Sudan University of Science and Technology

College of Graduate Studies

Assessment of Serum Testosterone and Low density Lipoprotein levels among Males Hypertensive Patients

تقييم مستويات التستوستيرون وكوليسترول البروتين الدهني منخفض الكثافة في مصل الدم عند الرجال المرضى بإرتفاع ضغط الدم

A dissertation submitted in partial fullfilment for the requirement of the degree of M.Sc. in Medical Laboratory Science (Clinical Chemistry)

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الآيـــــة

قَالَ تَعَالَىٰ:

﴿وَيَسْـَٔلُونَكَ عَنِ ٱلرُّوحِ ۖ قُلِ ٱلرُّوحُ مِنْ أَمْرِ رَبِّى وَمَآ أُوتِيتُم مِّنَ ٱلْعِلْمِ إِلَّا قَلِيلًا ﴿٨٥﴾﴾

صدق الله العظيم

سورة الإسراء الآية (85)

Dedication

I dedicate my work

To my parents

To my sisters and brothers

To my Husband

To my beauty family

To my lovely friends

To all teachers that give me a new knowledge

to reach this educational degree

.

Acknowledgements

Firstly, grateful thanks of Allah who enabled me to conduct this study by grace of him and donated strength and patience.

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Beautiful thanks to all research, lab staff of world medical center, all patients from whom blood samples were collected and colleagues for my assistance.

Finally, my wish to express my great thanks to all people who helped me in this work.

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Abstract

Background: **Hypertension is a serious public health concern. More than one-quarter of the adult population over the world has hypertension, a significant health burden in many countries.**

Objectives: To assess serum testosterone and low density lipoprotein cholesterol levels among males Hypertensive patients in Khartoum state.

Methods: This was a case control study was conducted at Khartoum state during the period from April to December 2019 . It was aimed hypertensive male of different Sudanese ethnic groups. The over all number of participants was 80 male, 40 males as hypertensive patients and 40 male normal subjects serve as control, the mean of age among case was 54 years and 51 years among control group. Testosterone tested by Automated immunoassay Tosoh, AIA 360 and low density lipoproteins cholesterol tested by Bio system, Data was collected using a questionnaire and analysis was done by statistical package of social since (SPSS) software.

Results: There was significant decreased of **testosterone in male hypertensive** patients when compared with male control group (p. value 0.000) and mean SD in male hypertensive patients 4.03± 0.75 and in male control group 6.1 ± 0.46 , and low density lipoproteins cholesterol was significantly higher in the hypertensive groups than control (P.value 0.040) and mean SD in male hypertensive patients 2.44 ± 0.24 and mean SD in male control group 2.34 ± 0.16. There were significant decrease of testosterone related to age and duration of disease , There was negative correlation related to age (p.value 0.030 R -0.725), duration of disease (p.value 0.035 R -0.334) and testosterone ,There was no correlation related between low density lipoprotein cholesterol and age (p.value 0.085 R 0.600) ,duration of disease (p.value 0.198 R 0.22) and diastolic blood pressure (p.value 0.29 R 0.171) and there was negative correlation related

between low density lipoproteins cholesterol and systolic blood pressure (p.value -0.085 R0.005) . Testosterone level was positive correlation related to systolic blood pressure (p.value 0.290 R 0.016) and diastolic blood pressure (p.value 0.375 R0.017) .

Conclusion: This study concludes that the male hypertensive patients had lower testosterone and higher low density lipoprotein levels

المستخلص

مرض ارتفاع ضغط الدم هو مصدر قلق خطير على الصحة العامة ،اكثر من ربع السكان في جميع انحاء العالم يعانون منه .

تهدف هذه الدراسة الي تقييم مستويات هرمون التستوستيرون وكوليسترول البروتين الدهني منخفض الكثافة في مصل الدم عند الرجال المرضى بإرتفاع ضغط الدم الموجودين بولاية الخرطوم ، وقد أجريت هذه الدراسة في ولاية الخرطوم خلال الفترة من ابريل الى ديسمبر 2019 ، وقد استهدفت مختلف القبائل العرقية السودانية .

كان العدد الاجمالي للمشاركين ثمانين رجل ،اربعون منهم يعانون من مرض ارتفاع ضغط الدم واربعين ذكور طبيعيون بمثابة المجموعة الضابطة ، وكان متوسط العمر بالنسبة للمجموعة الضابطة ما بين عمر واحد وخمسين عاما الي اربعة وخمسين عاما، تم اختبار التستوستيرون بواسطة جهاز توسو 360 ،والبروتين الدهني منخفض الكثافة بواسطة الاسبكتروفوتوميتر وجمع البيانات باستخدام استبيان واجراء التحليل بواسطة الحزمة الاحصائية الاجتماعية.

ومن النتائج التي تحصلت عليها كان هناك انخفاض كبير في مستوى الهرمون عند الرجال المصابون بمرض ارتفاع ضغط الدم مقارنة بالمجموعة الضابطة غير المصابة القيمة الاحتمالية (0،00) والانحراف المعياري والوسط الحسابي عند المصابون(03.4 ± 75.0) وفي المجموعة الضابطة (1.6 ± 46.0) ، والبروتين منخفض الكثافة كان اعلى بكثير في المجموعة المصابة مقارنة بالمجموعة الضابطة القيمة الاحتمالية (04.0) ،و الانحراف المعياري والوسط الحسابي عند المصابون(44.2 ± 24.0) وفي المجموعة الضابطة (34.2 ± 16.0).

وقد وجدت علاقة عكسية بين مستوى هرمون التستوستيرون والعمر (p.value 0.03 R -0.725) ومدة المرض (p.value 0.198 R 0.22) . لم يكن هنالك ارتباط بين مستوى البروتين منخفض الكثافة (p.value0.216 R 0.200) .

مدة المرض (p.value 0.198 R 0.22) وضغط الدم الانبساطي (p.value 0.29 R 0.171) ، وهنالك علاقة طردية بين مستوى البروتين منخفض الكثافة والضغط الانقباضي (p.value -0.085 R0.005) .

وهنالك علاقة طردية بين مستوى هرمون التستوستيرون وضغط الدم الانبساطي p.value (0.375 R0.017) والانقباضي (p.value 0.290 R 0.016) .

خلصت دراستي الي ان مستوى هرمون التستوستيرون منخفض كثيرا عند الرجال المصابين بمرض ارتفاع ضغط الدم ومستوى البروتين منخفض الكثافة مرتفع عندهم .

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List of abbreviations

ATP : adenosine tri phosphate

BP : Blood pressure

C-H: Carbon hydrogen bond

CHD: Coronary heart disease

Chylos : Chylomicrons

HDL: High density lipoproteins

HmG.COA: 3-hydroxy methyl 3- methyl glutaryl Co enzyme A

HTN: Hypertension

LDL-C :Low density lipoproteins cholesterol

LH: luteinizing hormone

mmHG: Milli meters of mercury

p value: probability value

RNA: Ribose nucleotide acid

SD: standard deviation

Shhg: sex hormone binding globulin

StAR: Steroidogenic acute regulatory protein

TG: Triglyceride

VLDL: Very low density lipoproteins

Who: world health organization

CHAPTER ONE

INTRODUCTION , RATIONALE and OBJECTIVES

Chapter one

Introduction , Rationale and Objectives

1.1 Introduction

The heart and blood vessels have a rich supply of testosterone receptors present

In the aorta and peripheral blood vessels as well as in the arterial cells and in the

normal male left ventricle. (Brash , Shereyl et al.,b 2017) .

Subjects with prevalent ischemic heart disease were reported to have significantly

lower serum testosterone levels than subjects without ischemic heart disease (Lichtenstein Met al., 1987) .

However, natural androgens inhibit male atherosclerosis, further, there is increased evidence in the literature to show that low levels of androgens is associated withadverse cardiovascular risk factors including an atherogenic lipid profile, systolic and diastolic hypertension, obesity, insulin resistance and raised fibrinogen in humans .Endogenous testosterone in males regulate the blood lipid metabolism , and the male with low plasma testosterone might be lead to lipid metabolism abnormality which is a risk factor for coronary disease.

Testosterone is an important hormone for men. It helps control growth and development and is linked to sex drive, muscle, and bone mass. Researchers are studying the idea that it's also linked to LDL in some way. Some think it might help prevent heart disease, but there's no proof of that yet. (David., 2016) ,Testosterone has been used since the 1930s for non medical, athletic

purposes, especially in male and female body builders and swimmers. Because testosterone abuse may increase arterial blood pressure, leading to left ventricular hypertrophy, it should be included among the differential diagnoses of secondary arterial hypertension. (Angell *et al.* , 2012) Moreover, testosterone abuse has been associated with myocardial infarction because of coronary vaso pasmor thrombosis.(Fanton *et al.* , 2009) Among the mechanisms of how exogenous, as opposed to endogenous, testosterone contributes to increase in cardiovascular risk, coagulators activation, as well as accelerated progression of coronary artery disease, have been described. (Angell *et al.*, 2012)

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., 2015).

Hypertension is a worldwide epidemic; accordingly, its epidemiology has been well studied in the United States and internationally.

1.2 Rationale

Hypertension is a health concern in the population According to American Heart Association, approximately 75 million adults in united states are affected by hypertension, the Rate of hypertension in the develop countries was 40.2% almost similar to rate of hypertension in UK (45.8%). Testosterone is the main hormonal agent used for cross-sex hormone therapy which is decrease with age as rapidly as 0.4–2% annually after age 30 years. Most Reports from show beneficial and detrimental effects of testosterone on blood pressure and other risk factors for cardiovascular disease, like lipid profiles In France, a study was done in 1997, they

found that men with low testosterone had a higher systolic blood pressure (Simon D et al., 2002) . Traditionally, high testosterone levels are thought to have a detrimental effect on lipid profiles.

1.3 Objectives

1.3.1 General objectives

To assess serum testosterone and LDL-C level among Hypertensive patients in Khartoum state.

1.3.2 Specific objectives

1- To measure serum testosterone and low density lipoprotein cholesterol (LDL-C) levels among hypertensive patients compared to control group.

2- To correlate between serum testosterone level and age, duration of disease and blood pressure (systolic and diastolic).

3- To correlate between serum LDL-C level and age, duration of disease and blood pressure (systolic and diastolic).

CHAPTER TWO

LITERTURE REVIEW

Chapter two

2.1 literature review

2.2.1 Hypertension

Blood pressure is measured in millimetres of mercury (mm Hg) and is recorded as two numbers usually written one above the other. The upper number is the systolic blood pressure - the highest pressure in blood vessels and happens when the heart contracts, or beat . The lower number is the

diastolic blood pressure - the lowest pressure in blood vessels in between heart beats when the heart muscle relaxes. Normal adult blood pressure is defined as a systolic blood pressure of 120 mm Hg and a diastolic blood pressure of 80 mm Hg ,(WHO).

Complication of hypertension are clinical outcomes that result from persistent elevation of blood pressure. Hypertension is a risk factor for all clinical manifestations of atherosclerosis since it is a risk factor for atherosclerosis it self .it is an independent predisposing factor for heart failure , coronary artery disease ,stroke ,renal disease and peripheral arterial disease . It is the most important risk factor for cardiovascular morbidity and mortality in industrialized countries (Hednesford ., 2007)

2.2.2 Epidemiology of Hypertension:

Hypertension is an important public health challenge world wide 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., 2014; Bishop et al., 2010).

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

2.2.3 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 pre hypertension and these patients are at increased risk for progression to hypertension (El-Guindy., 2015).

2.2.3.1 Essential hypertension

Is systemic hypertension of unknown cause that results from dis regulation 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., 2012).

2.2.3.2 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., 2012). 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).

2.2.4 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., 2012).

2.2.5 Diagnosis of hypertension

2.2.5.1 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., 2015).

2 .2.5.2 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., 2015)

Testosterone is an anabolic hormone that plays an important role in muscle anabolism (Cigarran *et al.,* 2017). Patients with end-stage renal disease are found to be at increased risk for hypo gonadism. In one report, testosterone deficiency (<10 nmol/L) was present in 44% of the men with renal failure, while 33% showed testosterone insufficiency (10–14 nmol/L), and only 23% had normal testosterone values (Thirumavalavan *et al*., 2015).

2.2.6 Testosterone

2.2.6.1 Definition and physiological function:

Testosterone is the principal androgenic anabolic steroid in human. It is produced primarily in the testis of male (Oscar *et al*., 2010). Small amount is also produced by the ovaries of female and, the adrenal cortex in both male and female. Testosterone is converted to two other important hormones. It is reduced to di hydro testosterone in specific tissues like the skin and the prostate and it is oxidized to estradiol. In men this oxidation mainly takes place in adipose tissue and in the testes. Testosterone and di hydro testosterone together are responsible for the typically male sex characteristics, but their function is different. In the adolescence testosterone induces the sex drive in men, enlargement of the penis, the production of sperm, increase of muscle mass and lowering of the voice, the so called anabolic effects. Di hydro testosterone is responsible for an increase of body hair, beard grow, acne, and at a later age for baldness and enlargement of the prostate. These are the androgenic effects (Aede and Willem., 2017).

2 .2.6.2 Biosynthesis and biochemistry

Testicular testosterone secretion is principally regulated by luteinizing hormone (LH) through its regulation of the rate-limiting conversion of cholesterol to pregnenolone within Leydig cell mitochondria by the cytochrome P-450 SCC enzyme located on the inner mitochondrial membrane. Cholesterol supply to mitochondrial steroidogenic enzymes is regulated by steroidogenic acute regulatory protein (stAR) which stimulates the flow of cholesterol from the outer mitochondrial membrane.to the inner mitochondrial membrane. All subsequent

enzymatic steps are located in the Leydig cell smooth endoplasmic reticulum (Eacker *et al*., 2018).

The cholesterol is predominantly formed by de novo synthesis from acetyl-CoA, although preformed cholesterol either from intracellular cholesterol ester stores or extracellular supply from circulating low-density lipoproteins also contributes (Miller and Auchus., 2011).

2.2.6.3 Metabolism

The metabolism of testosterone and di hydro testosterone takes place for 90% in the liver (Owing to the presence of steroid catabolic enzyme). Finally, testosterone undergoes inactivation by hepatic phase I and II metabolism to inactive oxidized and conjugated metabolites for urinary and/or biliary excretion (David., 2016).

Testosterone is converted to the most potent natural androgen DHT by the 5areductase enzyme that originates from two distinct genes (I and II). Type 1 5a-- reductase is expressed in the liver, kidney, skin, and brain, whereas type 2 5areductase is characteristically expressed strongly in the prostate but also at lower levels in the skin (hair follicles) and liver .The inactivation mechanism include the following: addition of two hydrogens (reduction) to a double bond or ketone group; removal of two hydrogens (oxidation) from a hydroxyl group; addition of hydroxyl group (hydroxylation) to a carbon in the steroid molecule; and conjugation of steroids by reaction of sulfuric acid or glucuronic acid with a hydroxyl group on the steroid molecule, forming steroid sulfates and glucuronides, respectively Both groups enhance the polarity of the whole molecule considerably and in this way the a polar steroids become soluble in water and can be excreted with the urine (frank., 2011).

2.2.6.4 Testosterone secretion and transport

Testosterone is released in the general circulation via the spermatic vein in a pulsatile way. In young males, this occurs in a circadian manner, with a Testosterone peak observed in the early morning. Aging is associated with progressive loss of circadian Testosterone secretion (alexander., 2017).

Once synthesized, the lipophilic androgens move out of the Leydig cells by passive diffusion, down the concentration gradient. Within the testis, testosterone and precursors diffuse freely into the interstitial space and enter the testicular blood capillaries that are immediately adjacent to Leydig cells (Stephen and Winters., 2017).

Interestingly, it is this process of testosterone release into the testicular vascular bed which might be altered in Klinefelter syndrome leading to reduced circulating testosterone levels. Once they are part of the systemic circulation, secreted testosterone binds to plasma proteins and is present in both bound and unbound forms. In adult humans, more than 95% of testosterone is complexed with proteins, both the high affinity sex hormone binding globulin (SHBG) and the low affinity albumin. The proportion of testosterone that is unbound or loosely bound represents the biologically active fraction, which freely diffuses from capillaries into cells. The SHBG-bound fraction is thought to act as a reservoir for the steroid, although SHBG bound steroids may also enter cells via endocytic receptors on the surface of target cells and contribute to hormone action. Increasing levels of SHBG during aging contributes to reduced free plasma testosterone during this period (Tuttelmann., 2014).

2.2.6.5 Pathophysiology

In general, a normal male testosterone level peaks at about age 20, and then it slowly declines. Sometimes significant changes in testosterone levels occur and is it termed hypogonadism, "male menopause" or andropause (Nayana., 2017)

2.2.7 Some causes of low testosterone levels

Some causes of low testosterone level are age**,** injury, Chemotherapy or radiation treatment, Klinefelter's Syndrome, Dysfunction of the pituitary gland, corticosteroid drugs, chronic kidney failure, Stress. Alcoholism and smoking, Obesity (especially abdominal) and Kallmann syndrome lead to decrease in testosterone production (Nayana., 2017).

2.2.8 Diagnosis of low testosterone:

Measure the amount of testosterone in your blood. Because testosterone levels fluctuate throughout the day, several measurements will need to be taken to detect a deficiency. Doctors prefer, if possible, to test levels early in the morning, when testosterone levels are highest (Nayana., 2017).

2.2.9 Treatment of low testosterone

Testosterone deficiency can be treated by: Intramuscular injections, given any where from two to 10 weeks apart, Testosterone gel applied to the skin or inside the nose, Muco adhesive material applied above the teeth twice a day, Long- acting subcutaneous pellet, Testosterone stick (apply like underarm deodorant (Nayana., 2017).

Testosterone deficiency is the common gonadal alteration in men mainly because of reduced prolactin clearance and uremic inhibition of

luteinizing hormone signaling at the level of the Leydig cells (Osman and Ismail, 2016). Moreover, other observation reported that, Low testosterone level is associated with endothelial dysfunction and the possibility of cardiovascular events (Cigarran *et al., 2*017).

2.2.10 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 non polar carbon-hydrogen (C-H) bonds are typically yield fatty acids and or complex alcohol after hydrolysis (Brutis., 2012).

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).

2.2.10.1 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. The remaining 2% of fat in food is composed of cholesterol, phospholipids, di glycerides, fat- soluble vitamins, steroids, and terpenes

(Hubbard., 2010).

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). (Hubbard., 2010) 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. Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible structural forms of triglycerides. (Hubbard., 2010)

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)

2.2.10.2 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-methylglutarylcoenzyme A (HMG-CoA) reductase is the committed step. (Bishop *et al*. , 2010)

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-C) (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 phosphor lipid molecules. The polar hydroxyl group on the cholesterol A-ring faces outward, away from the lipid layer, allowing it to interact with water by non covalent 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. (Bishop *et al*., 2010)

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- de hydro cholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight. (Bishop *et al*., 2010)

2.2.10.3 Lipoprotein

As the name implies, lipoproteins are composed of both lipids and proteins, called apo lipoproteins. 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. (Bishop *et al., 20*10).

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).

Apo lipoproteins are the protein components of lipoprotein. Each class of lipoprotein has several apo lipoproteins in differing proportions. Apo A1 is the major protein in HDL. Apo CI, CII, CIII and E are present in various proportions in all lipoprotein. Apo B100 is the main protein on LDL-C, and Apo B48 which is produced from Apo B100 by an RNA

editing process is on the chylomicron(Brutis., 2012).

Apo lipoproteins are primarily located on the surface of lipoprotein particles (Bishop et al., 2010) Apo lipoproteins 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., 2012, Bishop *et al*., 2010).

2.2.10.4 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).

2.2.10.5 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-Cholesterol 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).

2.2.10.6 Low-Density Lipoproteins

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

LDL-C 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-C is readily taken up by cells via the LDL-C receptor in the liver and peripheral cells. In addition, because LDL-C 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-C 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-C 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-C sub fractions, because small, dense, LDL-C particles have been shown to be more pro atherogenic and may be a better marker for coronary heart disease risk (Bishop *et al.*, 2010)

2.2.10.7 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 forma ring around a central lipid bi layer of phosphor lipid and cholesterol. (Hubbard., 2010).

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 anti atherogenic 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. HDL2particles are larger in size and richer in lipid than HDL3and may reflect better efficiency in delivering lipids to the liver (Bishop *et al*., 2010).

2.2.11The physiology of lipids

The physiology of lipids involves three phases:

2.2.11.1 Digestive phase

begins with chewing and swallowing. Triglycerides are digested by lipase, other enzymes, bile salts, and acid in the gut to form mono glycerides and di glycerides. Cholesterol becomes surrounded by bile to form a micelle package that is absorbed by the small intestine.

2.2.11.2Absorptive phase

occurs in the small intestine as triglycerides and cholesterol in the micelles are absorbed and broken down into fatty acids

2.2.11.3 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).

CHAPTER THREE

MATERIAL and METHODS

Chapter three

Material and Methods

3.1 Study Design

This was case-control study.

3.2 Study Area

This study was conducted in Khartoum State during the period from April to December 2019.

3.3 Study Population:

The study included 80 Sudanese males with age between 30 to 74 years ,40 of them who clinically diagnosed as hypertensive patients matched with 40 male normal subjects serve as control in same age with cases.

3.4 Inclusion Criteria

Healthy male individuals serve as control were included and also primary hypertensive male serve as case.

3.5 Exclusion Criteria

Diabetic, Renal disease, secondary hypertensive and patients with endocrinopathy were excluded.

3.6 Ethical Consideration

Ethical approval for conducting the research was obtained from the college of Medical Laboratory Sciences-SUST . A verbal consent was obtained from all the 80 participants after they had been informed about the aim of the study, expected outcome, confidentiality of the results and the procedure of blood collection.

3.7 Data Collection

The clinical data were obtained from clinical examinations and were recorded on sheeted a questionnaire .

3.8 Samples Collection and Processing:

About 4 ml of venous blood were collected from each participant (both cases and controls). The samples collected under aseptic conditions and placed in sterile Plain containers, and centrifuged for 10 minutes at 3000 rpm to obtain Serum, then the serum was kept at -20$_o$C till the time of analysis.

3.9 Estimation of Testosterone:

3.9.1 Principle of the Method:

Full automation technique is used Tosoh's AIA-360(Automated Immunoassay), the ST AIA-PACK Testosterone is a competitive Fluorescence Enzyme Immunoassay which is performed entirely in the ST AIA-PACK Testosterone test cup. testosterone present in the sample competes with enzyme-labeled testosterone limited number of binding sites on the testosterone specific monoclonal antibody immobilized on amagnetic solid phase. The magnetic beads are washed to remove unbound enzyme-labeled testosterone and are then incubated with a fluorogenic substrate, substrate 4-methylumbelliferyl phosphate (4MUP), the amount of-labeled testosterone that bind to the beads is inversely proportional to the testosterone concentration in the test sample. A standard curve is constructed, and unknown sample concentration are calculated using this curve.

3.9.2Calculations of results:

A standard curve which constructed by using calibrators with known concentrations, an unknown sample concentration is calculated using this curve.

3.10 Quality control:

The precision and accuracy of all methods used in this study were checked at least once per day with commercially available control, was done at least two levels of control (normal and abnormal).

3.11 Measurement of plasma low density lipoprotein:

I. Principle:

Low density lipoproteins (LDL-C) in the sample precipitate with polyvinyl

sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by mean of the coupled reactions described below.

Cholesterol esters + H2O cholesterol esterase cholesterol + Fatty acid

Cholesterol + 1/2 O2 + H2O cholesterol oxidase Cholestenone+ H2O2

H2O2 + 4-Aminoantipyrine + phenol peroxidase Quinoneimine + 4H2O

II. Procedures

Precipitation

pippetted into labeled test tubes **,** tacked 0.2 ml from serum sample and 0.2 ml of Reagent (A) low density lipoprotein cholesterol kit.

The tubes were mixed thoroughly and incubated for 15 minutes at room temperature and centrifuged at minimum of 4000 r.p.m for 15 minutes. The supernatant was carefully collected.

The reagent was brought to room temperature, pipetted in to labelled test tubes The tubes were mixed thoroughly and incubated for 30 minutes at room temperature (16-25C) or for 10minutes at 37C. The absorbance (A) of Standard and Sample was measured at 520 nm against the Blank.

The colour is stable for at least 30 minutes.

III. Quality Control

It is recommended to use the Biochemistry Control serum level to verify the performance of the colorimetry with the cholesterol reagent.

Each laboratory should its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances. **IV. Calculations:**

The cholesterol concentration in the supernatant is calculated using the formula.

$$\frac{\text{A of sample}}{\text{A of standard}} \text{ X Concentration of standard X Sample dilution factor}$$

The LDL cholesterol concentration in the sample is calculated as follow:

The LDL cholesterol = Total cholesterol – cholesterol in supernatant

CHAPTER FOUR

RESULTS

Chapter Four

Results

4.1. The epidemiological study

In the present study 40 of hypertension patients were included as case and 40 of healthy individual as control. all of them were males. The most affect edage group in the patients was more than 40 years followed less than 40 years which constituted 85% and 15%, respectively **(Table4-1),**also frequency of the duration of disease among hypertensive patients ,frequency of the occupation among hypertensive patient and control group and the frequency of the tribe among hypertensive patients and control group.

Age (years)	Case Frequency (%)	Control Frequency (%)
Less than 40	6 (15%)	7 (17.5 %)
More than 40	34 (85%)	33 (82.5 %)

Duration of disease:

More than 10	10 (25%)
Less than 10	30 (75%)

Occupation:

Teacher	7(8.75%)	7(8.75%)
Driver	11(13.75%)	6(7.5%)
Free worker	15(18.75%)	19(23.75%)
Trade	7(8.75%)	8(10%)

Tribe:

Bni Amer	11(13.75%)	7(8.75%)
Gaali	10(12.5%)	8(10%)
Kawahla	5(6.25%)	9(11.25%)
Mahasi	10(12.25%)	7(8.75%)
Shaygi	4(5%)	9(11.25%)

Table (4.2): comparing of blood pressure, testosterone and LDL-C of hypertensive patients versus control group

Parameter	Hypertensive patients (mean ±SD)	Control (mean± SD)	p.value
Systolic BP (mmHg)	139.4±7.6	116.2±4.7	0.00
Diastolic BP(mmHg)	91.4±5.3	78.4±4.3	0.00
Testosterone(nmol/L)	4.03±0.75	6.1±.46	0.00
LDL-C(mmol/L)	2.44±0.24	2.34±0.16	0.04

The table shows the mean ± SD and probability (P).

Independent T-test was used for comparison. P value ≤ 0.05 was considered significant.

There was significant difference in hypertensive patients and control group p. value ≤ 0.05.

Systolic blood pressure was significant increase in hypertensive patients group than control group the mean was 139.4 in hypertensive patients and 116.2 in control. While Diastolic blood pressure also significantly increase in hypertensive patients than control (P.value was 0.00), Testosterone level significant decrease in hypertensive patients than in control P.value was 0.00) and the LDL-C level also significant increase in hypertensive patients than control.

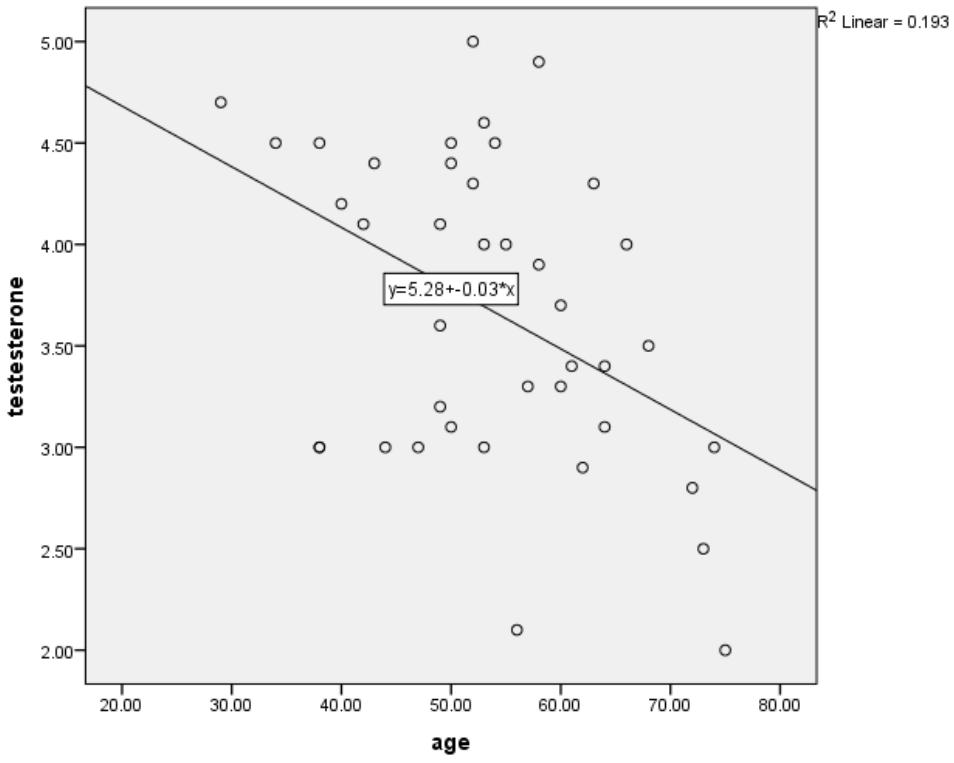
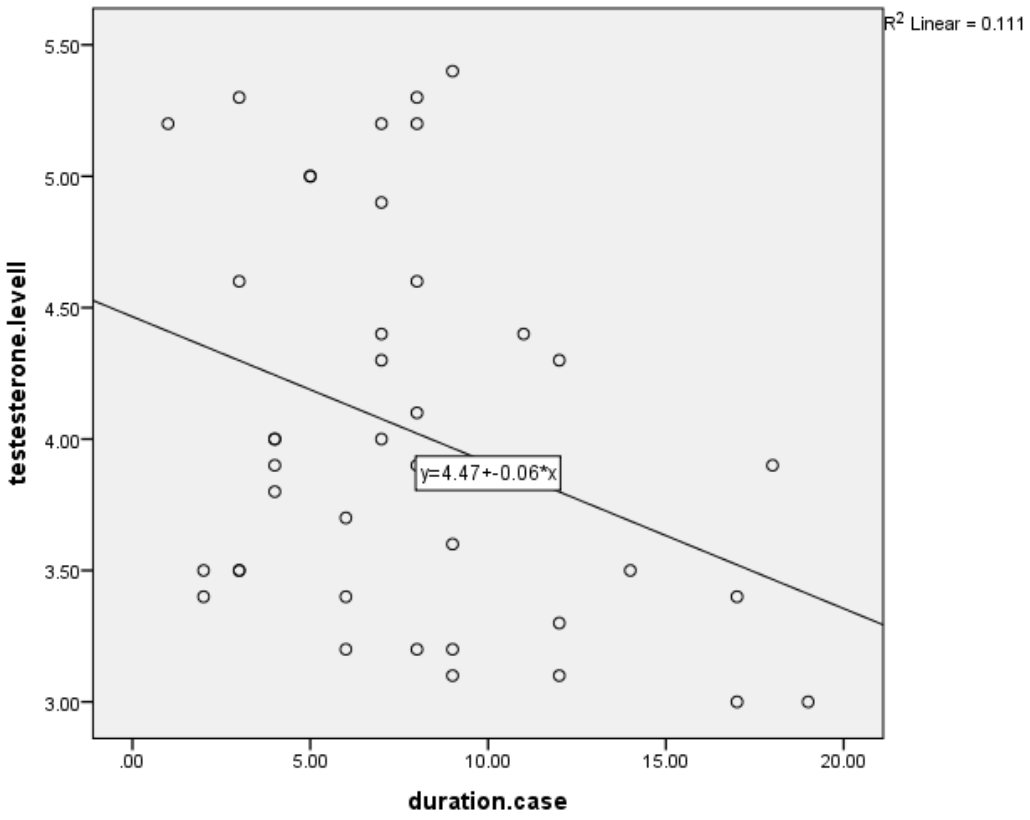


Figure (4-1): scatter plot showed the strong negative correlation between testosterone level and age in hypertensive patients

R = -0.725

P = 0.030



Figure(4-2):scatter plot showed the weak negative correlation between testosterone level and duration in hypertensive patients

R= -0.334

P= 0.035

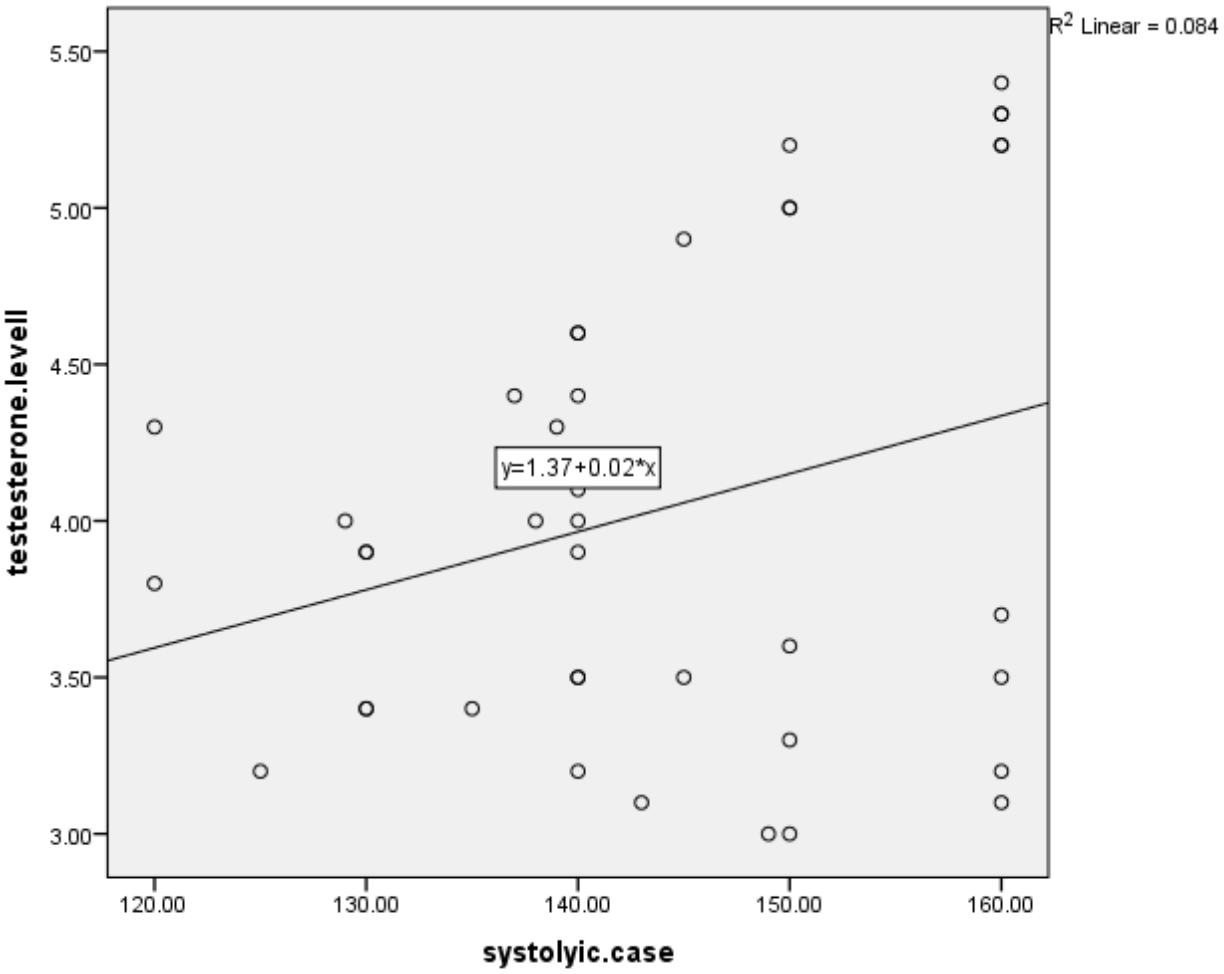


Figure (4-3): scatter plot showed the weak positive correlation between testosterone level and systolic blood pressure in hypertensive patients

R= 0.290

P= 0.016

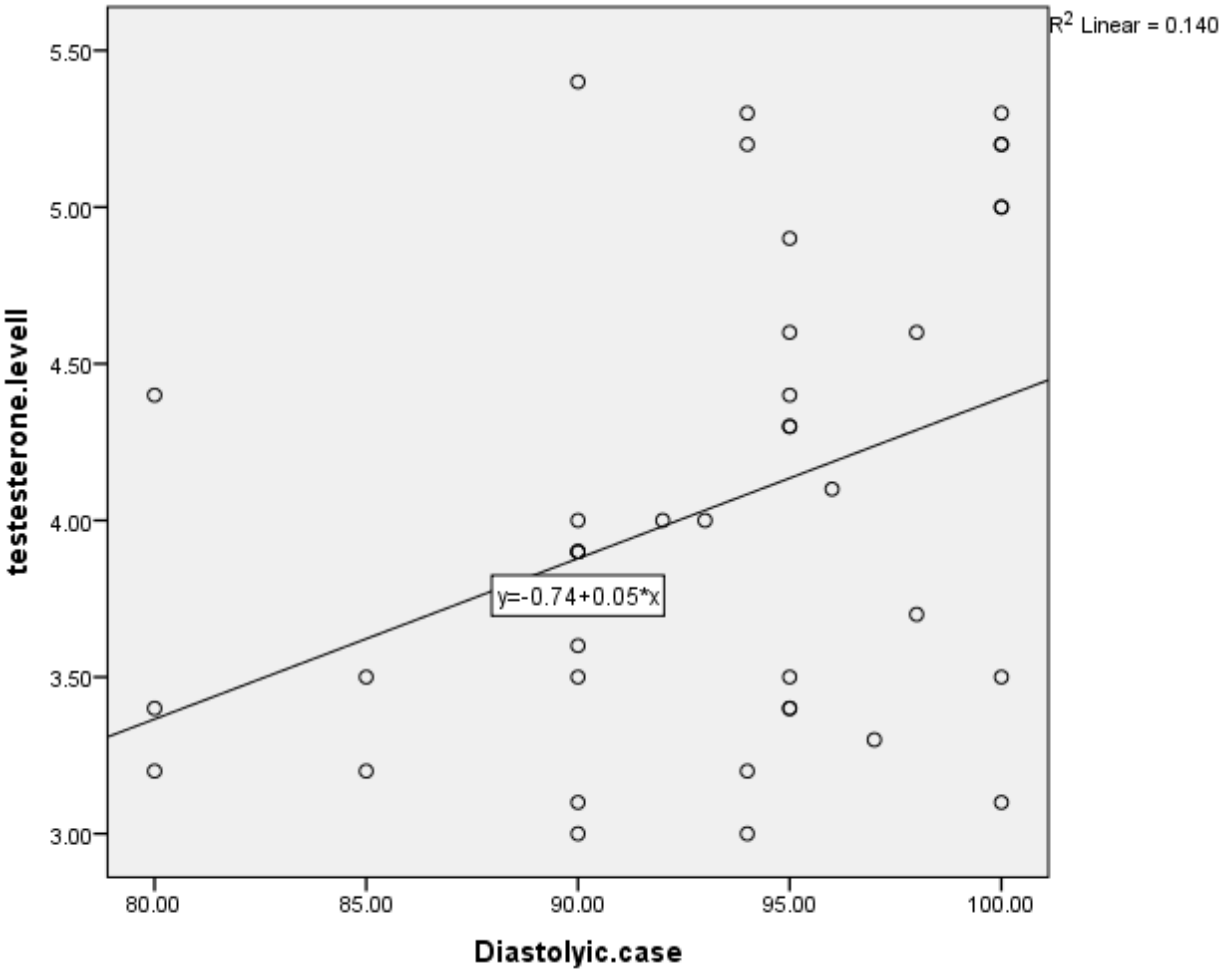


Figure (4-4):scatter plot showed the weak positive correlation between testosterone level and diastolic blood pressure

R = 0.375

P= 0.017

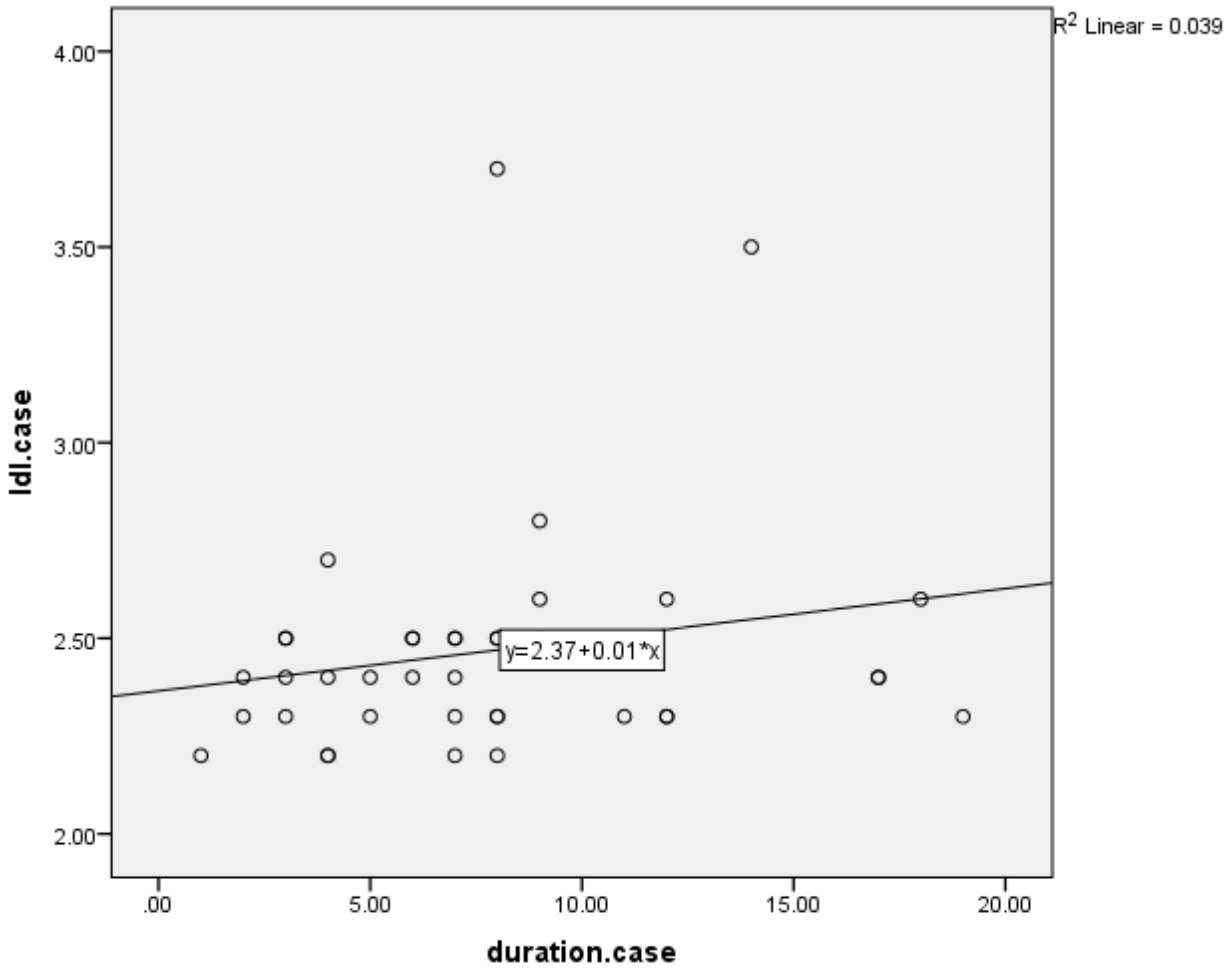


Figure (4-5): scatter plot showed no correlation between low density lipoprotein cholesterol level and duration in hypertensive patients
R = 0.198
P = 0.22

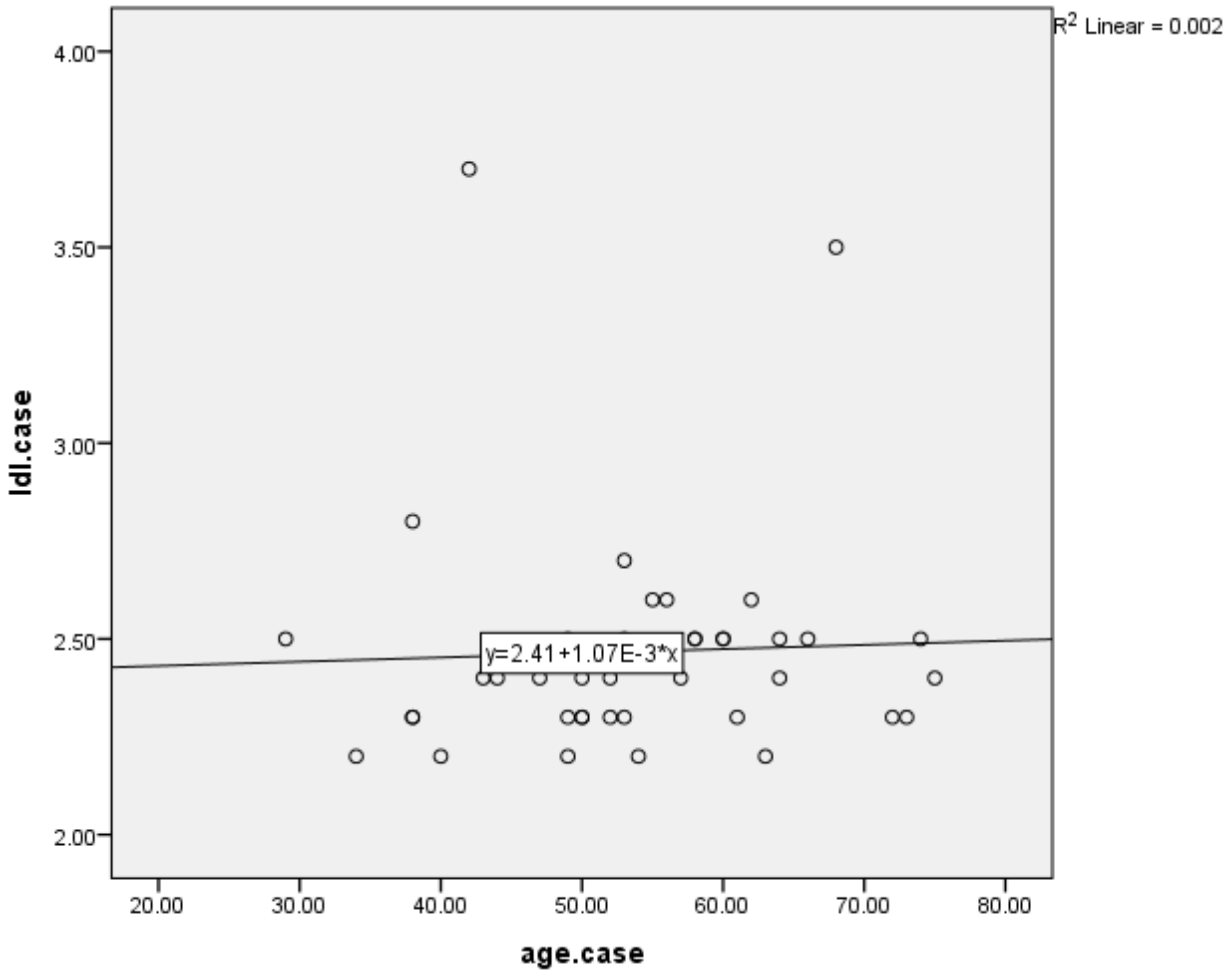


Figure (4-6): scatter plot showed no correlation between low density lipoprotein cholesterol level and age in hypertensive patients

R = 0.085

P = 0.600

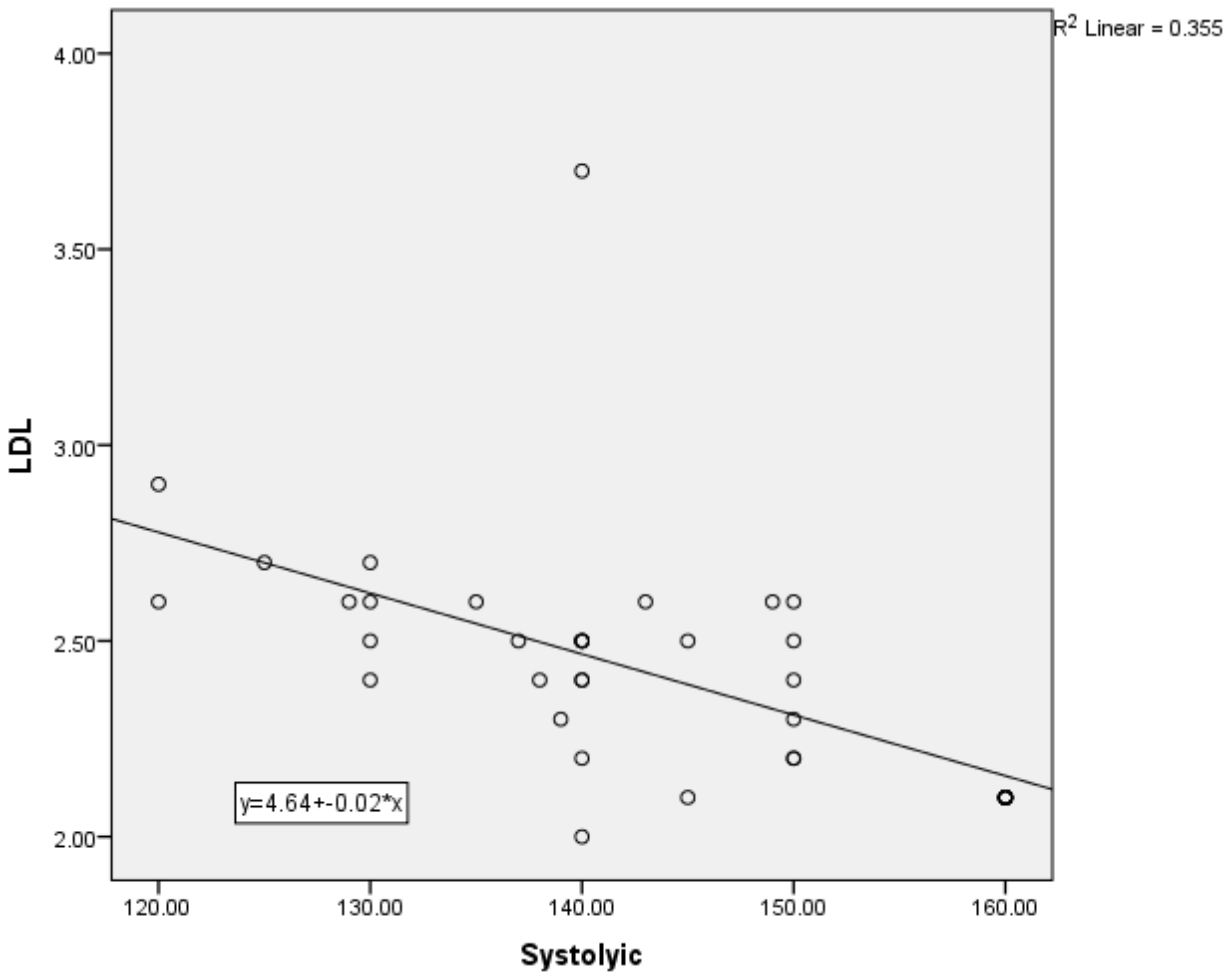


Figure (4-7): scatter plot showed the weak negative correlation between LDL-c level and systolic blood pressure in hypertensive patients
R = -0.085
P = 0.005

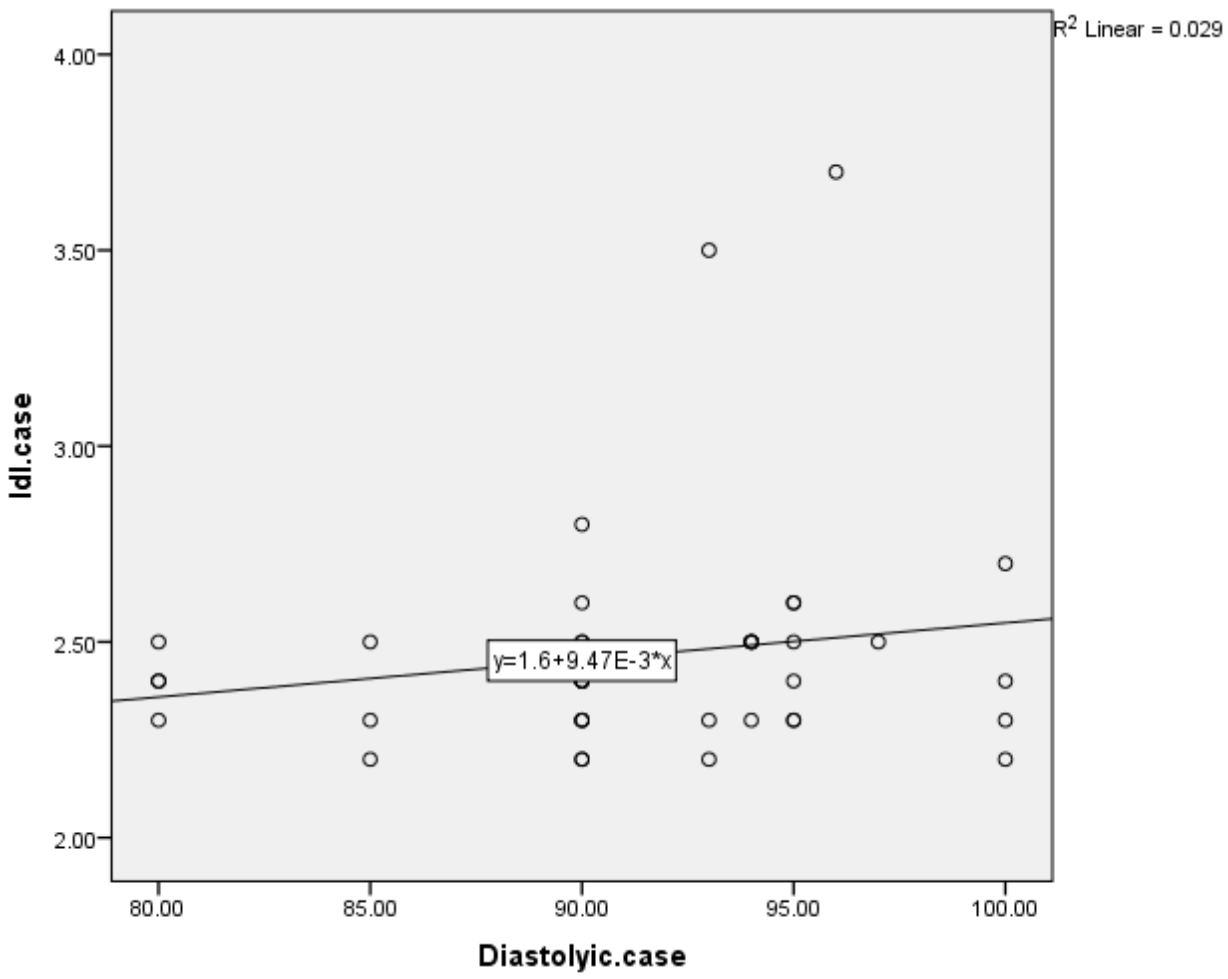


Figure (4-8): scatter plot showed no correlation between low density lipoprotein level and diastolic blood pressure in hypertensive patients

R = 0.171

P = 0.29

Table (4.3): Results of testesterone level among the different tribe

Tribe case	Mean±Std	Number
Bni Amer	3.87± 0.72	11
Gaali	3.86± 0.64	10
Kawahla	4.50± 0.65	5
Mahasi	4.25± 0.91	10
Shaygi	3.78± 0.75	4
Total	4.03± 0.75	40

According of this results no effect of testosterone level among the different tribe with hypertension

Table (4.4): Results of LDL-C among the different work

Work case	Mean± Std	Number
Driver	2.61± 0.41	7
Free worker	2.56± 0.32	19
Teachers	2.66± 0.49	7
Trader	2.44± 0.11	7
Total	2.47± 0.29	40

According to results no effect of LDL-C among the different work with hypertension .

CHAPTER FIVE

DISSCUSSION,CONCLUSION and RECOMMENDATION

Chapter Five

Discussion, Conclusion and Recommendation

5.1 Discussion

There was significant decreased of testosterone in hypertensive patients when compared with control group , that is agree with **(**Dudey *et al.*,2002).and (Ishikur*et al.*,2008) that found significant decrease of testosterone in hypertension this finding similar to our result that found testosterone level was significant decrease .other study also showed significant increase of testosterone levels in both men and women were higher in the hypertensive group than in the normotensive group (Huisman et al., 2006) that dis agree with our finding.

In addition to the low density lipoprotein cholesterol was significantly higher in the hypertensive groups than control this also similar to study done by **(**Huisman*et al.,* 2006) , low levels of testosterone may have effect on lipid profiles in male Africans, according to findings in the literature that a low testosterone level is associated with atheroma. But testosterone may also play a role in the development of hypertension, probably through elevated cortisol levels. (Hak *et al.*, 2002)

A significant inverse negative correlation is found between testosterone and age also the duration of disease that similar to Longitudinal studies in male

aging studies have shown that serum testosterone levels decline with age (Harman *et al* 2001; Feldman *et al*, 2002). Total testosterone levels fall at an average of 1.6% per year whilst free and bio available levels fall by 2%–3% per year.

Positive correlation is found between testosterone and systolic also diastolic blood pressure that similar to (Fogari *et al*, 2005).No correlation between low density lipoprotein cholesterol and age, duration of disease and diastolic blood pressure that dis agree to finding by (Isidori *et al*, 2005)

5.2 Conclusion

This study concludes `that the testosterone level is significantly decreased and high low-density lipoprotein cholesterol level in hypertensive patients.

5.3 Recommendations

- Follow up should be done.
- Designed cohort study for other studies.
- Needs further investigation at the molecular level.
- Abstinence from diet improve the androgen levels in hypertensive men.

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APPENDEIX (1)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Faculty of medical laboratory

CLINICAL CHEMISTRY

QUESTIONAIRE

Volunteer: Male

1-Age:…….

2-Duration of disease:……..years.

3-Blood pressure :…….

4-occupations :……

5-Tribe:……..

6-LDL-c (mmol/L) :……..

7-testosterone hormone (nmol/L) :……

Don’t suffer from:

1-Diabetes……..

2-Renal disease…….

3-Endocrine disease……..

Reference values of low density lipoprotein cholesterol :

Upto100 mg/dL= 2.59mmol/L	Optimal
100-129mg/dL = 2.59-3.34 mmol/L	Near optimal/above optimal
130-159mg/Dl = 3.37-4.12mmol/L	Borderline High
160-189mg/dl =4.14-4.90 mmol/L	High
>190 mg/dL = 4.92mmol/L	Very High

Reference values of testosterone level

Male 262-870 ng/dl / 5.3- 8.2 nmol/l

APPENDIX (2)

Tosoh aia-360 automated immunoassay analyzer


Flow cell
HEPOBIO
PRINT
HEPO INTERNATIONAL
DELETE
ENTER
PAPER FEED
ZERO
CALIB