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THE RELATIONSHIP BETWEEN TESTOSTERONE HORMONE AND LIPID PROFILE, PROTEINS AND SOME TRACE ELEMENTS IN THE SERA OF PATIENTS WITH PREECLAMPSIA

A thesis
submitted to the Council of the College of Medicine-Babylon University in partial fulfillment of the requirement for the degree of Master of Science in Clinical Biochemistry

BY

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AMEERA

بسم الله الرحمن الرحيم

((اللهُ يَعلَمُ ما تَحْمِلُ كُلُّ أُنثى
وَمَا تَغِيضُ الارْحَامُ وَمَا
تزْدَ اُدوَ كُلُّ شيءٍ عِندَهُ
بِمِقدَارٍ))

صدَقَ اللهُ

العلِيُّ العظِيم

DEDICATION

Supervision Certificate

We certify that this thesis has been prepared under our supervision at the Department of Biochemistry, College of Medicine / Babylon University, as a partial requirement for the degree of Master of Science in Clinical Biochemistry.

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Summary

Preeclampsia is one of the most common diseases which occurs during the second and third trimester of pregnancy. The rate of incidence of this disease is 2-5% among pregnant women.

The aetiology of this disease is still in debate and many theories were introduced in this field by many investigators in different countries. It is sometimes called disease of theories due to the contradictory issues concerning its causes and consequences.

In this study we tried for the first time to elucidate the relationship between testosterone and some biochemical constituents which vary during pregnancy (i.e , lipid profile, total protein, albumin and minerals (Ca & Mg).

This study was carried out on patients fifty five pregnant women referred to Babylon Hospital for Obstetric & Paediatrics for the period from November 2007 to May 2008 .The serum samples obtained from those patients and control groups (55 healthy pregnant) were analyzed for lipids, protein and minerals in addition to testosterone .The study group was subdivided into four subgroups as follows :

1.Group I is comprised of 25 preeclamptic patients in the second trimester of pregnancy .

2. Group II is comprised of 30 preeclamptic patients in the third trimester of pregnancy .

3.Group III includes 25 healthy pregnant women (2^{nd} trimester) which served as control group .

4.Group IV represents 30 healthy pregnant subject in their third trimester of pregnancy

The results revealed a significant increase in serum testosterone levels in Group I and Group II compared with Group III and IV ($p<0.01$). These were insignificant decrease in hormone level in Group IV in comparison with GroupIII ($p=0.36$).

The results showed a significant increase in serum level of total cholesterol, TG, LDL, VLDL in Group I and Group II compared with those in Group III and Group IV,($p<0.001$), ($p<0.01$) at respectively. However, there was a concomitant decrease in serum HDL level in Group I and Group II when compared with Group III and Group IV.

The results showed also a significant decrease in the levels of total protein ,albumin ,Ca and Mg in preeclamptic women compared with normotensive pregnants ($p<0.05$) .These changes were insignificant when the results of these component in Group IV were compared with Group III ($p>0.05$).

There were significant correlation between serum testosterone levels and lipid profile , protein and minerals .This

gives a preliminary idea about the role of testosterone in such changes. There were a positive correlation between testosterone and lipid profile except HDL-C which decrease at increase the testosterone in G1,G2 and G3 ($p<0.01$) and there were positive correlation between cholesterol / albumin ratio and testosterone in G1,G2 and G3 ($p<0.01$) but a negative correlation in G4 ($p<0.01$). There were inverse relationship between cholesterol and albumin ($p<0.001$).

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List of abbreviations

Symbol	Description
A	Absorbance
ACAT	Acyl-coA-cholesterol acyl transferase
ACTH	Adrenocorticotrophic hormone
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BMI	Body mass index
Ca	Calcium
CaSR	Calcium-sensing receptor
CBG	Cortisol-binding globulin
CE	Cholesterol esterase
CETP	Cholesteryl ester transefer protein
CHD	Coronary heart disease
CO	Cholesterol oxidase
CRH	Corticotrophin- releasing hormone
CV	Cardiovascular
cAMP	Cyclic adenosine monophosphate
DBP	Diastolic blood pressure
DGAT	Diglycerol acyl transferase
DHEA	Dehydroepiandrosterone
ECF	Exteracelular fluid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbant assay
GEDTA	Glycoletherdiamine-N,N,N′,N′-tetraacetic acid
GFR	Glomerular filteration rate
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
HDL-C	High density lipoprotein-cholesterol
HPL	Human placental lactogen
HRP	Horse radish peroxidase
IDL-C	Intermediate density lipoprotein-cholesterol
IgG	Immunoglobulin G
LCAT	Lecithin-cholesterol acyl transferase
LDL-C	Low density lipoprotein-cholesterol
LH	Luteinizing hormone
LPL	Lipoprotein lipase

Mg	Magnesium
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
PCO	Polycystic ovary
PET	Preeclampsia
POD	Peroxidase
PTH	Parathyroid hormone
RBC	Red blood cell
SBP	Systolic blood pressure
SHBG	Sex hormone-binding globulin
TBG	Thyroxine-binding globulin
TEBG	Testosterone-estrogen binding globulin
TG	Triglyceride
Tmax	Maximum transport
TMB	3,3′,5,5′ tetra methyl benzidine
TRH	Thyrotropin- releasing hormone
VLDL-C	Very low density lipoprotein-cholesterol
4-AA	4-amino antipyrine
11β-HSB	11β-hydroxy steroid dehydrogenase

Chapter One

Introduction

And

Literature Review

1.1.Preeclampsia

Hypertension in pregnancy is a significant problem , if it is associated with proteinuria (which indicates multisystemic disease, known as preeclampsia(PET)), it will be associated with increased morbidity and mortality for both mother and baby(1). Hypertension in pregnancy is a common accounting one from five women after 20 weeks of gestation (1).

Preeclampsia is divided according to severity into mild, moderate and severe forms depending on the level of the blood pressure and the degree of proteinuria , mild preeclampsia characterized by diastolic blood pressure of 90 mmHg with proteinuria less than 5gm/24hr (+) to (++) and edema in feet . In severe preeclampsia , blood pressure is more than 110 mmHg and proteinuria more than 5gm/24hr (+++) to(++++) and edema in hands and or face (2). Symptoms and signs include sudden rise in blood pressure , severe proteinuria , generalized edema, excessive weight gain , visual changes such as blurred or double vision , headache , nausea , vomiting , epigastric pain , oliguria, changes in liver or kidney function tests. These are signs and symptoms of immenant eclampsia. If these symptoms are associated with seizure, then the condition is called eclampsia (3).In PET an increase in the resistance of blood vessels may hinder blood flow in many different organs like the liver,

kidney, brain, uterus and placenta affecting their function or causing placental abruption which is a premature separating of the placenta after 20 weeks of gestation (3). PET can also lead to fetal complications including intrauterine growth restriction (poor fetal growth) and still birth (3).

Major preexisting risk factors for PET include primigravida state , history of PET in previous pregnancy , large body size, a family history of PET, multiple pregnancy , preexisting maternal hypertension, pregestational diabetes , antiphospholipid antibody syndrome , vascular or connective tissue disease and advanced maternal age (> 35 to 40 years) (4).

1.1.1.Aetiology

Preeclampsia was known as the disease of theories, as the exact course of events that leads to the clinical syndrome have not been elucidated(5). The first theory relates preeclampsia to immunogentic factors. Numerous studies suggest a genetic susceptibility to PET , daughters of women with PET are four to five times more likely to develop the syndrome than daughters in law (5). How the genotype result in the characteristic placental lesion is not known but may involve an immunological defect resulting failure to establish tolerance to the fetal allograft (5,6) .

The second theory relates the syndrome to the disturbance in different vasoactive compounds (6). Disturbance of endothelial cells in PET leads to alteration in the production of several vasoactive compounds producing a vasoconstrictor state: Prostacyclin (PGI_2) , the predominant vasodilator prostanoid is reduced while placental production of vasoconstrictor thromboxane A2 is increased. Plasma endothelin , a potent vasoconstrictor is also increased (5).

The third theory which relates the disease to uteroplacental ischemia, suggests the following :-

1- Preeclampsia begins with uteroplacental ischemia , which is an increase intramural resistance in the myometerial vessels, leads to hightened myometerial tension produced by large fetus in a primipara, twins or hydramnios (6).

2- The uteroplacental ischemia leads to the production of vasoconstrictor substance , which enters the circulation and produces renal vasoconstriction leading to increased production of renin - angiotensin and aldosterone (6) .

3-The renin-angiotensin system produces a generalized vasoconstriction and aggravates further the uteroplacental ischemia (6). It is followed by systemic of cytotoxic products that damage maternal vascular endothelium (7) .

4- Aldosterone leads to water and electrolyte retention and generalized edema(8) .

Women with cardiovascular (CV) risks are at increased risk for preeclampsia ,and those with history of preeclampsia are at increased risk for postpregnancy CV morbidity and mortality , compared with women with history of normal pregnancy, This suggests that preeclampsia and CV disease share common pathogenic mechanism(9). These changes may involve endothelial function deficient in preeclampsia , as seen from reduced prostacyclin and / or elevated endothelin-1 or thromboxane A2 production (9) .

1.1.2.Pathogenesis of preeclampsia :

There are three major pathologic lesions primarily associated with preeclampsia :

1- Hemorrhage and necrosis in many organs as liver , placenta and brain . In the liver , periportal necrosis and hemorrhage may occur with subcapsular haematoma , this leads to epigastric pain (10) . In the brain , focal areas of haemorrhage and necrosis may occur. In the retina the clinical window to the arterial vasculature vasospasm may be visualized on ophthalmoscopic examination (10) . Retinal haemorrhage is considered to be an extremely

ominous sign , since it may signal similar phenomena in other vital organs (10) .

2- The typical renal lesion of preeclampsia - eclampsia is glomerular capillary endotheliosis (10). This disorder is manifested by marked swelling of the glomerular capillary endotheliem , with deposits of fibrinoid material in and beneath the endothelial cells (10).

3- The uteroplacental pathology in preeclampsia is characterized by lack of decidualization of the myometrial segment of the spiral arteries(10).Vascular lesion in the uteroplacental vessels is characterized by prominent endothelial damage and proliferation of myointimal cells, followed by lipid accumulation in myointimal cell and in macrophage recruited to the vessel wall (11).

1.1.3.Investigations for preeclampsia [12] :

The following investigations help us in the diagnosis and in the follow up to know the severity of the condition, these include:

- Urinalysis by dipstick (semi- quantitative).
- 24 hour urine collection (total protein and creatinine clearance)
- Full blood count (platelets and haematocrit).
- Blood chemistry (renal function , protein concentration).

- Plasma urate concentration
- Liver function
- Coagulation profile .
- Ophthalmoscopic examination to check for retinal changes.
- Ultrasound assessment including fetal size , amniotic fluid volume, maternal and fetal dopplers .

1.1.4.Pathogenesis of proteinuria :

The pathogenesis of proteinuria in preeclampsia involves primarily glomerular changes . The normal absence of protein from urine is due both to a relative impermeability of glomeruli to large protein molecules and to the tubular reabsorption of smaller proteins that cross the glomeruli (13) . As glomerular damage occurs, permeability to proteins increases ,so the protein molecule that can cross the glomerular membrane increase. This increase in permeability results in a decrease in selectivity (13) . Microalbuminuria is a manifestation of endothelial dysfunction . Endothelial function in preeclamptic women is impaired (14). Loss of endothelial cell integrity results in an increase in vascular permeability and contributes to the formation of generalized edema , which is often found in women with preeclampsia . specifically in the face and hands and can be rapidly progressing without underlying pathology (12).

1.2.Endocrinology of pregnancy [15]:

Endocrine changes in pregnancy are largely dependent on the concerted production of protein and steroid hormones by the fetoplacental unite . Levels of hormones in pregnant women differ from those in nonpregnant women because of the presence of

1- Placenta which has a diverse secretory repertoire that surpasses any other endocrine organ.

2- Fetus whose endocrine structures (eg, pituitary gland , thyroid adrenal cortex , pancreas and gonad) function as early as the 11th week of pregnancy .

3- Increased levels of circulating estrogens .

1.2.1.Significant characteristics of hormones during pregnancy [12]:-

1- The chemical nature of hormones, including protein hormones (eg , hCG, hPL and prolactin) and steroid hormones (eg progesterone , estrogen and fetal adrenal steroid).

2- The source of certain hormones (such as estrogen and progesterone) early in pregnancy and by the end of the first trimester the fetus and placenta are important source of sex steroid and protein hormones .

3- Secretion patterns which recognize normal patterns of hormones activity throughout pregnancy can help to distinguish abnormal pregnancies and fetal compromise
4- Biologic functions understanding the function of particular hormone may illuminate its role in reproductive physiology particularly in maintaining pregnancy and fetal well being.

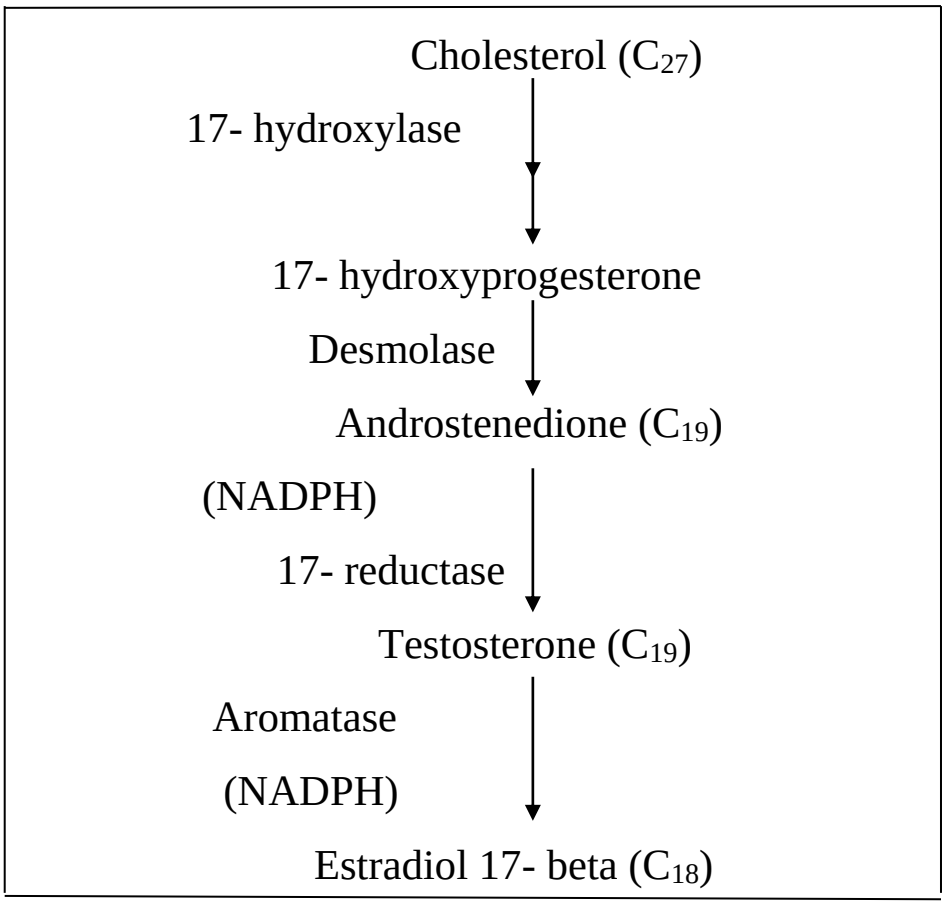
1.3.Androgens :

These hormones are responsible for the manifestation of primary and secondary sex characteristics and preservation of libido , sense of well being (16), lean mass and bone density (17). Androgens are involved in a negative biofeed back mechanism on the hypothalamic pituitary axis to inhibit gonadotropin secretion (18) . They are group of C_{19} steroid, androgen precursor by the adrenal cortex is dehydroepiandrosterone (DHEA) (19) . Adrenal androgen themselves are weak , they are converted in peripheral tissue to testosterone (as strong androgen) and to estradiol (19).

Testosterone is the major male hormone secreted from the leydig cells (interstitial cells), under the influence of luteinizing hormone (LH)(20,21). In female , the follicular theca cells produce C_{19} androgens . These are converted to C_{18} estrogens by

granulosa cells , by aromatization of ring A and loss of C19 methyl group according to Figure (1-1)(21) .

Testosterone is synthesized from cholesterol . Cholesterol is converted to pregnenolone . It is precursor for all steroid hormones (21). ACTH stimulates the rate limiting step for synthesis of all steroid hormones . Progesterone is the first steroid hormones formed from pregnenolone by 3-beta-ol Dehydrogenase . Progesterone is converted to testosterone by two reactions . These reactions are affected by dihydroxylation (21) . These specific hydroxylases are monooxygenase . Testosterone is converted to estradiol-17-beta by aromatase .The two latter reactions required NADPH . These steps are shown in Figure (1-1)



Fig(1-1): Synthesis of sex steroids

Theca cells are the source of androstenedione and testosterone . These are converted by aromatase enzyme in granulosa cell to estrone and estradiol . Significant amounts of estrogens are produced by the peripheral aromatization of androgens (22) . In human male the peripheral aromatization of testosterone to estradiol (E2) account for 80% of the production of the latter (22) In female , adrenal androgens are important substrates , since as much as 50% of the estradiol E2 produced

during pregnancy comes from the aromatization of androgens (22). Aromatase activity is present in adipose cells and also in liver, skin and other tissues (23) . Increased activity of this enzyme may contribute to the estrogenization that characterizes such disease as cirrhosis of the liver , hyperthyroidism and obesity (23) .

The relative percentage of bound and free hormone are determined by the binding affinity and binding capacity of the transport protein . Free hormones are the biologically active form (22) .

Most mammal including human being have a plasma β - globulins that binds testosterone with specificity, relatively high affinity and limited capacity (22). Binding protein , usually sex hormones binding globulin (SHBG) or testosterone-estrogen binding globulin (TEBG), is produced in the liver . Its production is increased by estrogens (women have twice the serum concentration of SHBG as men) (22) , in certain types of liver disease and by hyperthyroidism . It's decreased by androgens , advancing age , and hypothyroidism, since SHBG and albumin bind 97-99% of circulating testosterone, only a small fraction of the hormone in circulation in the free form (22). The primary function of SHBG may be to restrict the free concentration of testosterone in the serum . Testosterone binds

to SHBG higher affinity than does estradiol therefore change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level (22).

1.4. Plasma lipids profile [24] :-

Total plasma lipid is 400-600 mg/dl . One third is cholesterol, one third is triacylglycerol and one third is phospholipid . Since lipids are insoluble in water , they need the help to be carried in plasma . Therefore , they are complexed with proteins to form lipoprotein. The protein part of lipoprotein is called apolipoprotein .

1.4.1. Cholesterol [25]:

Cholesterol is present in tissue and in plasma lipoproteins either as free or combined with along-chain fatty acid , as cholesteryl ester . The source of cholesterol : dietary cholesterol (mostly triacylglycerol) and denovo synthesis from acetate. It is synthesized in many tissue from acetyl- coA and altimately eliminated from the body in the bile as cholesterol or bile salts. Cholesterol is the precursor of other steroid in the body such as corticosteroids , sex hormones ,bile acid and Vitamin D .It is typically a product of animal metabolism and therefore occurs in foods of animal origin such as egg yolk , meat , liver and brain .It is often found as cholesteryl ester where the hydroxyl group

on position 3 is esterified with along chain fatty acid .The structure of cholesterol is shown in Fig (1-2) .

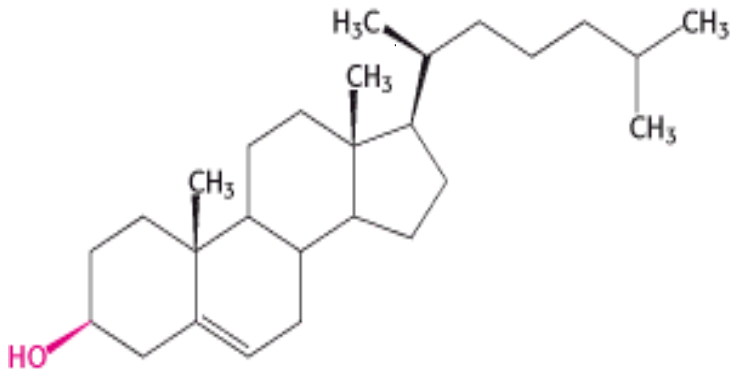


Fig (1-2) :Cholesterol , the most common animal steroid

1.4.2. Triacylglycerol[24]:-

Triacylglycerol consists of glycerol esterified with three long chain fatty acids. It's present in dietary fat and can be synthesized in the liver and adipose tissue to provide a source of stored energy which can be mobilized when required. For example , during starvation, triacylglycerol containing both saturated and unsaturated fatty acids are important components of cell membranes. It constitutes about ninety percent of the lipid. It is removed from chylomicrons by the action of the enzyme lipoprotein lipase (LPL) , located on the luminal surface of the capillary endothelium of adipose tissue, skeletal muscle and cardiac muscle and lactating breast , so that free fatty acids

are delivered to these tissues either to be used as energy sources or after reesterfication to triacylglycerol for energy storage . LPL is activated by apo C-II . Endogenous triacylglycerol synthesized in the liver is transported in VLDL is also available to tissues as an energy source for storage when occur hypertriglyceridaemia is also a risk factor for CHD.

1. 4. 3. Classification of lipoproteins :

Depending on its density (by ultra centrifuge) or on its electrophoretic mobility, the lipoproteins in plasma are classified into five major types (26) .

1.4.3.1. Chylomicrons :

Chylomicrons are the lipoprotein particles of the lowest density and largest in size and contain the highest percentage of lipid and the smallest percentage of protein (16) . Chylomicrons represent triacylglycerols and esters of cholesterol with coating of phospholipid, protein and cholesterol (27) . These enter the lymphatic system and are transported via the thoracic duct to the blood stream (27). The main sites for removal of chylomicrons are the muscle and liver (28), lipoprotein lipase , an enzyme bound to the capillary endothelium of extrahepatic tissues , hydrolyzes triacylglycerols in chylomicrons ,and VLDL into free fatty acids and glycerol as shown in Fig (1-3) (28). After

entering adipose tissue or muscle , these compounds are esterified and stored (28). The smaller remanant particles contain mainly cholesterol and pass to the liver where they are metabolized further (28).

1.4.3.2.Very low density lipoprotein (VLDL) [29]:

Very low density lipoprotein particles are produced by the liver and serve as vehicle for delivery of endogenous lipid to the peripheral tissue. Nascent VLDL-C is formed within the hepatocyte from the fusion of partially lipidated, newly synthesized apoB-100 with a triacylglycerol –rich lipid droplet , followed by addition of apoE , apo A-I and apoA-II . triacylglycerol and cholesterol ester used by hepatocytes for incorporation into VLDL are generated by the enzymes diglycerol acyltransferase (DGAT) and acyl-coA cholesterol acyltransferase (ACAT); respectively . The fatty acid and cholesterol supplies used for tissue process by the hepatocyte are derived from combination of denovo synthesis and uptake from a circulating blood .

1.4.3.3. Low density lipoprotein (LDL):

Low density lipoproteins (LDL) are synthesized from VLDL particles after delipidation by the action of lipoprotein lipase. LDL particles are transport cholesterol from the liver to the peripheral tissue (30). LDL may be removed from the

circulation by the LDL receptor or by other scavenger routes which are thought to be important to high LDL levels (30) . As shown in Fig (1-3), it is very important to keep LDL level low because high levels of LDL indicate that there is much more cholesterol in the blood stream than necessary because LDL causes atherosclerosis (31).

1.4.3.4.Intermediate density lipoproteins (IDL)[32]:-

Intermediate density lipoproteins (IDL) are intermediate between VLDL and LDL and are synthesized from VLDL after losing apart of triacylglycerols amount, this represent in Fig (1-3) . These compounds are either converted to other lipoprotein or transport to liver ,therefore their concentration in blood are very small or they are seemed to be as a transition step .

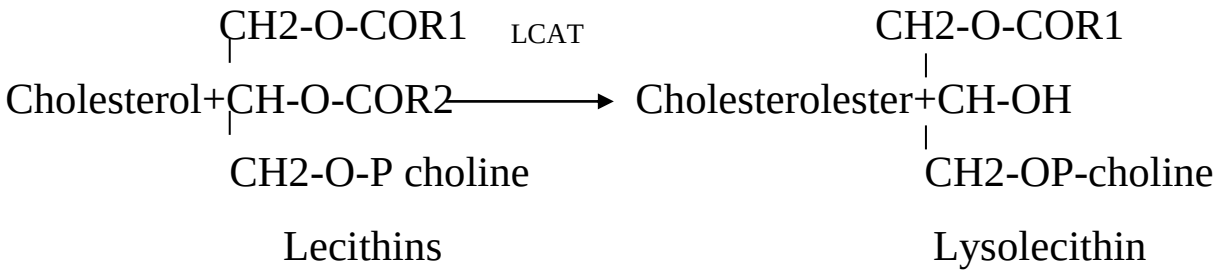
1.4.3.5. High density lipoproteins (HDL):-

High density lipoprotein (HDL) is the main transport form of cholesterol from peripheral tissue to liver , which is later excreted through bile , as shown in Fig (1-3) . HDL contains the highest protein concentration and it was known to be protective against heart attacks (21) .A group of patients have very high levels of HDL in blood . HDL subfractions are (21).

1- HDL-1 (density <1.063 gm/ml) :it is a bad type of HDL particles which contains only apo E . It's produced after ingestion of large amounts of cholesterol (21).

2- HDL-2 (density 1.063 - 1.125 gm/ml): It is rich in cholesterol ester .It's involved in inverse cholesterol transport. Its concentration in blood is inversely proportional to atherosclerosis or (HDL–2 is "good") (21).
3- HDL–3(density 1.125-1.21gm/ml): The function of HDL–3 is controversial . Some workers are of opinion that HDL-3 gets converted to HDL-2 on taking up cholesterol from tissues (21).

This result from the activity of plasma enzyme , lecithins cholesterol acyltransferase (LCAT) which transfers a fatty acid mainly linoleic acid from lecithins to free cholesterol (33). HDL particles are derived from both liver and gut (30). LCAT is associated with HDL and esterified choleslerol. In LCAT deficiency , free unesterified cholesterol accumulates in the tissues (28), as shown in following equation .



This equation shows reverse cholesterol transport which is critical for cellular cholesterol homeostasis and protection

against atherosclerosis, renal disease and other complication (34). Plasma HDL concentration is consistently reduced and maturation of cholesterol ester poor HDL-3 to cholesterol ester rich cardioprotective HDL-2 (35).

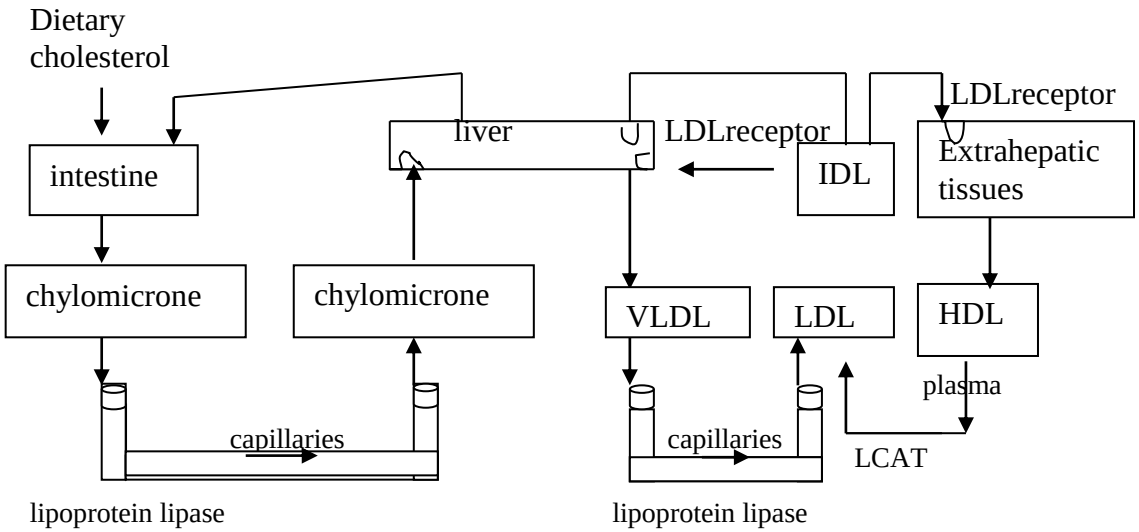


Fig (1-3): lipids transport and lipoproteins formation (28)

1.5. Total protein , albumin, globulin and albumin/ globulin (A/G ratio) :

The concentration of total protein in human plasma is approximately 6.2 -8.2 gm/dl , and comprises the major part of the solids of the plasma (22). The major types of protein in the plasma are albumin , globulin and fibrinogen . Albumin constitutes the major part of plasma proteins . It has one polypeptide chain with 585 amino acids and 17 disulfide bonds

(21). It has molecular weight of 69 KD . It is synthesized by hepatocytes . Half life of albumin is about 20 days (21) .

A major function of albumin is to provide colloid osmotic pressure in the plasma which prevents plasma loss from the capillaries. Another major function of albumin is to transport various hydrophobic substances. All proteins have buffering capacity and albumin may be considered as the transport form of essential amino acids from liver to extrahepatic cells (21) .

The globulins perform a number of enzymatic functions in the plasma , but equally important , they are principally responsible for the body's both natural and acquired immunity against invading organisms (36). A/G ratio is altered or even reversed by the reticuloendothelial system and decrease in albumin. This again leads to edema (37) . Fibrinogen polymerizes into long fibrin threads during blood coagulation , thereby forming blood clots that help repair leaks in the circulatory system (36) .

The total concentration of serum proteins decrease by about 1g/l during pregnancy . Most of the decrease occurs during the first trimester (38). The decrease is mainly in serum albumin . The maternal antibody (IgG) component , which is the major immunoglobulin transferred to the fetus , falls progressively, alteration that occurs in the levels of clotting factors and

plasminogen is probably brought about by estrogen action on the liver (38).

1.6. Minerals Metabolism :

1.6.1. Calcium : -

Calcium is found 46% free , 32% bound to albumin , 8% bound to globulins and 14% associated in freely diffusible calcium complexes. Calcium present in protein or inorganic complex (38). The skeleton contains 99% of calcium present in the body in the form of hydroxyapatite. The remainder is distributed in the soft tissue, teeth and extracellular fluid (ECF) (38). Many cells and organs functions are dependent on the tight control of extracelullar calcium concentration, these include neural transmission, cellular secretion, contraction of muscle cells , cell proliferation, the stability and permeability of cell membranes, blood clotting and the mineralization of bone (38).

Total serum calcium is maintained between 2.2 and 2.6 mmol /l (8.8-10.4 mg/dl) . Calcium which exists in the circulation in three forms including ionized calcium is the important physiologically active form (50% of total calcium), protein – bound is the majority of the remaining calcium, mainly bound to negatively charged albumin (40%) and complexed to substances. Such a citrate and phosphate are a smaller fraction

(10%) (38). Abnormal calcium metabolism contributes to the genesis of hypertension (39) .

1.6.2. Parathyroid hormones (PTH) responds to changes in ionized calcium [40] :-

Parathyroid hormone (PTH) is an 84-amino acid, a single-chain peptide hormones secreted by the chief cells of the parathyroid glands. A decrease in extracelluar ionized calcium stimulates it secretion , chronic severe magnesium deficiency can inhibit its release from secretory vesicles , and concentration of 1,25 dihydroxycholcalciferal ($1,25(OH)_2$ D_3) interfere with its synthesis . Most of the classical cellular actions of PTH are mediated by cyclic adenosine monophosphate (cAMP) which is generated through G-protein – stimulated adenyl cyclase . Hormonal control of circulating calcium involves bone , kidneys and the gastrointestinal tract , when plasma calcium decrease, PTH is released from the parathyroid glands , stimulating osteoclast . mediated bone resorption , reabsorption of calcium at the kidney and absorption of calcium at the small intestine (mediated by $1,25(OH)_2$ D_3). Increasing calcium decreases PTH secretion and stimulation of calcitonin release from the thyroid , which inhibits osteoclast resorption of bone . A decrease in plasma ionized calcium stimulates release

of PTH , this promote Ca^{+2} reabsorption from the kidney , resorption from bone, and absorption by the gut via increased production of $1,25(OH)_2 D_3$.

1.6.3. Magnesium:-

Magnesium is the fourth abundant cation in the body and the second most prevalent intracellular cation(41) . Total body magnesium is about 20 gm, 75% of which is complexed with calcium in bone . One third of skeletal magnesium is exchangeable with serum (41). Normal serum level Mg^{+2} is 0.8-1 mmol/l . Out of this 60% ionized 10% is complexed with other ions and 30% is bound with proteins . Inside the RBC , the magnesium contents is l0 mmol/l . In muscle tissue Mg^{+2} is 40 mmol/l . Homeostasis is maintained by kidney (41). Magnesium is acrucial intracellular element that is integral to many functions including the production of adenosine triphosphate (ATP) , proteins and nucleic acid as well as the activation of many enzymes that are essential for the sodium – potassium ATPase, calcium ATPase and protein pump (42) .

The placenta actively transports Mg and its levels in the neonate correlate with those in maternal serum (43). Several studies have documented the impact of prematurity on the alteration of Mg metabolism in the neonate (43). Lowering the

serum magnesium concentration decreases the threshold of axonal stimulation and increase nerve conduction velocity(44). Magnesium also influences the release of neurotransmitters at the neuromuscular junction by competitively inhibiting the entry of calcium in the presynaptic nerve terminal reducing the serum magnesium , therefore results in increase neuromuscular excitability (44) .

1.6.4. Mineral homeostasis and hypertension :

Magnesium ischemia is a term used to denote the functional impairment of the ATP – dependent sodium /potassium and calcium pumps in the cell membranes and within the cell itself (45) .The production of ATP and the functioning of these pumps are magnesium dependent and are critically sensitive to acidosis . Zinc and iron deficiencies may impair these pumps and thus contribute to magnesium ischemia as does acidosis (45) . It refers to functional magnesium deficiency whether actual or induced. It is argued that chronic acidosis is the most common inducing factor . It can also unify clinical thinking about pregnancy – induced hypertension , preeclampsia-eclampsia and acute fatty liver of pregnancy (45). as well as the coagulopathy of pregnancy . Mg can lead to important predictions about perinatal morbidty and suggests that early supplementation might prevent much pregnancy – induced disease (45) . On the basis of the therapeutic effects of

magnesium salts and the knowledge vasodilating properties of magnesium, it was suggested that a deficiency of magnesium contributes to the development of vasoconstriction in preeclampsia (46).

Calcium homeostasis is an important aspect of maternal and fetal physiology during gestation ,and recent evidence implicates alterations in calcium metabolism in the pathogensis of hypertension during pregnancy (47). Deficiencies in calcium intake have been linked to preeclampsia-eclampsia , and hypocalciuria and deviations in both 1,25 $(OH)_2$ D_3 and PTH have been shown in women with preeclampsia (47).

During the past 7 years , some progress has been made in the prevention of preeclampsia . Specifically , clinical studies have shown that calcium supplementation can significantly reduce the frequency of preeclampsia , especially in populations with a low calcium intake (48). They have suggested that in such population , calcium supplementation is a safe and effective measure for reducing the incidence of preeclampsia (48), as the levels of free intracellular calcium is a major determinant of vascular smooth muscle tone and consequently vascular resistance (49).

However ,the role of plasma calcium status in normal pregnancy is still discussed contraversally , as well as calcium

supplementation in preeclampsia (45) . Although epidemiologic studies have suggested a role for calcium deficiency in the development of preeclampsia , the published information regarding calcium metabolism in preeclampsia is scanty (49) .

Aim of the study

This study aims to :-

1- Determine the nature of relationship between the changes in the level of serum testosterone and the level of serum lipid profile in preeclamptic groups and normotensive pregnants groups in the second and third trimesters.

2- Know the relationship between the testosterone and the changes in serum total protein , serum albumin and some minerals like calcium and magnesium in preeclamptics groups and normotensive pregnants groups .

3- Elucidate the pathophysiological changes of preeclampsia in order to provide scientific basis in the prevention of such disease .

Chapter Two

Materials and Methods

2.1.Materials:

Chemicals	Suppliers

2.1.1.Instruments

All the instruments and tools which are used in this study are listed in the table below:

Table (2-1):Instruments and tools with their suppliers

Instruments	Suppliers
Centrifuge	Runne - Heidelberg
Spectrophotometer	Philips-W-Germany
water bath	Thermo-Germany
Incubator	Memerte-W-Germany
ELISA	Beckman coulter-USA
Freezer	Liebher-Austria
5 ml disposable syringes	Medicaljet , Syria
Eppendrof tube 1ml for serum	China
Centrifuge tube	China
Disposable test tube	China
Different grade of outomatic pipette	Different suppliers

2.1.2.Chemicals:

The chemicals and kits that are used in this study are listed in the table below with their suppliers:

Testosterone enzyme immunoassay test kit	Biocheck, Inc , foster city
Total cholesterol MR kit	Linear, Spain
HDLcholesterol kit	Linear, Spain
Triglyceride kit	BioMerieux®sa. France
Total protein kit	Spinreact.Spain
Albumin kit	Spinreact .Spain
Calcium kit	Human .Germany
Magnesium kit	Human .Germany
Urine strips for albumin	Linear, Spain

Table(2-2):Chemicals with their suppliers

2.1.3.Subjects:

2.1.3.1.Patients:

This study was conducted in Babylon Maternity and Pediatrics Teaching Hospital from November 2007 to the end of May 2008. Fifty five pregnant women with preeclampsia (twenty five of them in the second trimester of pregnancy while the rest of them were in the third trimester of pregnancy).

All the patients were nonsmokers, have no other diseases (ie, cardiac, hepatic, renal, endocrine and other disease)which may have effect on the measured parameters were excluded from the study. Details of anthropometric and clinical information for those patients were outlined in the questionnaire

form, Figure (2-1). Pregnancy is divided into 1st trimester (1-12 week), 2nd trimester (13-28 week) and 3rd trimester more than 28 weeks. Depending on the gestational age, the patients were divided into two groups:

2.1.3.1.1.Preeclamptics in the second trimester G1 :

They were twenty five preeclamptics in the second trimester of pregnancy. Age range 18-37 years (mean age ± SD=26.29 ± 5.12 year).Gestational age range 21-28 weeks (mean gestational age ± SD = 24.14 ± 3.63 week). Body mass index range = 24.7-50.4 kg/m^2 (mean body mass index ± SD = 36.7 ± 9.87 kg/m^2).Systolic blood pressure range 140-170 mmHg (mean Systolic blood pressure ± SD = 151.4 ± 10.7mmHg). Diastolic blood pressure range 90-120 mmHg (mean diastolic Blood pressure ± SD = 98.6 ± 10.3 mmHg). Mean proteinuria = 100 mg/dl. Details of their number, age, gestational age, body mass index, arterial blood pressure distributions are shown in Table (2-3).

2.1.3.1.2. Preeclamptics in the third trimester G2 :

They were thirty preeclamptics in the third trimester of pregnancy. Age range 18-44 years (mean age ± SD =24.86±5.4 year). Gestational age range 29-38 weeks (mean Gestational age ± SD = 35.57 ± 3.21 week).Body mass index range 32.1-61.2 kg/m^2 (mean body mass index ± SD =46.6±1.6 kg/m^2).Systolic

blood pressure range 140-200 mmHg (mean Systolic blood pressure ± SD = 157.1-13.8 mmHg). Diastolic blood pressure range 90-130 mmHg (mean diastolic Blood pressure ± SD = 101.4 ± 10.3 mmHg). Mean proteinuria = 300 mg/dl. Details of their number, age, gestational age, body mass index, arterial blood pressure distributions are shown in Table (2-3).

2.1.3.2.Control:

Fifty five apparently healthy pregnant women (twenty five of them were in the second trimester and thirty of them were in the third trimester). Pregnant women with chronic hypertension , diabetes mellitus, renal disease, multifetal gestation, and pregnancy less than 20 weeks of gestation were excluded from this study. Details of clinical manifestations were taken from each pregnant women according to the questionnaire form presented in Figure (2-1).

Depending on the gestational age, the pregnant women were divided into two groups:

2.1.3.2.1.Control pregnant women in the second trimester G3:

They were twenty five healthy (normotensive) women in the second trimester of pregnancy. Age range 19-35 years (mean age ± SD = 23.73 ± 3.73 year). Gestational age range 20-28 weeks (mean ± SD =23.43±3.2 week).Body mass index range

21.4-50.4 kg/m^2 (mean body mass index ±SD =35.8± 11.7 kg/m^2).Systolic blood pressure range 100-130 mmHg (mean Systolic blood pressure ± SD = 105 ± 15.1 mmHg). Diastolic blood pressure range 55-75 mmHg (mean diastolic Blood pressure ± SD = 64.3 ± 7.9 mmHg). Proteinuria range <30 mg/dl. Details of their number, age, gestational age, body mass index, arterial blood pressure distributions are shown in Table(2-3).

2.1.3.2.2.Control pregnant women in the third trimester G4:

They were thirty healthy (normotensive) women in the third trimester of pregnancy. Age range 19-42 year (mean age ± SD = 26.6 ± 3.74 year). Gestational age range 29-40 weeks (mean ± SD = 35.3±3.99 week). Body mass index range 29.5-45.1 kg/m^2 (mean body mass index ± SD = 37.7 ± 5.9 kg/m^2). Systolic blood pressure range 90-130 mmHg (mean systolic blood pressure ± SD = 110-14.1 mmHg). Diastolic blood pressure range 50-80 mmHg (mean diastolic Blood pressure ± SD = 62.9 ± 9.9 mmHg). Proteinuria range <30 mg/dl .Details of their number, age, gestational age, body mass index, arterial blood pressure distributions are shown in Table (2-3).

Name: Age: yr Bp: mmHg
Date: / /200 Serial No:
Height: cm Weight: kg BMI: kg/m^2

- Smoking: yes no Alcohol intake :yes no
- Familial dyslipidemia

G ─P ─ A gestational age: week

Parameters	G1	G2	G3	G4

Family history of preeclampsia:yes no

Medical history: DM HT: renal disease: cardiac disease:

Drug history:

Social history:

Laboratory tests:

A/Serum lipid profile:

- Serum triacylglycerol: mmol/l
- Serum total cholesterol: mmol/l
- Serum HDL-cholesterol: mmol/l

B/Serum electrolyte tests:

- Serum calcium: total mmol/l ionized mmol/l
- Serum magnesium: total mmol /l

C/Serum sex steroid:

- Serum testosterone: total ng/ml

D/General biochemical tests:

- Serum total protein : g/dl
- Serum albumin: g/dl
- Urine strip for albumin:

Figure (2-1) Details of clinical and biochemical state

Table (2-3): The number, age, gestational age, body mass index and blood pressure in different preeclampsic and normal pregnant groups.

Number of subjects	25	30	25	30
Age/year(Mean ±SD)	26.29± 5.12	24.86± 5.4	23.7± 3.73	26.6± 3.74
Age range/year	18-37	18-44	19-35	19-42
Gestational age/week (Mean ± SD)	23.14± 3.63	35.57± 3.21	23.43± 3.2	35.3± 3.99
Gestational age range/week	21-28	29-38	20-28	29-40
BMI Kg/m^2 (Mean ± SD)	36.7± 9.87	46.6± 16	35.8± 11.7	37.7± 5.9
BMI range Kg/m^2	24.7-50.4	32.1-61.2	21.4-50.4	29.5-45.1
SBP mmHg (Mean ± SD)	151.4± 10.7	157.1± 13.8	105.7±15.1	110± 14.14
SBP range mmHg	140-170	140-200	100-130	90-130
DBP mmHg (Mean ± SD)	98.6± 10.3	101.4± 10.3	64.3± 7.9	62.9± 9.9
DBP range mmHg	90-120	90-130	55-75	50-80
Mean of Proteinuria (mg/dl)	100	300	<30	<30
No. of women with edema (hands&face)	17	28	5	12

2.1.4. Collection of blood samples :-

Blood samples (5ml) were collected by venipuncture , fasting sample without tourniquet and were transferred into clean new plane tubes , the blood samples were allowed to clot at room temperature and sera were separated by centrifugation at l500 xg for 2 minutes , then divided into three parts and stored at – 20

1- A liquate of serum was transferred into eppendrof tube , which was used for measuring minerals (Ca ,Mg).

2-Another a liquate of serum was transferred into eppendrof tube, which was used for measuring total protein , albumin , cholesterol , HDL – cholesterol and triacylglycerol .

3-The rest of sera were transferred into eppendrof tube and was used for measurement of testosterone by enzyme Linked immunosorbant assay (ELISA) .

2.1.5. Urine samples :-

The urine samples were taken from each subject in the study , to determine proteinuria semi- quantitatively using dipsticks methods (50) .

2.2. Methods :

2.2.1.Testosterone enzyme immunoassay test kit[51] :

Serum testosterone was measured by colorimetric assay using kit supplied by Biocheck , Foster city .

- ***Principle:***

The testosterone EIA is based on the principle of competitive binding between testosterone in the test specimen and testosterone-horse radish peroxidase(HRP) conjugate for a constant amount of rabbit antitestosterone . In the incubation , goat antirabbit IgG – coated wells are incubated with , 10 µl of testosterone standards , controls , patient samples , 100 µl testosterone–HRP conjugate reagent and 50 µl testosterone reagent at 37C̊ for 90 minutes. During the incubation , affixed amount of the HRP- labeled testosterone competes with the endogenous testosterone in the standard , sample or quality control serum for affixed number of biding sites of the specific testosterone anti–body, thus the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases .

Unbound testosterone peroxidase conjugate is then removed and the well was washed. Next , a solution of 3,3′,5,5′ tetra methyl benzidine (TMB) reagent is the added and incubated at room temperature for 20 minutes, until the development of blue color. The color development stopped with the addition of 1N HCl, and the absorbance measured spectrophotometrically at 450 nm . The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled testosterone in the sample . A standard

curve is obtained by plotting the concentration of the standard versus the absorbance . The testosterone concentration of the specimens and control run concurrently with the standard can be calculated from the standard curve.

- ***Reagents :***

1- Goat anti – Rabbit IgG- coated microtiter well , 96 wells.

2- Testosterone reference standards : 0, 0.1, 0.5, 2.6 and 18.0 ng/ml liquids , 0.5 ml each , ready to use .

3- Rabbit anti- testosterone reagent (pink color)

4-Testosterone-HRP conjugate reagent (blue color)

5- Testosterone control 1, liquid , 0.5ml .

6- Testosterone control 2, liquid , 0.5ml.

7- TMB reagent (one – step).

8- Stop solution (1N HCl) .

- ***procedure :***

1-The desired number of coated wells were secured in the holder

2-A volume of 10 µl of standard, standards specimens and control were dispensed into appropriate wells .

3-A volume of 100 µl of testosterone – HRP conjugate reagent were dispensed into each well .

4-A volume of 50 µl rabbit anti-testosterone reagent were dispensed in to well.

5-The tubes were thoroughly mixed for 30 seconds . It is very important to mix them completely .

6- The wells were incubated of 37C° for 90 minutes .

7- The microwells were rinsed and flicked five times with distilled or deionized water .

8- A volume of 100 µL of TMB reagent were dispensed in to each well . Gently were mixed for 10 seconds .

9- The wells were incubated at room temperature (18-25C°) for 20 minutes .

10-The reaction was stopped by adding 100µL of stop solution to each well .

11- Gently the wells were mixed 30 seconds . It's important to make sure that all the blue color changes to yellow color completely .

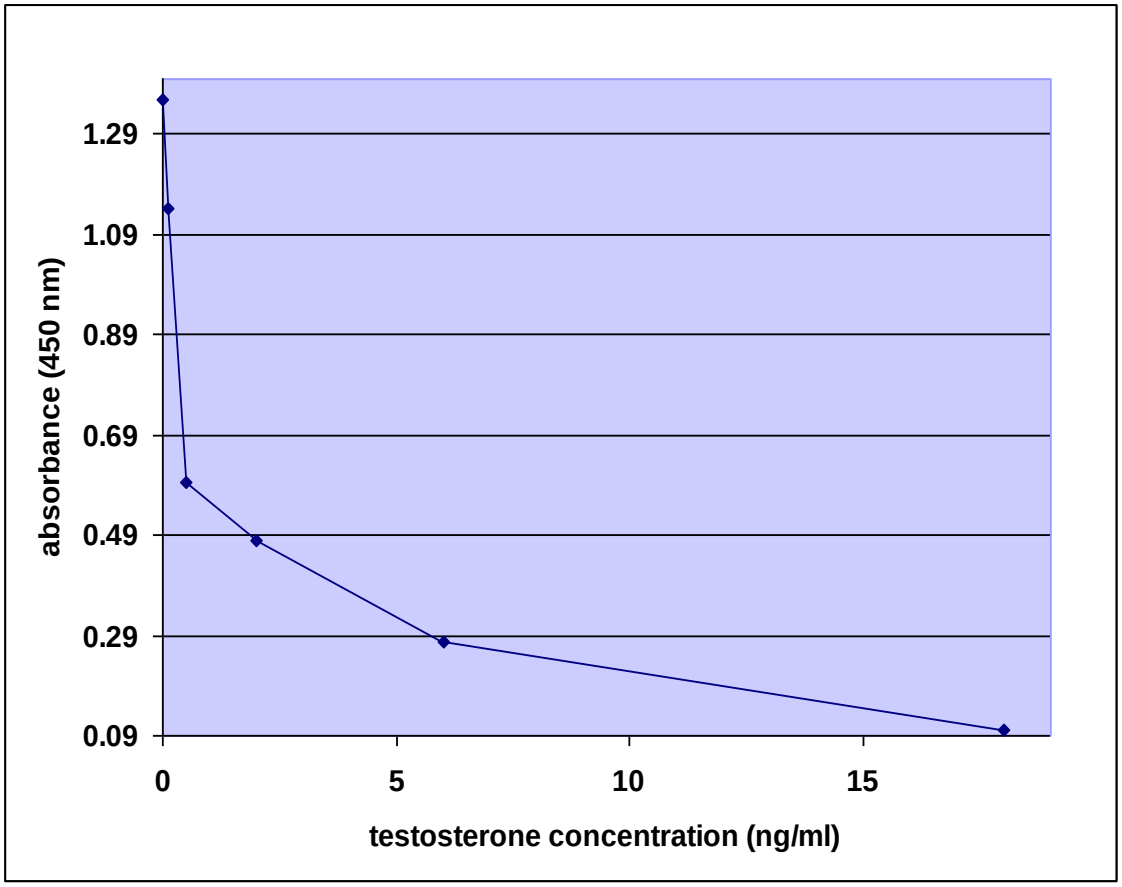
12- The absorbance was read at 450 nm with a microtiter well read within 15 minutes .

2.2.1.3. The standard Curve :

1- The absorbance value (A_{450}) for each standards, controls and samples were calculated .

2-A standard curve had been construct by plotting the absorbance obtained for each standard against concentration in ng/ml on linear – Linear graph paper , with absorbance values on the vertical or concentration on the horizontal or x axis .

3- The absorbance values was used for each specimen to determine the corresponding concentration of testosterone in ng/ml from the standard curve . A standard curve has been obtained as in Fig (2-2) .



Figure(2-2):Standard curve of testosterone determination

2.2.2.Lipid profile:-

2.2.2.1. Determination of serum total – Cholesterol [52] :

Serum total - Cholesterol was measured by colorimetric assay kit supplied by Linear Company (Spain).

- ***Principle***

This method for the measurement of total cholesterol in serum involves the use of three enzymes : cholesterol esterase (CE) cholesterol oxidase (CO) and peroxidase (POD) . In the presence of

the former the mixture of ADPS and 4- aminoantipyrine (4-AA) are considered by hydrogen peroxide to form a quinonoinine dye proportional to the concentration of cholesterol in the sample .

$$\text{Cholesteryl esters} \xrightarrow{CE} \text{Cholesterol + fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholestenone} + H_2O_2$$

$$4\text{–AA} + \text{ADPS} \xrightarrow[POD]{H_2O_2} \text{Quinoneimine} + 4H_2O$$

- ***Reagent***

1- Monoreagent 200 mmol/l . pH 7 , sodium cholate 1mmol/l , cholesterol esterase > 250 U/L , cholesterol oxidase >250 U/L peroxidase > KU/l , 4-aminoantipyrine 0.33 mmol/l ,ADPS 0.4 mmol/l , non ionic tensioactives 2g/l

2- Cholesterol standard : cholesterol 200 mg/dl (5.18mmol/l)

The monoreagent and standard are ready for use .

- ***Procedure***

1- Reagents and blank were brought to room temperature .

2-The following volumes were pipetted into test tube .

Tubes	Blank	Sample	Standard
Monoreagent	1mL	1mL	1mL
Sample	—	10µL	—
Standard	—	—	10µL

3-All tubes were shaked and incubated 10 minutes at temperature or 5 minutes at 37C°

4-The absorbance (A) of the samples and the standard were read at 550 nm against the reagent blank .

- ***Calculation***

The cholesterol concentration in the sample may be calculated form the following formula :

$$\text{Total-cholesterol mmol/l} = \frac{A_{\text{sample}}}{A_{\text{standard}}} * \text{Concentration of standard}$$

Normal values in serum < 5.18 mmol/l

2.2.2.2. Determination of serum HDL – Cholesterol [53] :

Serum HDL-cholesterol was measured by colorimetric assay using kit supplied by Linear Company (Spain) .

- ***Principle***

This technique uses a separation method based on the selective precipitation of apolipoprotein B-100 containing lipoproteins (VLDL, LDL and LPa) by phosphotungstic acid / $MgCl_2$, sedimentation of the precipitant by centrifugation , and subsequent enzymatic analysis of high density lipopotein (HDL) as residual cholesterol remaining in the clear supernatant .

- ***Reagent***

1- Precipitating reagent: Phosphotungstic acid 0.63 mmol/l magnesium chloride 25 mmol/l stabilizers .

2- HDL -cholesterol standard : Cholesterol 50 mg/dl (1.3mmol/l) **.**

- ***Procedure***

precipitation

1- The reagents and sample were brought to room temperature .

2- The following volumes were pipetted into test tubes .

Sample or standard	0.2ml
Precipitating reagent	0.4ml

3- The tubes were mixed and allowed to stand for 10 minutes at room temperature

4- Centrifuge for 10 minutes at 2000 xg

5- The clear supernatant were separated within 2 hours .

Colorimetry

1- The cholesterol MR Monoreagent and the cholesterol standard (50 mg/dl) of the kit were brought to room temperature

2- The following volumes were pipetted into test tubes .

Tubes	Blank	Sample Supernat	Standard Supernate
Monoreagent	1ml	1ml	1ml
Supernate	—	50 µL	—
Standard	—	–	50µL

3-The tubes were shaked and let stand for 10 minutes at room temperature or 5 minutes at 37C°.

4- The absorbance (A) of the supernatant and the standard read at 550 nm against the reagent blank .

- ***Calculations***

$$\text{HDL-Cholesterol mg/dl} = \frac{\text{A supernatant}}{\text{A standard}} * \text{Concentration of standard}$$

Normal values in serum >1.68 mmol/l

2.2.2.3. Determination of serum triacylglycerol [54]:-

Serum triacylglycerol was measured by coloremetric assay using kit supplied by Biomerieux (France)

- ***Principle***

Triglyceride are determined according to the following sequence lipase-glycerokinase-glycerol-3-phosphate-oxidase-peroxidase chromogen .

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerokinase}} \text{glycerol -3- phosphate + ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[\text{oxidase}]{\text{Glycerol-3- phosphate}} H_2O_2 + \text{dihydroxy acetone phosphate}$$

,

$$H_2O_2 + \text{parachlorophenol + 4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 2H_2O + HCl$$

The intensity of the coloration (quinoneimine)measured is proportional to the triglyceride content of the sample.

- ***Reagents :-***

The kit contains :

1- Reagent 1 (standard)(R1) : which contains glycerol (2.29mmole of triglycerides .

2- Reagent 2 (buffer) (R2) which contains Tris buffer pH 7,6 (100mmol/l)
Parachlorophenol (2.7 mmol/l)
Magnesium (4mmol/l).

3- Reagent 3 (reconstituted with R2) which contains protein base buffer.

4- Aminoantipyrine (0.4mmol/l)

Lipase (≥1000 U/l).

Glycerokinase (≥200 U/l)

Glycerol -3- phosphate oxidase (>2000U/I)

Peroxidase (>200U/I)

ATP (0.8mmol/l

Working reagent, which is prepared by reconstituting the content of one vial of reagent (3) with 25ml of reagent (2) (shake gently).

- ***Procedure***

	Blank	Standard	Sample
Standard	——	10 µl	——
Sample	——	——	10µL
Reconstituted Reagent3	1mL	1mL	1mL

Photometry was mixed and performed after incubation for 5 minutes at 37C° or 10minutes at 20- 25C° .

- ***Calculation***

Concentration of triglyceride = $\frac{\text{A sample}}{\text{A standard}}$ * concentration of standard

Normal value in serum 0.4- 1.5 mmol/l .

2.2.2.4.Calculation of very low density lipoprotein (VLDL-C) from triacylglycerol [55] :

The same data of the triacylglycerol can be used to calculate the (VLDL-C) according to the formula :

VLDL-C = TG/2.2 mmol/l.

2.2.2.5. Calculation of low density lipoprotein (LDL-C) from total cholesterol , VLDL-C and HDL – C[55] :

The same date of the total cholesterol , VLDL-C and HDL-C can be used to calculate the (LDL-C) according to the formula :

LDL-C = Total cholesterol – (HDL-C + VLDL-C)

2.2.3. Determination of serum total protein [56] :-

Total proteins in serum were measured by coloremetric assay using kit by supplied Spianreact (Spain) .

- ***Principle***

Proteins give an intensive violet – blue complex with copper salts in an alkaline medium . Iodide is included as an antioxidant the

intensity of the color formed is proportional to the total protein concentration in the sample .

- ***Reagents***

1- R Biuret :- sodium potassium tartrate 15 mmol/l , sodium iodide (100 mmol/l), Potassium iodide (5 mmol/l) and copper (II) sulphate 19 (mmol/l)

2-T- protein calibration :- Bovine albumin primary standard 7g/dl

- ***Procedure***

1- The instruments were adjusted zero with distalled water .

2- The following volume were pipette into test tube .

	Blank	Standard	Sample
R(ml)	1ml	1mL	1mL
Standard	——	25µL	——
Sample	——	——	25µl

3-All tubes were shaked and incubated 5min at 37C° or 10 minutes .

4-The absorbance (A) of the samples and standard were read against the blank at wave length 540 nm and temperature 15-25C°

- ***Calculation :***

The total protein concentration in the sample may be calculated from the following formula :

$$\text{Total protein g/dl} = \frac{\text{A sample}}{\text{A standard}} * \text{concentration of standard}$$

Normal value in serum 6.6-8.3 gm /dl

2.2.4. Determination of serum albumin [57]:-

Serum albumin was measured by colorimetric assay using kit supplied by Spinreact (Spain) .

- ***Principle***

Albumin in the presence of bromocresol green at a slightly acid PH , produces a colour change of the indicator from yellow – green to green blue . The intensity of the color formed is proportional to the albumin concentration in the sample .

- ***Reagents***

1-R. Bromocresol green pH 4.2 (0.12mmol/l).

2-Albumin calibration :- Albumin aqueous primary standard 5g /dl.

- ***Procedure:***

1-The instrument were adjusted to zero with distilled water

2- The following volumes were pipette in to test tube .

	Blank	Standard	Sample
R	1ml	1ml	1ml
Standard	—	5ml	—
Sample	—	—	5µL

3- All tubes were shaked and incubated for 10 min at room temperature (15-25C°) .

4- The absorbance (A) of the samples and standard were read against the blank at wave length 630 nm .

- ***Calculation :***

The albumin concentration in the sample may be calculated from the following formula .

$$\text{Albumin g/dl} = \frac{\text{A sample}}{\text{A standard}} * \text{concentration of standard (5g/dl)}$$

Normal value in serum 3.5-5 g/dl .

2.2.5. Calculation of globulin [58] :

The same data of the total protein and albumin can be used to calculate globulin according to the formula .

Globulin = Total protein – Albumin

2.2.6. Calculation of albumin : globulin ratio according to the formula[58] :

Albumin / Globulin ratio

2.2.7.Calculation of cholesterol/albumin ratio from measured total-cholesterol and albumin [59]:

The same data can be used to calculate total-cholesterol and albumin according to the formula:

Total-cholesterol/albumin ratio

2.2.8. Minerals

2.2.8.1. Determination of serum calcium [60] :

Serum calcium was measured by coloremetric assay using kit supplied by Human Company (Germany) .

- ***Principle***

Calcium ions react with o-cresolphthalin – complex in an alkaline medium to from a purple coloured complex. The absorbance of this complex is proportional to the calcium concentration in the sample .

- ***Reagents***

1- Avolume of 100 ml buffer solution (BUF): lysine buffer (pH 11.1, 0.2mmol/l) sodium azide (0.095%) .

2- Avolume of 100ml colour reagent (RGT): 8- Hydroxyquinoline (14mmol/l) O- cresolphthalein complex (0.1mmol/l) and hydrochloric acid 40 mmol/l .

3- Avolume of 3ml standard : calcium (II) (8mg/dl or 2mmol/l) and sodium azide (0.095%).

Working reagent : which is prepared by add RGT and BUF in equal volumes as required , then mixed and allowed to stand for 30 minutes at room temperature before use .

- ***Procedure.***

Pipette in to cuvettes	Blank	Sample	Standard
Sample	—	20µL	—
Standard	—	—	20µL
Working reagent	1000µl	1000µL	1000µL

3- All tubes were shaked and the absorbance of sample (A) and standard (A) were measured against the blank within 5 to 30 minutes at wave length 570 nm .

- ***Calculation***

The calcium concentration in the sample may be calculated from the following formula :

$$\text{Calcium concentration mmol/l} = \frac{\text{A sample}}{\text{A standard}} * 2$$

Normal value in serum 2.02- 2.6 mmol/l

2.2.8.2. Adjustment of total serum calcium for variations in serum protein (61):

As about 40 percent of the serum calcium is bounded to protein and as variations in protein concentration particularly of Albumin , are common in hospital patient , some adjustment of calcium is advisable if meaningful results are to be obtained ;thus corrected serum calcium can be calculated according to the formula

Corrected calcium (mmol/l) = measured calcium (mmol/l) +0.02 [40% - albumin (g/l)]

2.2.8.3.Calculation of ionized calcium from measured calcium , albumin and total protein (61) :

Instead of obtaining a crude correction for measured calcium the same data can be used to calculate the ionized calcium according to the formula

Ionized calcium(mmol/l)=[60*measured calcium(mmol/l)–k′/12]/k′+60

K= 0.19 * total protein g/l + albumin (g/l)

2.2.8.4. Determination of serum magnesium (62) : -

Serum magnesium was measured by coloremetric assay using kit supplied by Human Company (Germany).

- ***Principle :***

Magnesium ions in alkaline medium form a coloured complex with xylidyl blue . The absorbance increase is proportional to the magnesium concentration in the sample . Glycoletherdiamine . N,N,N′,N′ tetracetic acid (GEDTA) is used as masking agent for calcium ions

- ***Reagent :***

1- 100 ml color reagent (RGT) :CAPS(from kit) (49 mmol/l), GEDA (from kit) (0.13 mmol/l) xylidyl blue (0,09mmol/l) sodium azide (0.095%) and activators .

2- 3 ml standard (STD):- magnesium (II) (2.5mg/dl) or sodium azide (0.095%) (1.03 mmol/l).

- ***Procedure:***

Pipette in to cuvettes	Blank	Sample	Standard
RGT	1000µL	1000µl	1000µl
Distilled water	10 µL	—	—
Sample	—	10 µl	
Standard	—	—	10µl

3- All tubes were shaked and incubated for 10 minutes at 20- 25 C° .

The absorbance of the sample and the standard were measured against the reagent blank within 60 minutes at wave length 520 nm

- ***Calculation :***

The magnesium concentration in the sample may be calculated from the following formula:

$$\text{Concentration of magnesium (mmol/l)} = \frac{\text{A sample}}{\text{A standard}} * 1.03$$

Normal value in serum 0.8 - 1 mmol/l .

2.2.9.Calculation of body mass index(63):

Body mass index (BMI) calculated as weight (kg)/height$(m)^2$, normal value 18.5 - 24.9 kg/m^2 .

2.2.10.Statistical analysis:

The statistical analysis is based on ANOVA test to determine the differences between groups and within groups. Correlation, regression and correlation coefficient (r) , using SPSS (statistical product and service solutions) program for data analyses.

Chapter Three

Results

3.1.Testosterone:

Serum testosterone was significantly higher in preeclamptic groups(G1&G2) compared with normal pregnant women groups (G3&G4).Also serum testosterone was significantly higher in G2 compared with G1,and also shows nonsignificant decrease in G4 compared with G3 ,[Fig(3-1),Table(3-1),(3-2)]

Table (3-1): Serum data of testosterone in preeclamptic and normal pregnant women (2nd and 3rd trimester) (mean ± SD)

Measured parameter	G1	G2	G3	G4
Testosterone(ng/ml) ± SD	1.46±0.199	2.41±0.54	0.82±0.198	0.74±0.24

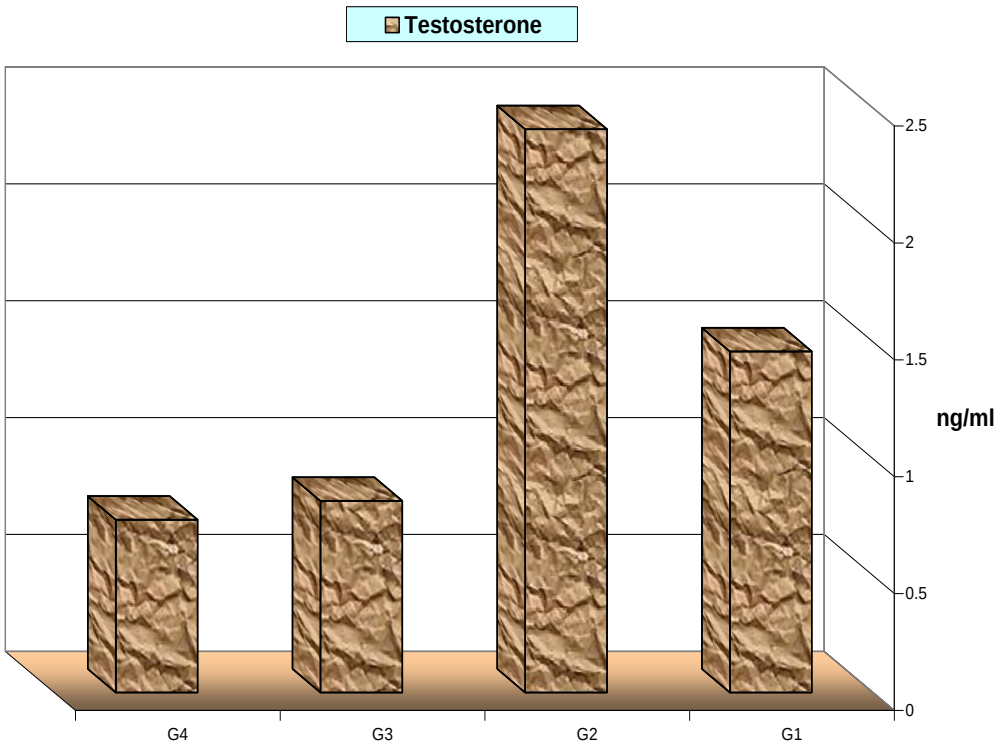


Figure (3-1): Serum data of testosterone in preeclamptic and normal pregnants women (2nd and 3rd trimester)

Table (3-2): Significance value for testosterone in different groups

Groups	P value
G1 vs G2	<0.001
G1 vs G3	<0.01
G1 vs G4	<0.001
G2 vs G3	<0.01
G2 vs G4	<0.01
G3 vs G4	=0.36

3.2. Lipid profile (total cholesterol, TG, LDL-C, VLDL-C & HDL-C):

Serum total cholesterol, TG, LDL-C and VLDL-C were significantly higher in preeclamptic groups (G1&G2) compared with normal pregnants groups (G3&G4). This parameters were significantly higher in G2compared with G1and in G4 compared with G3,but serum HDL-C was significantly lower in G2 compared with G1 and G4 compared with G3 [Fig (3-2), Table (3-3) ,(3-4)]

Table (3-3): Serum data of total cholesterol, HDL-C, TG, VLDL-C, LDL-C in preeclamptic and normal pregnant women (2nd and 3rd trimester) (mean ± SD)

Measured parameter	G1	G2	G3	G4
Cholesterol(mmol/l)	5.06±0.167	5.9±0.292	4.46±0.71	5.19±0.82
HDL-C (mmol/l)	1.226±0.061	1.05±0.166	1.57±0.116	1.21±0.29
TG (mmol/l)	1.59±0.117	2.72±0.54	1.28±0.39	1.89±0.68
VLDL-C (mmol/l)	0.72±0.053	1.24±0.24	0.58±0.17	0.86±0.31
LDL-C (mmol/l)	3.117±0.18	3.62±0.24	2.31±0.65	3.12±0.82

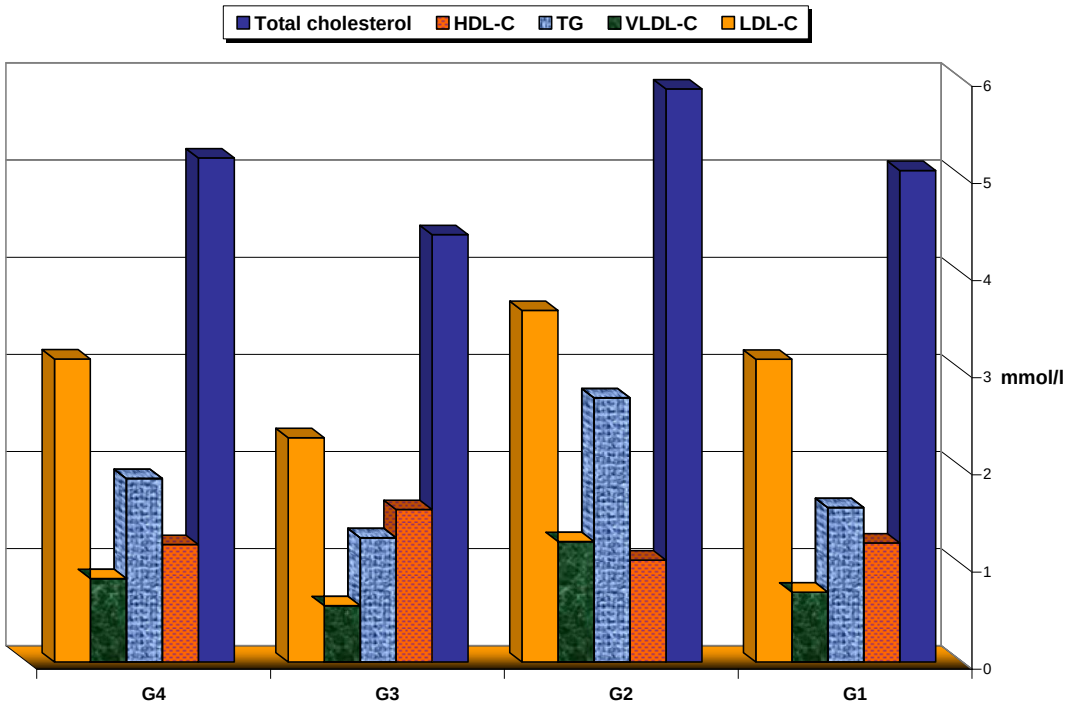


Figure (3-2): Serum data of total cholesterol, HDL-C, TG, VLDL-C, LDL-C in preeclamptic and normal pregnant women (2^{nd} and 3^{rd} trimester)

Table (3-4): Significance value for lipid profile in different groups

Groups	P value
G1 vs G2	<0.05
G1 vs G3	<0.001
G1 vs G4	>0.05
G2 vs G3	<0.05
G2 vs G4	<0.001
G3 vs G4	<0.05

3.3.Total protein, Albumin, Globulin and A/G ratio:

Serum total protein and albumin were significantly lower in preeclamptic groups (G1&G2)compared with normal pregnants groups (G3&G4), these results were significantly lower in G2 than G1 and there was insignificant decrease in G4compared to

G3. The results were reversed for globulin .A/G ratio was significantly lower in G2 than G1 and nonsignificant difference between G3&G4 [Fig(3-3),Table(3-5),(3-6),(3-7)].

Table (3-5): Serum total protein, albumin, globulin, albumin/globulin ratio in preeclamptic and normal pregnant women (2nd and 3rd trimester) (mean ± SD)

Measured parameter	G1	G2	G3	G4
Total protein(gm/dl)	6.076±0.34	5.06±1.22	6.7±0.13	6.56±0.28
Albumin (gm/dl)	3.14 ±0.31	2.5±0.54	3.58±0.12	3.44±0.22
Globulin (gm/dl)	2.94±0.091	2.56±0.69	3.04±0.082	3.12±0.21
A/G ratio	1.109±0.1	0.999±0.11	1.112±0.049	1.112±0.153

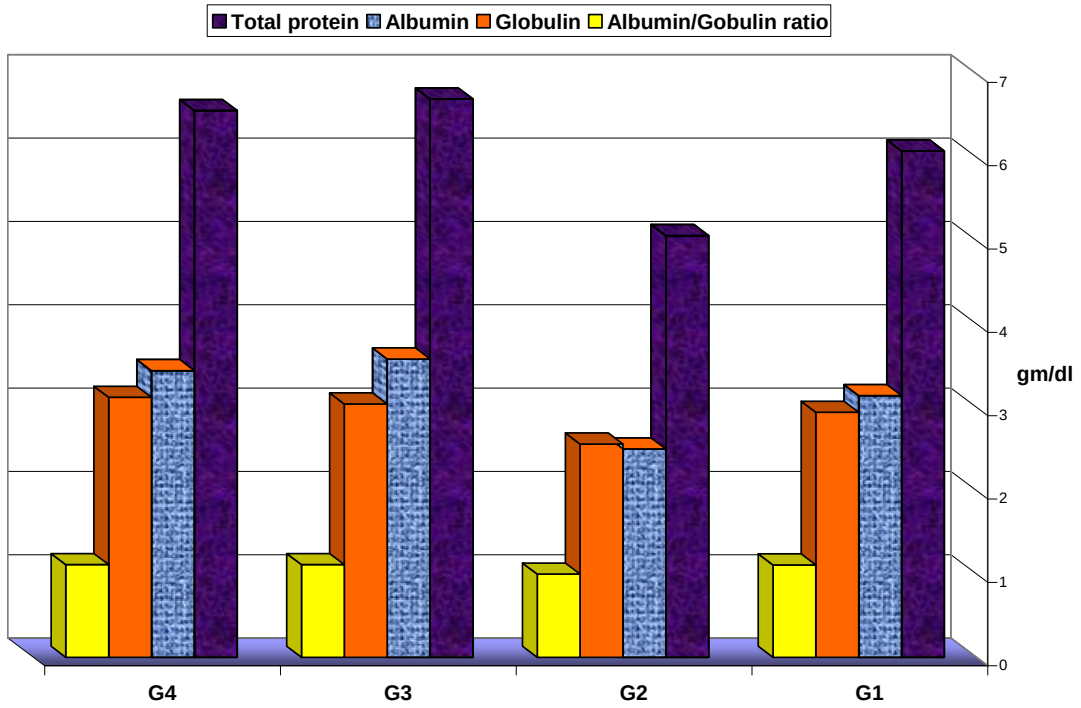


Figure (3-3): Serum total protein, albumin, globulin, albumin/globulin ratio in preeclamptic and normal pregnant women (2nd and 3rd trimester) .

Table (3-6): Significance value for total protein and albumin in different groups

Groups	P value
G1 vs G2	<0.001
G1 vs G3	<0.01
G1 vs G4	<0.05
G2 vs G3	<0.01
G2 vs G4	<0.01
G3 vs G4	>0.127

Table (3-7): Significance value for globulin and albumin/ globulin ratio at differrent groups

Groups	P value
G1 vs G2	<0.01
G1 vs G3	>0.01
G1 vs G4	>0.05
G2 vs G3	<0.01
G2 vs G4	<0.01
G3 vs G4	<0.01

3.4.Total cholesterol/albumin ratio:

Serum total cholesterol/albumin were significantly higher in preeclamptic groups (G1&G2) compared with normal pregnants groups (G3&G4). This parameter were higher in G2 compared with G1and in G4 compared with G3.[Fig(3-4),Table(3-8),(3-9)]

Table (3-8): Total cholesterol/albumin in preeclamptic and normal pregnants women(2nd and 3rd trimester) (mean±SD).

Measured parameter ± SD	G1	G2	G3	G4
Cholesterol/albumin ratio ± SD	0.063±0.009	0.096±0.028	0.048±0.009	0.059±0.0122

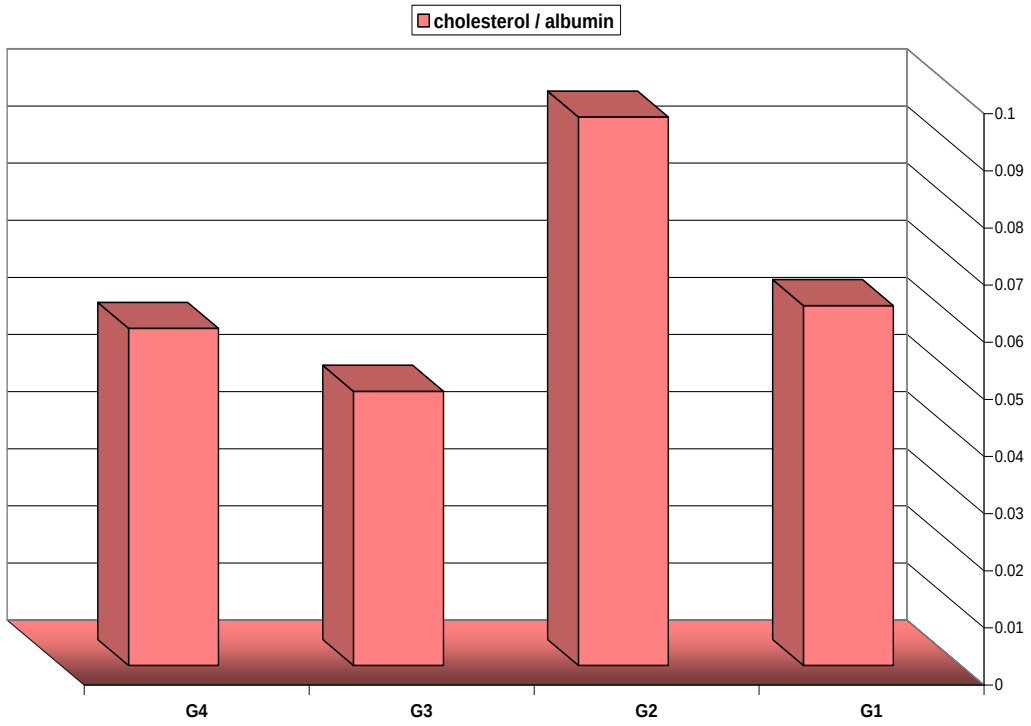


Figure (3-4): Total cholesterol/albumin in preeclamptic and normal pregnants women (2nd and 3rd trimester)

Table (3-9):Significance value for total cholesterol/albumin in different groups

Groups	P value
G1 vs G2	<0.001
G1 vs G3	<0.01
G1 vs G4	>0.05
G2 vs G3	<0.001
G2 vs G4	<0.001
G3 vs G4	<0.01

3.5.Minerals:

3.5.1.Total calcium, corrected calcium and ionized calcium

Total calcium, corrected calcium and ionized calcium were lower in preeclamptic groups (G1&G2) compared with normotensive groups(G3&G4). These parameters reversed a significant decrease in G2 in comparison with G4. The results showed insignificant decrease of total calcium in normotensive pregnants at the third trimester in comparison with those of 2nd trimester.Corrected calcium and ionized calcium were higher in G4 than G3, non significant difference [Fig(3-5), Table(3-10) , (3-11) ,(3-12)].

Table (3-10): Serum total calcium, corrected calcium, ionized calcium in preeclamptic and normal pregnants women (2nd and 3rd trimester) (mean ± SD)

Measured parameter	G1	G2	G3	G4
Calcium (mmol/l)	1.74±0.07	1.57±0.112	1.997±0.029	1.99±0.21
Corrected calcium (mmol/l)	1.91±0.018	1.87±0.009	2.08±0.009	2.101±0.197
Ionized calcium (mmol/l)	0.98±0.009	0.96±0.016	1.066±0.004	1.079±0.109

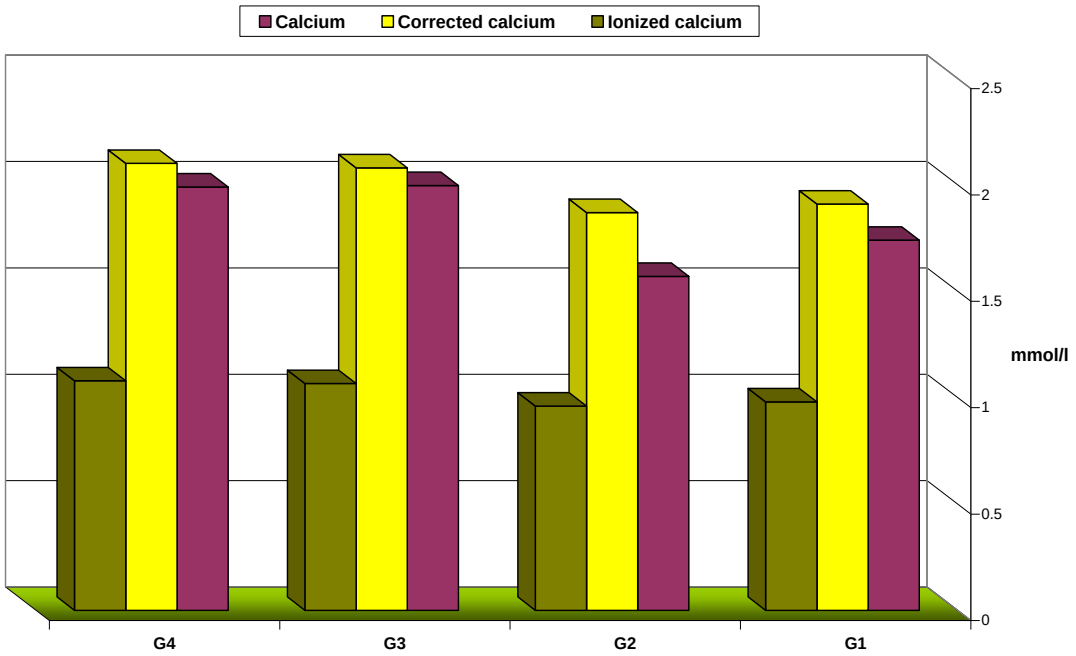


Figure (3-5): Serum total calcium, corrected calcium, ionized calcium in preeclamptic and normal pregnants women (2nd and 3rd trimester)

Table (3-11): Significance value for calcium in different groups

Groups	p Value
G1 vs G2	<0.05
G1 vs G3	<0.01
G1 vs G4	<0.01
G2 vs G3	<0.01
G2 vs G4	<0.001
G3 vs G4	=0.825

Table (3-12): Significance value for ionized calcium in different groups

Groups	p Value
G1 vs G2	=0.375
G1 vs G3	<0.05
G1 vs G4	<0.01
G2 vs G3	<0.001
G2 vs G4	<0.001
G3 vs G4	=0.386

3.5.2.Total magnesium:

Serum total magnesium was significantly lower in preeclampsia women (G1&G2)compared with normotensive pregnants women(G3&G4).These results showed nonsignificant difference between G3&G4 and nonsignificant difference between G1&G2[Fig(3-6),Table(3-13),(3-14)].

Table (3-13): serum magnesium in preeclamptic and normal pregnants women (2nd and 3rd trimester) (mean ± SD).

Measured parameter	G1	G2	G3	G4
Magnesium (mmol/l)	0.698±0.029	0.58±0.08	0.78±0.064	0.77±0.23

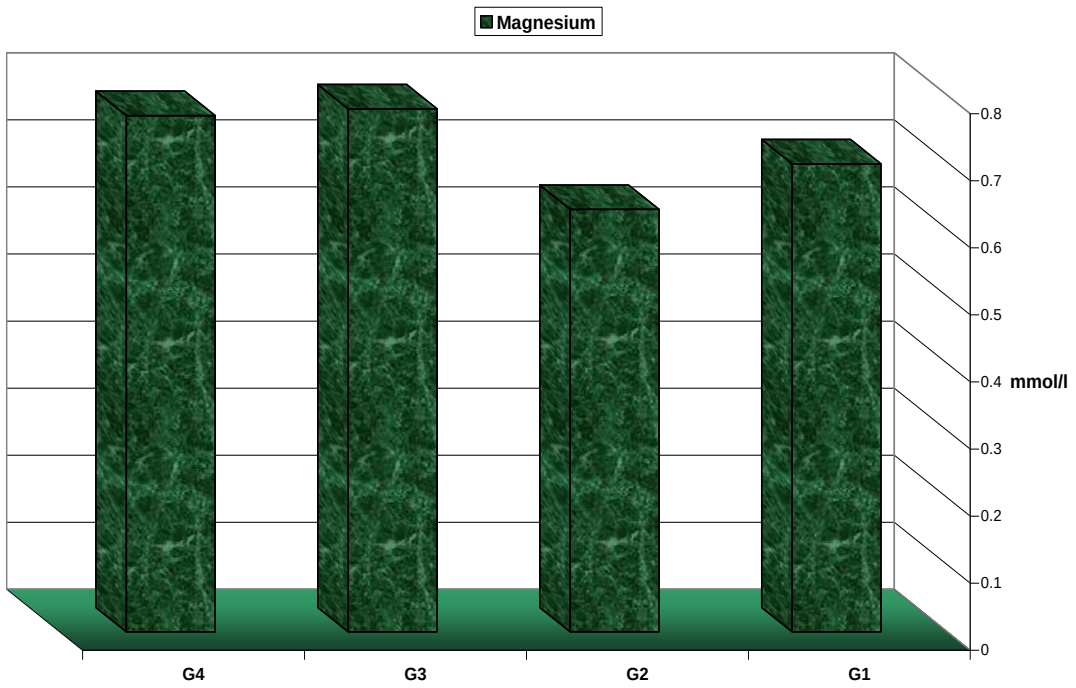


Figure (3-6): serum magnesium in preeclamptic and normal pregnants women (2nd and 3rd trimester)

Table(3-14): Significance value for magnesium at different groups

Groups	p Value
G1 vs G2	=0.085
G1 vs G3	=0.024
G1 vs G4	=0.48
G2 vs G3	<0.01
G2 vs G4	<0.001
G3 vs G4	=0.694

3.6.Correlation between serum testosterone and other parameters in different groups:

3.6.1. Correlation between serum testosterone and total cholesterol

A significant positive correlations between serum total-cholesterol and testosterone level was noticed in different groups except normal pregnants in third trimester(G4),which reversed negative correlation .Fig(3-7),(3-8),(3-9),(3-10).

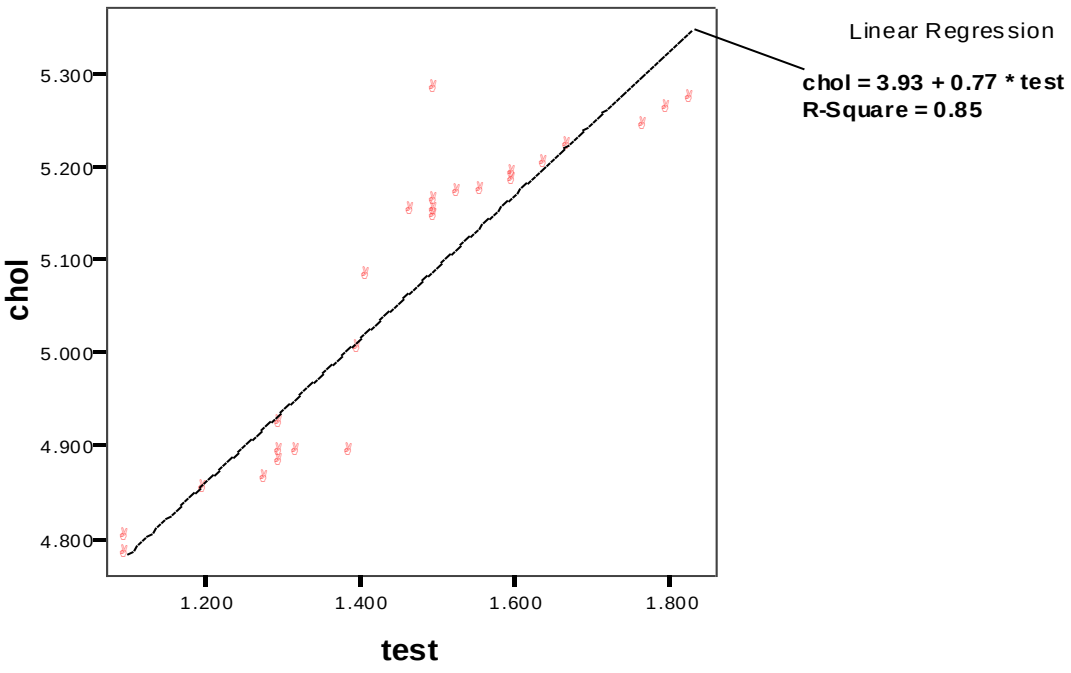


Fig (3-7): Correlation between testosterone and total cholesterol in G1 (r=0.92 ,p<0.01)

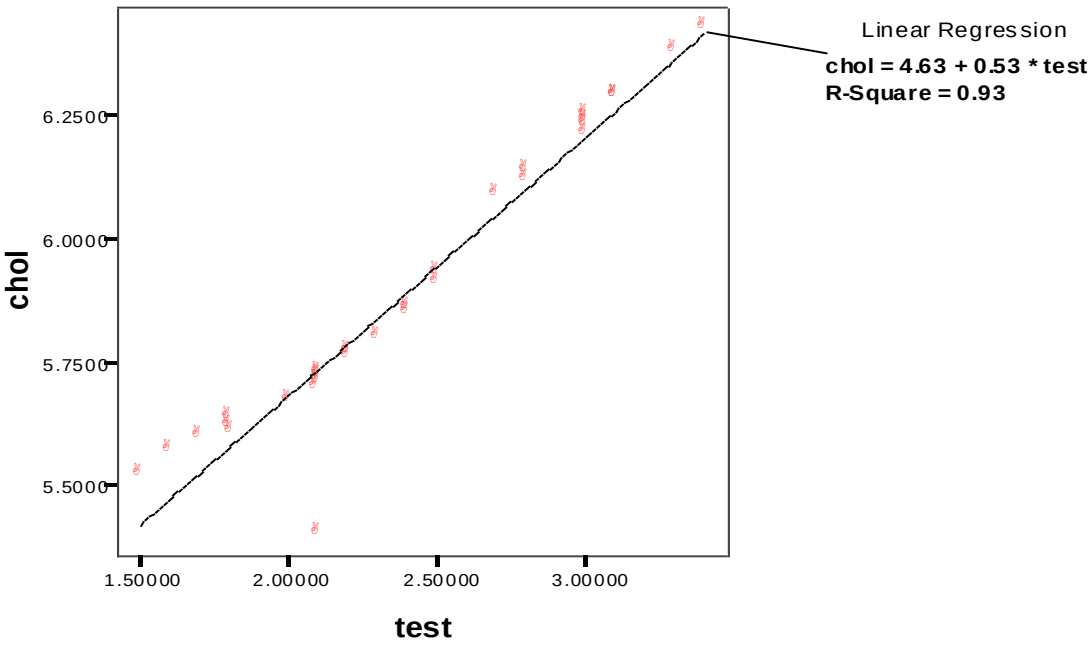


Fig (3-8): Correlation between testosterone and total cholesterol in G2 (r= 0.96,p<0.01)

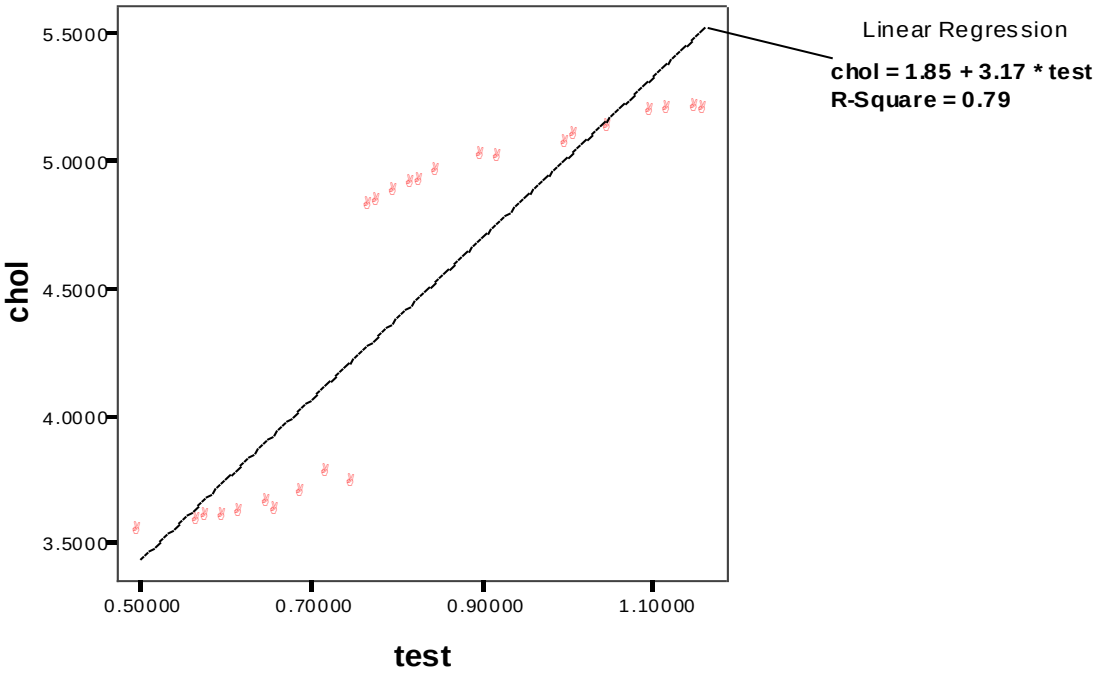


Fig (3-9):Correlation between testosterone and total cholesterol in G3 (r=0.89 ,p<0.01)

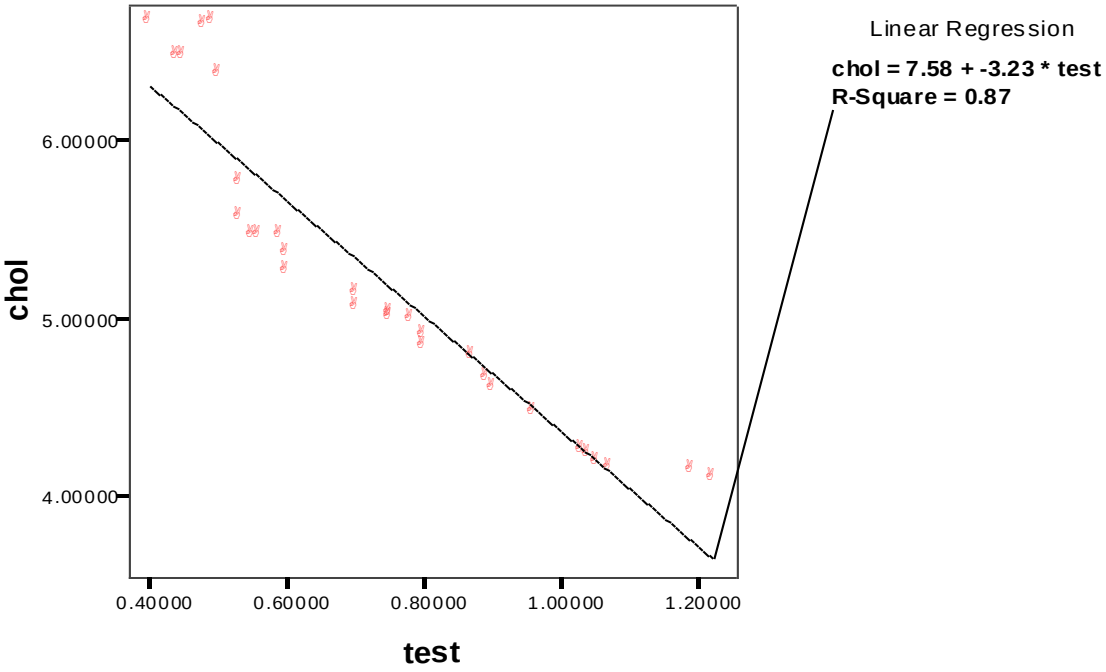


Fig (3-10):Correlation between testosterone and total cholesterol in G4 (r=0.93 ,p<0.01)

3.6.2. Correlation between serum triglyceride and testosterone

There were positive correlations between serum triglyceride and testosterone in different groups, but there was inverse correlation in G4 as in Fig (3-11),(3-12),(3-13),(3-14).

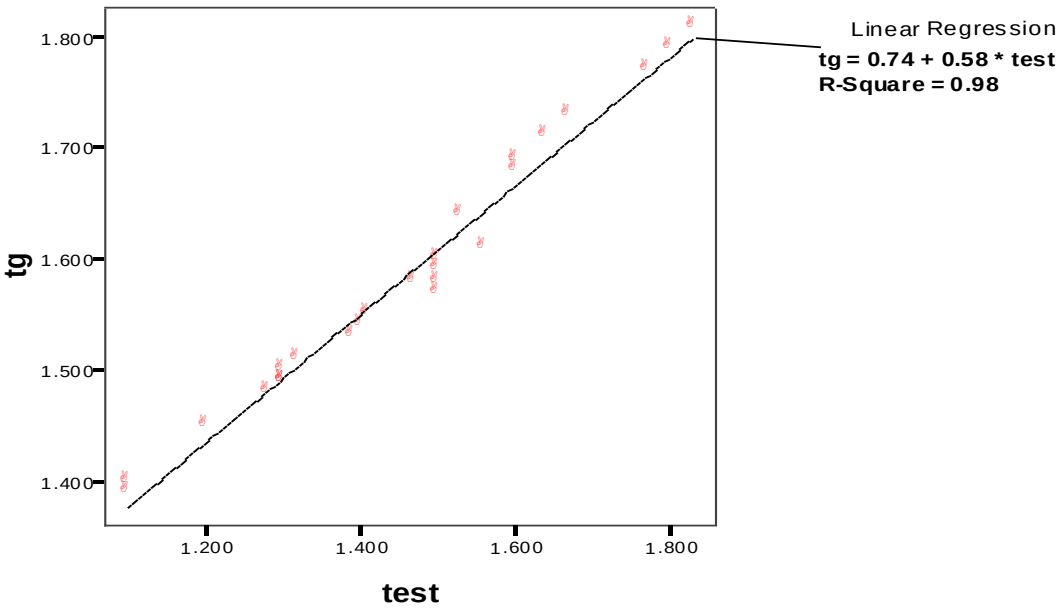


Fig (3-11): Correlation between testosterone and triglyceride in G1 (r=o.99 ,p<0.01)

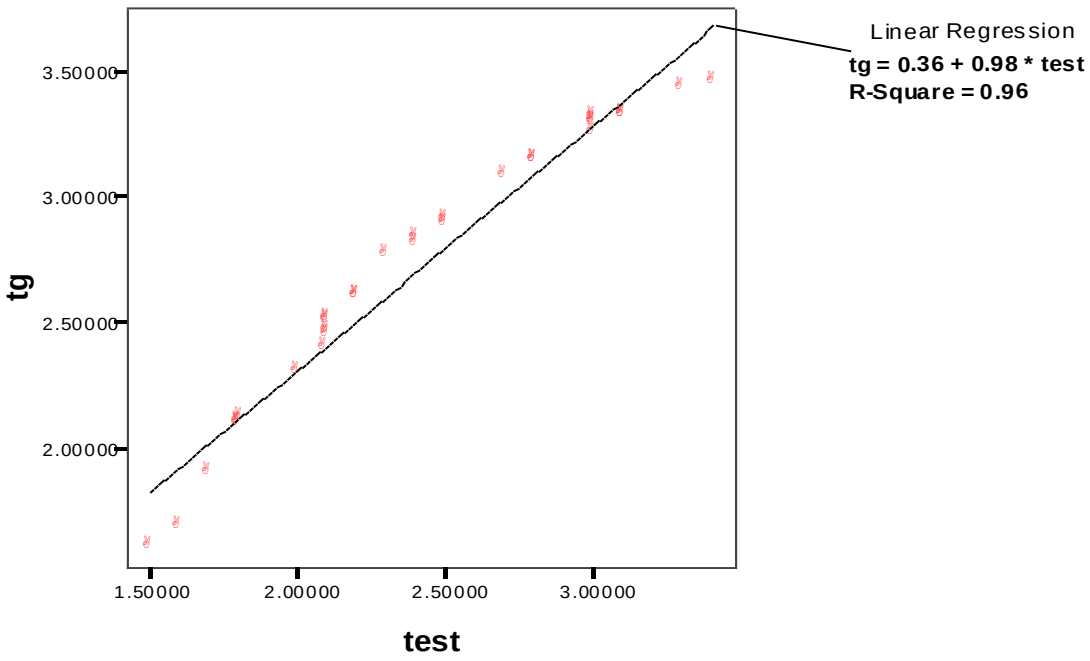


Fig (3-12): Correlation between testosterone and triglyceride in G2 (r= 0.98,p<0.01)

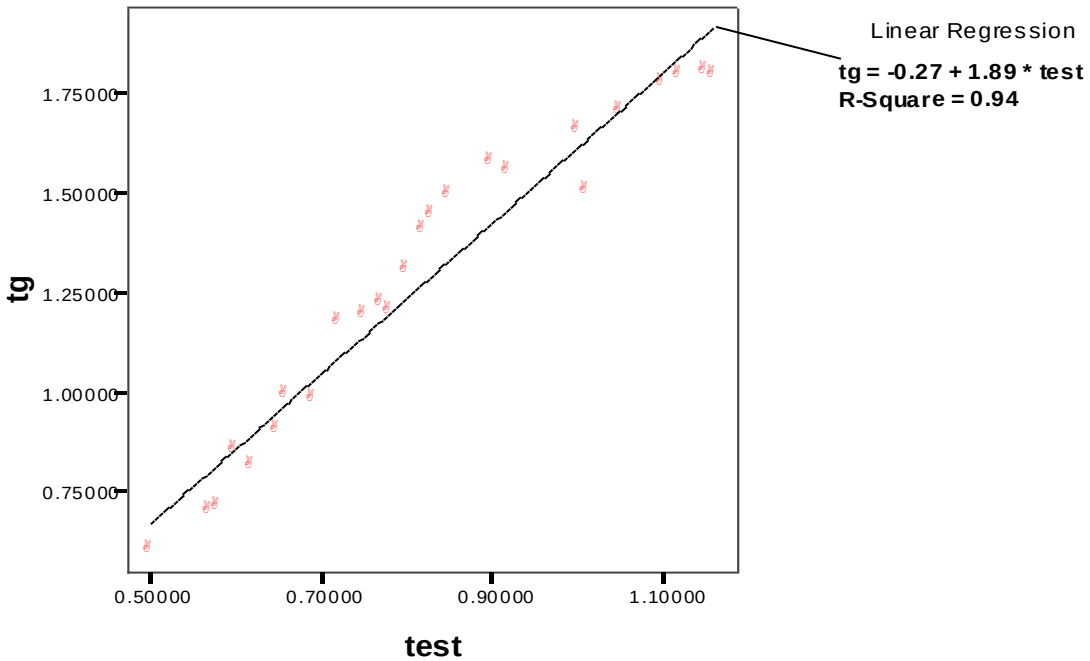


Fig (3-13): Correlation between testosterone and triglyceride in G3 (r= 0.97,p<0.01)

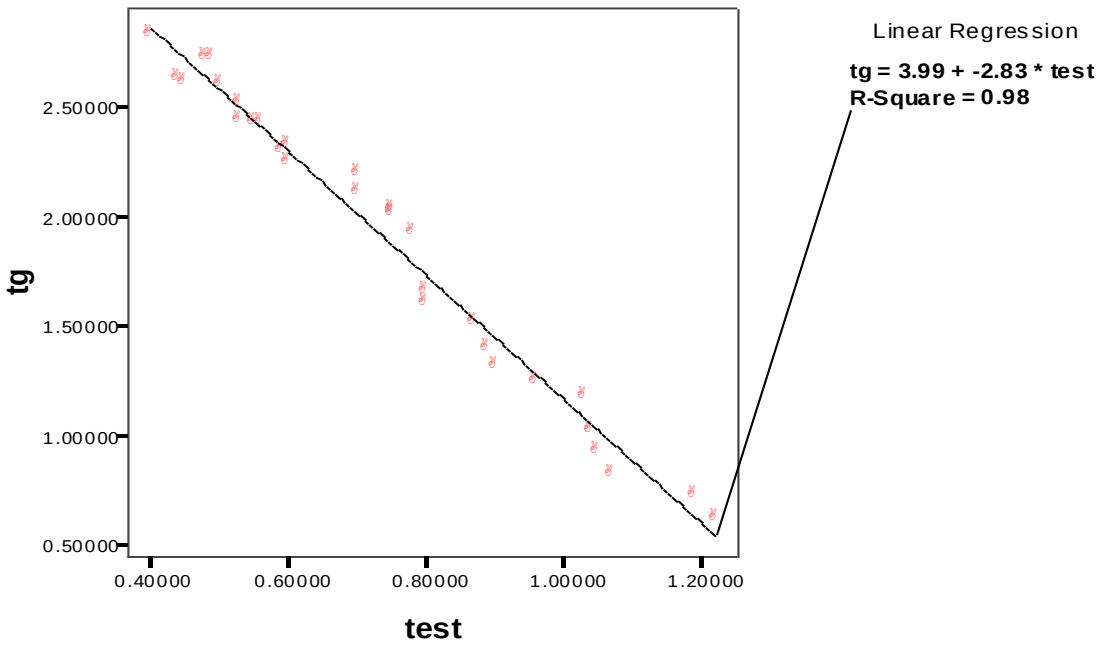


Fig (3-14): Correlation between testosterone and triglyceride in G4 (r=0.99 ,p<0.01)

3.6.3. Correlation between serum HDL-C and testosterone

There were negative correlations between serum HDL-C and testosterone in different groups, but there was inverse correlation in G4 as in Fig (3-15),(3-16),(3-17),(3-18).

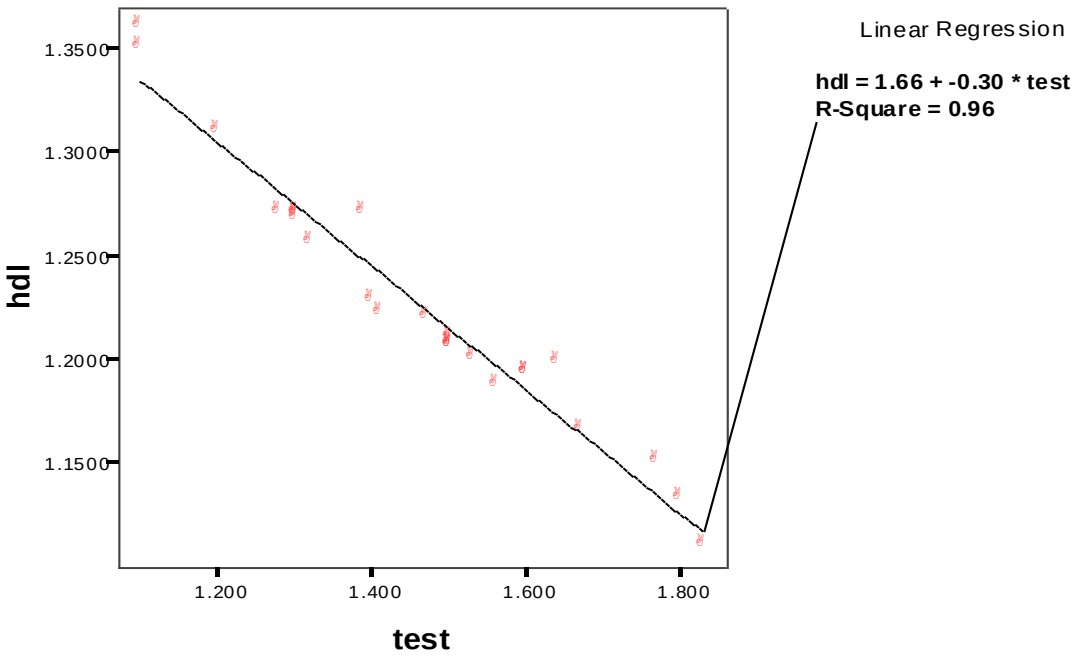


Fig (3-15): Correlation between testosterone and HDL-C in G1 (r=0.98 ,p<0.01)

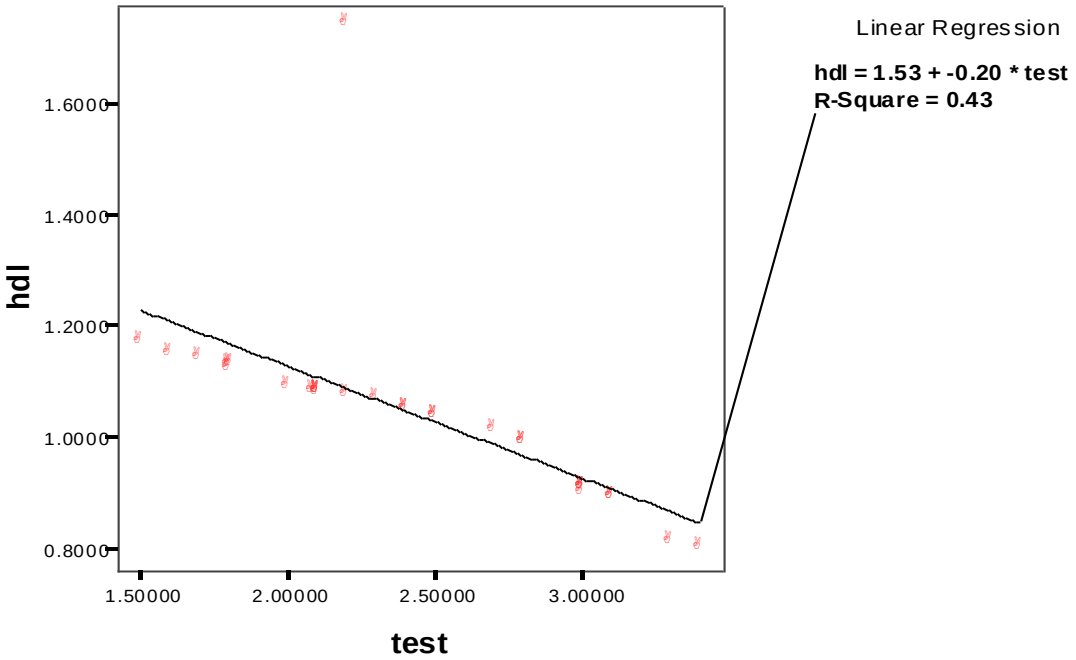


Fig (3-16): Correlation between testosterone and HDL-C in G2 (r=0.65 ,p<0.01)

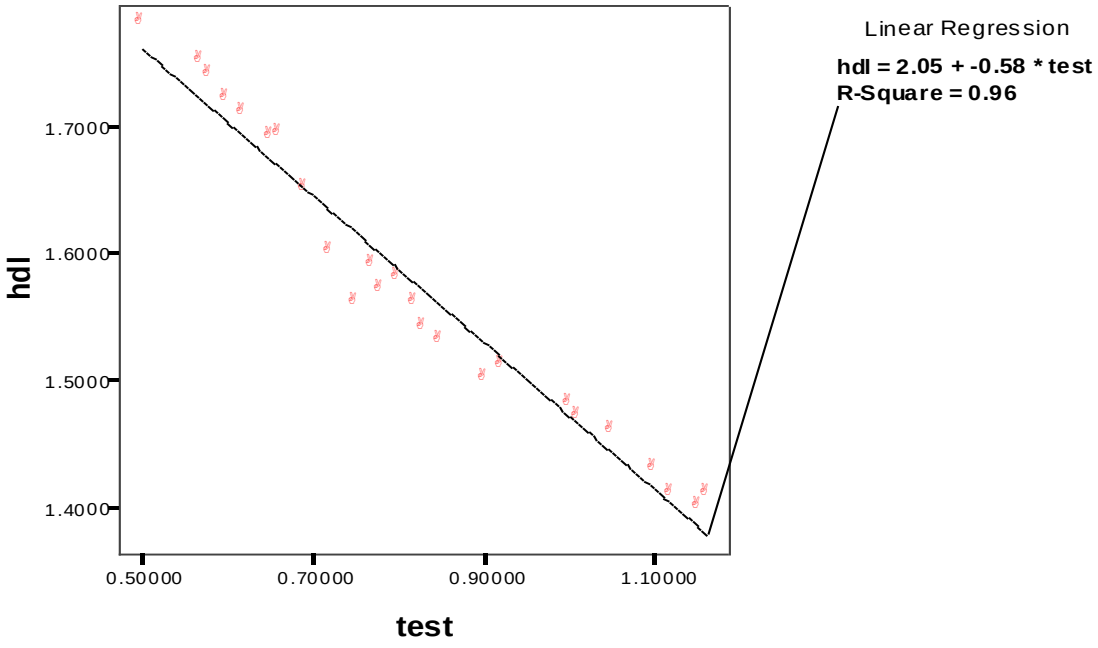


Fig (3-17):Correlation between testosterone and HDL-C in G3 (r=0.98 ,p<0.01)

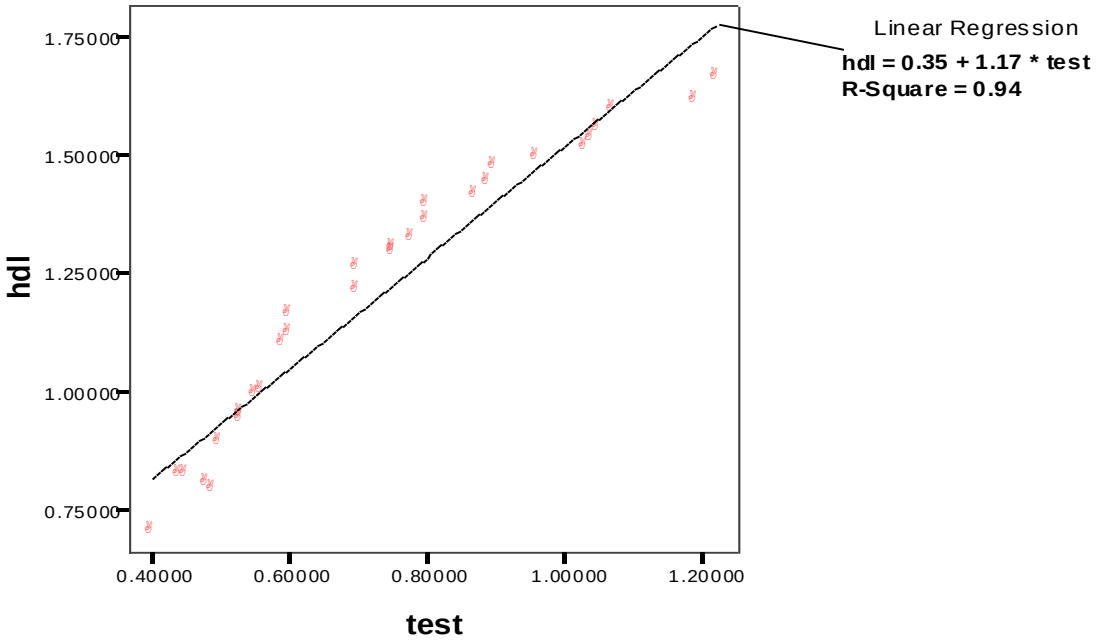


Fig (3-18): Correlation between testosterone and HDL-C in G4 (r=0.97 ,p<0.01)

3.6.4. Correlation between serum VLDL-C and testosterone

A significant positive correlations between serum total-cholesterol level was noticed in different groups except normal pregnants in third trimester G4,which reversed correlation as in Fig (3-19),(3-20),(3-21),(3-22).

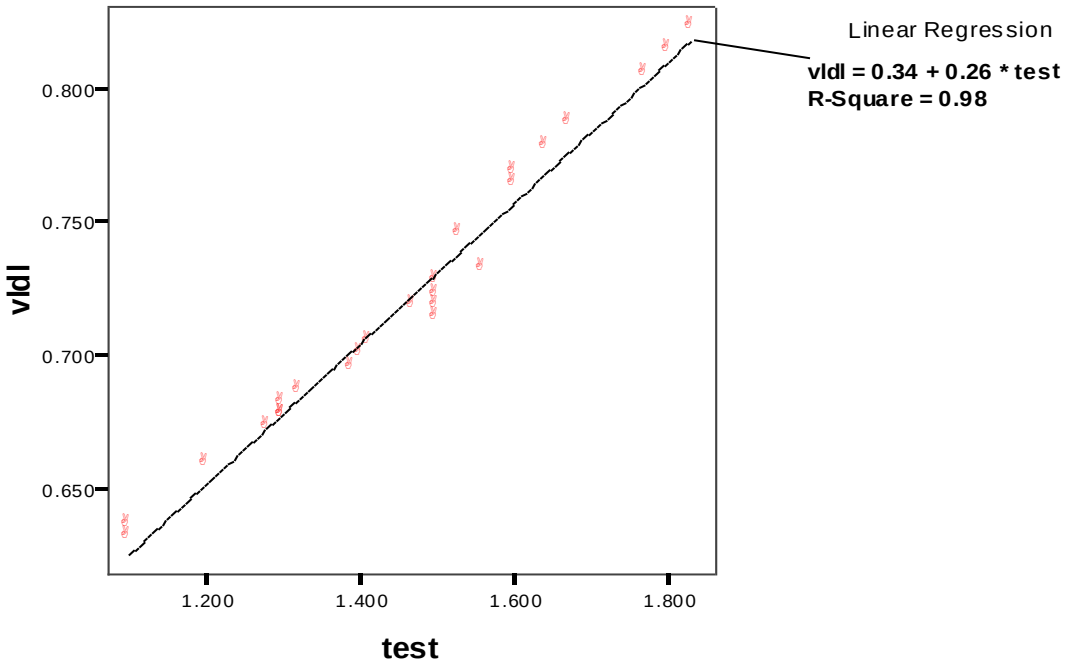


Fig (3-19): Correlation between testosterone and VLDL-C in G1 (r=0.99 ,p<0.01)

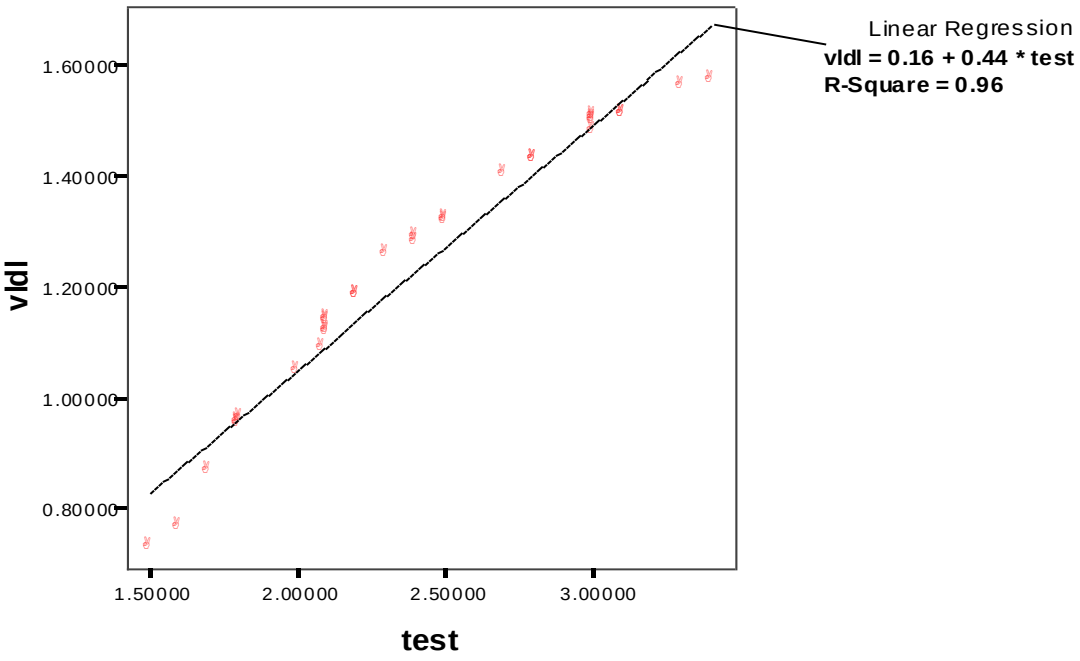


Fig (3-20): Correlation between testosterone and VLDL-C in G2(r=0.98 ,p<0.01)

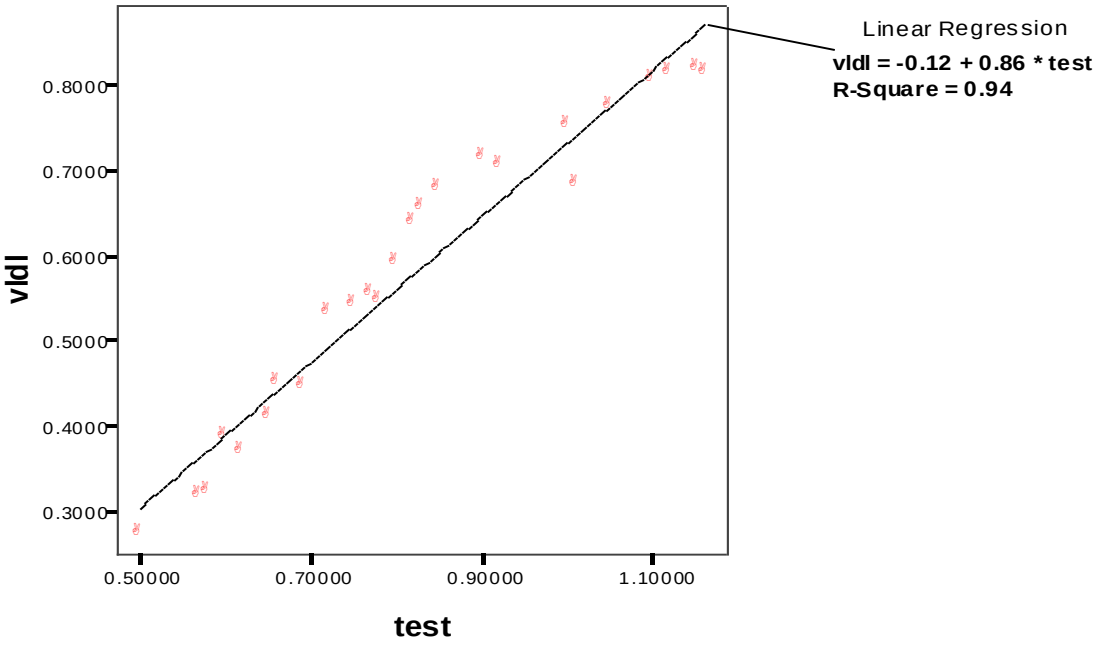


Fig (3-21): Correlation between testosterone and VLDL-C in G3 (r=0.97 ,p<0.01)

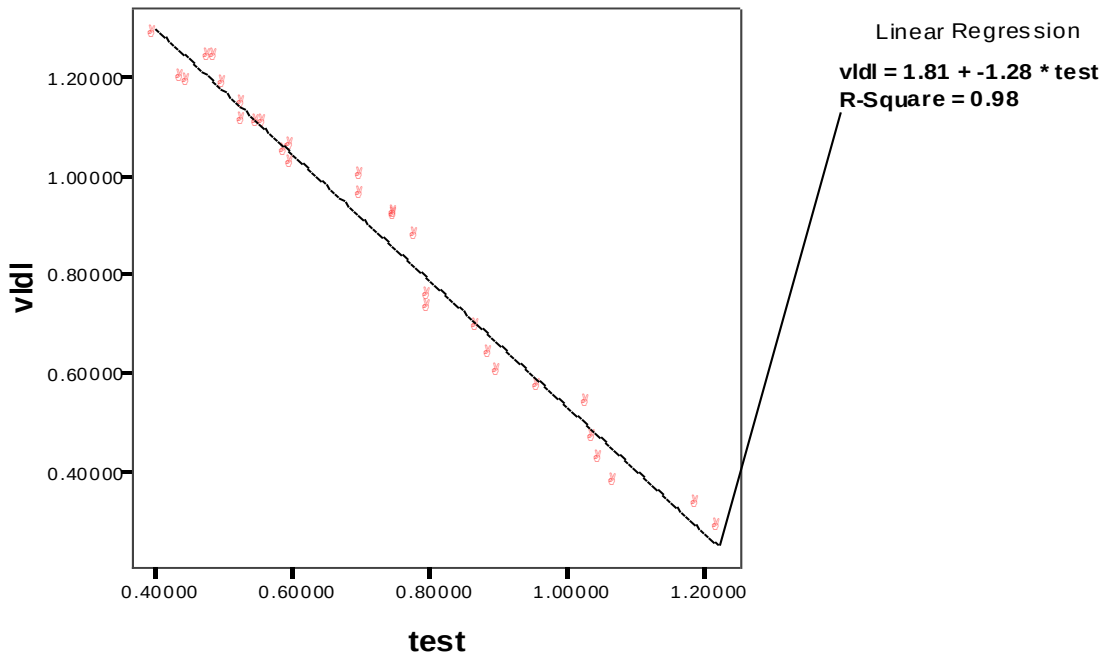


Fig (3-22): Correlation between testosterone and VLDL-C in G4 (r=0.99 ,p<0.01)

3.6.5.Correlation between serum LDL-C and testosterone in different groups:

A significant positive correlations between LDL-C and testosterone in G1,G2,G3 while there were inverse correlation in G4 as in Fig (3-23),(3-24),(3-25),(3-26).

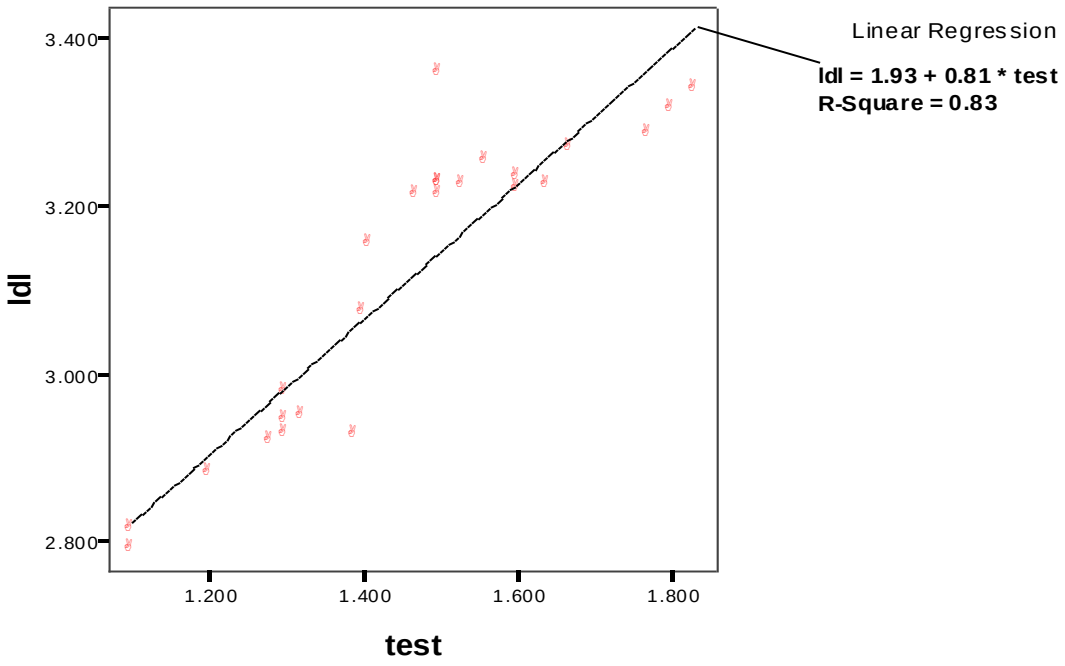


Fig (3-23): Correlation between testosterone and ldl-C in G1 (r=0.91 ,p<0.01)

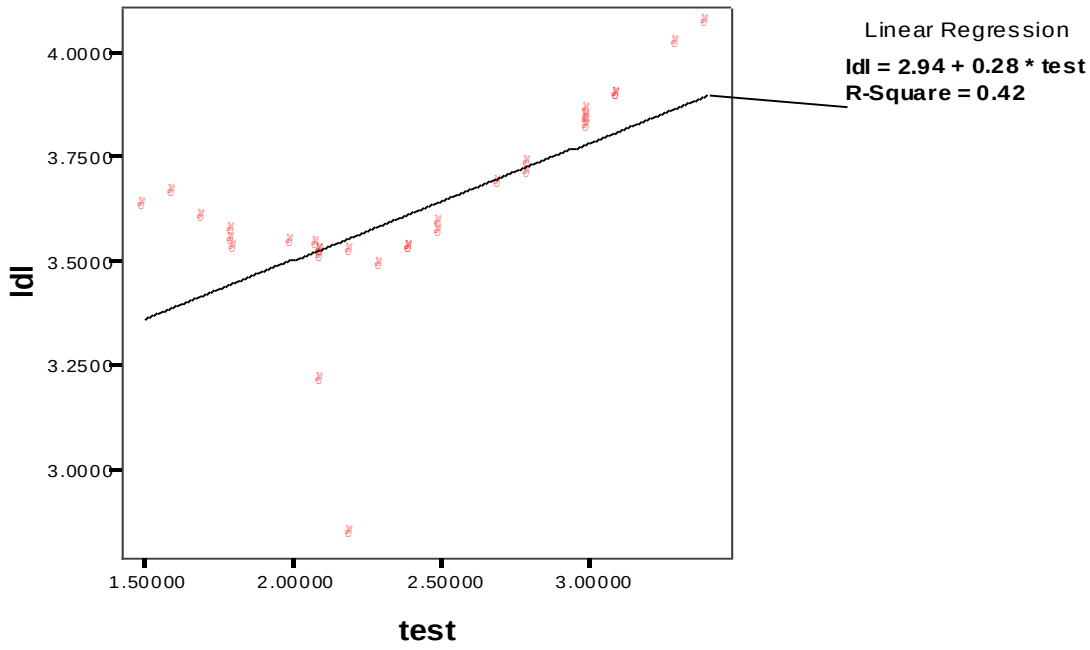


Fig (3-24): Correlation between testosterone and ldl-C in G2 (r=0.64 ,p<0.01)

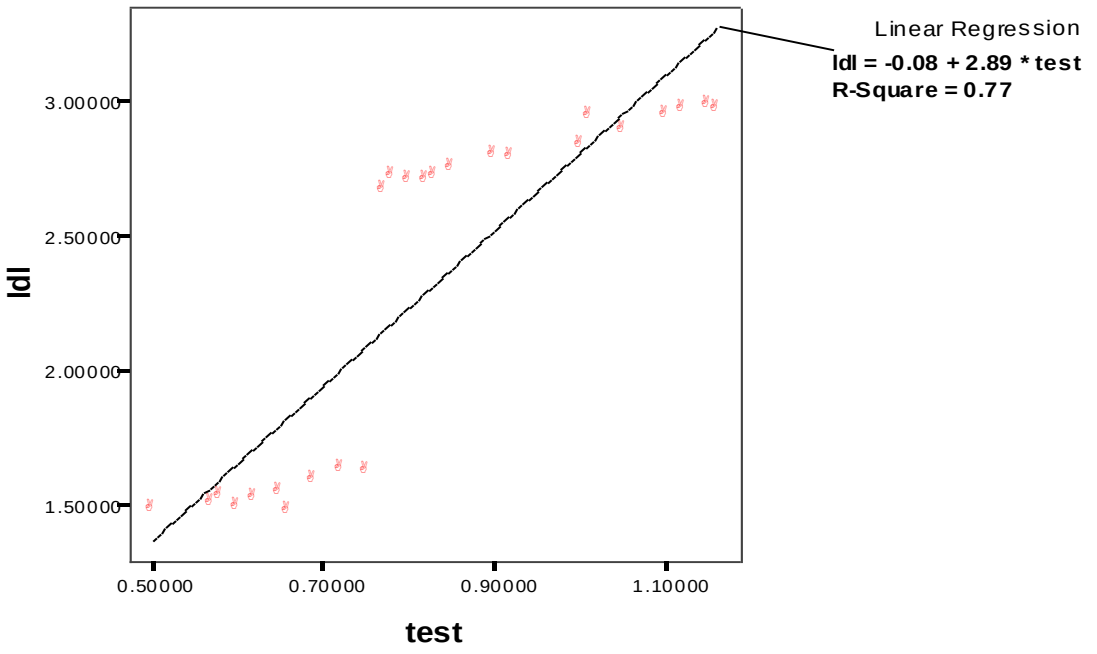


Fig (3-25): Correlation between testosterone and ldl-C in G3 (r=0.87 ,p<0.01)

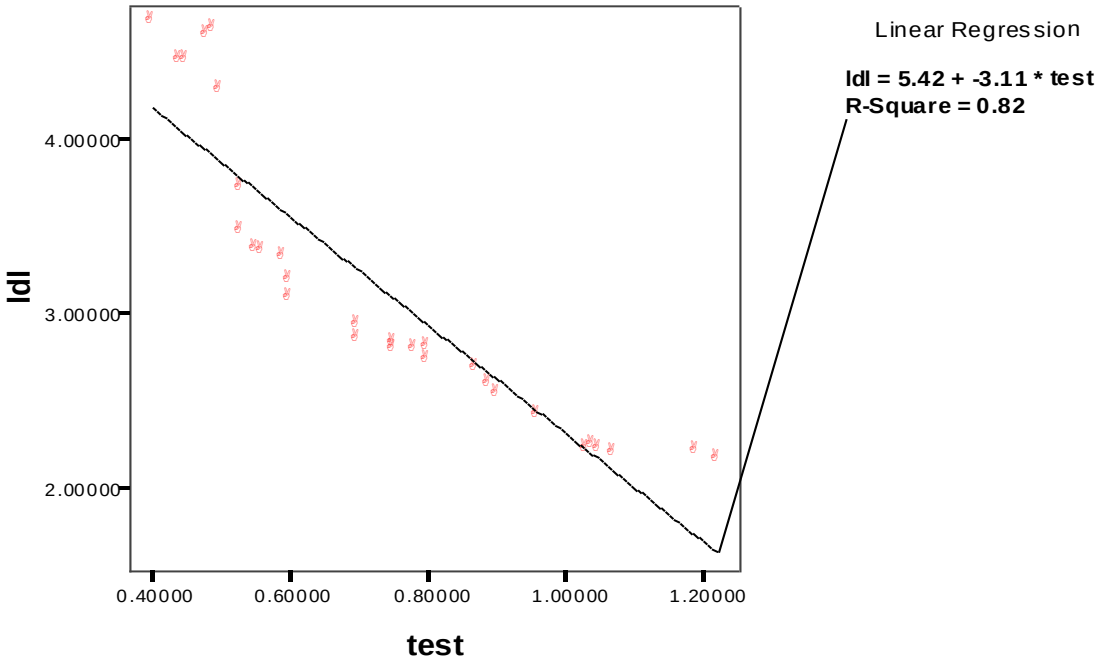


Fig (3-26):Correlation between testosterone and ldl-C in G4(r=0.9 ,p<0.01)

3.6.6.Correlation between serum total protein , albumin and testosterone in different groups:

There was **a** significant negative correlation**s** between total protein, albumin and testosterone in G1,G2,G3 while there were inverse correlation in G4 as in Fig(3-27),(3-28),(3-29),(3-30),(3-31),(3-32),(3-33),(3-34).

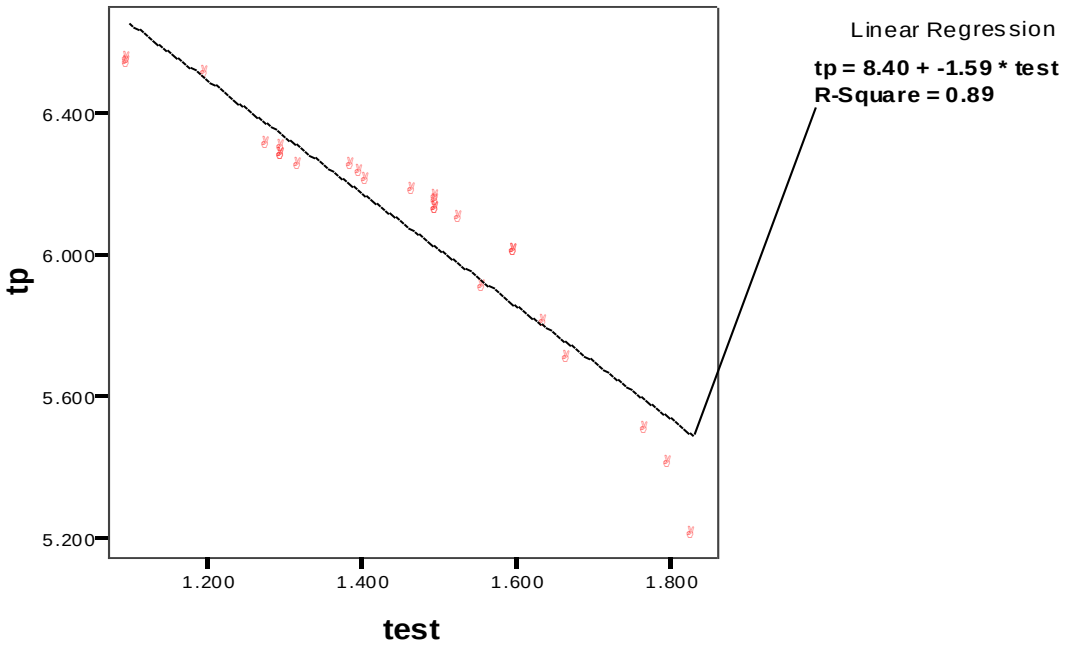


Fig (3-27):Correlation between testosterone and total protein in G1(r=0.94 ,p<0.01)

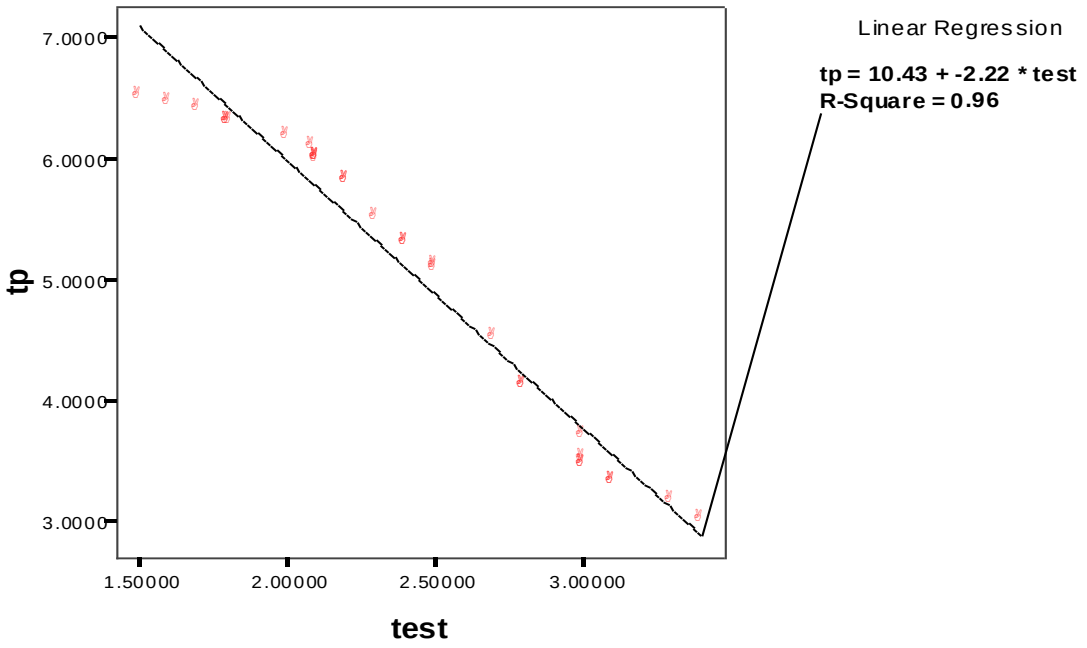


Fig (3-28):Correlation between testosterone and total protein in G2 ($p<0.001, r=0.98$)

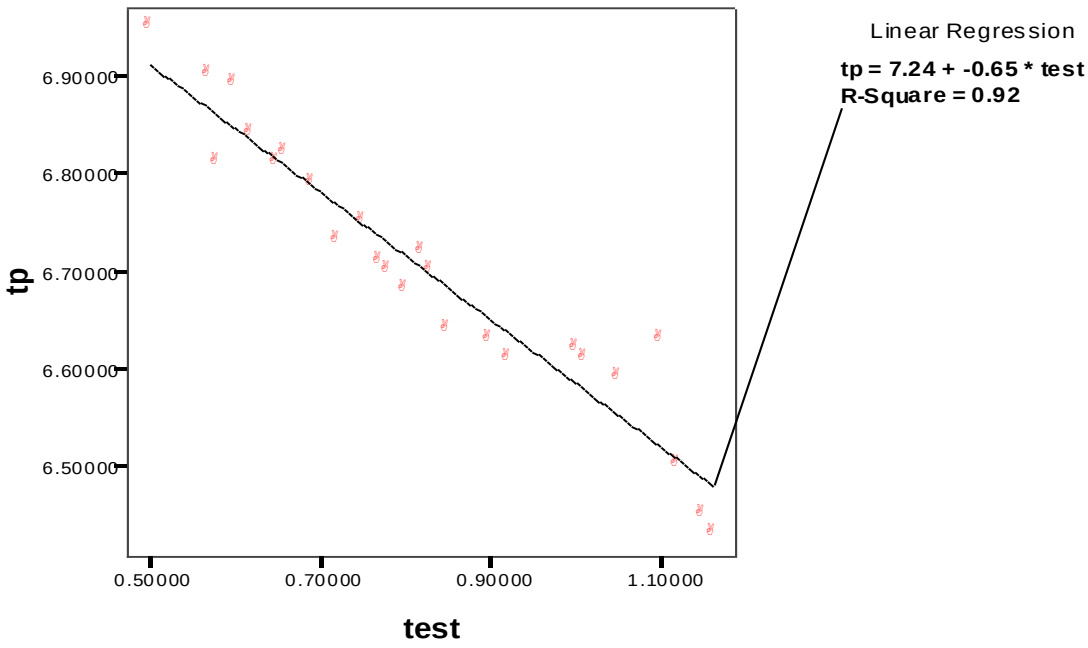


Fig (3-29): Correlation between testosterone and total protein in G3 ($r=0.96$,$p<0.01$)

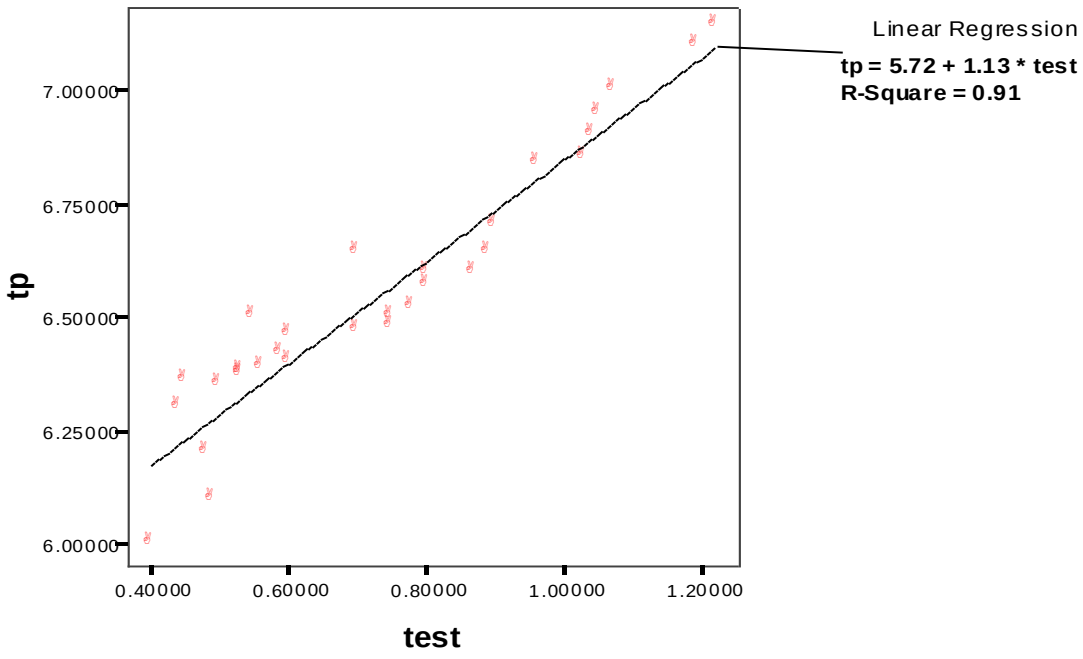


Fig (3-30): Correlation between testosterone and total protein in G4 (r=0.95 ,p<0.01)

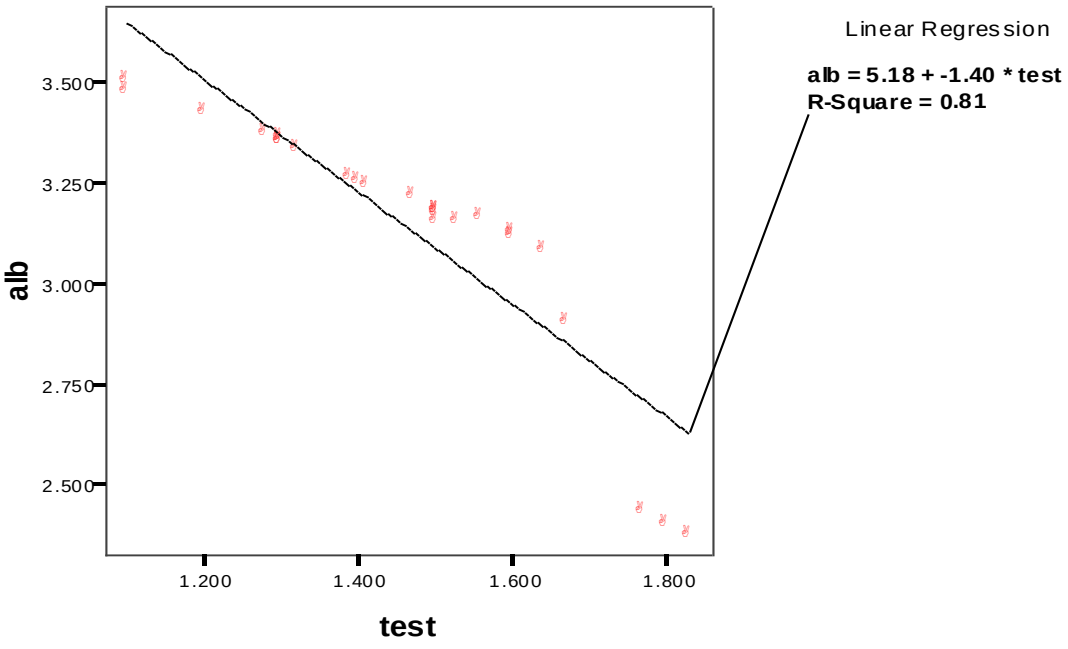


Fig (3-31): Correlation between testosterone and albumin in G1 (r=0.9 ,p<0.01)

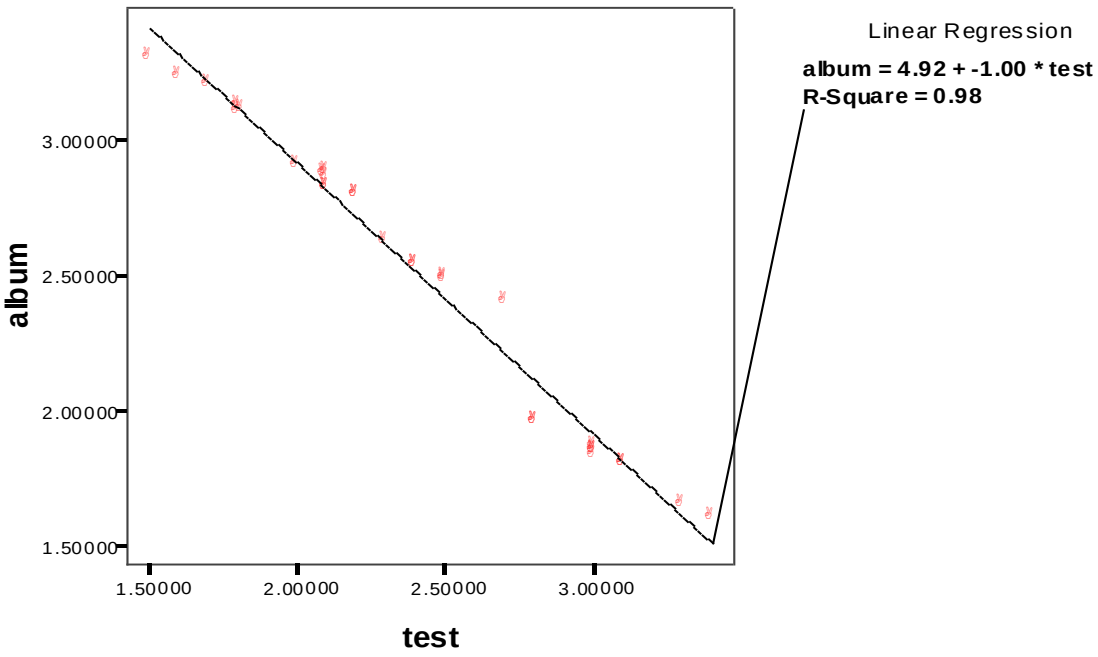


Fig (3-32): Correlation between testosterone and albumin in G2(r= 0.99, p<0.01)

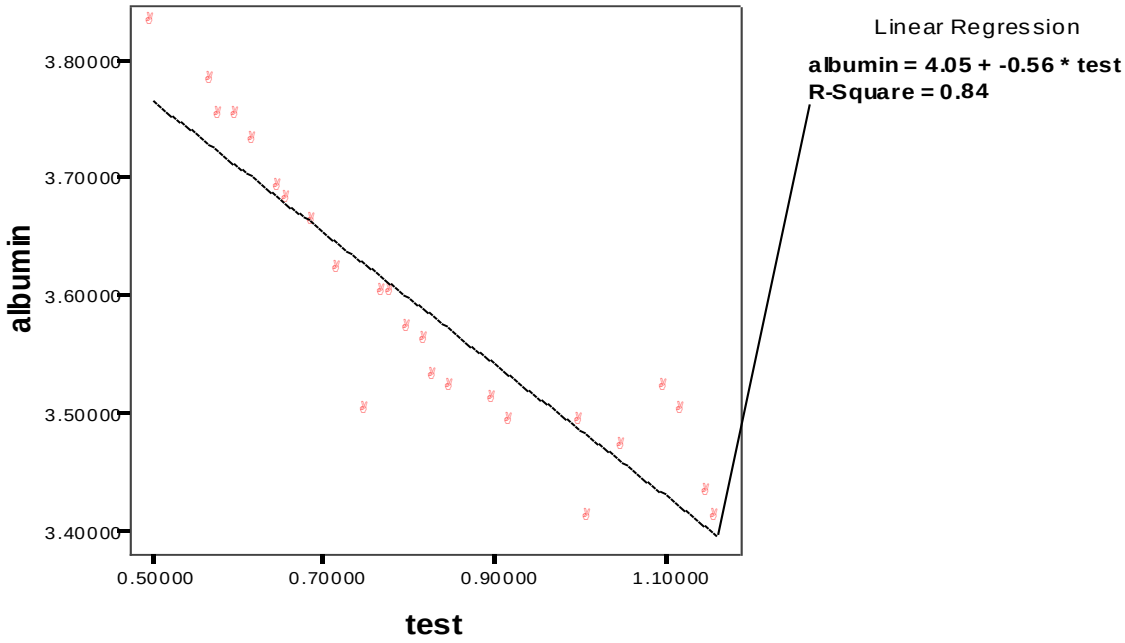


Fig (3-33): Correlation between testosterone and albumin in G3 (r=0.91 ,p<0.01)

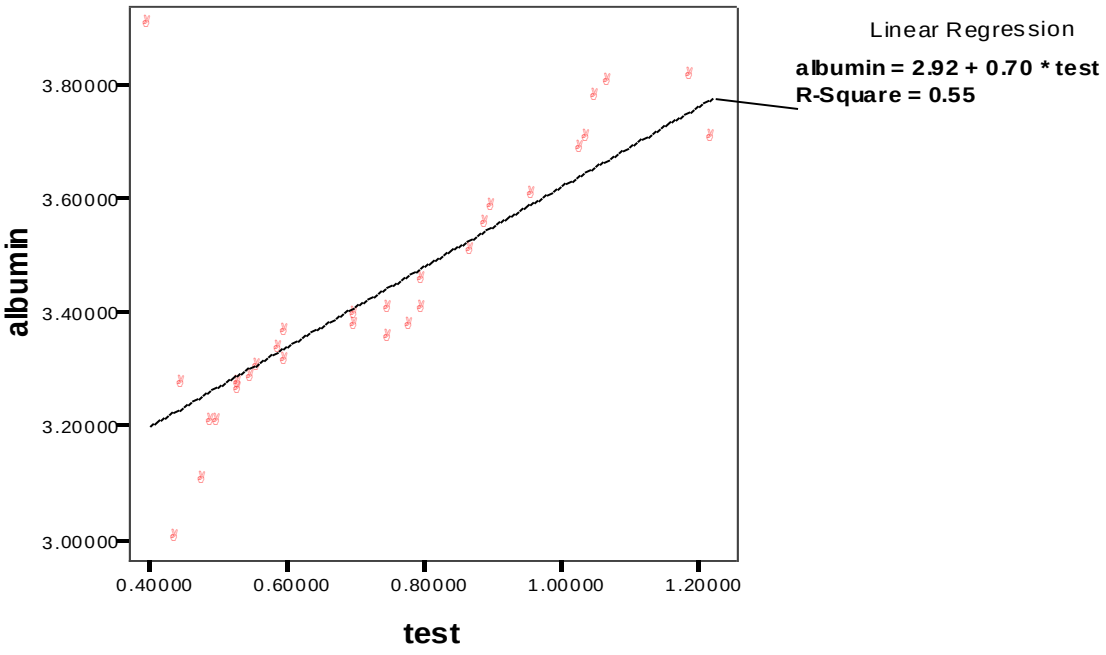


Fig (3-34): Correlation between testosterone and albumin in G4 (r=0.74 ,p<0.01)

3.6.7. Correlation between serum globulin and testosterone in different groups:

Significant negative correlations between serum globulin and testosterone levels were noticed in G2 but not significant in G1, G3 and G4 as in Fig (3-35),(3-36),(3-37),(3-38).

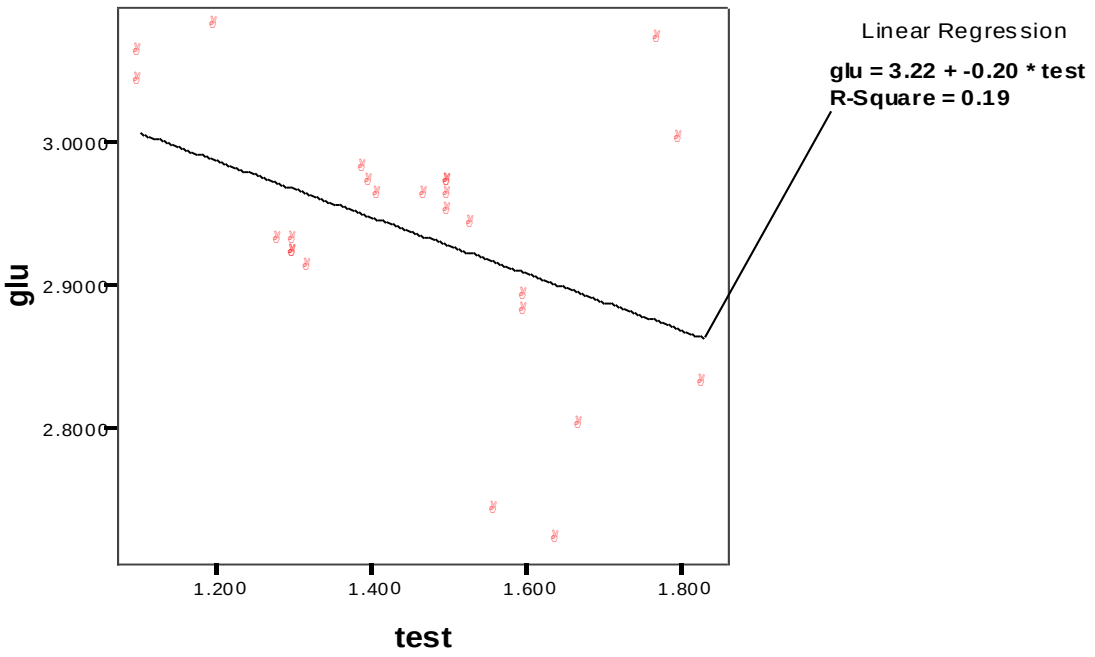


Fig (3-35): Correlation between testosterone and globulin in G1 (r=0.44 ,p>0.05)

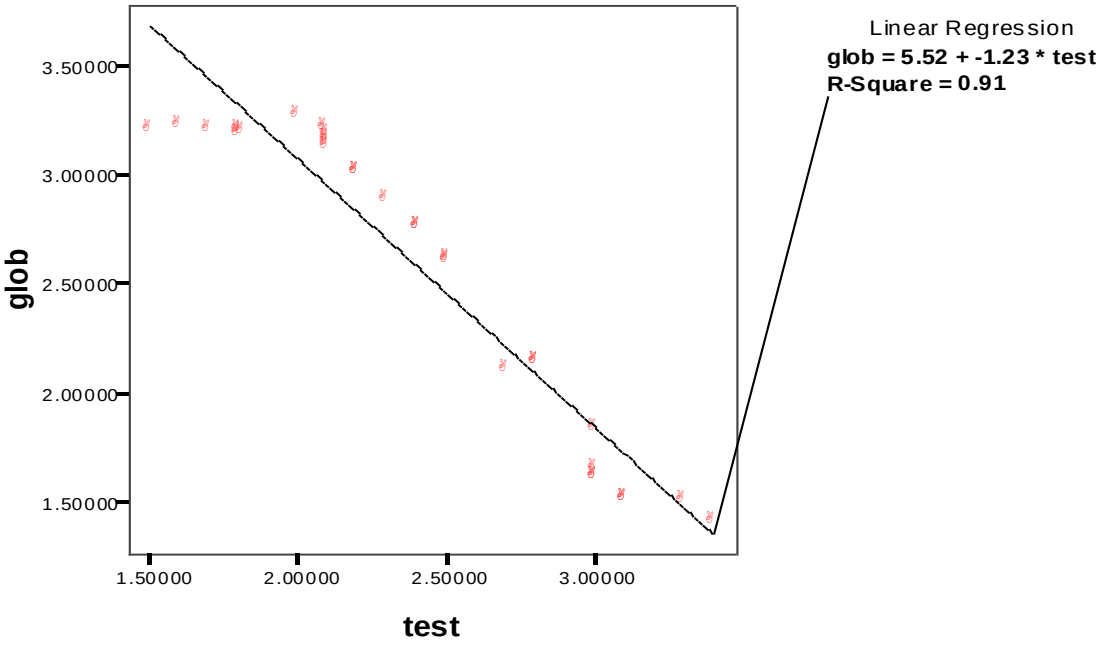


Fig (3-36): Correlation between testosterone and globulin in G2 (r=0.95 ,p<0.01)

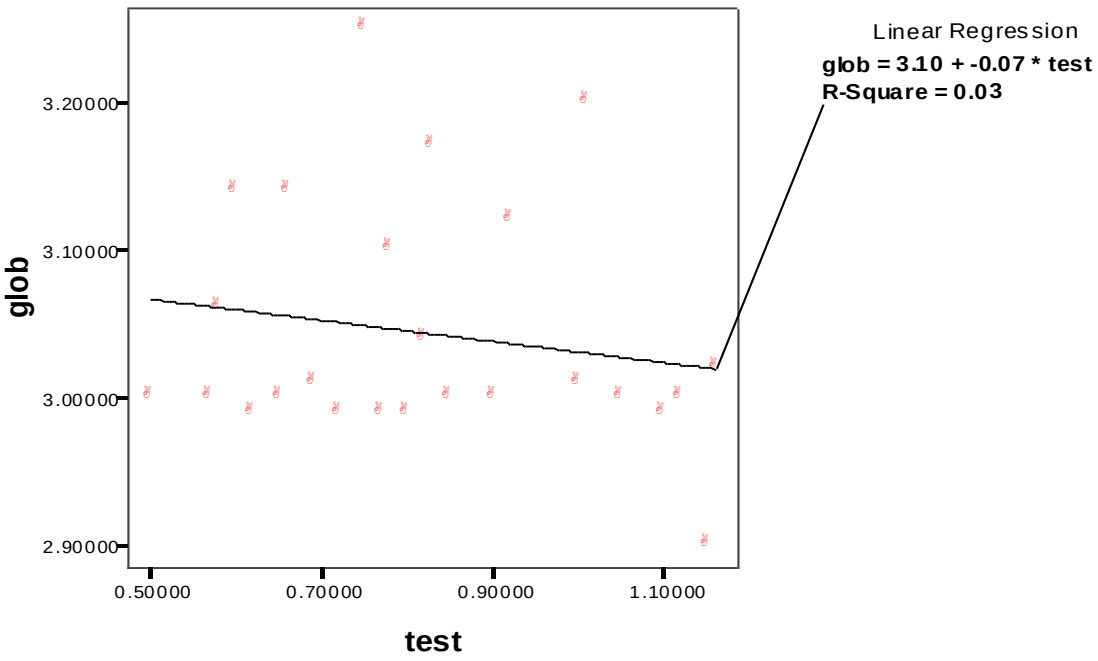


Fig (3-37): Correlation between testosterone and globulin in G3 (r=0.17 ,p>0.05)

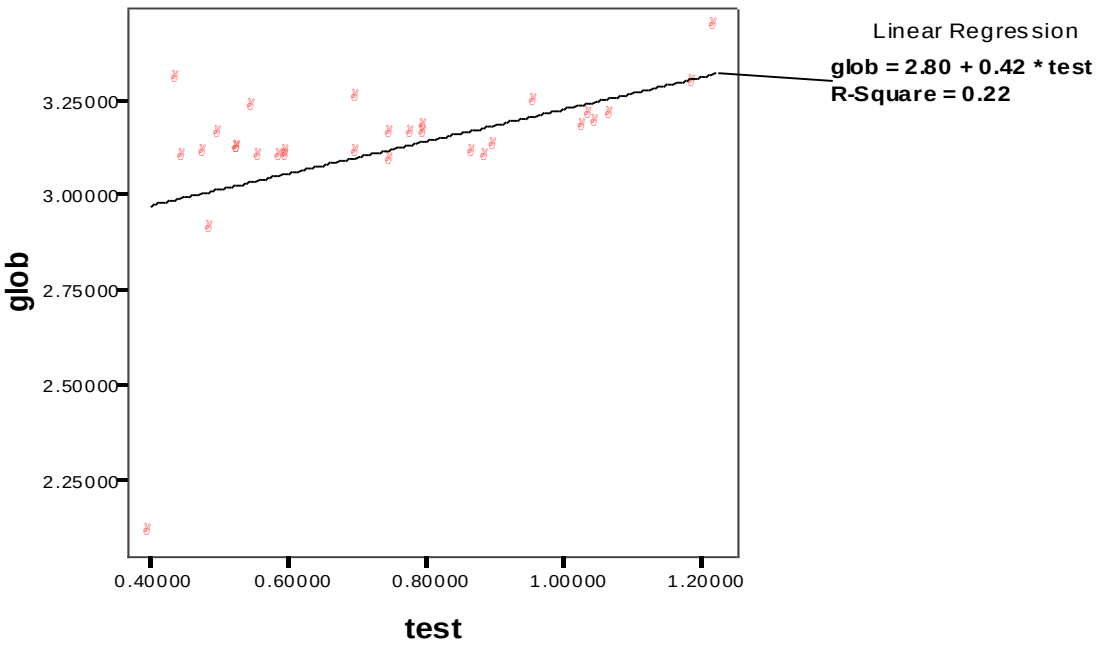


Fig (3-38): Correlation between testosterone and globulin in G4 (r=0.47 ,p>0.008)

3.6.8. Correlation between serum albumin/globulin ratio and testosterone in different groups:

There were a negative correlations between serum albumin/globulin ratio and testosterone levels was noticed in G3 but nonsignificant in G1. There was a positive correlation in G2 and no correlation in G4 as in Fig(3-39),(3-40),(3-41),(3-42).

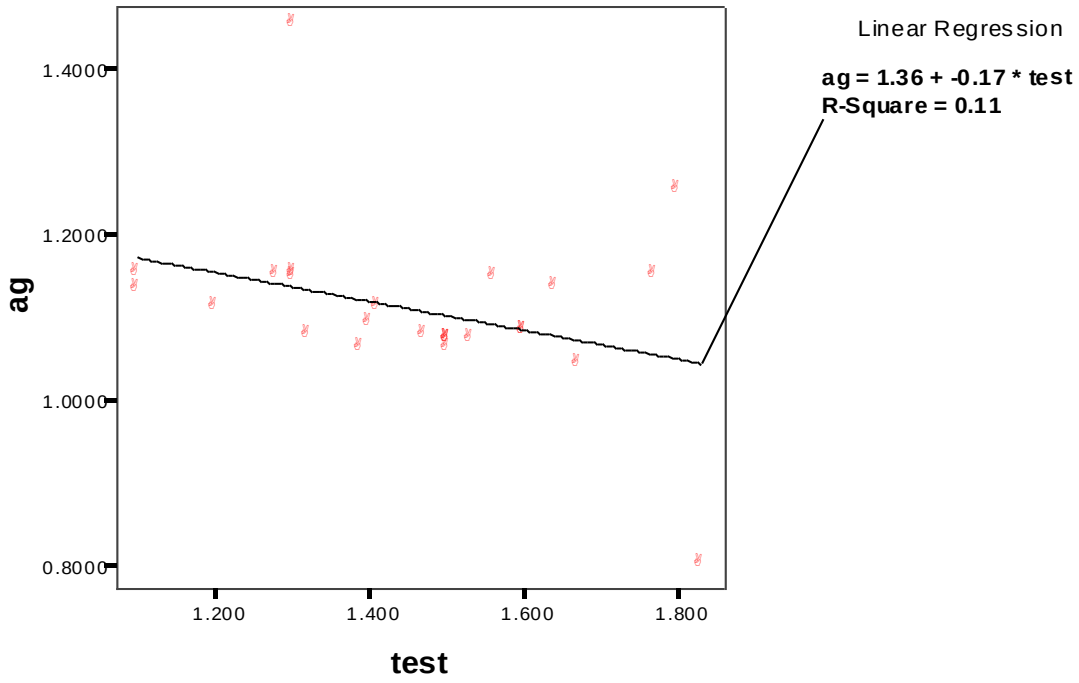


Fig (3-39): Correlation between testosterone and albumin/globulin ratio in G1(r=0.33 ,p>0.05)

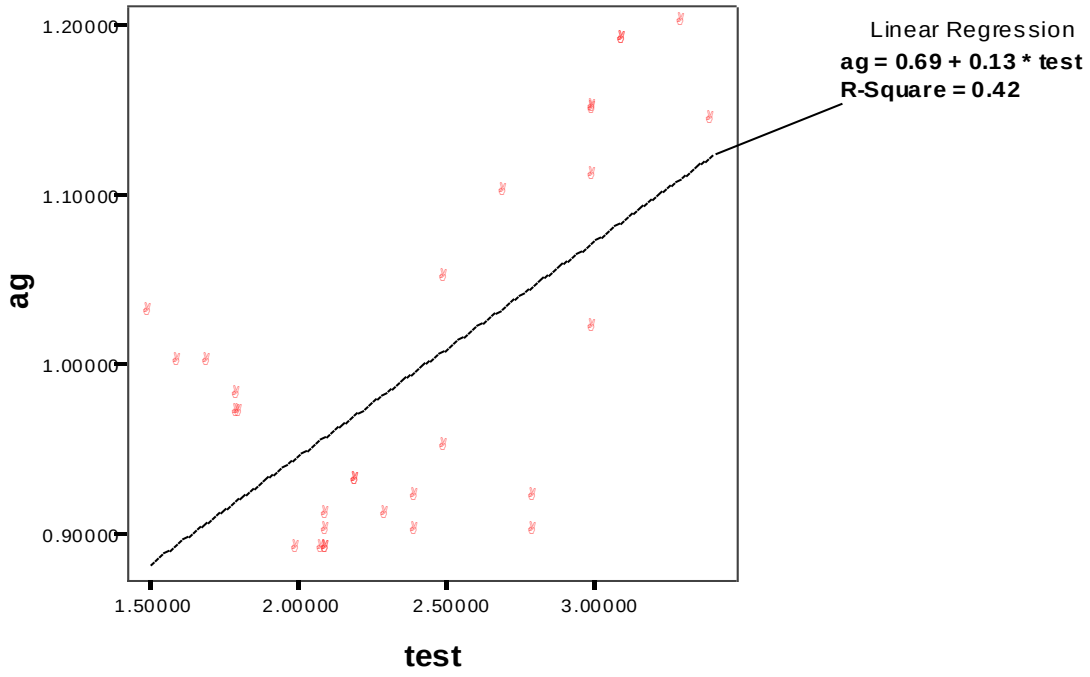


Fig (3-40): Correlation between testosterone and albumin/globulin ratio in G2 (r=0.64 ,p<0.01)

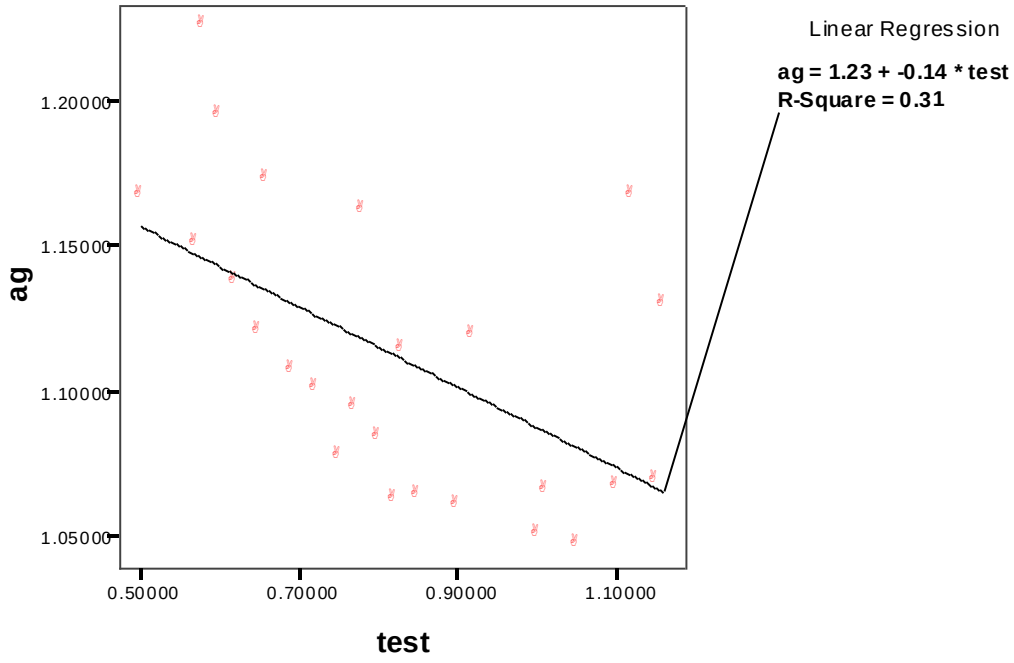


Fig (3-41): Correlation between testosterone and albumin/globulin ratio in G3 (r=0.58 ,p<0.05)

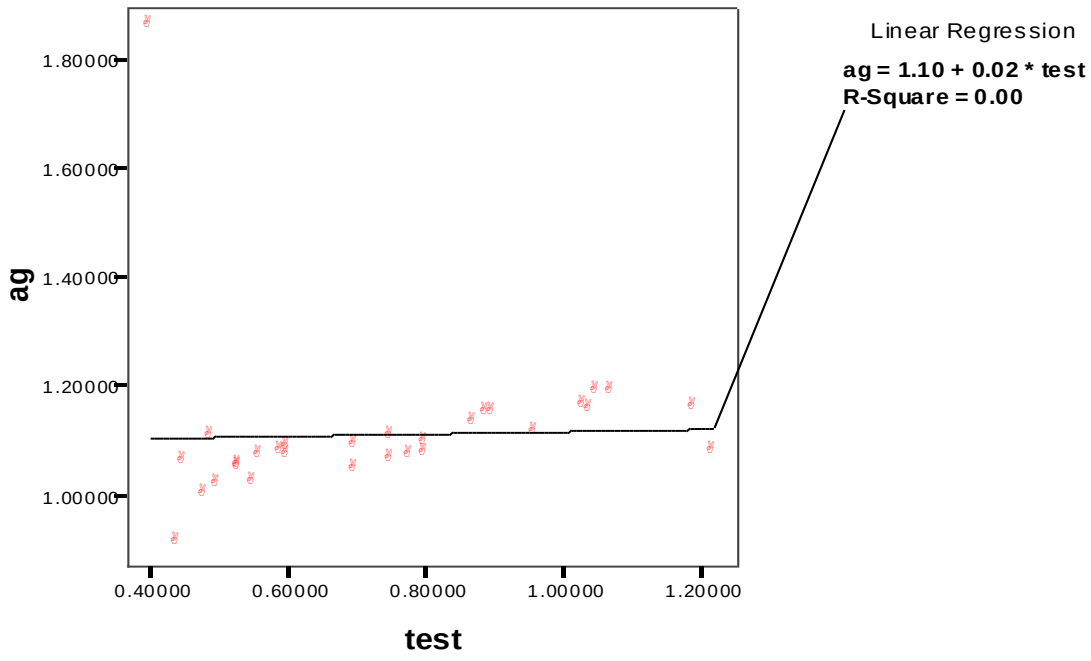


Fig (3-42): Correlation between testosterone and albumin/globulin ratio in G4(r=0 ,p>0.05)

3.6.9. Correlation between serum calcium and testosterone

There were a signifcant negative correlation between serum calcium and testosterone in different groups, but there was inverse correlation in G4 as in Fig (3-43),(3-44),(3-45),(3-46),

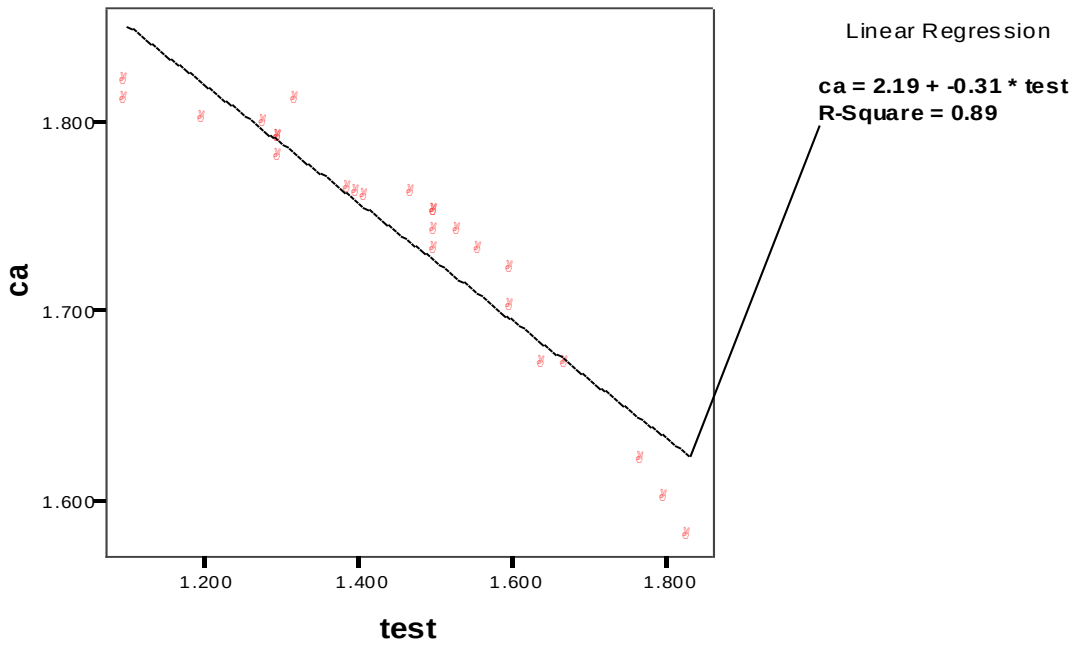


Fig (3-43): Correlation between testosterone and calcium in G1 (r=0.94 ,p<0.01)

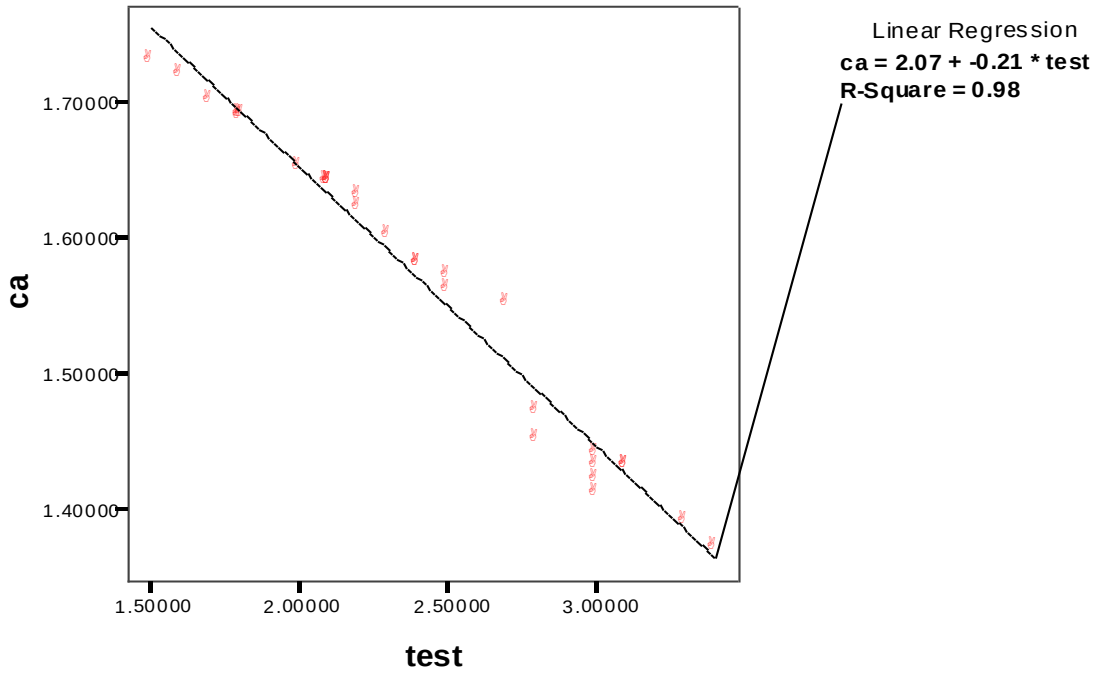


Fig (3-44): Correlation between testosterone and calcium in G2 (r=0.99 ,p<0.01)

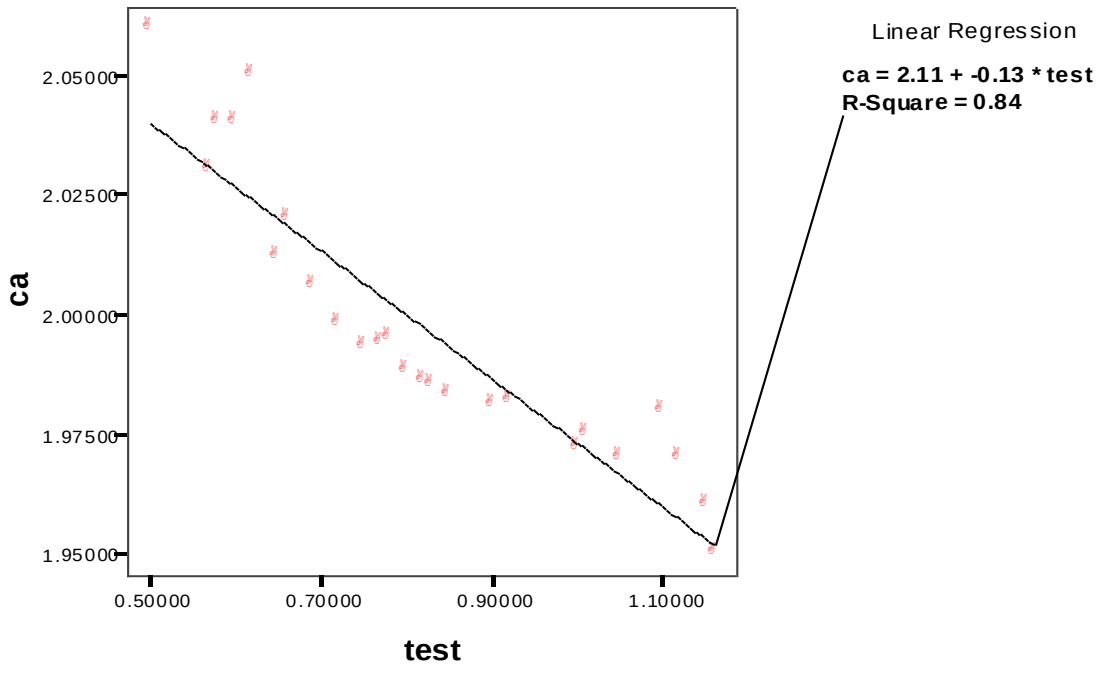


Fig (3-45): Correlation between testosterone and calcium in G3 (r=0.92 ,p<0.01)

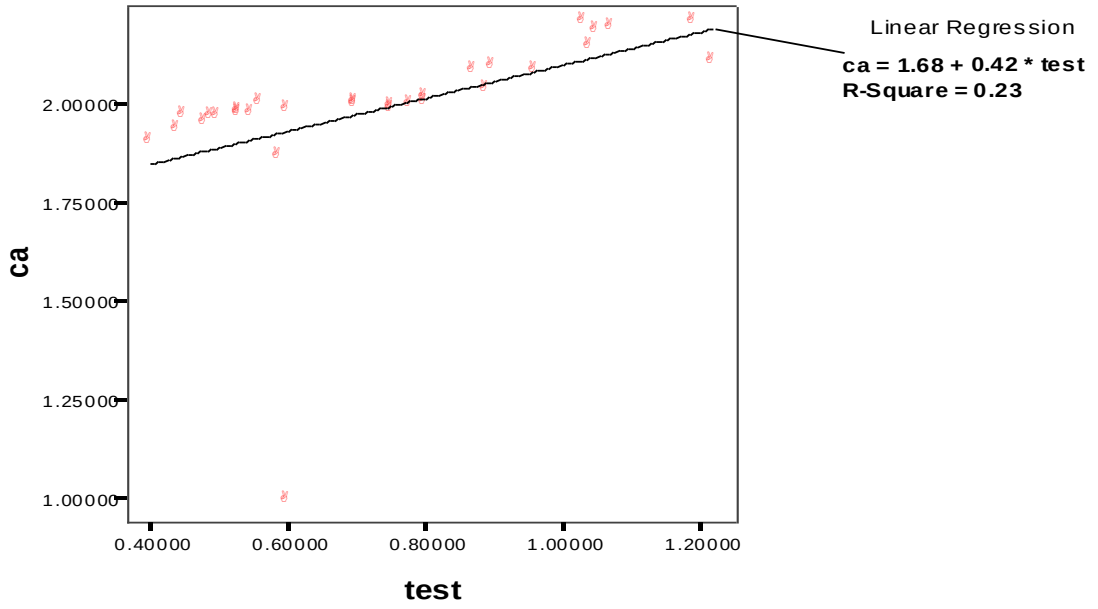


Fig (3-46): Correlation between testosterone and calcium in G4 (r=0.48 ,p<0.01)

3.6.10. Correlation between serum corrected calcium and testosterone

There were negative correlations between serum corrected calcium and testosterone in G3 ,but there was not correlation in G1, G2 and G4 as in Fig (3-47),(3-48),(3-49),(3-50),

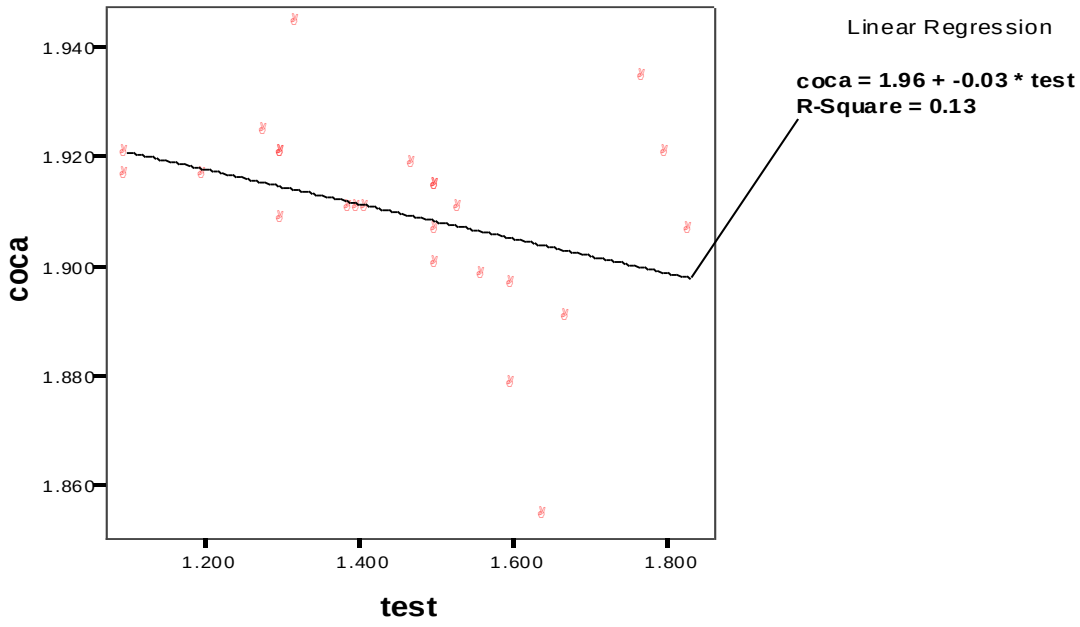
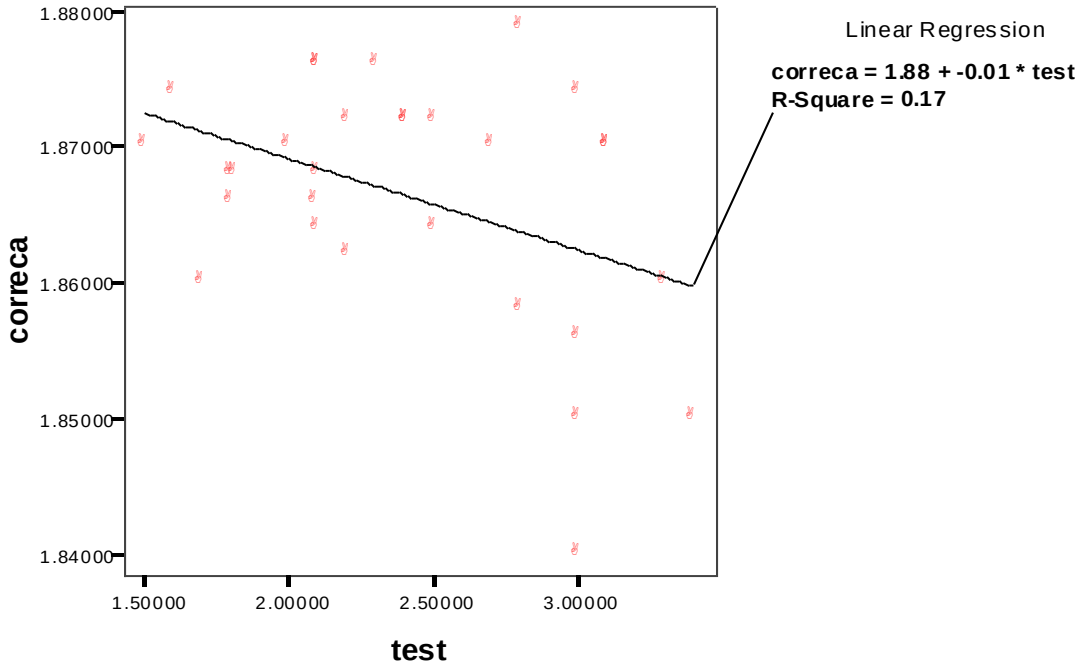


Fig (3-47): Correlation between testosterone and corrected calcium in G1 (r=0.36 ,p>0.05)



Fig(3-48): Correlation between testosterone and corrected calcium G2(r=0.41,p>0.05)

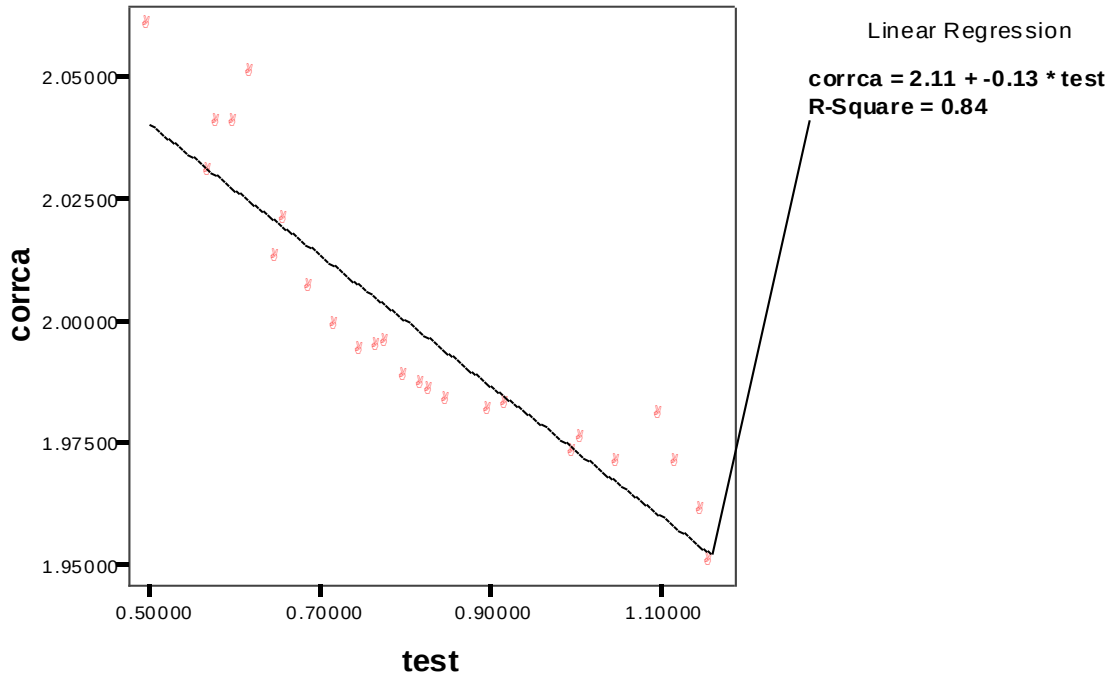


Fig (3-49): Correlation between testosterone and corrected calcium in G3 (r=0.91 ,p<0.01)

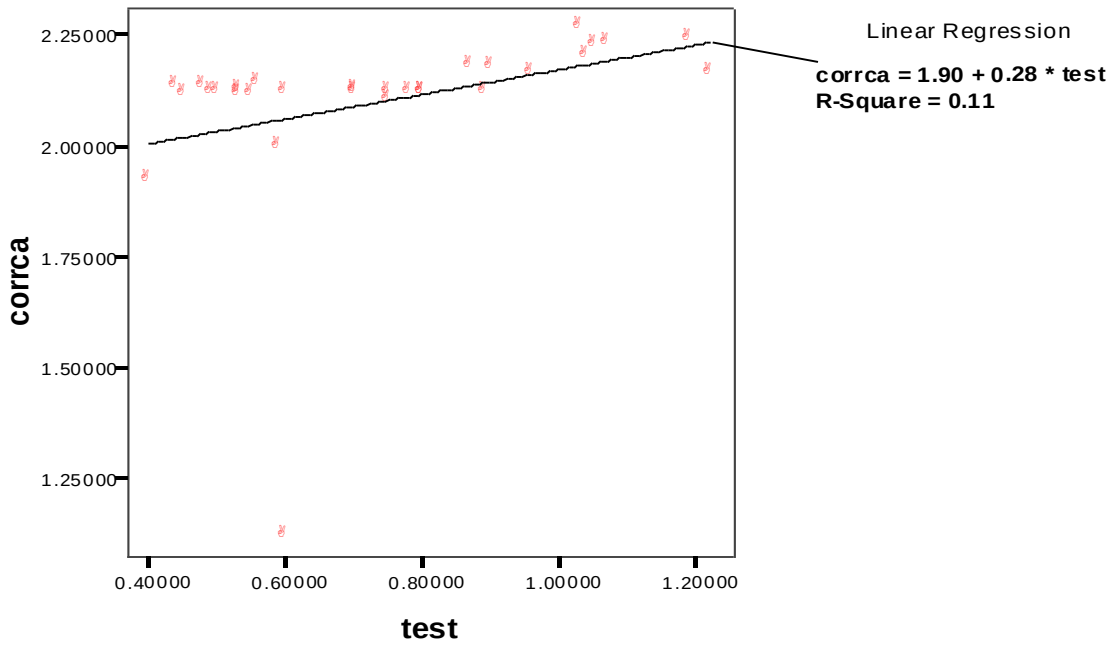


Fig (3-50): Correlation between testosterone and corrected calcium in G4 (r=0.33 ,p>0.05).

3.6.11. Correlation between serum ionized calcium and testosterone in different groups:

There were positive correlations between serum ionized calcium and testosterone in G2 and there were no correlations in G1,G3,G4. as in Fig (3-51),(3-52),(3-53),(3-54).

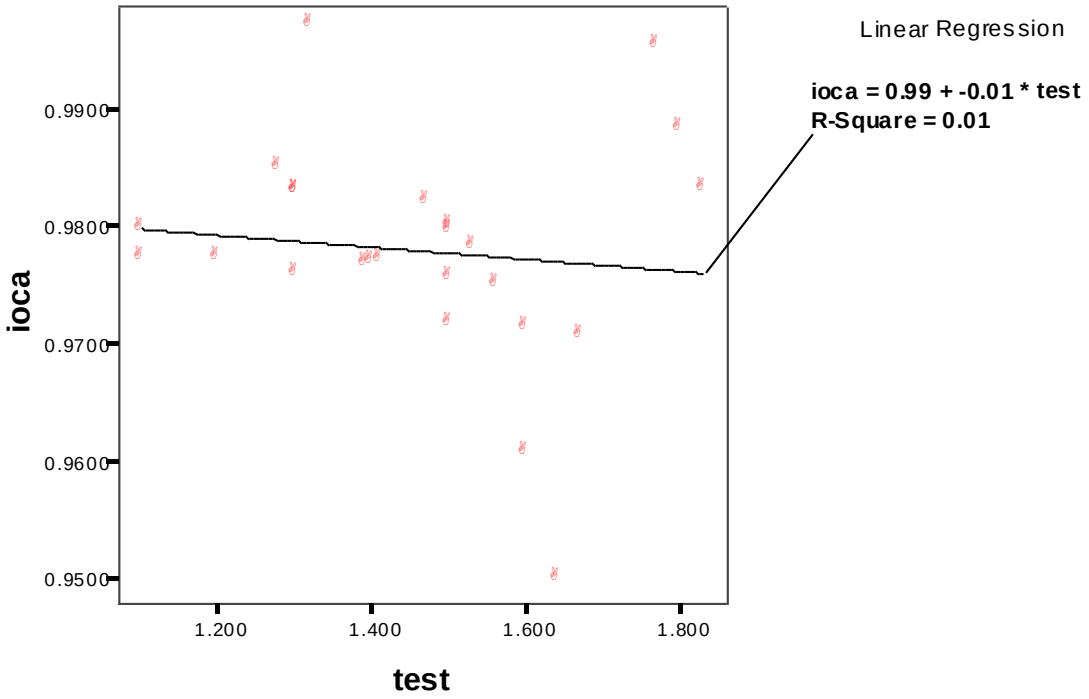


Fig (3-51): Correlation between testosterone and ionized calcium in G1(r=0.1 ,p>0.05)

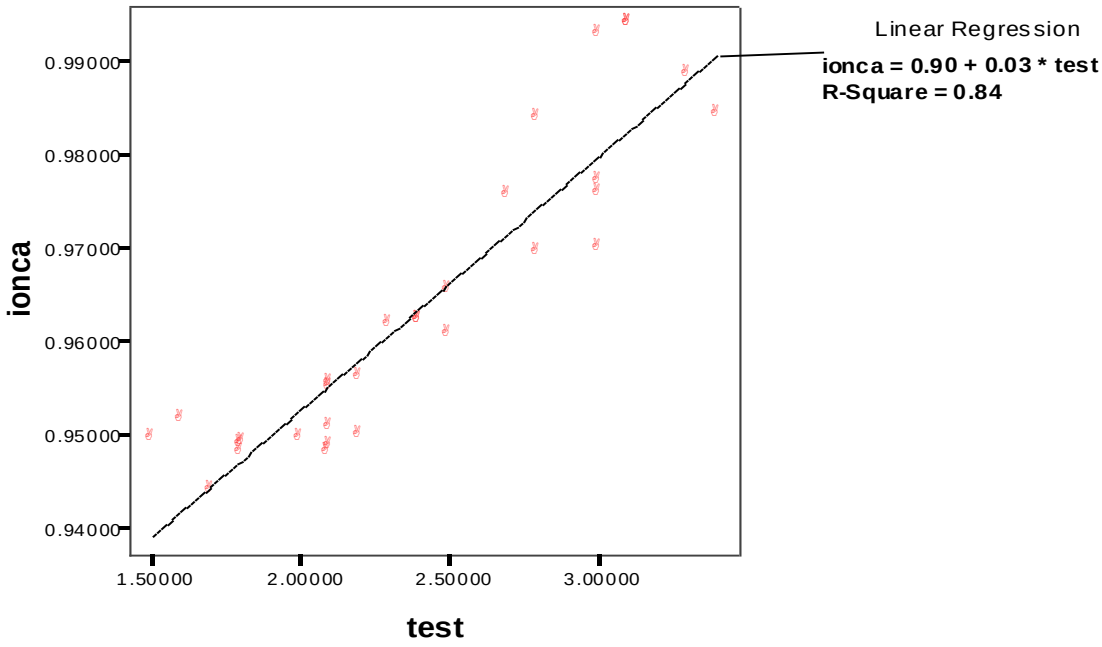


Fig (3-52): Correlation between testosterone and ionized calcium in G2 (r=0.92 ,p<0.01)

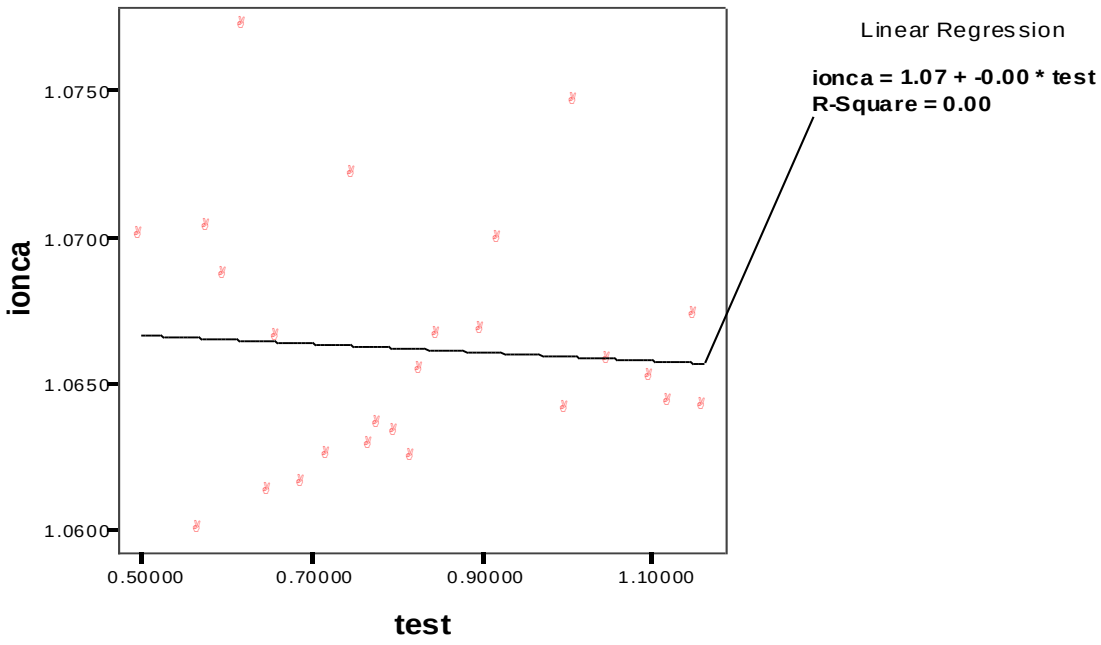


Fig (3-53): Correlation between testosterone and ionized calcium in G3(r=0 ,p>0.05)

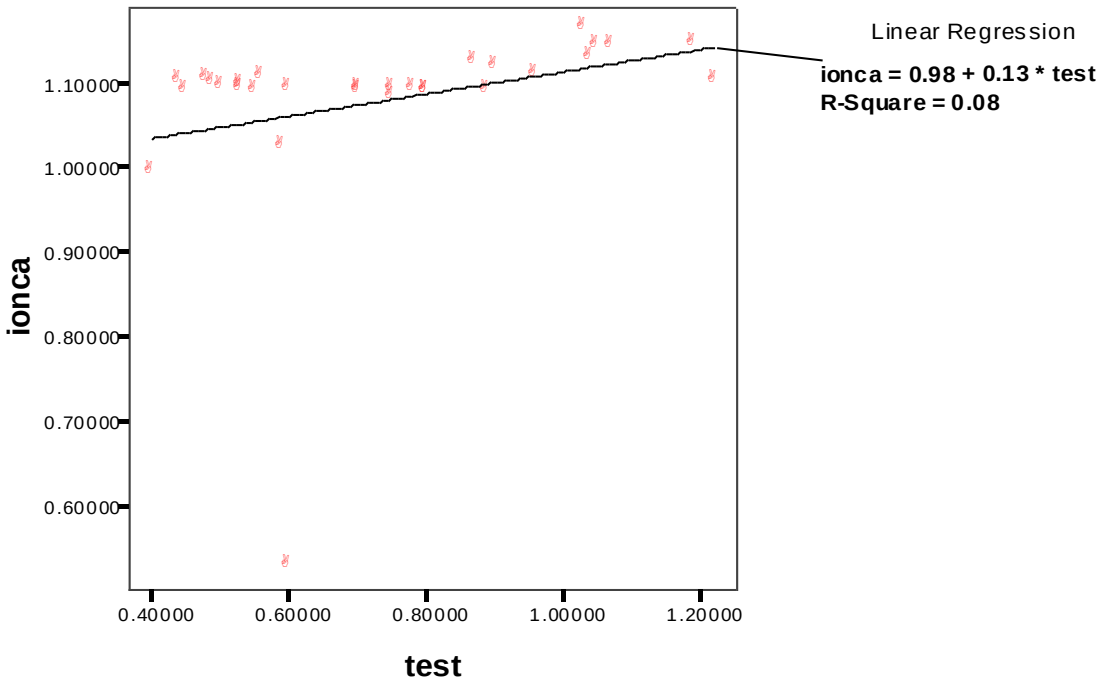


Fig (3-54): Correlation between testosterone and ionized calcium in G4 (r= 0.28,p>0.05)

3.6.12. Correlation between serum total-cholesterol / albumin and testosterone

A significant positive correlations between serum total-cholesterol/ level was noticed in different groups except normal pregnants in third trimester G4 ,there was a negative correlation as in Fig (3-55),(3-56),(3-57),(3-58).

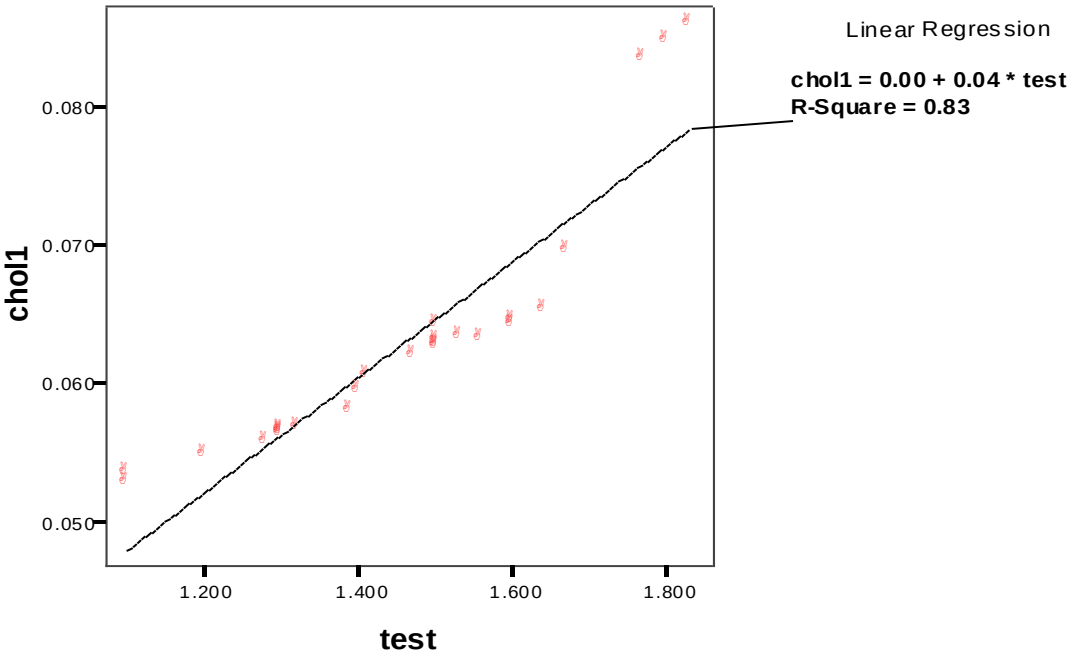


Fig (3-55): Correlation between testosterone and cholesterol / albumin ratio in G1 (r=0.91 ,p<0.01)

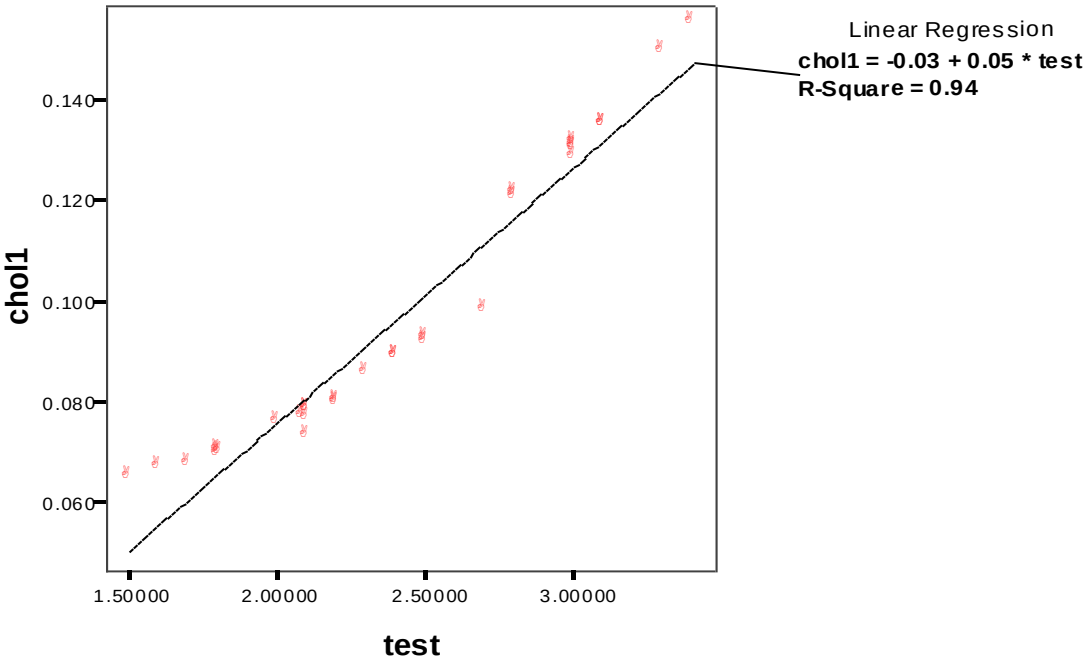


Fig (3-56): Correlation between testosterone and cholesterol / albumin ratio in G2(r=0.97 ,p<0.01)

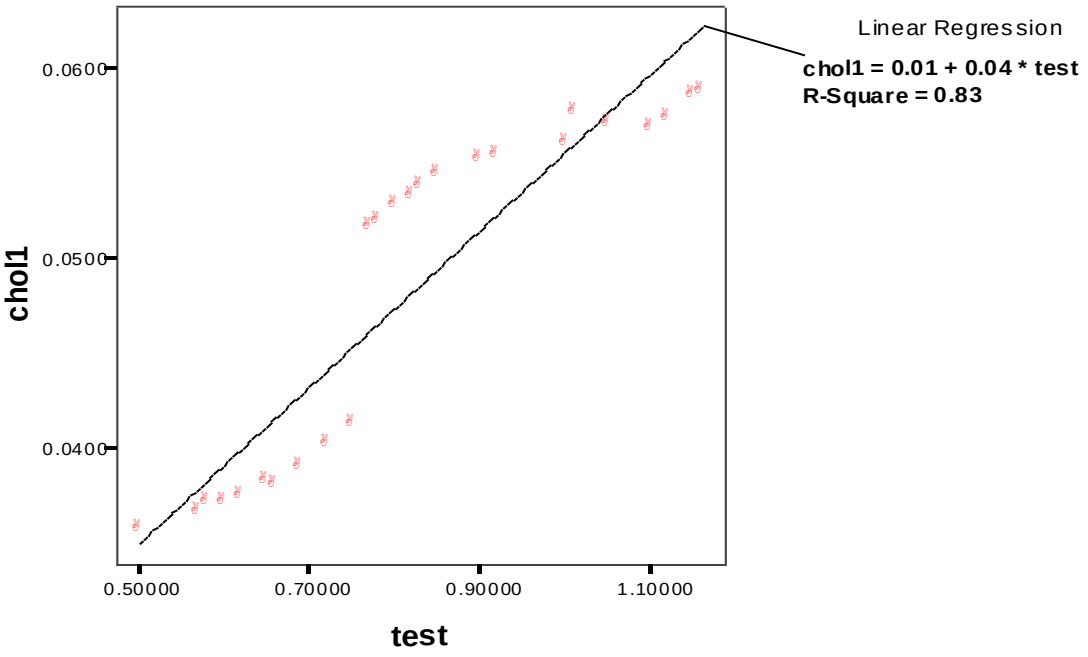


Fig (3-57): Correlation between testosterone and cholesterol / albumin ratio in G3 (r=0.91 ,p<0.01)

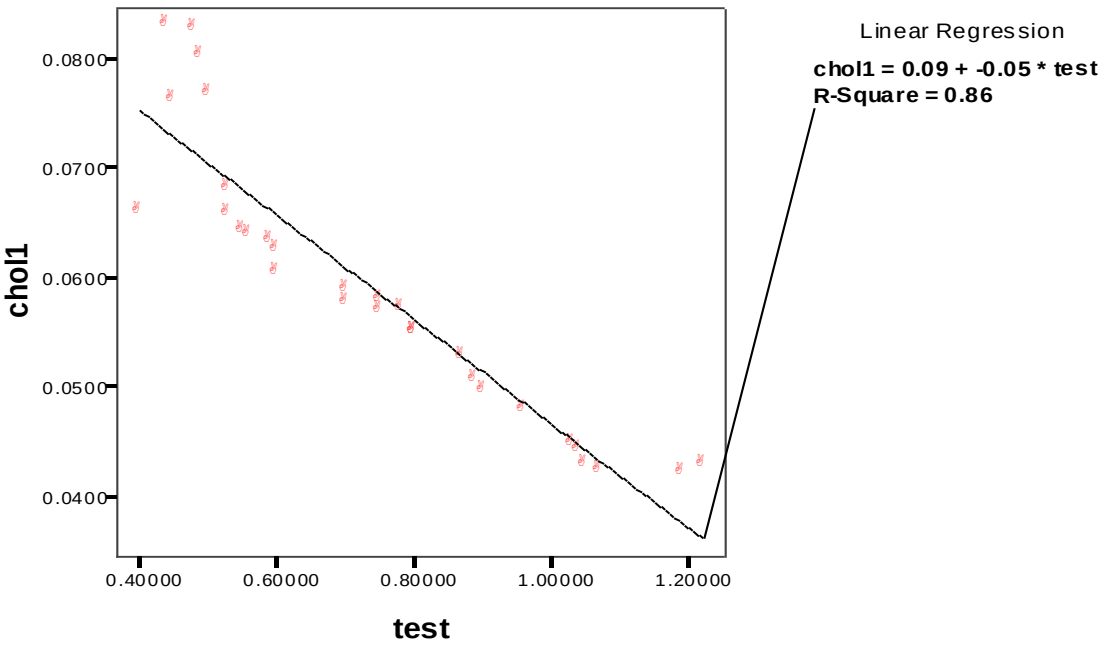


Fig (3-58): Correlation between testosterone and cholesterol / albumin ratio in G4 (r=0.92 ,p<0.01)

3.6.13. Correlation between serum magnesium and testosterone in different groups:

There was a significant negative correlation between magnesium and testosterone in G1,G2,G3 while there were inverse correlations in G4 as in Fig(3-59),(3-60),(3-61),(3-62).

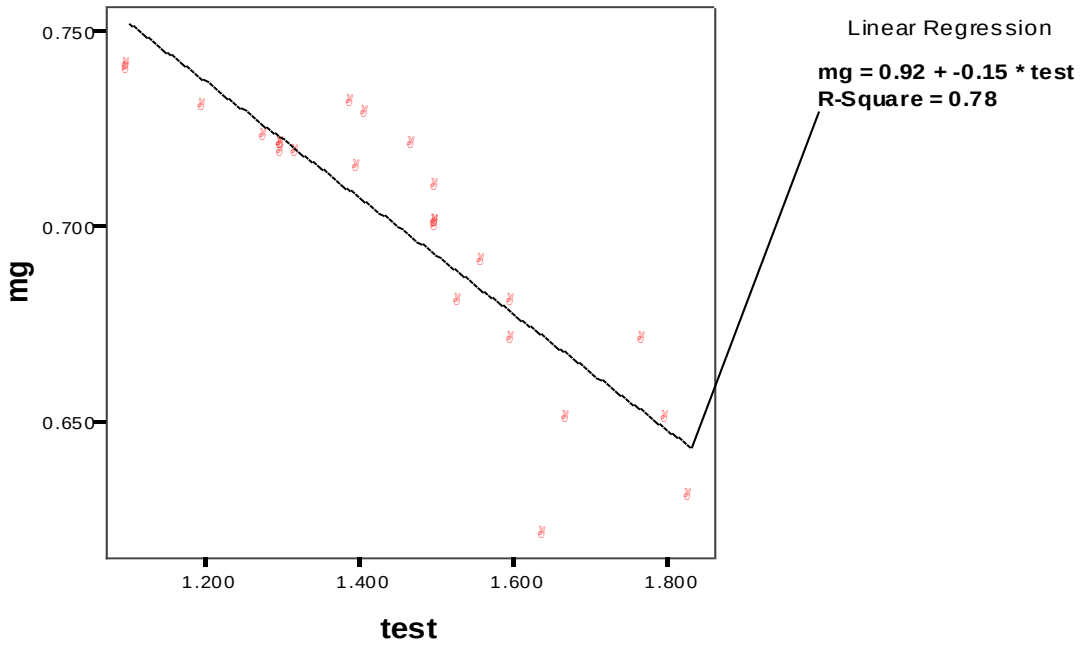


Fig (3-59): Correlation between testosterone and magnesium in G1 (r=0.88 ,p<0.01)

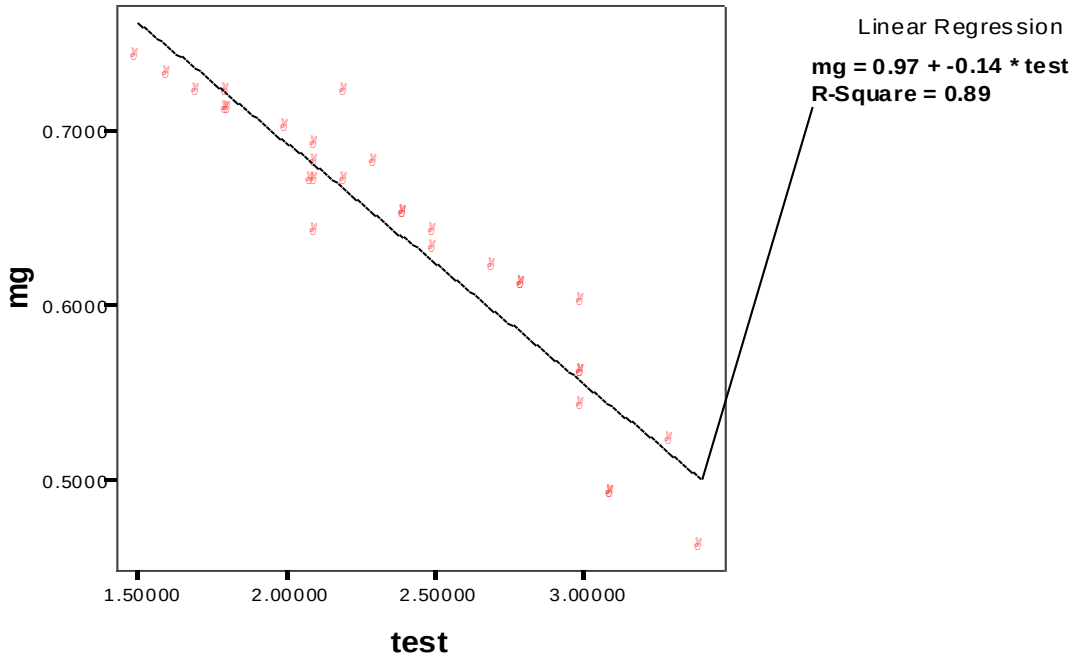


Fig (3-60): Correlation between testosterone and magnesium in G2 (r=0.94 ,p<0.01)

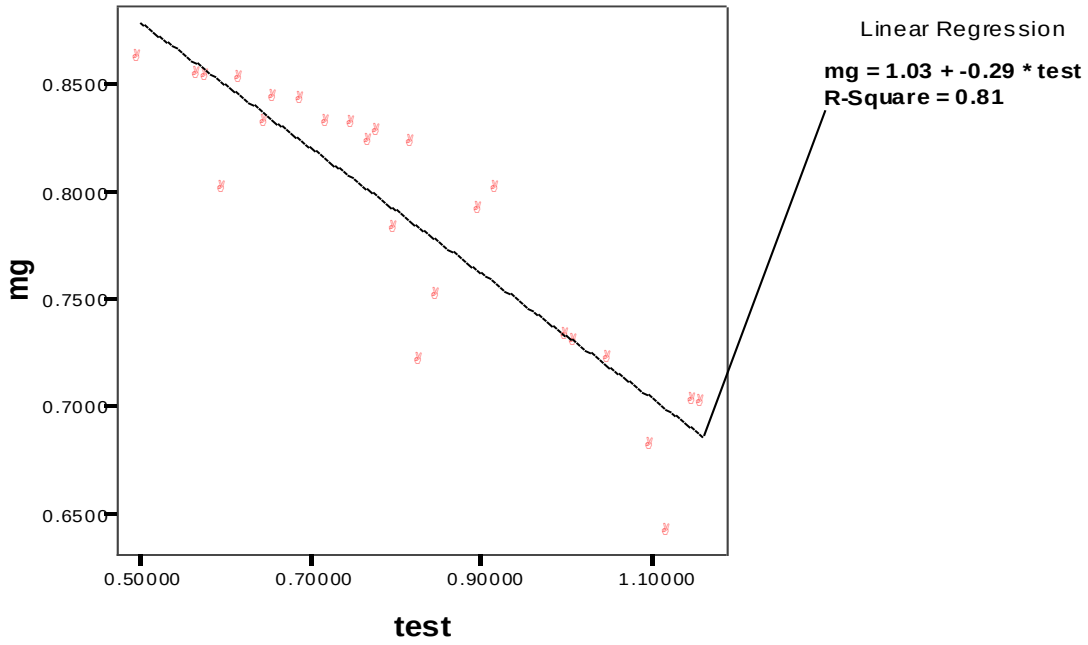


Fig (3-61): Correlation between testosterone and magnesium in G3 (r=0.9 ,p<0.01)

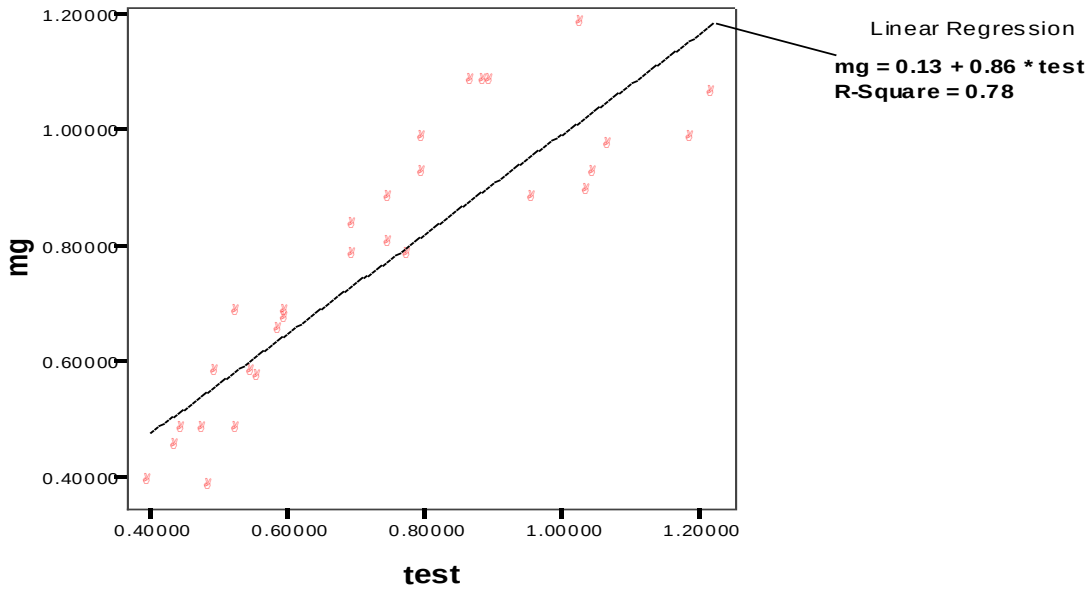


Fig (3-62): Correlation between testosterone and magnesium in G4 (r=0.88 ,p<0.01)

Chapter four

Discussion

Many previous studies reported the changes in oestrogen levels during normal and complicated pregnancy . Besides , there are numerous studies concerning the role of metabolic syndrome in the aetiology of preeclampsia (64,65,66) .

Recent studies revealed the association between the change of serum oestrogen levels and many biochemical parameters during the second and third trimesters of both normal and complicated pregnancy(67). Other studies connected between those biochemical changes and serum changes of hCG levels in different types of pregnancy(68) .

Depending on the available data, there is no study which link the changes in testosterone levels and consequent changes in lipid profile (as one of the criteria associated with metabolic syndrome).

In this study we tried to elucidate such relationship in order to pave the way for subsequent extensive studies concerning further understanding of the nature and mechanism of this disease and to provide a preliminary biochemical foundation for future treatment .

We found in our study that BMI are increase in G1& G2 more than G3&G4 ($P<0.001$) but non significant difference between G1 & G3 ($p>0.05$) and significant difference between G2 & G4 ($p<0.001$).

In this study, we found that ten patients out of fifty five preeclamptic patients give positive family history of

preeclampsia (18.1%) ; five patients had aprevious history of preeclampsia (9%) (both these factors are associated with more incidence of preeclampsia).

In this study , levels of serum testosterone were found to be significantly higher in women with preeclampsia than in normotensive women with similar gestational age . Such increase in hormone level in both 2nd and 3rd trimester can be attributed to :

1- Low expression of the aromatase gene due to small or impaired for the conversion of testosterone to estrogen . The decrease of enzyme activity lead to a subsequent increase in testosterone level (66) .
2- In the late pregnancy , when the fetal adrenal gland become mature it will result in further increment in the level of testosterone by conversion of DHEA to testosterone (66) .
3- Human chorionic gonadotropin increase in PET and this will stimulates the ovarian thecal cell to synthesis androstenedione and testosterone (69) .
4- Insulin stimulate the production of testosterone by ovarian tissue which suggests that hyperinsulinemia could be primary change that triggered the increased release of testosterone (70) . However, hyperinsulinemia should also stimulate the production of adrenal androgen (70) .

5- The decrease in testosterone clearance in normal pregnancy is intensified in PET patients . This will lead to increase in serum testosterone levels(71) .

Our results were in good agreement with the results reported by Golmahamed -Is (72) and Jasim – FG (73) .

The increase in serum testosterone levels in the second trimester of normal pregnancy in comparison with those values of the 3rd trimester can be attributed to the increase of aromatase activity with progressive course of pregnancy (66) .

A significant increase of the serum TC , TG and VLDL-C Levels in preeclamptic women , can be explained in the following points :

I- The endogenous female sex hormone have significant effect on serum lipid (74). Oestrogen is responsible for induction of TG synthesis (75). There is an increase in the hepatic lipase activity and decrease in lipoprotein lipase activity (74). Hepatic lipase is responsible for the increased synthesis of the triacylglycerols at the hepatic level, where the decreased activity of lipoprotein lipase is responsible for the decreased catabolism at the adipose tissue level. The net effect of this enzyme will be an increase in circulating triacylglycerol (74).The second stage of uptake of the remnant of chylomicrons by the liver is delayed so it lead to accumulation of triacylglycerol (74).

2-Serum VLDL increase follows serum TG increase, since the former was calculated from TG values (75). The increase in

triacylglycerol in gestation is estimated mainly in the VLDL, because it is synthesized in the liver and VLDL carries the endogenous triacylglycerol (76) .

The same trend of increase in the levels of those constituents were reported in the studies carried out by Demir-SC(77) and suzies – WJ (78) .

In this study , we found a significant increase in LDL-C and decrease in HDL–C in preeclamptic women (2^{nd} and 3^{rd} trimester) These changes can be attributed to : -

1-Increased triacylglycerols play a major role in decreasing HDL-C HDL particles carry cholesterol from peripheral tissues to the largest area of utilization (Liver) and this lead to decrease of HDL-C in serum (79) . There is a direct correlation between adipose tissue lipoprotein lipase activity and plasma HDL-C This direct correlation may be responsible for low levels of HDL- C (79) . Hypertriglyceridemia , leading to low HDL-C mainly due to the actions of cholestryl ester transfer protein (CETP)(79), which facilitates transfer of cholestryl ester from HDL to VLDL , IDL and in exchange for triacylglycerol , relieving product inhibition of LCAT activity in HDL-C. LCAT activity was lower in pregnancy induced hypertension (80).

2-Oestrogens were shown to increase serum HDL- C levels and decrease of LDL-C Levels (81). Therefore , the low level of HDL-C and a consequent increase in LDL-C level may be

attributed to hypoestrogenemia of preeclampsia . It may be also due to insulin resistance in the corresponding patients (82) .

3-The decrease in albumin lead to decrease in HDL- C because lysolecithin , one of the products of the lecithin cholesterol acyl transferase (LCAT) reaction , is removed by binding to serum albumin (53).

Our results were in good agreement with the results of Bulter-CL (83) .

The increase in TC, TG , VLDL- C and LDL-C in the third trimester of uncomplicated pregnancy may be attributed to the increased metabolic demand of the fetus with the advancing course of pregnancy (84) .

Our results are consistent with the results reported by Cekman-MB (85) and inconsistent with those reported by Tayanta–D (76) who found a significant decrease in the LDL-C level in the third trimester of pregnancy . The inconsistency can be attributed to dietary differences between the studied groups .

In this study, we found a significantly decrease in serum total protein and albumin in women with preeclampsia than in normotensive women with similar gestational age .

This decrease in serum total protein and albumin level in patients and healthy groups (2^{nd} and 3^{rd} trimester) may be attributed to :-

1-During normal pregnancy the hyperfiltration is largely due to profound resistance reduction in the renal afferent arterioles (86)

. In PET both glomerular filtration rate and renal plasma flow decrease by 30% to 40% compared with normal pregnancy of the same duration (87) . The basis for the hypofiltration in PET is largely secondary to structural changes into glomerulous as opposed to constriction of afferent arteriolar system and depression in renal plasma flow , which increasing permeability of glomerulous to protein (88) . Persistent microalbuminuria possibly reflects a state of increased renal endothelial permeability and is considered an early marker of diffuse endothelial dysfunction (89) .

2-The protein excretion was approximately four fold higher than that of nonpreeclamptic women (90). When preeclampsia is accompanied by proteinuria , there is a marked fall in albumin and an increase in α_2 – macroglobulin (91). It's believed that these changes are a result of urinary loss of the proteins of intermediate molecular weight , with a compensatory unselective increased synthesis of protein in the liver , and retention in the serum of macroglobulins , which are too large to pass through the defective glomerular basement membrane (92) . Metabolic studies have shown that albumin synthesis is significantly greater in preeclampsia than in normal pregnancy , and this is stimulated by the liver due to either decrease in estrogen production or low concentration of albumin in the blood (93) .

3-The increase in urinary protein excretion in preeclampsia occurs secondary to alterations in the size and / or charge selectivity of the glomerular filterate (94) . Loss of charge selectivity was likely the primary defect in the glomerular filtration barrier in women with preeclampsia (88) . Preeclampsia is associated with morphological changes in renal endothelial and mesangial cells have been noted enlarged due to their engorgement with lipid (95) . These lipid – induced changes have recently been named glomerular hitopathological endotheliosis (95) .

4-Aldosterone leads to water and electrolyte retention and generalized edema , including edema of intima of the arteriol (96). These changes produce arteriolar stiffness which increase the sensitivity to angiotensin, further vasoconstriction lead to capillary hypoxia and increased permeability of the glomerular membrane, leading to proteinuria and further edema (96).

5-Proteinuria lead to hypoalbuminemia , low plasma oncotic pressure and intravascular volume depletion , subsequent under perfusion of the kidney stimulates the renin- angiotensin – aldosterone axis , which causes increased renal sodium and volume retention which to increased extracellular fluid (97). The extracellular fluid expansion leads to a decrease in serum albumin (98) .

Our results were good agreement with these reported by Salako – BL (99) .

The decrease in serum total calcium and magnesium in preeclamptic pregnancy compared with control group can be attributed to :

1-During normal Pregnancy , there are many mechanism tend to promote lowering of maternal calcium concentration due to an increase in maternal estrogen production which blocks bone resorption and increases calcium excretion in urine (98)

2-The haemodilution occurs during the last trimester of pregnancy (100) . The extracellular fluid expansion leads to a decrease in serum albumin level and serum calcium because albumin is carrier for calcium (100). Jord-R found that were calcium was strong association between serum albumin with systolic and diastolic blood pressure (101). Because there is a strong correlation between total and ionized serum calcium , one would have to assume that the binding characteristics for calcium and its carrier proteins are abnormal in hypertension (101) .

3-The prevalence of magnesium deficiency may be due to the difference in the dietary pattern (100). The haemodilution could be another factor leading to a higher prevalence of deficiency of magnesium (100) .

4-Magnesium exclusively excreted in urine and reabsorbed in proximal convoluted tubules by a process called transport maximum (Tmax) its excretion increase as a filtered load increase above the transport maximum, in women with decrease

GFR, the filtered load is more excretion of magnesium in urine (102) . During normal pregnancy, the increase in GFR causing increase in calciuria (98) .

5-Magnesium homeostasis is linked with calciuria (103) . Studies from the first elucidated the nature of the effects of calcium and magnesium ions at the neuromuscular junctions (104). Magnesium competes for prejunctional site with calcium ions , the ions competed with each other , high magnesium concentration inhibit release of acetyl choline (Ach) and high calcium concentration increases of Ach from presynaptic nerve terminal (104) .During cellular injury and cellular death , there is influx of calcium ions and loss of calcium homeostasis . This mechanism precedes cell death and is also seen in reperfusion injuries (104) . In sever preeclampsia , there is vosospasm , ischemia as well as cellular hypoxia which may cause reperfusion injury following treatment (104) .

6-Magnesium is physiologically antagonist to calcium , it follows that in an attempt to mitigate cellular injury by calcium , there will also be influx of magnesium during reperfusion . This could explain why both calcium and magnesium were reduced in the blood of preeclamptic pregnant women (104). These extracellular changes could explain why some of patients suffered convulsion (eclampsia) (105).

Our results are in good accordance with the results reported by Sukonpan-K (106) and Sanders- GT (107) .

The correlation between serum testosterone Levels and different biochemical parameters.

There are many previous studies concerning the relationship between changes in oestrogen levels (during normal and complicated pregnancy) and many different biochemical parameters were carried out by many investigators .Such a relationship was not established in the case of testosterone changes during second and third trimester of pregnancy . In our study the following observations were noted .

1-There were significant correlation between serum testosterone levels and lipid profile , protein and minerals as shown in the corresponding Figures (3-7) to (3-62) . This gives a preliminary idea about the role of testosterone in such changes.

2-The trend of changes in the correlations between lipid profile and decreased testosterone level in G4 may be attributed to increase in estrogen levels in normal pregnancy . This increase is depressed in preeclamptic women leading to the reversal of this relationship in other groups . There were a positive correlation between lipid profile except HDL-C and testosterone in G1,G2 and G3, because increase in testosterone lead to increase in the activity of hepatic lipoprotein lipase (LPL) . an enzyme involved in HDL-C catabolism and increased synthesis of the triglyceride (108).

Hyperandrogenemia may lead to hyperinsulinemia because androgens are known to decrease both hepatic removal of insulin and peripheral sensitivity to insulin (70) . Similar coexistence of hyperinsulinemia and hyperandrogenism is present in polycystic ovary (PCO) and these patients appear to be at increased risk of preeclampsia. There is also evidence that women with androgen excess are at increased risk of cardiovascular disease (109) . Insulin resistance and high testosterone concentration might be signs of abnormalities in metabolizing fat, which would lead to increased risk of heart disease (109). Therefore the control of such changes is important for the follow up of complicated pregnancy.

In this study , we noted a positive correlation between cholesterol / albumin ratio and testosterone in G1, G2 and G3 but a negative correlation in G4 . There are inverse relationships between cholesterol and albumin because hypercholesterolemia occurs due to non specific increase in lipoprotein synthesis by liver in response to low albumin level (110).

References

1-Sheinam-A.Dewhurst's text book of obstetrics & Gynecology for Postgraduates. 8th ed. Graphicraft,Hong Kong.2007;227.

2-Symond-EA and Symond-LM. Essential obstetrics and Gynegology. 4th ed. Churchill livingstonec-philadelphia. 2004;163-168.

3-Reynold-C, Mabie –WC and Siba-BM, etal. Preeclampsia:hypertension State of pregnancy.Armeinian maedical network.2005.

4-Chan-PD and Hahnson-SM. Current clinical strategies publishing. 6th ed. Laguna Hills. California. 2006.

5- Edmond-DK.Dewhurst's text book of obstetrics & Gynecology for Postgraduates. 6th ed. Graphicraft,Hong Kong.1999.169.

6-Ficioglu-C and Kutlu-T. The role of androgens in the aetiology and pathology of preeclampsia. J Obstet Gynecol.2003;23:134-137.

7-Buhimschi-CB, Maglorri-L and funai-E,etal. Fractional excretion of Angiogenic factors in women with severe preeclampsia. Am College obstet & Gynecol. 2006;107(5):1103-11130

8-John-P. Evalution of priteinuria in pregnant women. Am J obstet & Gynecol.2008;57:611

.

9- Takeuchi-K , Zhang-B and ideishi-M , etal. Influence of age & Hypertension on the association between small artery Compliance & coronary artery disease. Am J Hypertension. 2004;17(12);1188-1191.

10-Charles-R and Brinkman. Essential obstetric & gynecology. 3rd ed. Churchill Livingstone-philadelphia.1999;164-168.

11-Dinesh-M and Shah. Role of the rennin-angiotensin system in the Pathogenesis of preeclampsia.Am J Rysiol Renal physiol.2005; 288:614-625.

12-Philp-N and Baker. Obstetric by ten teachers. 18th ed. Hodder Arnold. 2006;162-163.

13-Fauci, Braunwald and Kasper. Harrison,s internal medicine. 17th ed.

Mc Graw-Hill's.2008;841.

14-Agatisa-PK, Ness-RB and Reberts-JM,etal. Impairment of endothelial function in women with history of preeclampsia :An indicator of cardiovascular risk. Am J physiol. 2004;286:1389-1393.

15-Parry-S and Marchiana-D. National medical serias for independent Study),obstetrics & Gynecology.6th ed. Lippincott Williams & wilkins.2008;1-2.

16-Gronowki-AM and Levine-ML.Tietz text book of clinical chemistry. 3rd ed.Sunders company,philadeiphia. 1999;1601-1611.

17-Nini-AF,Riggs-BL and Alkinson-EJ,etal. Relative contributions of testosterone & estrogen in regulatating bone resorption & formation in normal elderly men. Am soci clin invest. 2000. 1553-1560.

18-Bagatell-CJ, Heiman-JR and Matsumate-AM,etal. Metabolic & Behavioral effects of high dose,exogenous testosterone in Healthy men. Am J clin chemistry.1994;8:212-215.

19-Chape-DC, Harvey-RA and Ferries-DR. Lippincott's Illustrated reviews:Biochemistry.3rd ed. Awolters Kluwer.2005;237-238.

20-Ivanova-EA.Effect of testosterone on rate of total protein synthesis in The fetal rabbit reproductive tract in vitro.Bulletin of Experimental Biology & medicine.1994;98(8):233-234.

21-Vasudevan and Sreekuman-S. Text book of biochemistry for medical Students.3rd ed.Jaypee brothers,New delhi. 2001;417-422,218.

22-Murray-RK, Granner-DK, Mayes-PA and Rodwell-VW.Harper's illusterated Biochemistry.26th ed. Mc Graw-Hill, New York. 2003;440-445.

23-Ojeda-NB, Grigore-O and Yanes-LL, etal. Testosterone contributes to marked elevations in mean arterial pressure in adlt male intrauterine grouth restricted offspring. Am J Physiol Regul Integr Comp Physiol. 2007;292:758-763.

24-William-J and Marshall.Illustraed text book of clinical chemistry.4th ed Sundard company,London.2000;222-229.

25-Murray-RK,Granner-DK and Radwell,etal. Harper's Illustrated Biochemistry .25nded. Stlouis,Sanfrancisco, New york. 2000; 285.

26-Whithy-L,Smith-AFand Beckett-GJ. Lecture notes on clinical chemistry. 6thed. Blak well scientific publication.1996.225.

27-Polo-A. Lipoproteins function & lipid transport. PhD thesis. College of Medicine. University of mustansiriya. 2007.

28-Philippa-J and Easterbrook. Basic medical sciences for MRCP part 1 . 2nd ed . Churchill Livingstone, New york.1999;245-247.

29-Vaziri-ND.Dyslipidemia ofchronic renal failure.A m J phsiol. 2006; 290(4):262-272.

30-Gaw-A,Gowan-RA,OReilly-DJ,Michael-J and Sewart-MJ.Clinical Biochemistry.2nded.Churchill Livingstone,London..2000;211 -212.

31-Castanza-CM,Ross-BM and Floherty-MS,etal. Association of extieme of blood lipid profile phenotypic variation with 11 revers cholesterol gens and 10 non genetic cardiovascular disease risk factor. J human Mol Genetic.2003;12(21):2733-2743.

32-Hornic-CA, Jones-AL and Rened-G. Lipoproteins.Am J physiol.1984; 246:187-194.

33-Genest-JJ,Maral-M and Denis-M. High density lipoprotein in health & In disease. J invist Med.1999;47:31-42.

34-Navab-M, Berlinear-JA and Subbangounder-G, etal. HDL & the Inflamatory response induced by LDL-derived oxidized Phospholipids. Arterioscler thromb Vasc.2001;21:481-488.

35-Majumdar-A and Wheeler-DL. Lipid abnormalities in renal disease. J R soc Med. 2000;93:178-182.

36-Guyton-AC and Hall-JE. Text book of medical physiology.11thed. Elsevier Saunders. 2006;855.

37-Ding-DC, Hsu-S and Chu-TW. Massive ascites in sever preeclampsia

.Taiwanes Jobstet Gynecol. 2005;44(4):384-386.

38-Kaplan-LA and pesce-AJ. Clinical chemistry,theory, analysis, Correlation. 4th ed. Elsever science,New York. 2002;387-348, 760.

39-Richards-SR, Nelson-DM and Zuspan-FP. Calcium levels in normal and hypertensive pregnant patient.Am J Obstet Gynecol. 1998;149(2):168-171.

40-Baynes-Jw and Dominiczak-MH. Medical Biochemistry. 2nd ed. Elsever. 2007.

41-Berg-JM,Tymoczko-JL and Stryer-L. Biochemistry. 5th ed. W.H Freeman.2005;115-117.

42-Gilroy-CV, Horney-BS, Burton-SA and Mackenzie-AL. Evalution of Ionized and total serum magnesium concentrations in Hyperthyroid cats. Can J Vet Res. 2006;137-142.

43-Creasy-RK and Resnik-R. Maternal-Fetal medicine . 4th ed.W,B saunders Company.1999;858.

44-Enders-DB and Rude-RK. Tietz text book of clinical chemistry.3rd ed. saunders Company,Philadelphia.1999;1395-1397.

45-Newman-JC and Amara-S. The pathogenesis of preeclampsia :the Magnesium ischemia hypothesis. Med Hypothesis(England). 1993;40(4):250-256.

46-Kisters-K,Barenbroka-M and Louwenb-F,etal.Membrane intracellular and magnesium,and calcium concentrations in preeclampsia Am J Hypertension.2000;13(7):765-769.

47-Hojo-M and August-P.Calcium metabolism in normal & hypertensive Pregnancy.Semin nephrol.1995;15(6):504-511.

48- LopezP. Preventation of preeclampsia with calcium supplementation And itsrelation with L-arginine ,nitric oxide pathway.Braz J Med Biol Res.1996;29(6)731-741.

49-Ray-J,Vasuhat-K and Kaura,etal.Calcium metabolism in preeclampsia .International J Gynecol obstet.1999;66(3):245-250.

50-Eigebh-JO, Abebe-J and Odike-MA. Protein/Creatinine ratio in Random urine specific quantitation of proteinuria in Preeclampsia. The Internet J Gynecol & Obest. 2007;8(1): 769-777.

51-Tietz-NW. Clinical Guide to laboratory tests.3rd ed. W.B. Saunders, Philadephia. 1995;578-580.

52-Stein-EA. Methods for clinical laboratory measurement of lipid and Lipoprotein risk factor. 3rded. Washington. 1991.

53- Weinstock-GR, Mayneld-C and Albers-J. Evaluation of quality – Control materials for high density lipoprotein cholesterol Quantitation. Clin Chem. 1991;27:116-133.

54-Bucolo-G and David-H. Quantitative determination of serum Triglycerides by use of enzymes. Clin Chem. 1973;19:475.

55-Cooper-GR, Myers-GL and Smith-SJ, etal. Blood lipid measurements: Variation and practical utility. J.A.M.A. 1992;267:1652-1660.

56- Schmidt-K. The plasma proteins. 2nd ed. New York,Academic Press. 1975;184-185.

57-Cooper-EH and Morgan-DB. Specfic protein laboratory improvement Program. Am Assoc Clin Chem. 1984;1:1-11.

58-Whicher-JT. Biochemistry in Clinical practice.William Heinemann , London. 1983;221-250.

59-Nanji-AM and Reddy-S. Use of total cholesterol/albumin ratio as an Alternative to High density lipoprotein - cholesterol Measurement. J clin pathol. 1983;36:716-718.

60-Henry-RJ, Cannon-DC and Winkelman-JW, etal.Clinical Chemistry Principles and techniques. 2nd ed. New York.Harper & Row. 1974;424-428.

61-Gowenlock-AH, Mc Marry-JR and Mc Lauchlan-D.Varley's practical clinical Biochemistry .6thed. Heinemann Medical Books, London. 1977;868-873.

62-Brilla-LR and Conte-V. Effect of a noval zink-magnesium Formulation on hormones and strength. Journal of Exercise Physiology. 2000;3(4):26-36.

63-Petridou-E, Katasouyanni-K and Spanos-E,etal. Tabacco smoking, Pregnancy,PET estrogens,and birth wieght. Epidemiology. 1999;1:247-250.

64-Iou-SG, Eskandari-M and Dabiri-A. Evaluation of estrogen level of Women with preeclampsia in third trimester. Medical J Islamic World Academy of Sciences. 2005;15(1):19-22.

65-Reyes-MR, Alvarez-SA and Lazalde-B. Estrogens are potentially the only steroids with an antioxidant role in pregnancy. Acta obstet Gynecol Scand. 2006;85(9):1090-1093.

66-Rebecca-T Postischman-N, Roberts-JM and Ness-R. Maternal serum estrogen and androgen concentrations in preeclamptic and uncomplicated pregnancies. Intern J Epidem. 2003;32: 455-460.

67-Michie-EA. Urinary oestriol excretion in pregnancies complicated by Suspected retarded intrauterine grouth, toxaemia or essential hypertension.J Obstet Gynecol Br Commonw. 1967;74:896-901.

68- Sorensen TK, Williams MA and Zingheim RW.etal. Elevated human chorionic gonadotropin and subsequent pregnancy-induced hypertension. Am J Obstet Gynecol. 1993;169:834–838.

69-Steier-JA, Ulstein-M and Myking-OL. Human chorionic gonadotropin and testosterone in normal and preeclamptic pregnancies in relation to fetal sex. Obstetric gynecol.2002;100:552-556.

70-Barbieri-RL,Makris-A and Randall-RW,etal. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J clin endocrinol.Metab.2005;62:204-210.

71-Bammann-BL, Goulam-CB and Jiang-NS.Androgen production and metabolism in normal pregnancy.Am J Obestet Gynecol.

1980;137(3):293-298.

72-Golmahammad-IS, Salari-S and Eskandari-M, etal. Evaluation 0f androgen and progesterone levels in women with preeclampsia. Iran J Med Sci. 2005;30(4):186-189.

73-Jasim-FG. Role of sex hormones in preeclampsia. PhD thesis.College of Medicine. Al-Nahrain University. 2008.

74-Patrizia-B, Giancarlo-Tand Francu-E, etal. Lipoprotein metabolism during normal pregnancy. Am J Obstet Gynecol. 1999;181 (2):430-434.

75-Friedewald,WT, Levy-RI and Fredrickson,DS. Estimation of the concentration of LDL-C without the use of preparative ultracentrifuge. Clin chem. 1972;18:499.

76-Jayanta-D, Mukhapadhyay-AK,and Saha-PK. Study of serum lipid Profile in pregnancy induced hypertension. Indian J Clin Biochem. 2006;21(12):165-166.

77-Demir-SC, Urunsak-IF and Ozgunen FT,etal. Comparison of lipid Profile in normal and hypertensive pregnant women. Ann Saudi Med. 2004;24(5):382-389.

78-Suzie-WJ, Sanchez-SE and Laraburre-G,etal. Plasma lipid concentrations in preeclamptic and normotensive Peruvian women. Intern J Gynecol Obstet.2008;100(9): 1059-1062.

79-Aziz-R and Mahboob-T. Preeclampsia and lipid profile. Pak J Med Sci. 2007;23(5):751-754.

80-Laukidi-B,Senhadji-L and Merzouk-S,etal.Serum Lecithin:cholesterol acyltrasferase activity, HDL_2 and HDL_3 composition in hypertensive mothers and their small for gestational age Eur J Pedia. 2008;167(5):525-532.

81-Bradley-R and Crook-D.Text book of clinical biochemistry:Metabolic and clinical aspects.1sted. Churchill livingstone,London. 1995;413-422.

82-Kaja-RTrikkanen-MJ and Vinnkka-L, etal.Serum lipoproteins, insulin

and urinary prostanoid metabolities in normal and hypertensive pregnant women,Ostet Gynecol. 1995;85(3): 356-359.

83-Bulter-CL, Williams-B and Raymond-SM, etal. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia Am J Hyper. 2004;17(7):574-581.

84- Winny-HT, Khoury-MY, and Takahashi-WH, etal. Assessment of serum lipids in pregnant women aged over 35 years and their relation with preeclamsia. Obstet Gynecol, 2008;6 (1):63-67.

85-Cekmen-MB, Erbagci-AB and Balat-A, etal. Plasma lipid and lipoprotein concentrations in pregnancy-induced hypertension. Clin Biochemis.2003;36(7):575-578.

86-Jeyabalan-Aand Conrad-KD. Renal function during normal pregnancy and preeclampsia. Front Biosci. 2007;1(12):2425-2437.

87-Joaquin-ZM, Armando-OCC and Hugo-MZ. Clinical evolution of hypertension and proteinuria in patients who developed preeclampsia. Inter J Gyneco Obstet.2005;5(1):1-90.

88-Hadunewich-M, Karumanchi-SA and Lafaette-R. Pathophysiology Of the clinical manifestations of preeclampsia. Cli J Am Sco Nephrol. 2007;2:543-549.

89-Hotha-B, Pappachan-JM and Pillai-HB,etal. Microalbuminuria in patients with essential hypertension and its relationship to target organ damage. Saudi J Kidney Dis Trans.2008;19: 411-419.

90-John MD, Homuth-V and Jeyabalan-A, etal. New aspects in the pathophysiology of preeclampsia. J Am Soc Nephrol. 2004; 15:2440-2448.

91-Horne CHW,Howie-PW and Goudie-RB.Serum alpha macroglobulin, tranferrin, albumin in preeclampsia. J Clin Payh. 2007;23: 514-516.

92-Studd-JWand Bailey-DE.Serum protein changes in the preeclampsia eclampsia syndrome. Inter J Obstet Gynecol. 2008;77(9): 796-801.

93-Woods-LL Ingelfinger-JR and Rasch-R. Modest maternal protein restriction fails to program adult hypertension in female rats.Am J Phsiol Renal Integer Comp Phsiol.2005;289(4): 1131-1136.

94-Brow-MA, Wang-MX and Buddle, etal. Albumin excretory rate in normal and hypertensive pregnancy. Clin Sci. 1994;86(3): 251-255.

95-Smean-AI, Bisalla-A and Ekele-BA. Comparison of metabolic syndrome variables among pregnant women with and without preeclamsia. J Nation Med Associ. 2008;100(9): 1059-1062.

96-David-B and Gordon . The role of renin substrate in hypertension. 1993;5:353-362.

97-Goldman and Ausiello. Cecil medicine. 23rded. Sunders Elsevier. 2007;867.

98-Nasser-O and Shurideh. Does serum calcium in preeclampsia and normal pregnancy differ. Saudi Med J .2001;22(10): 868-871.

99-Salako-BL, Odukagbe-AT and Olayemi-O, etal. Serum albumin, creatinine, uric acid, and hypertension disorder of pregnancy. East African Med J. 2003;80(8):424-428.

100-Pathak-P, Kapoor-SK and Kapil-V, etal. Serum magnesium level among Pregnant women in rural community of haryana state. Eur J Clin Nutrition. 2003;57:1504-1506.

101- Jord-R, Sunsfjard-J and Bonaa-KH. Serum calcium and cardiovascular risk factors and disease .Am heart Assoc.1999; 34:484-490.

102-Resnick-LM, Barbagallo-M and Bardicef-M,etal. Cellulr-free magnesium depletion in brain and muscle of normal and preeclampsia pregnancy.Am Heart Associ. 2004;44:322-326.

103-Mehta-R and Pertrova. Ionized magnesium and gestational age. Indian J Pediotrics. 2007;74:1025-1028.

104-Idogun-ES, Imarengiaye-CO and Momoh-SM. Extracellular calcium and magnesium in preeclampsia and eclampsia. African J rep Health. 2007;11(2):80-85.

105-Wang-,Cottrel-JE and Kass-IS. Small physiologic changes in calcium and magnesium alter excitability and burst firing of CIA pyramidal cell in rat hippocampal shees. J Neurosurg anesthesiol. 2004;16(3):201-209.

106-Sukopan-S and Phupong-V. Serum calcium and magnesium in Normal and preeclamptic pregnancy. Obstet Gynecol.2005; 273(1):12-16.

107-Sanders-GT, Huijgen-HJ and Sanders-R. Magnesium in disease: overview with special emphasis on the serum ionized magnesium. Clin Chem Lab Med. 1999;37(11):1011-1033.

108-Isidori-AM, Gianne-E and Greco-EA , etal. Effects of testosterone On body composition, bone metabolism and serum lipid profile In middle-aged men:ameta analysis.Clin Endo.2005;63:280-293

109-Laivuori-H, Kooja-R and Rutanen-EM, etal. Evidence of high circulating testosterone in women with prior preeclampsia. Jclin Endocrin Metab. 1998;83:2344-2347.

110- Goddard-J, Turner-AN, Cumming-AD and Steward-LH. Davidson's Principles and practice of medicine .20thed. Churchill Livingstone. 2006;480.

الخلاصة

مرض قبل الشنج هو واحد من الامراض الاكثر شيوعا يحدث اثناء الحمل في فصليه الثاني والثالث ونسبة معدل وقوع هذا المرض هي 2-5% بين النساء الحوامل و اسباب هذا المرض غامضة لحد الان و العديد من النظريات طرحت في هذا المجال من قبل بعض الباحثين وفي مختلف الدول .في بعض الاحيان يدعى هذا المرض بمرض النظريات بسبب تناقض الاصدارات المتعلقة باسبابه ونتائجه .

و في هذه الدراسة حاولنا ولاول مرة توضيح العلاقة بين هرمون الذكورة وبعض مكونات الكيمياء الحياتية التي تختلف اثناء الحمل (نطاق الدهون ,البروتين الكلي ,الالبومين, الكالسيوم, المغنيسيوم) .

وقد انتقينا 55 امرأة مريضة حامل احيلت الى مستشفى بابل للولادة والاطفال للفترة بين كانون الاول2007_أيار2008 لاجراء الدراسة وتم تحليل مصول من اولئك المرضى مع مجموعة السيطرة ال(55 امرآة سليمة حامل) وفحصت الدهون والبروتينات وبعض المعادن بالاضافة الى هرمون الذكورة .

قسمت الدراسة الى اربع مجاميع فرعية كما ياتي:

1- المجموعة الاولى: تشمل 25مريضة مصابة بمرض قبل الشنج في الفصل الثاني من الحمل .
2- المجموعة الثانية: تشمل30 مريضة مصابة بمرض قبل الشنج في الفصل الثالث من الحمل .
3- المجموعة الثالثة: تشمل 25 آمرآة سليمة حامل في الفصل الثاني من الحمل.
4- المجموعة الرابعة: تشمل 30امرآة سليمة حامل في الفصل الثالث من الحمل.

وقد اظهرت النتائج زيادة معنوية في مستوى هرمون الذكورة في المجموعة الاولى والثانية مقارنة بالمجموعة الثالثة والرابعة($p<0.001$) وهناك نقصان غير معنوي في هرمون الذكورة في المجموعة الرابعة مقارنة بالمجموعة الثالثة ($p=0.36$).

وبينت النتائج اَن‌هناك زيادة معنوية في الكوليسترول الكلي وتراي كليسرايد وكوليستيرول الليبوبروتن عالي الكثافة وكوليستيرول الليبوبروتين قليل الكثافة جدا في المجموعة الاولى والثانية بالمقارنة مع المجموعة الثالثة والرابعة ($p<0.001$), ($p<0.01$) بالتعاقب بينما هناك نقصان معنوي في كوليستيرول الليبوبروتين العالي الكثافة في المجموعة الاولى والثانية عند المقارنة بالمجموعة الثالثة والرابعة .

كما اظهرت النتائج نقصاناً معنوياً في مستوى البروتين الكلي والالبومين والكالسيوم والمغنسيوم في النساء المصابات بمرض قبل الشنج بالمقارنة مع الحوامل ذوات الضغط الطبيعي ($p<0.05$) وهذه التغيرات كانت غير معنوية في المجموعة الثالثة والرابعة ($p>0.05$).

وقد اظهرت النتائج وجود علاقة معنوية بين مستوى هرمون الذكورة في المصل و(نطاق الدهون والبروتين والمعادن).وهذا يعطي فكره حول دور هرمون الذكورة في هذه التغيرات.وتوجد علاقة موجبة بين هرمون الذكوره ونطاق الدهون ($p<0.01$) باستثناء كوليستيرول الليبوبروتين عالي الكثافة الذي يقل بزيادة هرمون الذكورة في المجموعة الاولى والثانية والثالثة.وهناك علاقة موجبة بين هرمون الذكورة ونسبة الكوليستيرول/الالبومين ($p<0.01$) في المجموعة الاولى والثانية والثالثة ولكن العلاقة موجبة في المجموعة الرابعة وبزيادة الكوليستيرول يقل الالبومين ($p<0.001$).

Conclusions & Recommendations

Conclusions and Recommendations

1- There were significant changes in lipid profile, total protein, albumin and minerals in the second and third trimesters of preeclamptic pregnancy in comparison with normal pregnancy.

2- Those up-mentioned changes were in good correlation with the changes in serum testosterone levels in corresponding patients and control group with the exception of the results in the third trimester of normal pregnancy. This necessitates the measurement of those parameters in relation to testosterone in women with preeclampsia.

3- The measurement of serum lipid profile is important in patients with preeclampsia in order to a void any consequent metabolic disturbances in those patients. It is more beneficial to measure such components in relation to serum testosterone level since the latter also increase significantly in the corresponding pateints and is well-correlated with measured parameters.

4- It is necessary to measure total cholesterol/albumin ratio since this ratio is important for the prediction of cardiovascular disease besides the changes in serum albumin levels which occur due to oedema accompany most case of preeclampsia.

5- It is highly beneficial to determine serum levels of calcium and magnesium during the second and third trimester of

pregnancy because these minerals are associated with the same clinical manifestations of the disease (i.e, neuromuscular irritability and seizure) in order to prevent further complications.

Future Studies

1-Determination of the changes in serum level of testosterone and insulin in relation to the changes in lipid profile, serum proteins, enzymes & minerals in order to clarify the chemical pathology of the disease.

2-Investigation of the changes in trace element in association with changes in female sex hormones.

3-Study of the enzymatic changes of this disease in association with testosterone.

4-Future studies are needed to establish the biochemical changes in RBC component in patients with PET.

5-Research work is needed to establish the relationship between the changes in steroid hormones levels in the urine samples and serum samples in PET patients . It is more beneficial to correlate those changes with the changes of other biochemical components which are normally excreted in urine.

6-Study of the association of increase in serum testosterone levels and antioxidant status in preeclamptic pregnancy. Determination of oxidative stress is also necessary to assign the degree of endothelial damage in preeclampsia.