more higher than females, females are more vulnerable to vitamin D deficiency than males, there is significant difference in the mean of vitamin D level of normal weight compared to overweight, as well as CRP level, and there is a significant decrease in the mean of vitamin D level in long duration term of disease compared to short duration term, in contrast no significant differences were found regarding age of the patients.

Mean of hs CRP showed insignificant difference in study variables classified according to (gender, age and duration of hypertension), while there was significant increased in overweight subjects when compared to normal weight, there was weak negative correlation between vitamin D and hs CRP level.

Recommendations

- 1- More studies recommended to underlying the mechanism of association between hypertension, vitamin D and CRP with estimation of more related parameters, ex: PTH, total calcium, ionized calcium and vitamin D receptor.
- 2- More research should be performed among large number of patients to determine the correlation between vitamin D, hs CRP and study variables (gender, BMI, age, and duration).
- 3- Possibly provide solutions and supplementations to correct depletion in risk groups (females and overweight) should be evaluated and monitored in order to avoid the complications of vitamin D deficiency and vitaminosis D.

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Sudan University of Science and Technology
College of Graduate Studies
Msc of medical Laboratory

Questionnaire

Date:
.....

ID NO:

Patient Name:

Age:

Sex: Male ☐ Female ☐

Phone Number:

Weight:kg

Height:meter

Body Mass Index (BMI):kg/m²

Duration of Disease:

Diseases:

Diabetes Mellitus: Y

Renal Faliure : Ye

Others:

Vitamin D Result:ng/ml

Hs CRP Result :mg/l