

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. 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; Bishop *et al.*, 2010). Earlier studies of hypertension prevalence in the Sudan were estimated at 7.5% (Elzubier *et al.*, 2000).

1.1.2 Classification of hypertension

The classification is based on the mean of two or more properly measured seated blood pressure readings on two or more office visits. Normal blood pressure is defined as levels <120/80 mmHg. Systolic blood pressure of 120–139 mmHg or diastolic blood pressure 80–89 mmHg is classified as prehypertension and these patients are at increased risk for progression to hypertension (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 (Chionget *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)

1.2 Vitamin D

The generic term vitamin D designates a group of chemically related compounds that possess antirachitic activity. The two most prominent members of this group are vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol); vitamin D does not meet the classical definition of a vitamin. A more accurate description of vitamin D is that it is a prohormone; thus, vitamin D is metabolized to a biologically active form that functions as a steroid hormone (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)-cholestatatriene- 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, 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 $1\alpha, 25(\text{OH}) 2\text{D}_3$ 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. $1\alpha, 25(\text{OH}) 2\text{D}_3$ is the most active metabolite known, but there is evidence that $24, 25(\text{OH}) 2\text{D}_3$ 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 $25(\text{OH})\text{D}_3$, the major circulating form of vitamin D, the production of $25(\text{OH}) \text{D}_3$ is catalyzed by the cytochrome P450 enzyme, vitamin D_3 25-hydroxylase, the kidney is considered the primary source of circulating $1\alpha, 25(\text{OH})2\text{D}_3$. The major controls on the production of $1\alpha, 25(\text{OH}) 2\text{D}_3$ are $1\alpha, 25(\text{OH}) 2\text{D}_3$ itself, PTH, and the serum concentrations of calcium and phosphate (Bender *et al.*, 2003; 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, $1, 25-(\text{OH}) 2\text{D}$, increases active intestinal calcium (and phosphate) absorption, when calcium concentrations decrease below normal, even slightly, coupled to a G protein system, stimulate the secretion of parathyroid hormone. Parathyroid hormone then proceeds to the osteoblasts and to the proximal convoluted tubule cells within seconds. Most importantly, in the convoluted tubule cells that serve as the endocrine gland for the vitamin D hormone, 1- hydroxylase concentrations are markedly elevated. This signals the vitamin D hormone, which by itself stimulates intestinal

absorption of calcium or together with parathyroid hormone, at higher concentrations, stimulates mobilization of bone calcium and renal reabsorption of calcium, the increase in serum calcium concentrations exceeds the set point of the calcium sensing system, shutting down the parathyroid gland-induced cascade of events (Norman, 2008; Katsilambros *et al.*, 2010; Harvey and Ferrier, 2011).

1.2.12.2 Non genomic action of vitamin D

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

1.2.12.3 Vitamin D in non-classical system

Nuclear receptors for $1\alpha, 25(\text{OH})_2\text{D}_3$ are found in a variety of tissues and cells not directly involved in calcium homeostasis, thus, the role of the vitamin D endocrine system has expanded to include a broader range of effects on cell regulation and differentiation, the expression of more than 100 proteins is known to be regulated by $1\alpha, 25(\text{OH})_2\text{D}_3$, including several oncogenes by far extending the classical limits of vitamin D actions on calcium homeostasis, the presence of muscle weakness or myopathy during metabolic bone diseases related to vitamin D deficiency (Zempleni *et al.*, 2007).

1.2.12.4 Specific functions of active vitamin D

Active vitamin D ($1\alpha, 25(\text{OH})_2\text{D}_3$) and minerals metabolism, the classical target tissues for $1\alpha, 25(\text{OH})_2\text{D}_3$ are those that are directly involved in the regulation of mineral homeostasis, serum calcium and phosphorous, actions on Intestine, deficiency of vitamin D severely impairs intestinal transport of both calcium and phosphorus, although calcium uptake is usually accompanied by phosphate uptake, the two ions are transported by independent mechanisms, both of which are stimulated by $1, 25(\text{OH})_2\text{D}_3$. Actions on bone, although the most obvious consequence of vitamin D deficiency is decreased mineralization of bone, $1, 25(\text{OH})_2\text{D}_3$ apparently does not directly increase bone formation or calcium phosphate deposition in osteoid, actions on kidney, $1, 25(\text{OH})_2\text{D}_3$ increases reabsorption of both calcium and phosphate. PTH

secretion is increased in vitamin D deficiency, and hence tubular reabsorption of phosphate is restricted. actions on the parathyroid glands, the chief cells of the parathyroid glands are physiological targets for 1, 25(OH) 2D3 and respond to it in a manner that is characteristic of negative feedback Immunoregulatory Roles of 1 α , 25(OH) 2D3, 1 α , 25(OH) 2D3 has been shown to affect cells of the immune system in a variety of ways. 1 α , 25(OH) 2D3 reduces the proliferation of HL-60 cells and also induces their differentiation to monocytes and macrophages. The actions of 1 α , 25(OH) 2D3 on normal monocytes is controversial but it appears that it may enhance monocyte function. 1 α , 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 $1\alpha, 25(\text{OH})_2\text{D}_3$ 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 Vitaminosis D

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

1.2.17 Vitamin D and hormone D

The steroid hormone $1\alpha, 25$ -dihydroxyvitamin D_3 [$1\alpha, 25(\text{OH})_2\text{D}_3$] and its receptor, the vitamin D receptor (VDR), has resulted in significant contributions to good bone health in addition to the kidney's endocrine production of circulating $1\alpha, 25(\text{OH})_2\text{D}_3$ a paracrine production of this steroid hormone in extra renal organs. This article identifies the fundamentals of the vitamin D endocrine system, including its potential for contributions to good health (DeLuca, 2004).

1.2.18 Biological mechanisms relating vitamin D with hypertension

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

Dietary sodium and increased activity of the RAS are known to contribute to hypertension; salt restriction and inhibition of RAS activity reduce blood pressure. vitamin D as a proximal inhibitor of the RAS 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, 2012).

1.3 Lipid and lipoprotein

1.3.1 Lipid chemistry

The term lipid applies to a class of compounds that are soluble in organic solvents, but nearly insoluble in water. Chemically lipids contain primarily nonpolar carbon-hydrogen (C-H) bonds are typically yield fatty acids and or complex alcohol after hydrolysis (Brutis, 2008). Lipids, commonly referred to as fats, have a dual role. First, because they are composed of mostly carbon-hydrogen (C-H) bonds, they are a rich source of energy and an efficient way for the body

to store excess calories. Because of their unique physical properties, lipids are also an integral part of cell membranes and, therefore, also play an important structural role in cells. The lipids transported by lipoproteins, namely triglycerides, phospholipids, cholesterol, and cholesteryl esters, are also the principal lipids found in cells (Bishop *et al.*, 2010). Lipids and lipoproteins, which are central to the energy metabolism of the body, have become increasingly important in clinical practice, primarily because of their association with coronary heart disease (CHD) (Bishop *et al.*, 2010).

1.3.2Lipid composition of food

a. Triglycerides comprise 98% of fat found in food and are made up of 95% fatty acid and 5% glycerol. Fatty acids are long carbon chains joined by single (saturated) or double bonds (unsaturated) and a terminal carboxyl group.

b. The remaining 2% of fat in food is composed of cholesterol, phospholipids, diglycerides, fat-soluble vitamins, steroids, and terpenes (Hubbard, 2010).

1.3.3Triglycerides

As can be inferred from the name, triglycerides contain three fatty acid molecules attached to one molecule of glycerol by ester bonds. Triglyceride is partly synthesized in the liver hepatocyte. It is transported through the bloodstream by chylomicrons and very low-density lipoproteins (VLDLs). Triglyceride provides energy to cells as it loses its fatty acid and forms ATP, thus acting as an energy store in the form of fat, and it insulates organs through fat deposits. (Hubbard, 2010) Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible structural forms of triglycerides. Triglycerides-containing saturated fatty acids, which do not have bends in their structure pack together more closely and tend to be solid at room temperature. In contrast, triglycerides, containing cis unsaturated fatty acids, there are no charged groups or polar hydrophilic groups, making it very hydrophobic and virtually water insoluble. Because it has no charge, triglyceride is classified as a neutral lipid. (Bishop *et al.*, 2010)

1.3.4 Cholesterol

Cholesterol is a sterol (steroid with long side chains), which is a four-ringed structure made in liver hepatocytes from two acetate units. The process is long, and 3-hydroxy-3-methylglutaryl

coenzyme A (HMG-CoA) reductase is the committed step. Cholesterol is an important constituent of cell membranes and a precursor of many hormones. Most serum cholesterol is in the form of cholesterol esters, which are transported through the blood by low-density lipoproteins (LDL) (Hubbard, 2010).

The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is, therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. Cholesterol is oriented in lipid layers so that the four rings and the side chain tail are buried in the membrane in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer, allowing it to interact with water by noncovalent hydrogen bonding. Cholesterol can also exist in an esterified form called cholesteryl ester, with the hydroxyl group conjugated by an ester bond to a fatty acid, in the same way as in triglycerides. In contrast to free cholesterol, there are no polar groups on cholesteryl esters, making them very hydrophobic. Because it is not charged, cholesteryl esters are classified as a neutral lipid and are not found on the surface of lipid layers but instead are located in the center of lipid drops and lipoproteins, along with triglycerides. Cholesterol is almost exclusively synthesized by animals, but plants do contain other sterols similar in structure to cholesterol. Cholesterol is also unique in that, unlike other lipids, it is not readily catabolized by most cells and, therefore, does not serve as a source of fuel. Cholesterol can, however, be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents. A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary, to steroid hormones, such as glucocorticoids, mineralocorticoids, and estrogens. Finally, a small amount of cholesterol, after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight. (Bishop *et al.*, 2010)

1.3.5 Lipoprotein

As the name implies, lipoproteins are composed of both lipids and proteins, called apolipoproteins. The amphipathic cholesterol and phospholipid molecules are primarily found on the surface of lipoproteins as a single monolayer, whereas the hydrophobic and neutral triglyceride and cholesteryl ester molecules are found in the central or core region. Because the

main role of lipoproteins is the delivery of fuel to peripheral cells, the core of the lipoprotein particle essentially represents the cargo that is being transported by lipoproteins. The size of the lipoprotein particle correlates with its lipid content. The larger lipoprotein particles have correspondingly larger core regions and, therefore, contain relatively more triglyceride and cholesteryl ester. The larger lipoprotein particles also contain more lipids relative to protein and thus are lighter in density. The various lipoprotein particles were originally separated by ultracentrifugation into different density fractions (chylomicrons [chylos], VLDL, LDL, and HDL), which still form the basis for the most commonly used lipoprotein classification system (Bishop *et al.*, 2010).

Apolipoproteins are the protein components of lipoprotein. Each class of lipoprotein has several apolipoproteins in differing proportions. Apo A1 is the major protein in HDL. Apo C I, C II, C III and E are present in various proportions in all lipoprotein. Apo B100 is the main protein on LDL, and Apo B48 which is produced from Apo B100 by an RNA editing process is on the chylomicron (Brutis, 2008).

Apolipoproteins are primarily located on the surface of lipoprotein particles (Bishop *et al.*, 2010). Apolipoproteins have the following major function: (1) modulating the activity of enzymes that act on lipoproteins. (2) Maintaining the structural integrity of the lipoprotein complex. (Hubbard, 2010) facilitating the uptake of lipoprotein by acting as ligands for specific cell surface receptors (Brutis, 2008, Bishop *et al.*, 2010).

1.3.5.1 Chylomicrons

Chylomicrons, which contain Apo B-48, are the largest and the least dense of the lipoprotein particles, having diameters as large as 1200 nm. Because of their large size, they reflect light and account for the turbidity of postprandial plasma. Because they are so light, they also readily float to the top of stored plasma and form a creamy layer, which is a hallmark for the presence of chylomicrons. Chylomicrons are produced by the intestine, where they are packaged with absorbed dietary lipids. Once they enter the circulation, triglycerides and cholesteryl esters in chylomicrons are rapidly hydrolyzed by lipases and, within a few hours, they are transformed into chylomicron remnant particles, which are recognized by proteoglycans and remnant receptors in the liver, facilitating their uptake. The principal role of chylomicrons is the delivery of dietary lipids to hepatic and peripheral cells (Bishop *et al.*, 2010).

1.3.5.2 Very Low Density Lipoproteins

Very Low Density Lipoproteins (VLDLs) are smaller than chylomicrons. They contain equal amounts of phospholipids and cholesterol, and degrade to LDLs in the circulation (Hubbard, 2010).

VLDL is produced by the liver and contains Apo B-100, apo E, and Apo Cs; like chylomicrons, they are also rich in triglycerides. They are the major carriers of endogenous (hepatic-derived) triglycerides and transfer triglycerides from the liver to peripheral tissue. Like chylomicrons, they also reflect light and account for most of the turbidity observed in fasting hyper-lipidemic plasma specimens, although they do not form a creamy top layer like chylomicrons, because they are smaller and less buoyant. Excess dietary intake of carbohydrate, saturated fatty acids, and *trans* fatty acids enhances the hepatic synthesis of triglycerides, which in turn increases VLDL production (Bishop *et al.*, 2010).

1.3.5.3 Low-Density Lipoproteins

Low-Density Lipoproteins (LDLs) contain mostly cholesterol, with equal amounts of phospholipid and protein and some triglyceride. They are taken into cells via a special cell-surface receptor the apoprotein B (Apo B) receptor and are degraded into component parts and this is considered “**bad**” cholesterol (Hubbard, 2010).

LDL primarily contains Apo B-100 and is more cholesterol rich than other Apo B-containing lipoproteins. They form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells. In addition, because LDL particles are significantly smaller than VLDL particles and chylomicrons, they can infiltrate into the extracellular space of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors. Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells, which are the predominant cell type of fatty streaks, an early precursor of atherosclerotic plaques. LDL particles can exist in various sizes and compositions and have been separated into as many as eight subclasses through density ultracentrifugation or gradient gel electrophoresis. The LDL subclasses differ largely in their content of core lipids; the smaller particles are denser and have relatively more triglyceride than cholesteryl esters. Recently, there has been great interest in measuring LDL sub fractions,

because small, dense, LDL particles have been shown to be more proatherogenic and may be a better marker for coronary heart disease risk (Bishop *et al.*, 2010)

1.3.5.4 High-Density Lipoproteins

HDLs contain mostly protein, some cholesterol, and a little triglyceride and they remove excess cholesterol from cells. HDL is considered the “good” lipoprotein (Hubbard, 2010). HDL is smallest and most dense lipoprotein particle is synthesized by both the liver and intestine. HDL can exist as either disk-shaped particles or, more commonly, spherical particles. Discoidal HDL typically contains two molecules of Apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol. Discoidal HDL is believed to represent nascent or newly secreted HDL and is the most active form in removing excess cholesterol from peripheral cells. The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport, is one of the main mechanisms proposed to explain the antiatherogenic property of HDL. When discoidal HDL has acquired additional lipid, cholesteryl esters and triglycerides form a core region between its phospholipid bilayer, which transforms discoidal HDL into spherical HDL. HDL is highly heterogeneous separable into as many as 13 or 14 different sub-fractions. There are two major types of spherical HDL based on density differences: HDL2 and HDL3. HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver (Bishop *et al.*, 2010).

1.3.5.5 Lipoprotein (a)

Lipoprotein (a) (Lp (a)) particles are LDL-like particles that contain one molecule of Apo (a) linked to Apo B-100 by a disulfide bond. Lp(a) particles are heterogeneous in both size and density, as a result of a differing number of repeating peptide sequences, called kringles, in the Apo (a) portion of the molecule. The concentration of Lp (a) is inversely related to the size of the isoform. Plasma levels of Lp (a) vary widely among individuals in a population but remain relatively constant within an individual. Elevated levels of Lp (a) are thought to confer increased risk for premature coronary heart disease and stroke. Because the kringle domains of Lp(a) have a high level of homology with plasminogen, a protein that promotes clot lysis, it has been proposed that Lp(a) may compete with plasminogen for binding sites, thereby promoting clotting, a key contributor to both myocardial infarction and stroke (Bishop *et al.*, 2010).

1.3.6 The physiology of lipids

The physiology of lipids involves three phases: a. Digestive phase begins with chewing and swallowing. Triglycerides are digested by lipase, other enzymes, bile salts, and acid in the gut to form monoglycerides and diglycerides. Cholesterol becomes surrounded by bile to form a micelle package that is absorbed by the small intestine. b. Absorptive phase occurs in the small intestine as triglycerides and cholesterol in the micelles are absorbed and broken down into fatty acids. c. Transport phase occurs as long fatty acids reassemble into chylomicrons (water soluble Macromolecules) and enter the lymphatic system. Short fatty acids enter the blood bound to albumin, and these head to all tissues, including adipose tissue (Hubbard, 2010).

1.3.7 Lipoprotein Physiology and Metabolism

The four major pathways involved in lipoprotein metabolism. The lipid absorption pathway, the exogenous pathway, and the endogenous pathway, which all depend on Apo B-containing lipoprotein particles, can be viewed as means to transport dietary lipid and hepatic-derived lipid to peripheral cells. In terms of energy metabolism, these three pathways are critical in the transport to peripheral cells of fatty acids, which are generated during the lipolysis of triglycerides and, to a lesser degree, cholesteryl esters on lipoproteins. In regard to the pathogenesis of atherosclerosis, the net result of these three pathways is also the net delivery or forward transport of cholesterol to peripheral cells, which can lead to atherosclerosis when the cells in the vessel wall accumulate too much cholesterol. Peripheral cells are prone to accumulating cholesterol because they also synthesize their own cholesterol, and, unlike liver cells, they do not have the enzymatic pathways to catabolized cholesterol. Furthermore, cholesterol is relatively water insoluble and cannot readily diffuse away from its site of deposition or synthesis (Bishop *et al.*, 2010).

One principal way that peripheral cells maintain their cholesterol equilibrium is the reverse cholesterol transport pathway, which is mediated by HDL. In this pathway, excess cholesterol from peripheral cells is transported back to the liver, where it can be excreted into the bile as free cholesterol or after being converted to bile acids. The liver is, therefore, involved in both forward and reverse cholesterol transport pathways and, in many ways, acts as a buffer in helping the body maintain its overall cholesterol level (Bishop *et al.*, 2010).

1.3.7.1 Exogenous Pathway

The role of exogenous pathway is the transport of dietary lipids that are absorbed by the intestine to the liver and peripheral cell is largely mediated by chylomicrons (Brutis, 2008).

The newly synthesized chylomicrons in the intestine are initially secreted into the lymphatic ducts and eventually enter the circulation by way of the thoracic duct. After entering the circulation, chylomicrons interact with proteoglycans, such as heparin sulfate, on the surface of capillaries in various tissues, such as skeletal muscle, heart, and adipose tissue. The proteoglycans on capillaries also promote the binding of lipoprotein lipase (LPL). The free fatty acids and glycerol generated by the hydrolysis of triglycerides by LPL can then be taken up by cells and used as a source of energy. Excess fatty acids, particularly in fat cells (adipocytes), are re-esterified into triglycerides for long-term storage in intracellular lipid drops. Hormone-sensitive lipase inside adipose cells can release free fatty acids from triglycerides in stored fat when energy sources from carbohydrates are insufficient for the body's energy needs. The hormones epinephrine and cortisol play a key role in the mobilization and hydrolysis of triglycerides from adipocytes, whereas insulin prevents lipolysis by adipocytes and promotes fat storage and glucose utilization (Bishop *et al.*, 2010).

During lipolysis of chylomicrons, there is a transfer of lipid and apolipoproteins onto HDL, and chylomicrons are converted within a few hours after a meal into chylomicron remnant particles. Chylomicron remnants are rapidly taken up by the liver through interaction of Apo E with specific remnant receptors on the surface of liver cells. Once in the liver, lysosomal enzymes breakdown the remnant particles to release free fatty acids, free cholesterol, and amino acids, while some cholesterol is converted to bile acids. Both bile acids and free cholesterol are directly excreted into the bile but not all of the excreted cholesterol and bile salt exit the body (Bishop *et al.*, 2010).

1.3.7.2 Endogenous Pathway

The primary function of the endogenous pathway is to transfer the hepatic derived lipids, especially triglyceride to the peripheral cells for energy metabolism. It is mediated by the Apo 100-containing lipoprotein (Brutis, 2008).

Most triglycerides in the liver that are packaged into VLDL are derived from the diet after recirculation from adipose tissue. A small fraction is synthesized *de novo* in the liver from dietary carbohydrate. VLDL particles, once secreted into the circulation, undergo alipolytic process similar to that of chylomicrons. VLDL loses core lipids causing dissociation and transfer of apolipoproteins and phospholipids to other lipoprotein particles, primarily by the action of LPL. During this process, VLDL is converted to VLDL remnants, which can be further transformed by lipolysis into LDL. About half of VLDL is eventually completely converted to LDL, and the remainder is taken up as VLDL remnants by the liver remnant receptors. LDL particles are the major lipoproteins responsible for the delivery of exogenous cholesterol to peripheral cells due to the efficient uptake of LDL by the LDL receptors. Once bound to LDL receptors, they are endocytosed by cells and transported to the lysosome, where they are degraded. The triglycerides in LDL are converted by acid lipase into free fatty acids and glycerol and further metabolized by the cell for energy or are re-esterified and stored in lipid drops for later use. Free cholesterol derived from degraded LDL can be used for membrane biosynthesis, and excess cholesterol is converted by acyl-CoA: cholesterolacyltransferase (ACAT) into cholesteryl esters and stored in intracellular lipid drops (Bishop *et al.*, 2010).

1.3.7.3 Reverse cholesterol transport pathway

As previously described, one of the major roles of HDL is to maintain the equilibrium of cholesterol in peripheral cells by the reverse cholesterol transport pathway. The function of the reverse cholesterol is to remove excess cellular cholesterol from peripheral cells and return it to the liver for excretion. Because most peripheral cells do not catabolize cholesterol and do not secrete cholesterol on lipoprotein, cholesterol under certain circumstances will accumulate and become toxic to the cell (Brutis, 2008).

HDL aids cell in their cholesterol homeostasis by removing it from cells by several different mechanism. Cholesterol is actively pumped out of the cells by the ABCA1 Transporter onto lipid-poor Apo A1, which binds to cells. This process results in the formation of disc-shape nascent HDL, which is made in the liver and intestine. Discoidal HDL also interacts with ABCA1 in peripheral cells; such as macrophage, and removes additional cholesterol. LCAT, which esterifies cholesterol on HDL, plays a key role in reverse-cholesterol transport because cholesteryl ester are much more hydrophobic than cholesterol and remain trapped in the core of

HDL until they are removed by the liver. The esterification of cholesterol on HDL converts the disc-shaped nascent HDL to spherical HDL. Spherical HDL, the main form of HDL in the circulation, also acts as an extracellular acceptor for cholesterol that may be removed from cells by the ABCG1 transporter or by a passive-diffusion mechanism (Brutis, 2008).

In the next stage of the reverse-cholesterol transport pathway, the liver selectively removes cholesteryl ester from the lipid-rich spherical HDL and lets the lipid-depleted HDL return to the circulation for additional rounds of cholesterol removal from peripheral cells. CETP also plays an important role in the pathway because a significant fraction of cholesterol that is removed from cells by HDL is transferred as cholesteryl-ester onto LDL by CETP and eventually removed from the circulation by hepatic LDL receptors. Besides promoting the efflux of cellular cholesterol, HDL also has antioxidant, anti-inflammatory and anti-clotting properties, which are not as well understood but are also likely beneficial in reducing atherosclerosis (Brutis, 2008).

1.3.8 Lipid and Lipoprotein Population Distributions

Serum lipoprotein concentrations differ between adult men and women, primarily as a result of differences in sex hormone levels, with women having, on average, higher HDL cholesterol levels and lower total cholesterol and triglyceride levels than men. The difference in total cholesterol, however, disappears after menopause as estrogen decreases. Men and women both show a tendency toward increased total cholesterol, LDL cholesterol, and triglyceride concentrations with age. HDL cholesterol concentrations generally remain stable after the onset of puberty and do not drop in women with the onset of menopause (Bishop *et al.*, 2010).

1.3.9 Lipid disorders

Diseases associated with abnormal lipid concentrations are referred to as dyslipidemias. They can be caused directly by genetic abnormalities or through environmental/lifestyle imbalances or they can develop secondarily, as a consequence of other diseases. Many, but not all, dyslipidemias, regardless of etiology, are associated with CHD, or arteriosclerosis (Bishop *et al.*, 2010).

Lipid disorders usually lead to abnormal lipid deposits on walls of vasculature (atherosclerosis) and skin (xanthomas). Hyperlipidemia obstruction leads to lack of bile, so cholesterol cannot be adequately absorbed by the small intestine (Hubbard, 2010).

Disease states associated with abnormal serum lipids are generally caused by malfunctions in the synthesis, transport, or catabolism of lipoproteins. Dyslipidemias can be subdivided into two major categories: hyperlipoproteinemias, which are disease associated with elevated lipoprotein levels, and hypolipoproteinemias, which are associated with decreased lipoprotein levels. The hyperlipoproteinemias can be subdivided into hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia with elevations of both cholesterol and triglycerides (Bishop *et al.*, 2010).

1.3.9.1 Hyperlipoproteinemia

Hypertriglyceridemia

Triglyceride levels are most affected by diet, but high triglyceride levels are often caused by diabetes or pancreatitis. Lipoprotein lipase (LPL), present in the capillary wall, hydrolyzes triglycerides for use in the tissues and can be affected by various hormones. If LPL does not function properly, serum triglyceride levels rise (Hubbard, 2010).

Hypertriglyceridemia is generally a result of an imbalance between synthesis and clearance of VLDL in the circulation. Hypertriglyceridemia can be a consequence of genetic abnormalities, called familial hypertriglyceridemia, or the result of secondary causes, such as hormonal abnormalities associated with the pancreas, adrenal glands, and pituitary, or of diabetes mellitus or nephrosis. Triglycerides are influenced by a number of hormones, such as insulin, glucagon, pituitary growth hormone, adrenocorticotrophic hormone (ACTH), thyrotropin, and adrenal medulla epinephrine and nor epinephrine from the nervous system. Epinephrine and nor epinephrine influence serum triglyceride levels by triggering production of hormone-sensitive lipase, which is located in adipose tissue.⁹⁵ Other body processes that trigger hormone sensitive lipase activity are cell growth (growth hormone), adrenal stimulation (ACTH), thyroid stimulation (thyrotropin), and fasting (glucagon). Each process, through its action on hormone-sensitive lipase, results in an increase in serum triglyceride values. (Bishop *et al.*, 2010)

Hypercholesterolemia

Hypercholesterolemia is the lipid abnormality most closely linked to heart disease. One form of the disease, which is associated with genetic abnormalities that predispose affected individuals to elevated cholesterol levels, is called familial hypercholesterolemia (Bishop *et al.*, 2010)

Cholesterol levels are affected mostly by genetic defects in the liver or by lack of Apo B receptors on cell surfaces, which leads to elevated cholesterol levels (Hubbard, 2010).

Combined Hyperlipoproteinemia

Combined Hyperlipoproteinemia is generally defined as the presence of elevated levels of serum total cholesterol and triglycerides. Individuals presenting with this syndrome are considered at increased risk for CHD. In one genetic form of this condition, called familial combined Hyperlipoproteinemia (FCH), individuals from an affected kindred may only have elevated cholesterol, whereas others only have elevated triglycerides, and yet others, elevations of both.

1.3.9.2 Hypolipoproteinemia

Hypolipoproteinemia is caused by a genetic defect leading to absent or decreased LDL and HDL.

(1) **Absent LDL** and low serum cholesterol leads to a failure to thrive, steatorrhea, central nervous system degeneration, and malabsorption of fat and vitamins.

(2) **Decreased LDL** leads to an increased life expectancy and decreased risk of myocardial infarction.

(3) **Reduced HDL** leads to an increased risk of atherosclerosis.

(4) **Absent HDL** (Tangier disease) leads to an accumulation of cholesterol esters in tonsils, adenoids, and spleen. It is considered a benign disease (Hubbard, 2010).

1.3.10 Methods of lipid analysis

a. Total cholesterol analysis involves either:

(1) **Formation of free cholesterol**, which is oxidized to form hydrogen peroxide, which then reacts with a dye to form a colored product

(2) **Selective oxygen electrode**, which measures the rate of oxygen consumption when an enzyme specific for cholesterol is added to serum

b. HDL cholesterol analysis involves precipitation of LDL and VLDL, followed by measurement of HDL in the supernatant.

c. LDL cholesterol analysis involves one of the following:

(1) **Calculation** by the following formula:

$$\text{LDL} = \text{total cholesterol} - (\text{HDL} + \text{triglyceride} / 5)$$

(2) Ultracentrifugation

(3) Immunoseparation using an ag-ab reaction

d. Triglyceride analysis uses either an:

(1) Enzymatic method that involves three enzymes—lipase, glycerol kinase, and glucose-6-phosphate-dehydrogenase (G6PD)—to form NADH

(2) Colorimetric method involving the formation of hydrogen peroxide (Hubbard, 2010).

1.3.11 The Biological mechanisms link Hypertension with lipid

The relationship between heart disease and dyslipidemias stems from the deposition of lipids, mainly in the form of esterified cholesterol, in artery walls. This lipid deposition first results in fatty streaks, which are thin streaks of excess fat in macrophages in the subendothelial space. Fatty streaks can develop over time into plaques that contain increased number of smooth muscle cells, extracellular lipid, calcification, and fibrous tissue, which can partially block or occlude blood flow. Plaque formation involves repeated cycles of cell injury, followed by infiltration and cell proliferation to repair the site. LDL is believed to play a central role in initiating and promoting plaque formation. It is deposited into the subendothelial space where it is taken up by various cells, including macrophages. This alters the gene and protein expression pattern of these cells and can promote an inflammatory response, particularly when LDL becomes oxidized. Injury signals from the evolving plaque trigger the expression of adhesion proteins on endothelial cells and the production of soluble chemo tactic proteins from resident macrophages, which promotes the attachment and infiltration of additional macrophages, lymphocytes, and platelets to the plaque. Continual injury and repair lead to additional narrowing of the vessel opening, or lumen, causing the blood to circulate in a non-laminar manner under greater and greater pressure. Because lipid deposits in the vessel walls are frequently associated with increased serum concentrations of LDL cholesterol or decreased HDL cholesterol, lowering LDL is an important step in preventing and treating CHD (Bishop *et al.*, 2010).

1.4 General objective

To evaluate lipid profile (Cholesterol, Triglycerides, LDL-Cholesterol and HDL-Cholesterol) levels among hypertensive vitamin D deficient patients in Khartoum State.

1.5 Specific objectives

1. To estimate vitamin D level and lipid profile in study group.
2. To compare between lipid profile and vitamin D level in Vitamin D deficient and severe deficient with control group (normal vitamin D).
3. To correlate between vitamin D levels and study variables (age, BMI, gender and duration).
4. To correlate between lipid profile level and study variables (age, body mass index, gender and duration).

1.6 Rational

Hypertension is known to be associated with alterations in lipid metabolism which gives rise to abnormalities in serum lipid and lipoprotein levels. So abnormalities in serum lipid and lipoprotein levels are recognized major modifiable cardiovascular disease and essential hypertension risk factors.

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. In the Sudan little information yet found in previous study evaluated lipid profile in vitamin D deficient hypertensive patients. Accordingly current study carried out to appraise the total cholesterol, triglyceride, HDL and LDL values in vitamin D deficient hypertensive patients for providing information to the health-policy planners and also to the clinical practitioners about the importance of routine monitoring of lipid profile and vitamin D in hypertensive patients for prevention of coronary heart disease and other consequences and introduce vitamin D as supplementation to hypertensive patient to combat morbidity and mortality and to reinforce the need to consider these parameters in daily clinical practice.

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 (Nile east model hospital, Khartoum teaching hospital, Alfaroug medical center, Alnelain diagnostic 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 (>30 ng/ml) considered as control, group two diffident vitamin D ($20 -30$ ng/ml) , group three sever diffident vitamin D (< 20 ng/ml).

2.1.4 InclusionCriteria

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, Fasting blood samples (5ml) was collected in plane containers from each volunteer under septic condition. All blood samples were allowed to clot at room temperature and then they were centrifuged at 4000 rpm to obtain the serum samples, and stored in -20° until the analysis.

2.1.7 Ethical Considerations

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

2.2 Methods

2.2.1 Vitamin D Estimation

2.2.1.1 Principle

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

2.2.1.2 Procedure

Briefly according to manufactured and 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

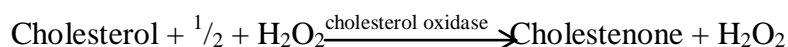
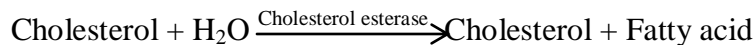
The standard curve from which the 25-OH vitamin D in the serum samples can be taken was obtained by point-to-point plotting of the extinction values measured for six calibration sera

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

2.2.2 Cholesterol measurement

2.2.2.1 Principle of method

Free and esterified cholesterol in the sample originates, by means of coupled reactions described below, a colored complex that can be measured by spectrometry



2.2.2.2 Procedure

Briefly according to manufactured and prior to use in the assay, reagents and samples were stand at room temperature. Pipetted into labeled test (B, ST, SP) tube 10 microliter of sample and cholesterol standard with 1 mililiter of reagent in each tube. Mixed thoroughly and incubate the tubes for 10 minutes at room temperature, then measure the absorbance (A) of the standard and sample at 520 nm against the blank

2.2.2.6 Calculation

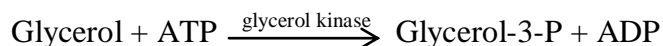
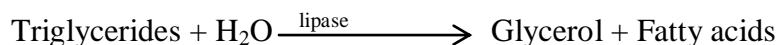
The cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{\text{A sample}}{\text{A standard}} \times \text{Standard concentration} = \text{mg/dl cholesterol}$$

2.2.3 Triglyceride measurement

2.2.3.1 Principle of method

Triglyceride in the sample originates, by means of coupled reactions described below, a colored complex that can be measured by spectrometry



2.2.3.2 Procedure

Briefly according to manufactured and prior to use in the assay, reagents and samples were stand at room temperature. Pipetted into labeled test (B, ST, SP) tube 10 microliter of sample and triglyceride

standard with 1 milliliter of reagent in each tube. Mixed thoroughly and incubate the tubes for 10 minutes at room temperature, then measure the absorbance (A) of the standard and sample at 520 nm against the blank

2.2.3.6 Calculation

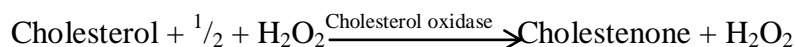
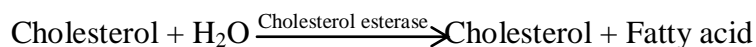
The Triglyceride concentration in the sample is calculated using the following general formula:

$$\frac{A \text{ sample}}{A \text{ standard}} \times \text{Standard concentration} = \text{mg/dl cholesterol}$$

2.2.4 Cholesterol LDL measurement

2.2.4.1 Principle of method

Low density lipoprotein LDL in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between serum total cholesterol and the cholesterol in the supernatant after centrifugation the cholesterol is spectrometry measured by means of coupled reactions described below.



2.2.4.2 Procedure

Briefly according to manufactured and prior to use in the assay, reagents and samples were stand at room temperature. Firstly Pipetted into centrifuge tube 0.2 ml of each sample and cholesterol LDL reagent. Mix thoroughly and let stand for 15 minutes at room temperature. Centrifuge at a minimum of 4000 r.p.m for 15 minutes. Carefully collect the supernatant. Secondly Pipetted into labeled test (B, ST, SP) tube 20 microliter of supernatant and cholesterol standard with 1 milliliter of reagent in each tube. Mixed thoroughly and incubate the tubes for 10 minutes at room temperature, then measure the absorbance (A) of the standard and sample at 520 nm against the blank

2.2.4.6 Calculation

The cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A \text{ sample}}{A \text{ standard}} \times \text{Standard concentration} = \text{mg/dl cholesterol in supernatant}$$

LDL cholesterol mg/dl = total cholesterol – cholesterol in supernatant

2.2.5 Cholesterol HDL

$$\text{HDL Cholesterol} = \text{Total cholesterol} - \frac{\text{LDL cholesterol} + \text{Triglyceride}}{5}$$

2.2.6 Statistical Analysis

Statistical analysis was performed using SPSS 14.0 window software (statistical package of social sciences). Continuous variables were presented in titer of mean \pm SD. Categorical variable were expressed as proportions. The student's – test was perform to test the differences in continuous variables, and test was used to study the association in proportions. All tests were two sides and value ≤ 0.05 was considered statistically significant

3 Results

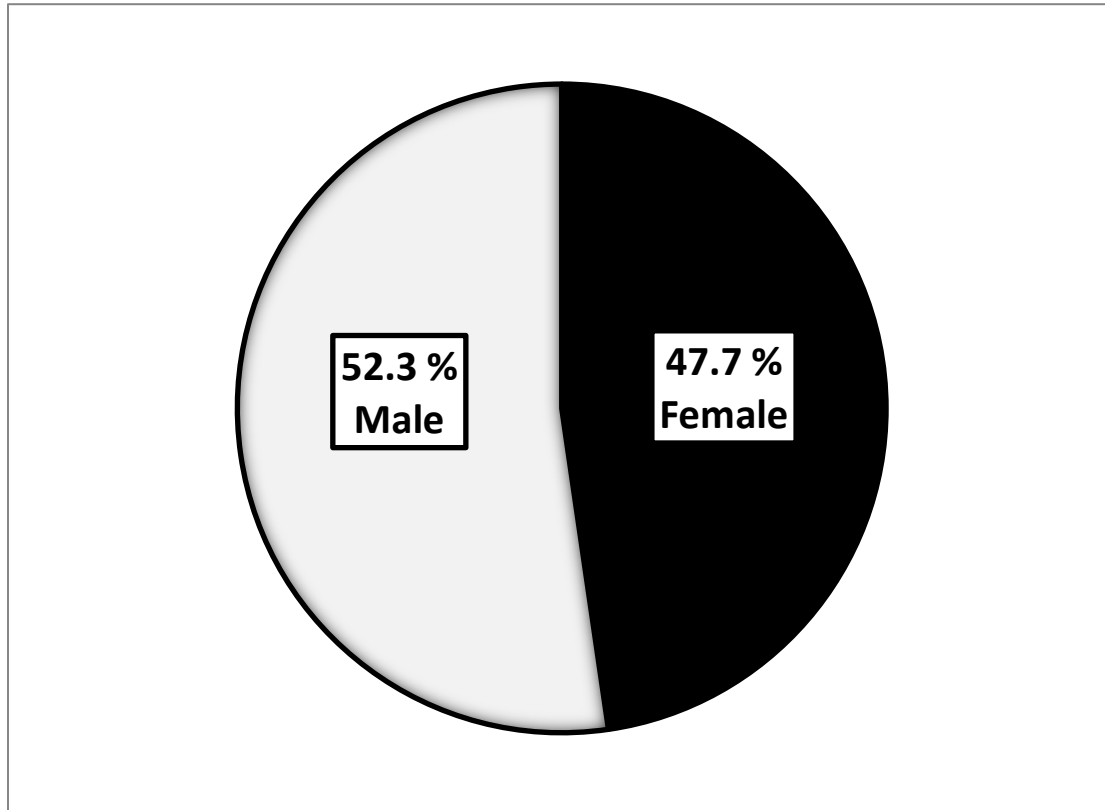


Fig. 3.1 Shows frequencies of gender among hypertension patients, results expressed as percentage (%).

Table 3.1 shows frequencies of BMI {(normal weight ($\text{BMI} \leq 25 \text{ kg/m}^2$) and over weight ($\text{BMI} > 25 \text{ kg/m}^2$))} in study group classified as male and female, result expressed as percentage (%).

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

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

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

Table 3.4 Shows frequencies of vitamin D level in study group classified as gender that have normal weight and other who have over weight, result expressed as percentage (%).

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

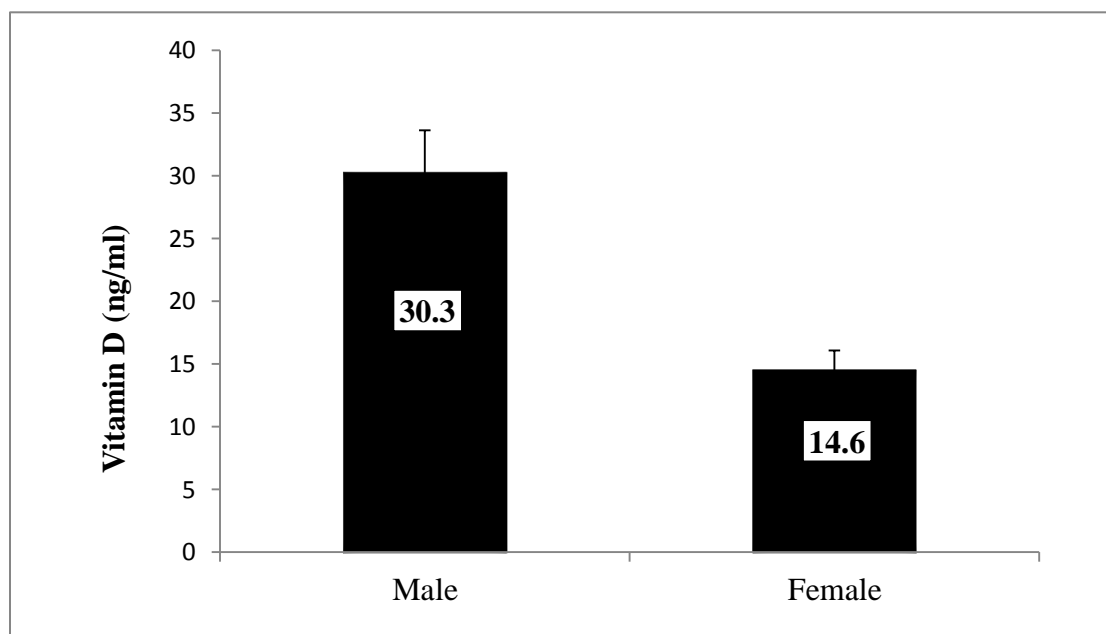


Fig. 3.2 Shows mean of vitamin D level in study group classified as male and female, result expressed as ($M \pm SD$) with P-value 0.000.

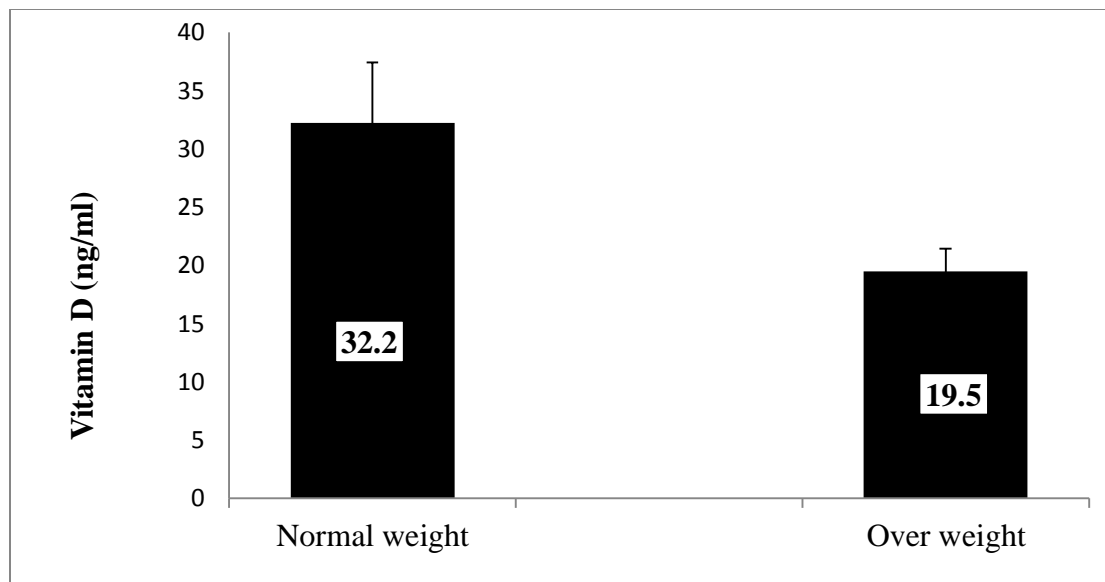


Fig 3.3 Shows mean of Vitamin D level in study group classified as normal weight ($\text{BMI} \leq 25 \text{ kg/m}^2$) and over weight ($\text{BMI} > 25 \text{ kg/m}^2$), result expressed as ($M \pm \text{STD}$), with P-value 0.033.

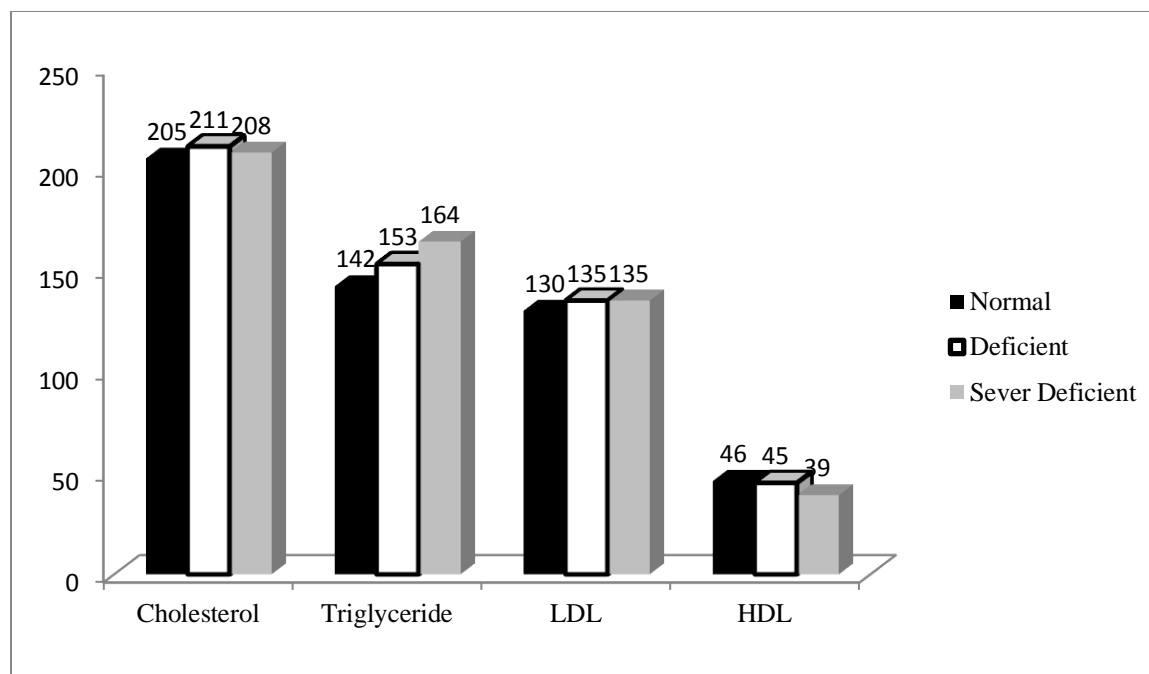


Fig 3.6 Shows mean of Cholesterol, Triglyceride, LDL and HDL level in study group classified as normal, deficient and sever deficient vitamin D group, result expressed as ($M \pm STD$) with P-value (0.882, 0.555, 0.927, 0.576) respectively.

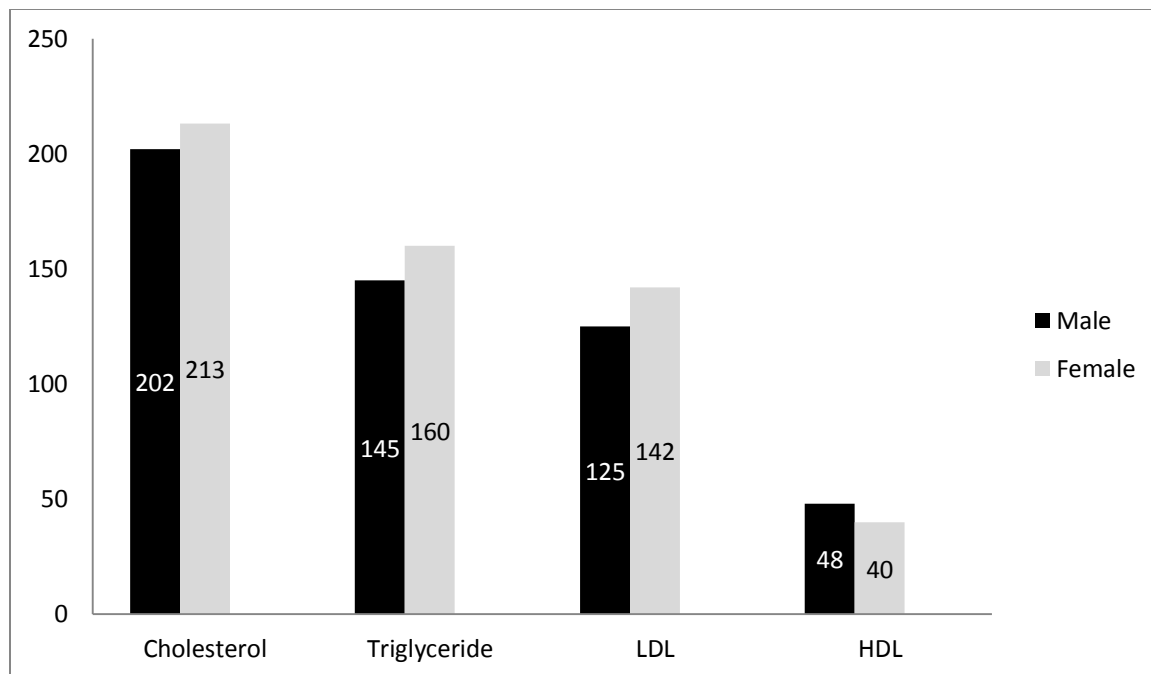


Fig. 3.6 Shows mean of Cholesterol, Triglyceride, LDL and HDL level in study group classified as male and female, result expressed as ($M \pm STD$) with P-value (0.251, 0.386, 0.123, 0.092) respectively.

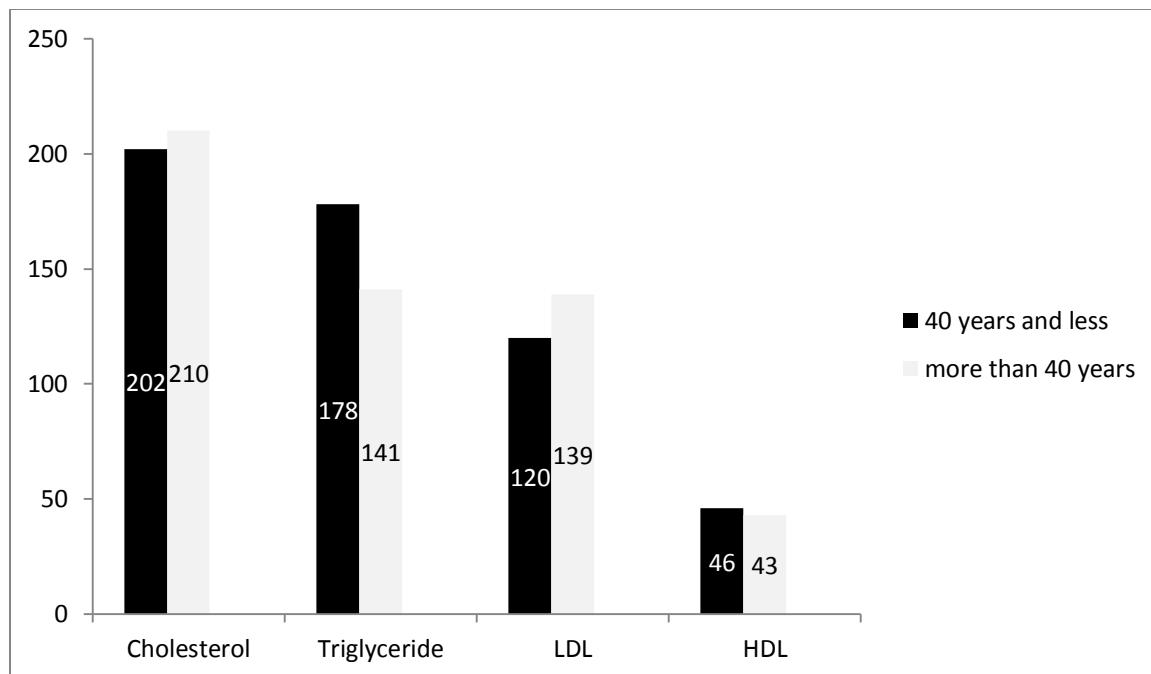


Fig. 3.7 Shows mean of Cholesterol, Triglyceride, LDL and HDL level in study group classified by age as 40 years and less and more than 40 years, result expressed as ($M \pm \text{STD}$), with P-value (0.494, 0.045, 0.127, 0.508) respectively.

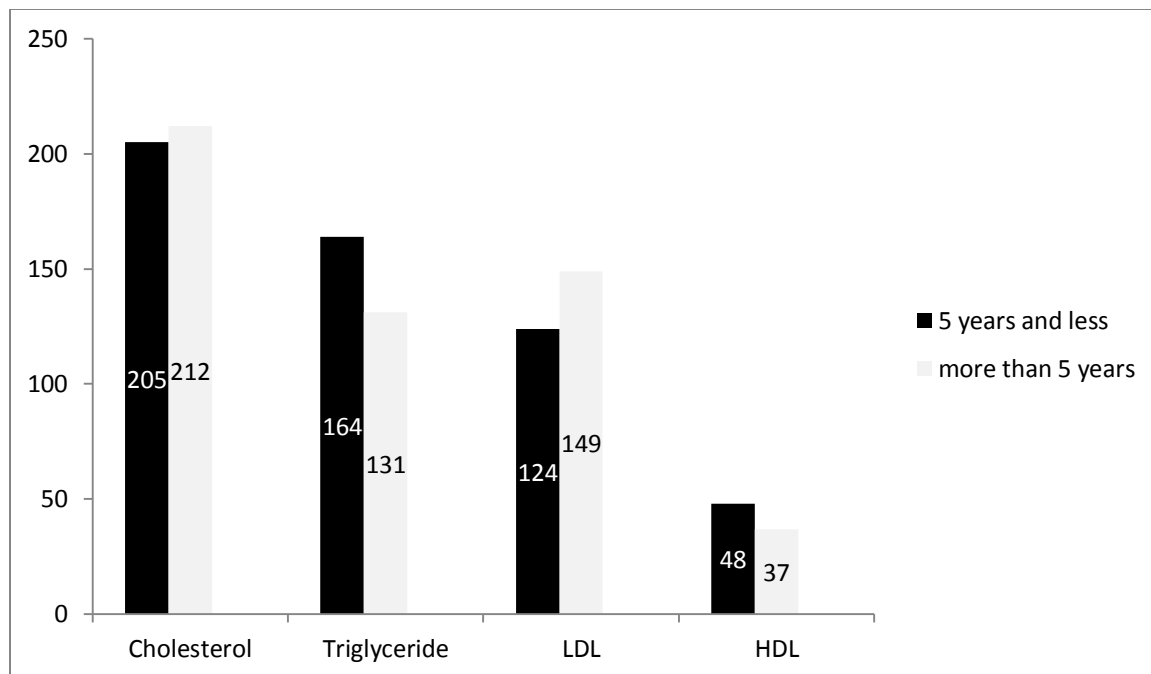


Fig. 3.8 Shows mean of Cholesterol, Triglyceride, LDL and HDL level in study group classified by duration of disease as 5 years and less and more than 5 years, result expressed as ($M \pm STD$), with P-value (0.509, 0.054, 0.037, 0.025) respectively.

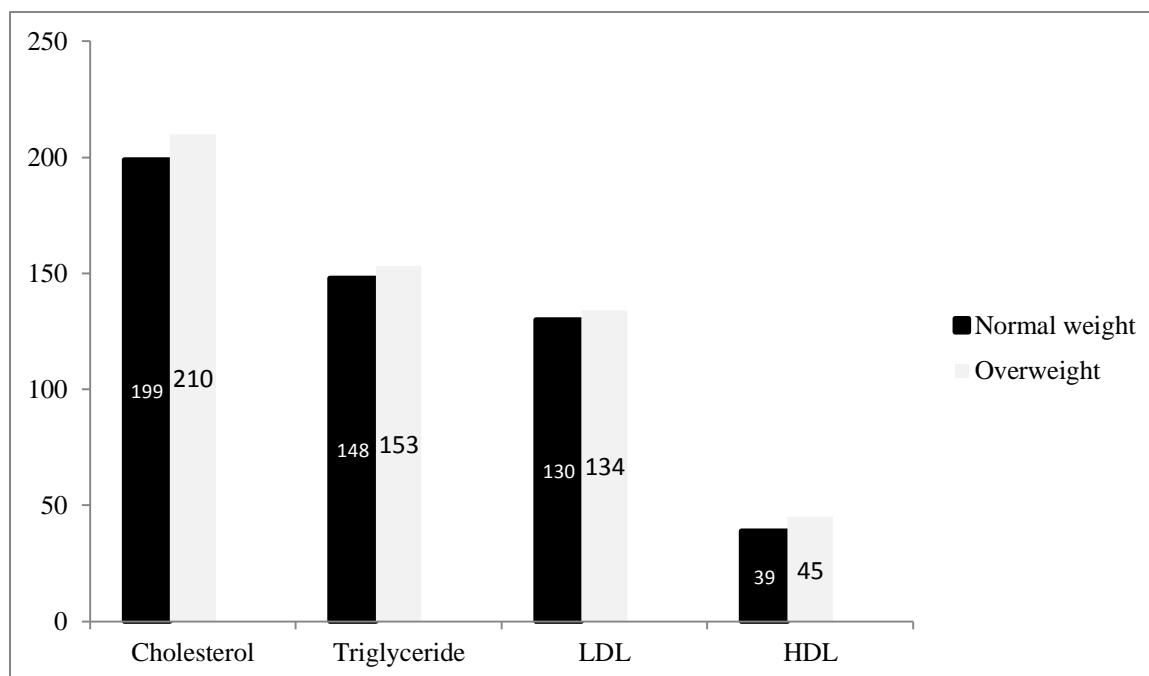


Fig. 3.9 Shows mean of Cholesterol, Triglyceride, LDL and HDL level in study group classified as normal weight ($BMI \leq 26.5 \text{ kg/m}^2$) and over weight ($BMI > 26.5 \text{ kg/m}^2$), result expressed as ($M \pm STD$), with P-value (0.333, 0.786, 0.694, 0.286) respectively.

Discussion

Hypertension is the most common causes of cardio-vascular diseases thus increase morbidity and mortality in the industrial world as well as becoming an increasing common disease in the developing countries. Recently, researchers emphasized in the association of vitamin D and hypertension, which link the vitamin deficiency with early risk of hypertension complications (Lim *et al.*, 2012), in addition changes in serum lipids metabolism strongly influence the early complication in hypertensive patients which lead to cardiovascular disease, accordingly current study aims to evaluate lipid profile level in vitamin D deficient hypertensive patients in addition to study relationship between serum lipids levels and vitamin D status in hypertensive patients in order to prevent early complication.

Results of frequency showed, gender variations are approximately equal in hypertension patients (52.3%) male and (47.7%) female. In addition study observed that female are more susceptible to vitamin D deficient than male (81%) female and (45.7%) male with vitamin D deficient, it is possible that due to life style changes such as working indoors, occlusive clothing, and increase use of sunscreen creams (John, 2005), so female expose less to the sun less often than male, resulting in reduced synthesis of vitamin D.

There is a consistent association in the published literature between increasing BMI and lower serum vitamin D concentrations. In our study over weight patients are more frequent in both sexes male and female (80.4%, 66.7%), but study observed percentage of male have increased overweight are more than female, and also serum vitamin D levels inversely correlated with BMI, as fact noted that body fat act as a reservoir for storage of the fat soluble vitamin D, reducing its bioavailability, therefore same time therelease of vitamin D from the fat is extremely slow and proportional to the concentration of the vitamin D in the adipose tissue. However, excess body fat results in its increased sequestration and low availability and, as a consequence, low serum vitamin D levels (Bischof *et al.*, 2006; Zoya *et al.*, 2009; Vanlint, 2013).

Few studies have been carried out on the relationship between serum vitamin D levels and lipid profiles, However the mean values seem to be similar in comparison with available literature data. In our studies results of One Way ANOVA test showed there were no significant differences between mean of serum lipid profile level cholesterol, triglyceride, LDL and HDL in both cases groups (vitamin D deficient and sever deficient) in comparison with control with *P*-

value (0.882, 0.555, 0.927, 0.576) respectively. Surprisingly, HDL cholesterol observed to be decrease in sever deficient followed by deficient groups when compared with control group, perhaps HDL have a direct relationship with serum vitamin D. This postulate is supported by previous findings of correlations between serum vitamin D concentrations and both HDL-cholesterol concentrations and ApoA-I concentrations (Auwerx *et al.*, 1992). In addition mean of triglyceride observed to be higher in case group (sever deficient group followed by deficient group) than normal group whereas Cholesterol and LDL tend to be lower in normal than both cases groups. These results are in agreement with recent study which stated that, vitamin D mediated reduction in serum triglycerides through regulatory action that increases the activity of lipoprotein lipase in adiposity, thus decreased absorption of fat, particularly saturated fatty acids, it is expected that serum levels of total and LDL cholesterol will be reduced but it does not have a significant effect on lipid profile (Chaudhuri *et al.*, 2013; Saedisomeolia *et al.*, 2014). According to other studies it is suggest that vitamin D deficiency is associated with change in serum levels of apolipoproteins, but not with fasting levels of lipids. Therefore more studies are needs to confirm the possible effect of vitamin D on serum lipids concentration (Saedisomeolia *et al.*, 2014).

Notably, there were no significant difference in mean lipid profile of male in comparison female hypertensive patients, but slightly higher levels of lipid profile was observed among women suggested to be due to variation in HDL reference range. Presumably these differences are due to different level of sex hormone, specially estrogen and androgens in women versus men. Cross sectional study data from large-scale population studies suggest that around the time of menopause LDL level increase by approximately 15-25% (Habib *et al* 2005). These findings are in agreement with other author (Arderin *et al.*, 2004, Saha, 2006, Okecka-Szymanska, 2011).

Out of all fraction of lipid profile, there was significant difference in mean triglyceride level among age groups (*P*-value 0.049). As we mentioned above triglyceride comprise 98% of fat found in food (Hubbard, 2010), we can suggest that elderly patient take less fat content food.

Obviously, we have observed that, patients with long history of disease (> 5 years) have significant increase in LDL levels, in contrast they have significant decrease in HDL level compared with those (< 5 years) group, and this increased the risk of cardiovascular diseases.

The results of our study revealed that, means of total lipid profile were higher in overweight hypertensive patients than normal weight patients whereas, even though we could not observe significant differences between fasting serum lipid profile and BMI was observed.

In conclusion: Female are more susceptible to vitamin D deficiency than male, also serum vitamin D levels inversely correlated with BMI. Although in hypertensive patients with vitamin D deficiency, serum levels of total cholesterol, TG, and LDL were higher and HDL was lower compared to patients with vitamin D sufficiency, this association was statistically insignificant. There was significant difference in mean triglyceride level among age groups, patients with long history of disease (> 5 years) have significant increase in LDL levels in contrast they have significant decrease in HDL level compared with those (< 5 years) group. In general hypertensive patients with vitamin D deficiency tend to develop cardiovascular disease more than those with normal vitamin D.

Recommendations

1. Monitoring of serum vitamin D should be useful and recommended in hypertensive patients
2. Exposure to sunlight and vitamin D supplementation recommended preventing hypertension complication. especially for women.
3. Physical exercises are recommended to the hypertensive patient, especially with overweight.
4. Recommended dietary intake for hypertensive patient exclusively with long history of disease should be performing.
5. More studies are needs to study the underline mechanism of associations between lipids metabolism, vitamin D in hypertensive patients.
6. Dietary habits, life style and hereditary along with other parameter such as (PTH Apo a lipoprotein, calcium and CRP) also need to be considered.

5. References

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