Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Chemistry and Biochemistry Department

Assessment of Electrolytes and Some Trace Elements Levels After Administration of Atorvastatin Drugs in Sera of Patients with Hyperlipidemia in Al-Hilla City

A Thesis
Submitted to the Council of the College of Medicine / University of Babylon as a partial fulfillment for the requirements of the Degree of Master in Science /Clinical Biochemistry

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2023 A. **1444**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ ۚ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا ۗ وَمَا يَذَّكَّرُ إِلَّا أُولُو الْأَلْبَابِ ٢٦٩﴾

Supervisor Certification

We certify that this thesis entitled " **Electrolytes and Some Trace Elements Levels After Adminastartion of Atorvastatin Drugs in Patients with Hyperlipedemia in Al-Hilla City** " was carried out under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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In review of the available recommendation, I forward this thesis for debate by the examining committee.

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Dedication

To my kind father....my role model, and my ideal in life; He taught me how to live with dignity and dignity.

To my loving mother... I cannot find words that can give her due, as she is the epic of love and the joy of life, and an example of dedication and giving.

To my brothers.... my support, my joys, and my sorrows.

To my husband... the highest symbols of sincerity, loyalty, and companion on the path

To my children..... the pleasure of my liver.

To all brothers, I dedicate my scientific research to you

Huda

Acknowledgment

I wish to express my deepest and special acknowledgment with great deal of gratitude to my supervisor Professor Dr. Mufeed Jalil Ewadh and Assistant Professor Dr. Tariq Hussein Mgheer

Dr. Mgheer for suggesting and supervising this study with an endless support, encouragement and invaluable advice throughout the different steps of this work.

I want to thank the staff of the Department of Biochemistry in the College of Medicine at the University of Babylon Furthermore, much appreciation is dedicated to the Dean of the college of Medicine for his crucial role, and to the Head of the Biochemistry Department, Dr. Abdulsamie Hassan Hammod Altaee who offered a permission to use all the required equipments and the necessary materials for the successful completion of the present work.

I want to give special praise and recognition to all staff members of the Al-Suwayda hospital, Dr. Emad Q. Kadhem, and Wasan Ghanim Abed.

I would like to express sincere gratitude to my family for their support and for helping me to achieve this thesis in the best way.

Many thanks to everyone who helps me and supports me to complete this project

Summary

Hyperlipidemia is a term that encompasses various genetic and acquired disorders that describe elevated lipid levels within the body. It is a very common disorder, especially in the Western area, but also throughout the world. The disease is detected by measuring blood levels (cholesterol triglyceride HDL LDL VLDL) .exercise, changes in diet, and medications can be effective in helping with treatment, the most important of which is statins.

There is insufficient evidence to suggest a relationship between high fats, electrolytes, and trace elements. So, this study aimed to use evalete to measure, estimate and evaluate this relationship, after taking statin therapy.

The examination was performed on 100 subjects, 50 of whom were hyperlipidemia patients and 50 were control. Samples were collected from October to December 2022, and their ages ranged from (20-65)year . When sampling, several things must be taken into account, including medical history, treatment period, and patients not taking some treatments that could affect the concentrations of elements in the body, including diuretics, and treatments that interfere with the action of statins.

There is no significant difference between studied groups according to age. However, there are a significant differences between sex and means of body mass index according between studied groups.

Cholesterol, triglyceride, and VLDL were significantly lowered after administration of atorvastatin, HDL was significantly raised after administration of atorvastatin. While there is no significant difference in LDL level after administration of atorvastatin.

There is a significant decrease in the sodium and magnesium levels with $P < 0.001$ in patient as compared to control. However, our results didn't show any significant difference between patients and controls as regard to potassium($P = 0.51$) or chlorine ($P= 0.80$).

There is a significant decrease in Cr, Se, and Zn levels in patients group compared to control group with ($P < 0.001$). on the other hand, there is a significant increase in Cu level in patients group compared to control group with ($P < 0.001$).

There is a significant positive correlation between chromium, zinc, selenium, and BMI, the decrease in chromium, selenium and zinc in hyperlipidemia patients can cause an increase in weight.

There is a significant positive correlation between selenium and chromium, and there is an inverse relationship between chromium and copper, and copper and selenium.

There is an inverse relationship between chromium, zinc, selenium proportional to the high lipids profile.

There is a moderate significant positive correlation between Zink and chromium. Also, between Zink and Selenium. However, there is a moderate significant inverse correlation between Zink and copper.

ROC curve of Cr for diagnosis of hyperlipidemia showed (Cut-off point was $\leq$ 0.55 (ng/mL)), AUC=0.98, P= <0.001*, 95% CI (0.755-0.904), the sensitivity and the specificity was 94.0%, 96.0 % respectively.

ROC curve of Cu for diagnosis of hyperlipidemia showed (Cut-off point was ≥ 147 (ng/mL)), AUC=0.91, P= <0.00*, 95% the sensitivity and the specificity was 90.0%, 92.0 % respectively .

ROC curve of Se for diagnosis of hyperlipidemia showed (Cut-off point was ≤ 70.0 (ng/mL)), AUC=0.98, P= <0.00*, 95% the sensitivity and the specificity was 88.0%, 92.0 % respectively .

ROC curve of Zn for diagnosis of hyperlipidemia showed (Cut-off point was ≥ 71.5 (ng/mL)) , AUC=0.94, P= <0.001*, 95% CI (0.755-0.904), the sensitivity and the specificity was 92.0%, 90.0 % respectively .

High triglycerides, cholesterol and VLDL are closely associated with hyperlipidemia . Finally Low sodium and magnesium are positively associated with hyperlipidemia patients after taking statin treatment .Low selenium, chromium and zinc are closely associated with hyperlipidemia patients undergoing statin treatment. High copper is associated with hyperlipidemia patients undergoing statin therapy.

List of Contents

List of Tables

List of Figures

List of Abbreviations

AAP	American Academy of Pediatrics
APOC-2	Apo-lipoprotein c-2
ATP	Adenosine Triphosphate
BMI	Body mass index
BP	Blood pressure
Ca	Calcium
CAD	Coronary artery disease
CHD	Coronary heart disease
Chol	Cholesterol
CK	Creatine kinase
Cl	Chloride
Cr	Chromium
Cr pic	Chromium picolinate
Cu	Copper
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
ECG	electrocardiogram
FH	Familial hypercholesterolemia
GFR	glomerular filtration rate
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
HDL	High Density Lipoproteins
HeFH	Heterozygous Familial Hypercholesterolemia
HMG-CO2	3-hydroxy-3-methylglutaryl coenzyme A reductase
IDL	Intermediate Density Lipoproteins
IHD	Ischemic heart disease
ISE	selective electrode
K	Potassium
LDL	Low-Density Lipoproteins
LFT	Liver function test
Lp (a)	lipoprotein abide
LPL	lipoprotein lipase

MCT	Medium-chain triglycerides
Mg	Magnesium
MI	Myocardial infarction
Na	Sodium
PL	Protein lipase
POD	Peroxidase
RDA	Recommended dietary allowance
ROC	Reactive oxygen species
SAMSs	Statin-associated muscular symptoms
Se	Selenium
TG	Triglyceride
UAE	United Arab Emirates
VLDL	Very Low-Density Lipoproteins
Zn	Zinc

Chapter One

Introduction

1.1. Introduction

Hyperlipidemia is a common disease that affects both developed and developing countries, in this disease blood level of lipids or lipoproteins is elevated more than normal range. Elevated lipids levels (cholesterol, fats, and triglyceride) predispose the patient to various serious and sometimes lethal complications such as cardiovascular disease, cerebral strokes, hepatic and renal dysfunction [1]. This disease is usually asymptomatic, and the patient discover the disease by routine blood analysis. At the advanced stages of the disease, patients may suffer multiple complications such as hypertension and angina [2].

Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs) resulting from the increase in one or more of the plasma lipids, including triglycerides (TGs), cholesterol (CHOL), cholesterol esters and phospholipids and or plasma lipoproteins including very low density lipo-protein (VLDL) and low-density lipoprotein (LDL), and reduced high-density lipoprotein (HDL) levels [3]. The deposition of these lipids (especially LDL cholesterol) in the arteries walls resulting in narrowing of these vessels and insufficient blood flow through the affected vessels and formation of atherosclerosis as seen in figure (1-1). High cholesterol, especially high blood LDL cholesterol and low blood HDL cholesterol, poses a significant risk of atherosclerosis, the leading cause of cardiovascular mortality and morbidity worldwide [4].

Throughout the last two decades, mortality rate due to CVD has been raised Globally, there were 17 million deaths due to CVDs in 2008 that increased to 17.9 million CVD deaths in 2019, and the number expected to be 20 million. Therefore, hyperlipidemia treatment plays a vital role in managing coronary artery disease (CAD) patients or those at increased risk of CAD worldwide [5].

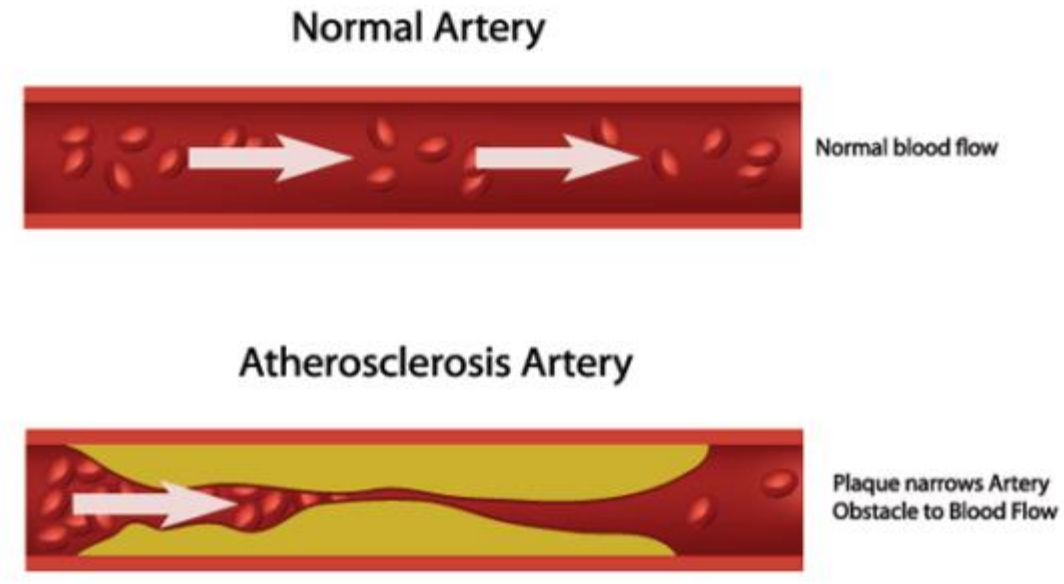


Figure (1- 1) Effect of high lipid levels on blood flow [6].

Lipoproteins are macromolecules comprising lipids and proteins. Their structure enables the lipids to combine well with other aqueous body fluids. Plasma lipoproteins are important for lipid solubilization in order to transport TGs, an important energy source, which synthesized and absorbed to places of utilization and storage; and to transport CHOL between different places of absorption, synthesis, catabolism, and elimination. lipoproteins are categorized into non-polar lipids, polar lipids, and specific proteins also known as apolipoproteins. Non-polar lipids include cholesteryl esters and TGs while polar lipids include unesterified cholesterol and phospholipids. Lipoproteins are also classified according to their densities. There are HDL and non-HDL such as chylomicrons (CM), VLDL, LDL, and intermediate-density lipoproteins (IDL) [7].

1.2. Classification of Hyperlipidaemias

Hyperlipidemia can be classified depending on types of elevated lipids or the causing factors as shown in figure (1-2)

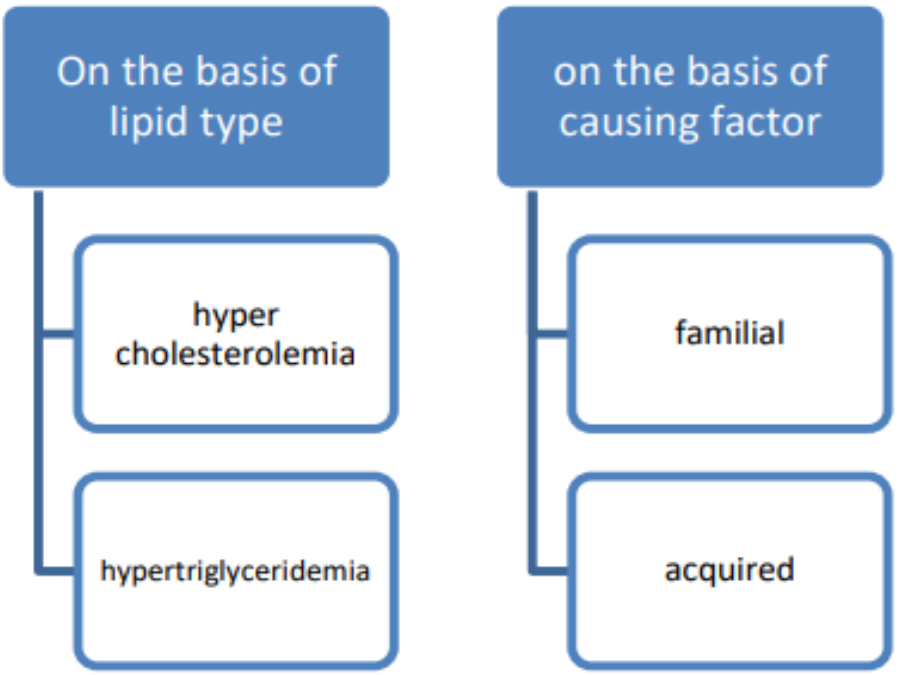


Figure (1- 2) Classification of Hyperlipidemia [8]

1.2.1 On the basis of types of elevated lipids [9]

a- Hypercholesterolemia -In this the level of cholesterol is elevated.

b- Hypertriglyceridemia-It is defined as an elevated level of triglycerides.

1.2.2 On the idea of causing factor

1.2.2.1. Primary (Familial hyperlipidemia)

It is also called familial due to a genetic defect; it may be monogenic: a single gene defect or polygenic: multiple gene defects. Primary hyperlipidemia can usually be resolved into one of the abnormal lipoprotein that are summarized in table 1-2.

Type I–Raised cholesterol with high triglyceride levels.

Type II–High cholesterol with normal triglyceride levels.

Type III–Raised cholesterol and triglycerides.

Type IV–Raised triglycerides, atheroma and uric acid.

Type V–Raised triglycerides [8].

Type I hyperlipidemia, also known as familial chylomicronemia syndrome (FCS), familial LPL deficiency, or Apo C-II deficiency. As its name suggests, FCS is the only true monogenic form of hypertriglyceridemia that characterized by greatly elevated concentrations of exogenous TGs levels ≥10 mmol/L (≥885 mg/dL), and it is the result of impaired lipolysis of chylomicrons due to a deficiency of LPL of its activator, the Apo C-II. Several genetic mutations at the LPL and APOC2 genes have been reported to cause this autosomal recessive disorder [10].

Former Frederickson hyperlipidemia type II B, also known as homozygous familial hypercholesterolemia that is characterized by markedly elevated levels of LDL cholesterol. The condition is most often caused by the presence of loss-of-function variants in LDL receptor, which leads to low or absent hepatic clearance of LDL cholesterol from the circulation. Genetic alterations that cause a virtually complete absence of LDL-receptor expression (null homozygotes) result in higher

LDL cholesterol levels than alterations that partially reduce LDL-receptor activity with either two non-null alleles or one null and one non-null allele (non-null homozygotes) [11].

Fredrickson type III hyperlipoproteinemia, also known as dysbetalipoproteinemia (HLP3) is the second most common monogenic dyslipidemia, with an estimated prevalence of 1 in 1000 to 1 in 2500 individuals that is characterized by excess cholesterol-enriched, triglyceride-rich lipoprotein. HLP3 in individuals who are homozygous for a common recessive allele APOC2 via the substitution of cysteine for arginine at position 158. These mutations lead to defective removal of chylomicron remnants and VLDL, promoting premature atherosclerotic cardiovascular disease [12].

Familial type IV and type V hypertriglyceridemia may have some overlapping phenotypes. In type IV or familial endogenous hypertriglyceridemia, triacylglycerol, specifically VLDL concentrations, is increased, even on a regular diet, and HDL is usually decreased. This disorder appears to be autosomal dominant and relatively frequent in populations consuming high-fat diets. The precise molecular defect(s) has not been identified; however, the increase in triacylglycerol is associated with overproduction of triacylglycerol by the liver and often with the subsequent reduced clearance. Precocious atherosclerosis, abnormal glucose tolerance, and atheroeruptive xanthoma may occur. The disorder is undoubtedly heterogeneous, and the phenotype is strongly influenced by environmental factors, particularly carbohydrate and ethanol consumption [13].

Type V hyperlipidemia, also known as combined hyperlipidemia, is a much rarer disorder associated with increased susceptibility to atherosclerosis. VLDL concentrations are high, and chylomicrons are present in the fasting states. this is

mainly caused by mutations in APOA5. APOA5 plays a role in stabilizing the APOC2-LPL complex, which is needed to hydrolyze VLDL and chylomicrons [14].

1.2.2.2. Secondary (Acquired hyperlipidemia)

It is acquired because it is caused by another disorder like diabetes, glomerular syndrome, chronic alcohol intake, hypothyroidism and with use of drugs like corticosteroids, beta blockers and oral contraceptives. Secondary hyperlipidemia combined with significant hypertriglyceridemia can cause pancreatitis [15].

Secondary dyslipoproteinemias may mimic primary forms of hyperlipidemia and can have similar consequences. They may result in increased predisposition to premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to the development of pancreatitis and other features of the chylomicronemia syndrome. Diabetes mellitus and use of drugs such as diuretics, beta blockers, and estrogens are commonly encountered causes of secondary dyslipoproteinemia [9]. Other conditions leading to acquired hyperlipidemia include hypothyroidism, renal failure, nephrotic syndrome, alcohol usage, and some rare endocrine and metabolic disorders. When secondary and familial forms of hypertriglyceridemia coexist, triglyceride removal mechanisms may be saturated and marked hypertriglyceridemia with fasting chylomicronemia might ensue. Treatment of the underlying condition, when possible, or discontinuation of the offending drugs usually leads to an improvement in the hyperlipidemia. Specific lipid-lowering therapy may be required in certain circumstances [16].

Table (1- 1) Fredrickson classification for hyperlipidemia [17]

Hyperlipo-proteinemia	Synonyms	Defect	Increased lipoprotein	Symptoms	Treatment
Type I	Familial hyperchylomicronemia Familial apoprotein CII deficiency	Decreased lipoprotein lipase (LPL) Altered ApoC2 LPL inhibitor in blood	Chylomicrons	Acute pancreatitis, lipemia retinalis, xanthomas, hepatosplenomegaly	Diet control
Type II	Familial hypercholesterolemia	LDL receptor deficiency	LDL	Xanthelasma, arcus senilis, tendon xanthomas	Bile acid sequestrants, statins, niacin
	Familial combined hyperlipidemia	Decreased LDL receptor and increased Apo B	LDL and VLDL		Statins, niacin, fibrate
Type III	Familial dysbetalipoproteinemia	Defect in Apo E2 synthesis	IDL	Tuboruptive xanthomas and palmar xanthomas	Fibrate, statins
Type IV	Familial hypertriglyceridemia	Increased VLDL production and decreased elimination	VLDL	Can cause pancreatitis at high triglyceride levels	Fibrate, niacin, statins
Type V		Increased VLDL production and decreased LPL	VLDL and chylomicrons		Niacin, fibrate

The most common causes of acquired hyperlipidemia are given below .

- Diabetes Mellitus
- Use of drugs such as diuretics, β-blockers and estrogens.
- Alcohol consumption.
- Some rare endocrine disorders and metabolic disorders.
- Hypothyroidism
- Renal failure
- Nephrotic syndrome

Major primary and secondary forms of hyperlipidemia, their lipoprotein abnormalities and drugs used for their treatment are listed in table 2 and table 3.

Table (1- 2) Common forms of primary hyperlipidemia [7].

Disorder	Lipoprotein abnormality	Drug therapy
Familial hypercholesterolemia	↑↑LDL	Lovastatin
Familial defective apolipoprotein B	↑↑LDL	None
Polygenic hypercholesterolemia	↑LDL	Lovastatin
Familial lipoprotein lipase deficiency	↑Chylomicrons	Nicotinic acid
Familial hypertriglyceridemia	↑VLDL	Gemfibrozil
Familial combined hyperlipidemia	↑VLDL, ↑LDL, ↓HDL	Nicotinic acid, clofibrate
Familial dysbetalipoproteinemia	↑Chylomicrons, ↑LDL, ↓IDL, ↓HDL	Gemfibrozil

Table (1- 3) Common forms of secondary hyperlipidemia [18].

Condition	Lipid abnormalities	Lipoprotein abnormalities
Diabetes mellitus	↑TG	↑VLDL, ↓HDL
Nephrotic syndrome	↑Chol	↑LDL
Uremia	↑TG	↑VLDL, ↓HDL
Hypothyroidism	↑Chol	↑LDL
Obstructive liver disease	↑Chol	↑Lp(a)
Alcoholism	↑TG	↑VLDL
Oral contraceptive	↑TG	↑VLDL, ↓HDL
β-Adrenergic blocking agents	↑TG	↑VLDL, ↓HDL
Isotretinoin	↑TG	↑VLDL

1.3. Complications of Hyperlipidemia

1.3.1. Atherosclerosis

It is a common disorder and occurs when fat, cholesterol, and calcium are deposited in the arterial linings. This deposition results in the formation of fibrous plaques. A plaque normally consists of three components:

1) atheromas, which is a fatty, soft, yellowish nodular mass located in the center of a larger plaque that consists of macrophages, which are cells that play a role in immunity;

2) a layer of cholesterol crystals.

3) calcified outer layer. Atherosclerosis is the leading cause of cardiovascular disease [19].

1.3.2. Coronary Artery Disease (CAD)

Atherosclerosis is the major cause of CAD. It is characterized by the narrowing of the arteries that supply blood to the myocardium and results in limiting blood flow and insufficient amounts of oxygen to meet the needs of the heart. The narrowing may progress to the extent that the heart muscle would sustain damage due to lack of blood supply. Elevated lipid profile is correlated to the development of coronary atherosclerosis [20].

1.3.3. Myocardial Infarction (MI)

MI is a condition which occurs when blood and oxygen supplies to the cardiac arteries are partially or completely blocked, resulting in damage or death of heart cells. The blockage is usually due to the formation of a clot in an artery. This condition is commonly known as heart attack. The studies show that one-fourth of survivors of myocardial infarction were hyperlipidemic [21].

1.3.4. Angina Pectoris

Angina is not a disease but a symptom of an underlying heart condition. It is characterized by chest pain, discomfort, or squeezing pressure. Angina occurs because of a reduction or a lack of blood supply to a part or the entire heart muscle. Poor blood circulation is usually due to Coronary heart disease (CHD) when partial or complete obstruction of the coronary arteries is present [22].

1.3.5. Ischemic stroke or Cerebrovascular Accident (CVA)

It occurs when blood circulation in part of the brain is blocked or diminished. When blood supply, which carries oxygen, glucose, and other nutrients, is disrupted, brain cells die and become dysfunctional. Usually, strokes

occur due to blockage of an artery by a blood clot or a piece of atherosclerotic plaque that breaks loose in a small vessel within the brain. Clinical trials revealed that lowering of LDL and total cholesterol by 15% significantly reduced the risk of first stroke [23].

1.4. Lipid Metabolism

Lipid metabolism can be divided into two basic pathways: the exogenous pathway, which is associated with the metabolism of exogenous (dietary) lipids Figure 1, and the endogenous pathway, which is associated with the metabolism of endogenously produced lipids [24]. Exogenous pathway the first step in dietary lipid metabolism is digestion. Dietary lipids that reach the intestine duodenum then undergo emulsification, then hydrolyzed by the pancreatic and intestinal lipases. Hydrolysis products (mainly free fatty acids and monoglycerides) are then transferred to the intestinal epithelial cell, where they diffuse through the epithelial cell membranes into the intestinal mucosal cells. In the intestinal mucosal cell, free fatty acids and monoglycerides reassemble to form triglycerides, which then combine with phospholipids, free and esterified cholesterol [25].

Chylomicrons are the lipoprotein class responsible for transfer of dietary lipids. After formation in the enterocytes, chylomicrons, which mainly contain triglycerides, are secreted into the lacteals, and enter first the lymphatic and later the blood circulation [26]. Lipoprotein, which is exposed on the chylomicron surface, activates the lipoprotein lipase attached to the capillary beds in adipose and skeletal muscle tissues, which then hydrolyzes triglycerides into free fatty acids and glycerol. Free fatty acids enter the muscle cells (where they are used for energy production) and/or adipocytes (where they are re-esterified into triglycerides for storage) [27]. The cholesterol-rich remaining particles (chylomicron remnants), return to HDL and are recognized by specific hepatic

receptors that rapidly remove them from the circulation by endocytosis [28]. The cholesterol found in chylomicron remnants can be used for lipoprotein (VLDL) and/or bile acid formation or stored as cholesteryl esters [29].

Endogenous pathway while chylomicrons are responsible for transport of dietary lipids, VLDL, LDL and HDL is mainly involved in the metabolism of endogenously produced lipids. Endogenously synthesized TGs, and cholesterol and cholesteryl esters. Lipoprotein species VLDL, LDL, HDL. Classification and properties of major human plasma lipoproteins. Lipoproteins Major metabolic function chylomicrons structural, IDL, LDL, Lp (a): lipoprotein abide with phospholipids to form VLDL. After VLDL molecules reach the vasculature. VLDL activates lipoprotein lipase located in the capillary beds, which in turn leads to hydrolysis of VLDL triglycerides and the production of free fatty acids and glycerol [30]. The VLDL molecules remaining after hydrolysis of VLDL triglycerides (VLDL remnants) are either removed from the circulation by the liver or undergo further transformation by lipoprotein lipase and/or hepatic lipase to form LDL. LDL which contains mainly cholesteryl esters and phospholipids, circulates in the blood and binds to specific receptors that are widely distributed throughout tissues in order to deliver cholesterol, which can be used for the synthesis of steroid hormones and cell membranes as well as for hepatic metabolism [31].

HDLs have a critical role in the reverse cholesterol transport pathway; it is well known that a new attempt to reduce the absorption of free fatty acids is by delaying triglyceride digestion with the inhibition of pancreatic lipase. Pancreatic cholesterol esterase plays a pivotal role in hydrolyzing dietary cholesterol esters. The hydrolysis of cholesterol esters in the lumen of the small intestine is catalyzed by pancreatic cholesterol esterase, which liberates free cholesterol [32].

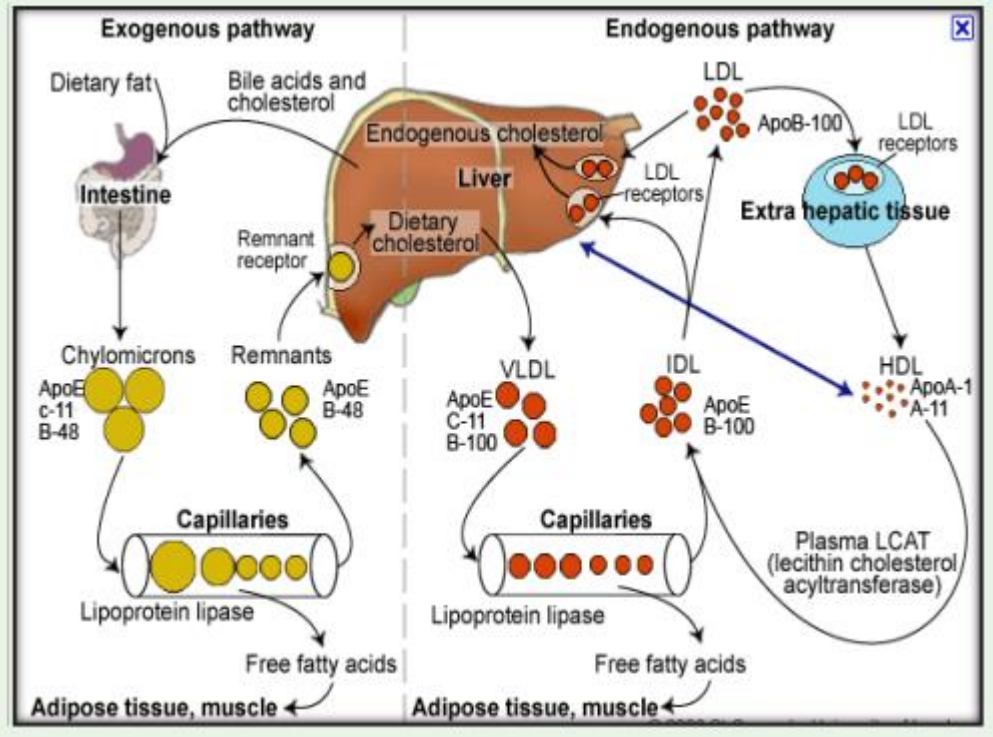


Figure (1- 3) The lipid metabolism can be divided into two basic pathways : the exogenous pathway and endogenous pathway [33].

1.5. Management

1.5.1. Inpatient Management

Pancreatitis is a well-documented complication of severe hypertriglyceridemia occurring in 1% to 4% of patients with pancreatitis, and mild to moderate elevation in serum TG levels are found in one-third of all patients with acute pancreatitis. Severe hypertriglyceridemia with concentrations of TGs greater than 1,000 mg/dL (11.3 mmol/L) with abdominal pain/pancreatitis require hospital admission and aggressive medical management, which can include initiation of lipid-lowering medications in addition to intravenous insulin to facilitate clearance of TGs by activation of LPL [34].

Plasmapheresis is typically reserved for severe cases with additional findings of lactic acidosis, severe hypocalcemia, acute respiratory distress, and/or organ failure, where immediate reduction of serum TG levels is necessary. During hospitalization, possible causes of secondary hypertriglyceridemia, such as diabetes, medication adverse effects, poor diet or alcohol consumption, or other comorbidities, should be investigated. Patients can be discharged from the hospital when the TG level is less than 1,000 mg/dL (<11.3 mmol/L) or less than 500 mg/dL (<5.7 mmol/L) in monogenic hypertriglyceridemia. The patient should be tolerating a nonfat diet before discharge with a goal to increase to less than 10% to 15% of total calories from fat after discharge [35]. Hulgan

1.5.2 Outpatient Management (Lifestyle Modification.)

The first-line therapy for an elevated lipid profile remains recommending a healthy lifestyle, which includes dietary modification, improving body weight, avoiding tobacco smoking or, if smoking, beginning smoking cessation, and 30 to 60 minutes of daily physical activity with moderate to vigorous intensity. Although dietary modifications remain under debate, decreasing intake of total, saturated, and trans fats may have a lipid-lowering effect, particularly on TG levels, and is a current recommendation despite modest declines in LDL-C levels. A dietitian may be helpful in making dietary adjustments needed to reduce LDL-C levels without compromising appropriate growth and development [36]. A 5% to 10% reduction in excess weight has been shown to be beneficial for reducing CVD risk in obesity. For patients with hypertriglyceridemia, dietary fat should be restricted to less than 10% to 15%, with 30% to 40% of daily calories from protein and 50% to 60% from complex carbohydrates. The dietary fat restriction can be useful once TG levels decrease below 500 mg/dL (5.7 mmol/L). A 6-month trial of intensive lifestyle modification with weight management and exercise using a

comprehensive team has been suggested before medication use in patients with intermediate TG levels in the 150 to 499 mg/dL (1.7–5.6 mmol/L) range. In addition to dietary modifications, physical activity may be useful in increasing HDL-C values but, more importantly, may assist with weight reduction [37].

1.5.3. Pharmacologic Management

1. Statins

Statins prevent the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme. HMG-CoA is an enzyme that controls the rate of cholesterol biosynthesis in the body. Statins act by lowering LDL-C and TGs and slightly elevating HDL levels, thereby providing intermediate care for dyslipidemia management. Statins should be initiated at the minimum dose, and dosing should be adjusted to achieve a target LDL-C level of 140 mg/dL or less (£3.6 mmol/L) by increasing the dose, changing to a more potent statin, or adding another lipid-lowering drug, such as ezetimibe and resins [38].

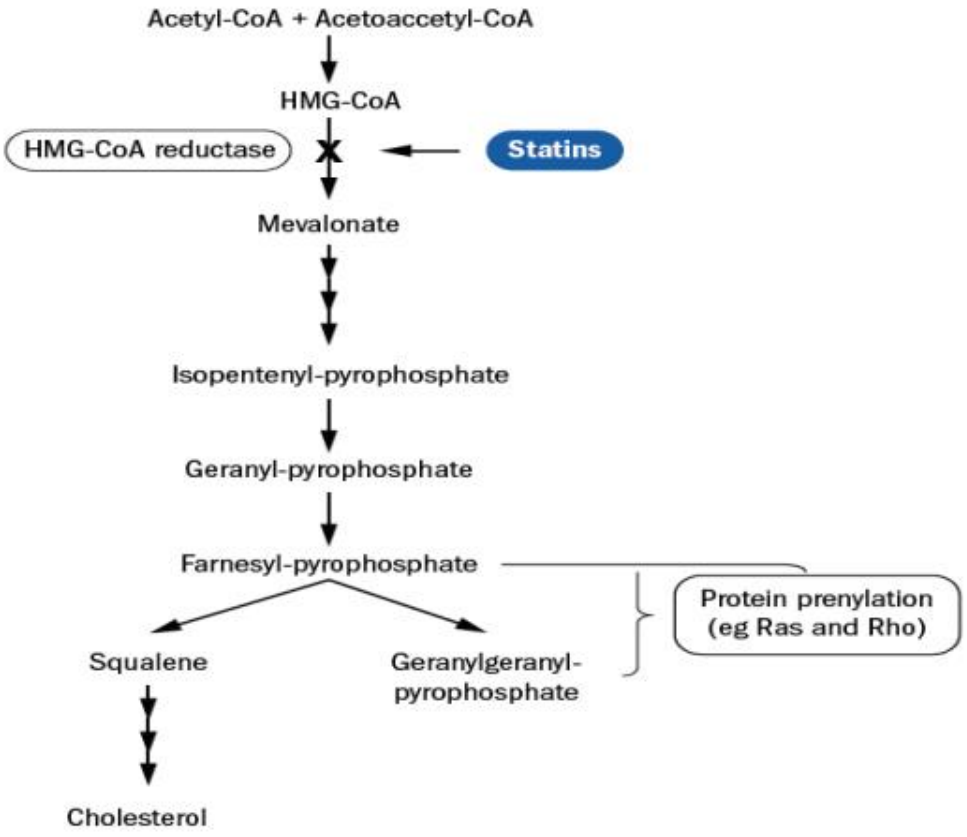


Figure (1- 4) Statins and the cholesterol synthesis pathway – the mevalonate pathway [39].

Unfortunately, statins have several side effects, such as liver damage, type 2 diabetes mellitus, and muscle pain. Fortunately, the benefit (dyslipidemia management) outweighs statin-related risks. Statins are contraindicated in pregnancy due to potential as a teratogen, so appropriate contraceptive measures and/or counseling are required in adolescent females.Statins avert smooth muscle cell relocation and spread and obstruct the stimulation of tumor necrosis factor-alpha (TNF-alpha), interleukin 1 (IL-1) beta, and other interleukins, which contribute to inflammation, a common symptom of hyperlipidemia [7]. Therefore,

these medication's side effects and costs should be weighed against the individual patient's potential benefit from taking the drug as follow:

As recommended by the AAP and the American Heart Association: SORT evidence rating system [40] :

- Patients with a high risk of ASCVD (>7.5% 10-year risk) should receive statin therapy for primary prevention: Rating B.
- Statin therapy should be initiated for secondary prevention in patients with known ASCVD, absent any contraindication: Rating A
- Niacin, fibrates, and omega-3 fatty acids should not be routinely given for primary or secondary prevention of ASCVD: Rating A
- A moderate-intensity statin plus ezetimibe should merit consideration as an alternative in patients with acute coronary syndrome who cannot tolerate high-intensity statin therapy: Rating B.
- Moderate-intensity statins include: lovastatin 40 mg, pravastatin 40 mg, simvastatin 40 mg, atorvastatin 10 to 20 mg, and rosuvastatin 5 to 10 mg
- High-intensity statins include: atorvastatin 40 to 80 mg, rosuvastatin 20 to 40 mg

Statins have been studied in large randomized controlled trials to reduce morbidity and mortality of coronary events in adults in the high-risk category, and some studies have shown reduction in LDL-C levels of 21% to 39% in pediatrics as well [41]. Higher doses of statins have also been studied, with their use resulting in a 38% to 50% reduction in LDL-C levels by some reports [42].

Although adverse effect profiles and efficiency have shown promise in short-term studies, there are no published studies on the long-term effect of statins on morbidity, mortality, and adverse effects in children, including relationship to diabetes mellitus and kidney disease. There is evidence for the use of statins in

children with FH, and statins are recommended as treatment for both types of FH. Fluvastatin and pitavastatin have been studied in children and adolescents with FH. A systematic review in 2017 of the FH literature found reductions in mean LDL-C concentrations at any time point in studies from 6 weeks to 2 years [43].

2. Bile Acid Sequestrants (Resins).

Bile acid sequestrants (resins) act to decrease LDL-C levels with primary use for familial or severe hypercholesterolemia. The adverse effects are usually gastrointestinal, including bloating abdominal discomfort, and constipation. Resins are generally considered safe and are approved for patients older than 10 years of age. However, resins can interfere with absorption of fat-soluble vitamins and folic acid, so supplementation may be needed [44]. The figure (1-5) shows mechanism of action of resins.

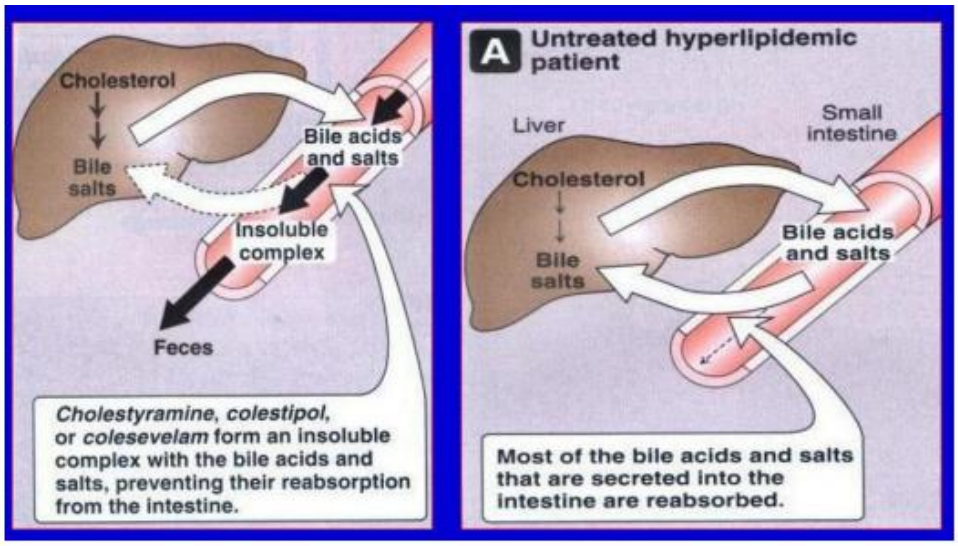


Figure (1- 5) Mechanism of action of resins[44]

3. Cholesterol Absorption Inhibitors

Cholesterol absorption inhibitors (ezetimibe) act to decrease LDL-C levels with primary use for familial or severe hypercholesterolemia. Adverse effects include gastrointestinal symptoms, hepatotoxicity, and myopathy. It is approved in the United States and Europe for patients older than 10 years and in some studies to be effective in treating HeFH. However, it is often used as an adjunct to other medications and has not been fully evaluated as monotherapy [45].

4. Fibric Acids.

Fibric acids (fenofibrate and gemfibrozil) are used to decrease LDL-C and TG levels and increase HDLC levels in hypertriglyceridemia (TG levels >600–1,000 mg/dL [>6.8–11.3 mmol/L]). There are limited data in children, but fibric acids seem to be generally well tolerated as monotherapy and may be used with caution when combining with a statin. Fibrates are first-line pharmacologic agents for severe hypertriglyceridemia despite limited data for the pediatric population [46].

5. Nicotinic Acid

Nicotinic acid (niacin) is used to decrease LDL-C and TG levels and increase HDL-C levels in familial or severe hypercholesterolemia. Common adverse effects are flushing, glucose intolerance, headaches, hepatotoxicity, and myopathy. It is not used frequently due to the potential for severe adverse effects.Flushing, caused by release of prostaglandin E2 in the skin, can be reduced by giving aspirin 15 to 30 minutes before taking the drug. However, caution should be exercised in the use of aspirin to prevent Reye syndrome. Niacin has not been fully studied in the pediatric population [47].

6. Omega-3 Fatty Acids.

The long chain omega-3 fatty acids (fish oils) are used to lower TG levels so that they may be used in severe hypertriglyceridemia as an adjunct. These work by reducing hepatic secretion of VLDL-C and enhancing chylomicron metabolism. It is more effective in adults than in the pediatric population and is not suggested as a single therapy [48].

1.5.4 Monitoring

Patients should receive follow-up testing of serum aspartate aminotransferase, alanine aminotransferase, creatine kinase, and lipid levels 1 month after initiating therapy Table (1-4). Once the dose is stable, testing should continue every 3 to 6 months while monitoring for adverse effects such as growth abnormalities or secondary sexual characteristics. On high-dose statins, fasting plasma glucose and/or glycated hemoglobin levels should be measured because statins have been found to increase the risk of new-onset diabetes in adults [49].

Table (1- 4) Drugs used to treat hyperlipidemia [50].

DRUG	MECHANISM	ADVERSE EFFECTS	MONITORING	SPECIAL CONSIDERATIONS
Statins	Block cholesterol synthesis	Myopathy	LFT, CK	Contraception in adolescent females as teratogenic
Bile acids	Bind with cholesterol	Diarrhea, constipation	None	Can reduce absorption of fat-soluble vitamins
Cholesterol absorption inhibitors	Block absorption	GI adverse effects, myopathy	None	Not approved in children but can be used if necessary
Fibrates	Decreasing production of VLDL and clearing triglycerides	GI adverse effects	None	Can cause muscle damage when used with statins
Nicotinic acid	Antilipolytic effect	Flushing, glucose intolerance, myopathy	LFT	Flushing can be reduced by giving aspirin 15–20 min before

CK=creatine kinase, GI=gastrointestinal, LFT=liver function test, VLDL=very low-density lipoprotein.

1.6 Sex and age effect on hyperlipidaemia

The original observations on the responsiveness of plasma lipids to dietary fat and cholesterol made over 25 years ago by Keys and associates, were restricted to men, but the quantitative relation developed from these results has been freely generalized to women as well. However, men and women have differences in the distribution of lipoprotein lipids as well as some enzymes that control lipoprotein metabolism [51]

In women the activity of hepatic lipase, one of the most important determinants HDL cholesterol levels, is about 60-70% of the activity in men. Before puberty, HDL cholesterol is similar in boys and girls but then falls in boys and remains unchanged in girls. This fall in HDL cholesterol level is probably related to the activation of hepatic lipase activity by testosterone [52].

Low density lipoprotein (LDL) cholesterol level is lower in women, and this is due to the increased activity of the LDL receptor induced by estrogens. The gender-related difference in HDL cholesterol level persists throughout adult life, whereas the lower LDL cholesterol level found in women disappears after menopause. Estrogens regulate lipid metabolism in the liver by increasing the hepatic synthesis of LDL receptor, resulting in decreased LDL circulating levels. Estrogens also increase the activity of the enzyme lipoprotein lipase, raising HDL levels. There is some evidence that women experience greater changes in HDL cholesterol concentration than do men, but this finding has not been consistent. Although there are large interindividual differences in response to both dietary cholesterol and fatty acids, the response of groups is reasonably predictable [53].

1. 7 Electrolytes

It is impossible to overstate the significance of proper measurement of electrolytes like Na^{+}, K^{+}, Ca^{++}, etc. in clinical practice. Therefore, one of the most common diagnostic procedures in a medical context is the measuring of electrolytes in the blood. Although analysis of electrolytes like sodium and potassium have long been conducted in the clinical chemistry laboratory, they are increasingly being performed at the point-of-care [54].

Pseudohyponatremia may occur when serum water is displaced by higher quantities of serum lipids or proteins due to a measurement error. Two of the most frequent approaches of detecting sodium levels (indirect potentiometry and flame photometry) require diluting the sample, which might result in inaccurately low sodium results and lead to a condition known as pseudohyponatremia. This might lead to spurious electrolyte diseases, when blood electrolyte values are erroneously high or low compared to what they should be [55].

The third technique, direct potentiometry, does not need diluting the sample and seems to be immune to the effects of hyperlipidemia and hyperproteinemia on sodium determinations. Direct ISE relies on a sample of whole blood or plasma that has not been diluted in any way. Nonaqueous and water fractions make about 7 and 93 percent, respectively, of serum volume., respectively [56].

The sole occurrence of sodium in the body is in the watery phase of serum. The direct ISE uses an undiluted sample from a blood gas machine, whereas the indirect ISE requires preanalytical serum dilution, which is performed in most high-throughput labs. Both devices use essentially the same methodology to test electrolytes. Rather of gauging electrolyte content in plasma (mmol/L), this method assesses plasma water activity (mmol/kg H2O) [54].

A constant (ion-specific) multiplier converts the electrochemical activity of the ions in the water to the readout concentration. The solids content of a sample

has no effect on the results obtained using a direct ion selective electrode (ISE). Some research has shown a difference in outcomes between direct and indirect ISE. When comparing the findings of direct ISE with indirect ISE, however, one research showed a significant discrepancy. Different protein concentrations in the samples were shown to be the root cause of the errors in the sodium concentration estimates, which was the primary focus of the research. Protein levels that begin to affect electrolyte measurement were not specified [57, 58].

However, there is a dearth of research on the reliability of electrolyte measurements using direct ISE when an abnormal lipid or protein concentration is present, and most of the time, the error in measurements in hyperlipidemia or hyperproteinemia is associated with the use of an indirect method involving dilution rather than undiluted serum [59].

1. 7.1 Sodium

Sodium represents more than 90 percent of cations in the extracellular fluid (its concentration is 140 mmol/l in ECF and 3–35 mmol/l in ICF, depending on type of tissue). That is why sodium determines the osmotic pressure together with corresponding anions. As a result, the amount of sodium is responsible for the volume of extracellular fluid. As there exists a balance between extra and intracellular fluid, any change of Na+ concentration causes the shift of water between cells and extracellular environment. Sodium has an important role in the maintenance of acid-base balance. The concentration of sodium determines the number of needed bases. The presence of Na+ is important for the maintenance of normal membrane potentials and normal cardiac function [60].

1. 7.2 Chlorides

Daily food intake of chlorides is 140–260mmol. Their total amount in organism is around 1400 mmol. Elimination of chlorides via urine is only a bit lower than their intake, because nearly 10 mmol Cl^-/day is excreted by sweating. Nearly the same amount is excreted by stool. Chlorides are distributed in ECF, forming its main anion. Plasma Cl^- concentration is around 100 mmol/l. The shift of chlorides between ICF and ECF appears in pathological conditions, such as heart failure and disturbances of acid base balance. The concentration of chlorides in organism is regulated similarly as sodium concentration. In opposite, chlorides are not resorbed actively in kidneys, but along the electrochemical gradient of Na+. Chlorides are resorbed actively only in ascendent arm of Henley's loop [61].

1. 7.3 Potassium

The daily food intake of potassium ranges between 30–80 mmol. The resorbed K+ is distributed in ECF in a low concentration (4.5 mmol/l). Despite this fact, the concentration of K^+ in ICF is high and variable in different tissues. Potassium concentration in ICF ranges between 100–160mmol/l. About 90 percent of potassium in organism is excreted by urine and 10 per cent is excreted by stool. These values can be variable, for example the amount of K+ in stool considerably increases in diarrhea. To maintain the K^+ balance in the organism intact kidneys is necessary. Potassium is the main intracellular cation. Nearly all processes in cells are depended on gradients of potassium and natrium on both sides of the cellular membrane [62].

1. 7.4 Magnesium

Chlorophyll is the most important source of daily magnesium requirement. Magnesium is resorbed in the upper part of intestine, and is excreted via bile and large intestine, and to a less extent via urine. The same carrier assists in absorption of both magnesium and calcium from the intestine. So, a low calcium intake stimulates the reabsorption of magnesium and vice versa. The parathyroid hormone increases magnesium resorption from intestine, whereas calcitonin has an opposite effect. Magnesium is an important cofactor for many enzyme systems participating in the metabolism of glycides and in muscle contraction. It activates plasma and bone alkaline phosphatase, inhibits calcification and modulates the neuromuscular excitability [63].

1.8 Relationship between Hyperlipidemia and trace element and Electrolytes

Among cardiovascular diseases, ischemic heart diseases (IHD) are the leading cause of death worldwide. In 2017, IHD affected about 126 million individuals, with an estimated prevalence of 1655 per 100,000. However, the incidence rate of IHD is decreasing worldwide. The discrepancy between IHD prevalence and incidence is likely explained by improved treatment of patients with IHD, hence the improved survival rate. IHD is strongly linked to hyperlipidemia. It is possible to considerably lower the incidence of IHD-related mortality and morbidity by the treatment and prevention of hyperlipidemia [64].

Hepatotoxicity has been reported as adverse effect in individuals using a number of lipid-regulating drugs, including atorvastatin, simvastatin, fluvastatin, pravastatin, and rosuvastatin. On the other hand, lipophilic statins may passively diffuse across the cell membrane and enter the liver without being actively taken up by a carrier protein, but hydrophilic statins must be taken up by a carrier protein and so lack hepatoselectivity. active acid forms of [65]. Numerous

enzymes, which may play a crucial role in preventing atherosclerosis, need copper, zinc, and selenium as part of their functional groups [66].

The mineral chromium (Cr) is essential for maintaining healthy glucose and cholesterol levels. Cr was assumed to reduce cardiovascular disease risk factors such as increasing levels of total blood cholesterol, LDL cholesterol, and serum triacylglycerols; lipid abnormalities and chronic inflammation play essential roles in the onset and development of atherosclerosis. Chrome picolinate is an easily absorbable and effective chromium supplement (CrPic) [67].

Some of the present epidemic of IHD may be attributable to widespread copper deficiency. Consumption of copper has been on the decline, and it seems that a sizable proportion of the population does not even eat the RDA for copper3 (0.9 mg per day) (2.6 mg per day). So, it seems like a lot of people might be getting less than they need of copper. Many of the dangers or symptoms of inflammatory heart disease (IHD) may be traced back to copper's involvement in several biological processes, and a lack of copper can have serious consequences. Transcriptional regulators, chaperones, oxidoreductases, mitochondrial electron transport, and free radical scavenging are only a few examples of copper-dependent proteins. Copper's role in the immune system is also crucial [68].

Glucose intolerance, hypercholesterolemia, irregular electrocardiogram (ECG), hyperuricemia, and hypertension are all risk factors for ischemic heart disease (IHD), just as they are in animals with copper deficiency. Copper insufficiency sheds new insight on the lipid theory of cardiovascular disease [69].

Inadequate copper levels make lipoproteins (LDL, HDL, VLDL) more vulnerable to oxidation. Lipoproteins from copper-deficient animals generate more thiobarbituric acid reactive compounds when subjected to oxidative processes involving iron, suggesting that copper may provide a protective effect against iron-induced oxidation. Copper is a part of enzymes that protect against oxidative

modification, such as copper, zinc superoxide dismutase, and may also catalyze the oxidation of lipoproteins [70].

It has been suggested that increased lipoprotein oxidation may be due to changes in the composition of lipoprotein components result from a drop in copper-dependent antioxidant enzyme levels. It has not been thoroughly defined how copper controls cholesterol homeostasis, but animal studies provide some insights. Diet-induced copper deficiency increases hepatic glutathione (GSH) levels and subsequently increases the activity of cardiac hydroxy methyl glutaryl-coenzyme A (HMG-CoA) reductase, which controls the rate-limiting step of cholesterol biosynthesis [71].

Independent of contemporaneous changes in lipid parameters, short-term therapy with atorvastatin is linked with a considerable decrease in the erythrocyte Na^+/Li^+ CT activity, BP levels, and insulin resistance in individuals with hypercholesterolemia, with or without essential hypertension. However, the most plausible explanation for a possible inverse connection is that lipid-lowering medicines, by preserving glomerular filtration rate, also reduce the reduction in capacity for electrolyte free water clearance, hence decreasing the risk of fluid retention and hyponatremia [72].

The effect of statins on the electrolyte level including sodium, potassium, and chloride has been investigated in only a few of studies. However, results are inconsistent. Therefore, more extensive investigations are needed to evaluate the effect of a class-agent regimen for management of hypertriglyceridemia (which has been shown to effectively decrease CVD risk) on electrolyte activity, and determine whether a greater dose may result in any alterations to the electrolyte balance.

Aim of the study

1 - Investigate the level of some trace element after administration of atorvastatin drug in patients with hyperlipidemia.

2 - Investigate the level of some serum electrolyte after administration of atorvastatin drug in patients with hyperlipidemia.

3 - Existing the statistical relation with all the values with hyperlipidemia patients.

Chapter Two

Material and Methods

2.1. The instruments and equipment

The instruments and equipment used in this study were shown in table (2-1).

Table (2- 1) Instruments and equipment used in the study.

NO	Instruments and equipment	Company/Country
1	Spectrophotometer	Cecil7200
2	Gel tube	AL Rawan
3	Centrifuge	Japan
4	Freezer	Japan
5	Eppendorf tube (1.5ml)	China
6	Micropipettes (100-1000)	Germany
7	Pipettes tips (0.02-ml)	China
8	Refrigerator	Ashtar / Iraq
9	Disposable syring (5 ml)	China
10	Distillator	GFL/Germany
11	Fully Auto Biochemistry Analyzer	Selectra ProXL/Netherlands
12	Electrothermal atomizers (ETAs)	Japan

2.2. Chemicals

All the chemicals and the standard kits utilized in the present study were shown in table (2- 2).

Table (2- 2) Chemical used in the study.

1	Cholesterol Kit	Giesse /Italia
2	Triglycerides Kit	Giesse /Italia
3	LDL Cholesterol Kit	Giesse /Italia
4	HDL Cholesterol Kit	Giesse /Italia
5	Sodium Kit	Giesse /Italia
6	Potassium Kit	Giesse /Italia
7	Chloride Kit	Giesse /Italia
8	Magnesium Kit	Biosam/UAE
9	Zinc Kit	Biosam/UAE
10	Copper Kit	Biosam/UAE
11	Chromium Kit	Biosam/UAE
12	Selenium Kit	Biosam/UAE

2.3. Patients

This study was carried out on 50 patients suffering from hyperlipidemia our patients include both male and female, female 27 (54 %) and male 23 (46%). Healthy control was chosen in this study include both male and female carried out on 50 healthy controls.

These healthy control and patients aged from (20-65) years and they admitted to Al-Emam Al-sadik hospital between October to December 2022., sample size was calculated by sample size equation with the aim of the community health department in the college. A questionnaire was designed to obtain the

information from patients and control group, which included name, age, gender, BMI history and the presence of chronic diseases, as shown in table (2-3)

Table (2- 3) Patient in information

Name:			**Age:**	
Height:	Weight:		BMI:	
Gender: **Male:**		**Female:**	Family history of disease:	
Treatment:			Clinical history of hyperlipidemia:	
Other disease:				
Lipped profile	Ch:	Tg:	HDL:	LDL
Electrolyte:	Na:	K:	CL:	Mg:
Trace element	Copper:	Zinc:	Cr:	Selenium:

2.4. Exclusion Criteria

1-Alcoholic cirrhosis of liver with ascites

2-pregnant women

3-sever kidney disease

4-hypertensiv patients

2.5. Blood samples collection and storge

Three to five milliliters of blood were obtained from hyperlipidemia patients and control, then collected in tube without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged for 10 minutes. Then the sera were aspirated and stored at (-20 ºC) until time of use.

2.6. Calculation of BMI

Body mass index is calculated by dividing weight (Kg) by height square (m^2), (BMI=Kg/ m^2) [73].

- BMI < 18kg/m^2 is underweight.
- BMI 19-24.9 kg/m^2 is normal.
- BMI 25 - 29.9kg/m^2 is overweight.
- BMI 30 - 34.9 kg/m^2 is class I obesity
- BMI 35 - 39.9 kg/m^2 is class II obesity.
- BMI 40 kg/m^2 and above is Class III obesity.

2.7. Methods

2.7.1. Spectrophotometric Determination of serum Triglyceride Concentration

1. Principle [74]

Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to produce glycerol and free fatty acids. The glycerol participates in a series of coupled enzymatic reactions. in which glycerol kinase (GK) and glycerol phosphate oxidase (GPO) are involved and H_2O_2 is generated. The Hydrogen peroxide reacts with TOOS and 4-AAP to form a colored complex, whose color intensity is directly proportional to the concentration of triglycerides in the sample.

2. Sample

Serum, obtained from patients fasted for at least 10-12 hours. Do not use highly hemolyzed or icteric samples. Store samples at 2-8°C before analysis, do not store samples at room temperature as phospholipids may hydrolyze, releasing free glycerol and falsely elevating triglycerides value. Freeze the sample if not tested within 24 hours.

Table (2-4) Kit components for Determination of serum Triglyceride Concentration.

Reagent(A) **Volume** **50/100/250/1000ml**	**Good buffer** **Magnesium chloride** **ATP** **4-AAP** **TOOS** **LPL(lipoprotein lipase)** **POD(peroxidase)** **GK(glycerol kinase)** **GPO**	**100mmol/l** **15mmol/l** **4 mmol/l** **1 mmol/l** **0.1mmol/l** **2500 U/L** **1800 U/L** **1000 U/L** **5500 U/L**
Standard **Volume =10 ml**	Glycerol	200 mg/dl (2.28 mmol/l)

The reagents are stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not freeze. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided. Keep bottles closed when not in use.

3. Reagents Preparation

Liquid Reagent, bring to room temperature (15-25°C) before use. The light color of the reagent (< 0.050 O.D.) due to air or light does not affect their operation.

Table (2- 5) Procedure for Determination of serum Triglyceride Concentration

Wavelength	**546 nm (520-570)**
Light path	1 cm
Temperature	37°C
Reading	against blank reagent
Method	Increasing End Point
Sample/Reagent	1/100

Pipette	**Blank**	**Sample**	**Standard**
Reagent(A)	1000µl	1000µl	1000µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mix, incubate at 37 °C for 5 minutes, read against blank reagent absorbance of the sample (Ax) and the standard (As). Volumes can be proportionally modified; this method describes the manual procedure to use the kit.

4. result Calculation

Triglycerides mg/dl = Ax/As x 200 (standard value)

5. Expected values

Serum/plasma <200 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

2.7.2. Spectrophotometric Determination of serum cholesterol Concentration

1. Principle [75]

Esterified cholesterol is hydrolysed into free cholesterol and fatty acid by cholesterol esterase (CHE). Cholesterol oxidase (CHOD) oxidizes the free presence of peroxidase (POD), hydrogen peroxide reacts with a derivative of phenol and 4-aminoantipyrine to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the cholesterol into cholestene-3-one with formation of hydrogen peroxide. In sample.

4. Sample

Serum, avoid samples with high concentrations of ascorbic acid. Cholesterol in the sample is stable 3 days at 2-8°C and one month at -20°C.

Table (2- 6) Kit components for Determination of serum cholesterol Concentration.

Reagent (A) **Volume =** **50/100/250/1000 ml**	**Buffer** **4-AAP** **CHE** **CHOD** **POD** **Derivative of phenol**	**100 mmol/l** **1 mmol/l** **300 µ/l** **300 µ/l** **1500 µ/l** **1 mmol/l**
Standard **Volume =5 ml**	Cholesterol Sodium aside	200 mg/dl 14 mmol/l

The reagents are stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not use over expiry date. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided. Do not freeze. Keep bottles closed when not in use. A slight colour of the reagent (less than 0.040 O. D) due to air or light does not affect its operation.

5. Reagent preparation

Liquid Reagent, bring to room temperature (15-25°C) before use.

Table (2- 7) Procedure for Determination of serum cholesterol Concentration.

Wavelength	**510 nm (500-520)**
Light path	1 cm
Temperature	37°C
Reading	against blank reagent
Method	Increasing End Point
Sample/Reagent	1/100

Pipette	**blank**	**Sample**	**Standard**
Reagent(A)	1000 µl	1000 µl	1000 µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mix, incubate at 37°C for 5 minutes, and read against blank reagent the absorbance of the sample (Ax) and the standard (As).

Reaction volumes can be proportionally varied. This method describes the manual procedure to use the kit. For automated procedure, ask for specific applications.

6. Results calculation

Serum/plasma:

Cholesterol mg/dl = Ax/As x 200 (standard value)

7. Expected values

Serum, plasma:

Low Risk: < 200 mg/dl

Moderate Risk: 200 -240 mg/dl

High Risk: >240 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

2.7.3. Spectrophotometric Determination of serum HDL Concentration

2.7.3.1. Intended use.

Quantitative determination of HDL Cholesterol in serum and plasma.

2.7.3.2 Principle

Specific polyanions in the first phase block the interfering lipoproteins (LDL, VLDL and chylomicrons) and a specific surface-active agent inhibits the coloration of VLDL, LDL and chylomicrons in the second phase. The intensity of color produced is directly proportional to the HDL cholesterol in the sample.

2.7.3.3 Sample

Fresh serum. Centrifuge and collect serum as soon as possible. Avoid samples with high concentrations of ascorbic acid. HDL in the sample is stable 3 days at 2-8°C and one month at -20°C.

Table (2- 8) Kit components for Determination of serum HDL Concentration.

Reagent (A) **Volume = 90 ml**	**Good Buffer** **Polianions** **4-AAP**	**100 mmol/l** **1 mmol/l** **4 mmol/l**
Reagent (B) **Volume =30 ml**	Cholesterol esterase Cholesterol oxidase Peroxidase HDAOS Detergent	800 U/L 500 U/L 1500 U/L 1 mmol/l 4 mmol/l

The reagents are stable until the expiration date indicated on the label if stored at 2-8°C and protected from light. Do not use over expiry date. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided. Do not freeze. Keep bottles closed when not in use.

2.7.3.4 Reagents preparation

Liquid Reagents, bring to room temperature (15-25°C) before use. Reconstitute the Calibrator (included in the kit Ref. 0026T) with 3.0 ml of distilled water.

Table (2- 9) Procedure for Determination of serum HDL Concentration.

Wavelength	**600 nm**
Light path	1 cm
Temperature	37°C
Reading	against blank reagent
Method	Increasing End Point
Sample/Reagent	1/100

Pipette	**Blank**	**Sample**	**Calibrator**
Reagent (A)	300 µl	300 µl	300 µl
Water	4 µl		
Sample		4 µl	
Calibrator			4 µl
Reagent (B)	100 µl	100 µl	100 µl

Mix, incubate at 37°C for 5 minutes, read the absorbance of blank sample (Abx) against blank reagent, then add reagent (B) and Mix, incubate at 37°C for 5 minutes and read the absorbance sample (Ax) and the calibrator (Ac) against blank reagent.

Volumes can be proportionally modified. This method describes the manual procedure to use the kit.

2.7.3.5 Results calculation

HDL (mg/dl) = (Ax - Abx)/ (Ac - Abc) x Calibrator Value

mg/dl x 0.02586= mmol/l

For the calculation of LDL cholesterol use the following formula:

$$\textbf{LDL (mg/dl)} = \text{Cholesterol} - \left(\text{HDL} + \frac{\text{Triglycerides}}{5}\right)$$

2.8 Quantitative determination of magnesium in serum, plasma .

2.8.1. Principle

With xylidyl-blue dye, magnesium forms a blue-violet complex, whose color intensity is proportional to the magnesium concentration in the sample.

2.8.2. Sample

Serum,Avoid hemolyzed samples. Remove serum from clot as soon as possible. Urine/24h adjusted to pH 3+4 with hydrochloric acid. Dilute the urine sample 1:5 with distilled water and multiply the result by 5.

Table (2- 10) Kit components for determination of magnesium in serum .

Reagent (A) Mg **Volume 50/100 ml**	**Good buffer** **Xylidyl blue** **EDTA**	**100Mm** **0.20Mm** **0.15Mm**
Standard Mg **Volume =10 ml**	Magnesium solution	2mEq/l (2.5 mg/dl)

The reagents are stable until the expiration date indicated on the label if stored at 15-25°C and protected from light. Do not use over expiry date. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided. Do not freeze. Keep bottles closed when not in use.

2.8.3. Reagent preparation

Liquid Reagent, ready to use.

Table (2- 11) Procedure for determination of magnesium in serum.

Wavelength	**512 nm (500-520)**
Light path	1 cm

Temperature	25,30, 37°C
Reading	against blank reagent
Method	Increasing End Point
Sample/Reagent	1/150

Pipette	**Blank**	**Sample**	**Standard**
Reagent (A)	1500 µl	1500 µl	1500 µl
Water	10 µl		
Sample		10 µl	
Standard			10 µl

Mix, incubate at 25, 30, 37°C for 3 minutes. Read the absorbance of the standard (As) and the sample (Ax) against blank reagent.

Volumes can be proportionally modified. This method describes the manual procedure to use the kit.

2.8.5. Results calculation

Serum, plasma:

Magnesium mg/dl = Ax/As x 2.5 (standard value)

2.8.6. Expected values.

Serum/plasma: 17-25 mg/dl

Urine: 50-150 mg/24h

Each laboratory should establish appropriate reference intervals related to its population.

2.9. Quantitative determination of Chlorides in serum.

2.9.1. Principle

The chloride ions present in the sample react with mercuric ions, releasing the same quantity of thiocyanate ions which, in the presence of ferric ions, forms a

red colored complex. The color intensity is proportional to the chloride concentration present in the sample.

2.9.2 Sample

Serum, Chlorides in the sample are stable up to 7 days at 2-8°C and one month at -20°C.

Table (2- 12) Kit components for determination of Chlorides in serum, plasma and urine.

Reagent (A) Cl **Volume 100 ml**	**Mercury thiocyanate** **Mercury nitrate** **Iron nitrate** **Nitric acid**	**25 mmol/l** **0.2 mmol/l** **25 mmol/l** **60 mmol/l**
Standard **Volume =100 ml**	Sodium Chloride Sodium azide	100 mEq/l (100 mmol/l) 14 mmol/l

The reagents are stable until the expiration date indicated on the label it stored at 15-25°C and protected from light. Do not use over expiry date. Once opened reagents are stable for 2 months if contamination is avoided. Keep bottles closed when not in use.

2.9.3. Reagent preparation

Liquid Reagent ready to use.

Table (2- 13) Procedure for determination of Chlorides in serum

Wavelength	**480nm (450-500)**
Light path	1 cm
Temperature	25,30, 37°C

Reading	against blank reagent
Method	Increasing End Point
Sample/Reagent	1/100

Pipette	**Blank**	**Sample**	**Standard**
Reagent (A)	2000 µl	2000 µl	2000µl
Water	20 µl		
Sample		20 µl	
Standard			20 µl

Mix, incubate at 25, 30, 37 °C for 5 minutes. Read the absorbance of the sample (Ax) and the standard (As) against the blank reagent. The reaction volumes can be proportionally modified. This method describes the manual procedure to use the kit.

2.9.4. Results calculation

Serum, plasma: Chloride mEq/l = Ax/As x 100 (Standard Value)

Urine 24/h: Chloride mEq/l = Ax/As x 100 x 2 (dilution factor) x Urine Vol. 24/h

2.9.5. Expected values.

Serum/plasma: 95-110 mEq/L , Urine: 160 - 250 mEq/24h

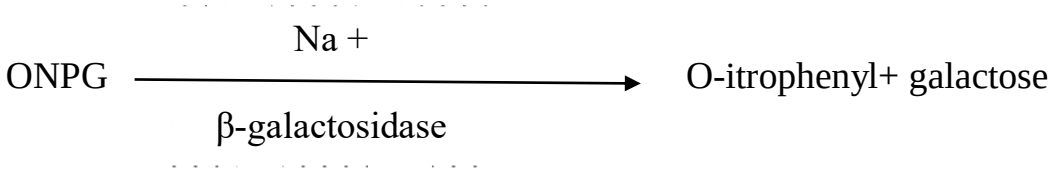
Each laboratory should establish appropriate reference intervals related to its population.

2.10. quantitative determination of sodium in serum

The Enzymatic Sodium method was developed using the specificity of the sodium-dependent ß-D-galactosidase, being a handful alternative to flame photometry and ion-selective electrode (ISE) methodologies, which require specific equipment. [76].

2.10.1. Principle

Sodium is determined enzymatically via sodium dependent β-galactosidase activity with ONPG as the substrate. The absorbance at 405 nm of the product O-nitrophenyl is to the sodium concentration.



ONPG = o-nitrophenyl-8-D-galactopyranoside

2.10.2 Sample

Serum.

Table (2- 14) Kit components for determination of sodium in serum.

Reagent (A) Sodium **Volume = 40 ml**	**Good's Buffer pH B5** **Encrypting (> 0.4 mM), B-D-galactosidase (< 8 U/ml),** **Proclin 300 (0.02%)**
Reagent (B) Sodium **volume = 20 ml**	"Good's Buffer - pH 65 O.niropheny B-0-glycoside (> 0.5 mM), Proclin 300 (0.02%)
low Calibrator '	Buffered Sodium

Volume = 1 ml	batch-specific value indicated on the bottle)
High Calibrator **Volume = 1 ml**	Buffered Sodium (Batch-specific value indicated on the bottle)

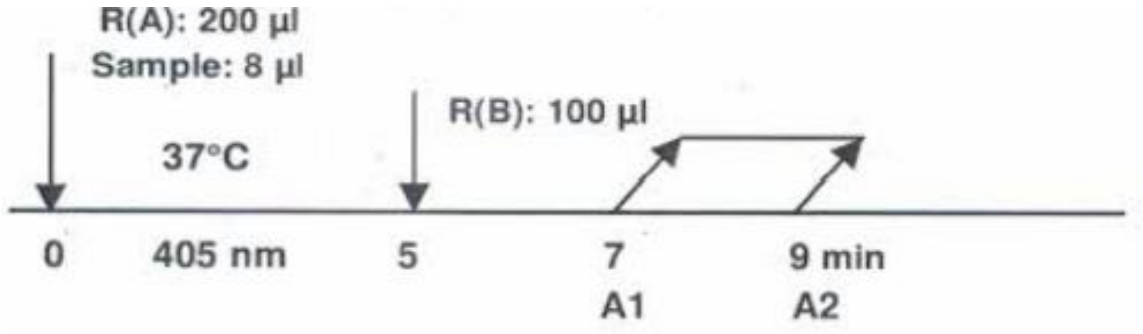
Reagents and calibrators are stable until the expiration date indicated on the label if stored at 2-8°C. Do not use over the expiry date. Once opened reagents are stable for 2 months if contamination is avoided. Keep bottles closed when not in use.

2.10.3. Reagent preparation

Liquid Reagents, ready to use.

2.10.4. Procedure

Diagram of the test to automated chemistry analyzers:



2.10.5. Calibration

For the calibration s recommended 10 Use the calibrators High and Low. Itis advisable to perform a 2-point calibration every week. This test uses a linear calculation and a blank reagent.

2.10.6. Reference range

136-146 mmol/L (313-336 mg/dl)

Each laboratory should establish appropriate reference intervals related to its population.

2.11. Quantitative determination of potassium in serum.

Measurements obtained by this assay are used to monitor electrolyte balance in the diagnosis and treatment of diseases conditions characterized by low or high blood potassium levels.

2.11.1 Principle

Potassium is determined spectrophotometrically through a kinetic coupling assay system using potassium dependent pyruvate kinase. Pyruvate generated is converted to lactate accompanying conversion of NADH analog to NAD analog. The corresponding decrease of optical density at 380 nm is proportional to the potassium concentration in the serum.

2.11.2.Sample

Non-hemolyzed serum

It is advisable to collect the samples 50 you can test them as soon as possible and within five days from samples collection.

Table (2- 15) Kit components for determination of potassium in serum.

Reagent (A) Potassium Volume = 2 x 40 ml	**LDH (< 50KUIL), Analog (< 10 mmol/L), NADH, Substrate, Azide(0.05 96), Stabilzers.**
Reagent (B) Potassium volume = 2x10 ml	Pyruvate kinase (< 50 KUJL), Azide (0.05 %), Stabilizers
low Calibrator ' Volume = 1 -2 ml	Potassium (batch-specific value indicated on the bottle)
High Calibrator Volume = 1-2 ml	Potassium (Batch-specific value indicated on the bottle)

Reagents and calibrators are stable until the expiration date indicated on the label if stored at 2-8°C. Do not use over the expiry date. Once opened reagents are

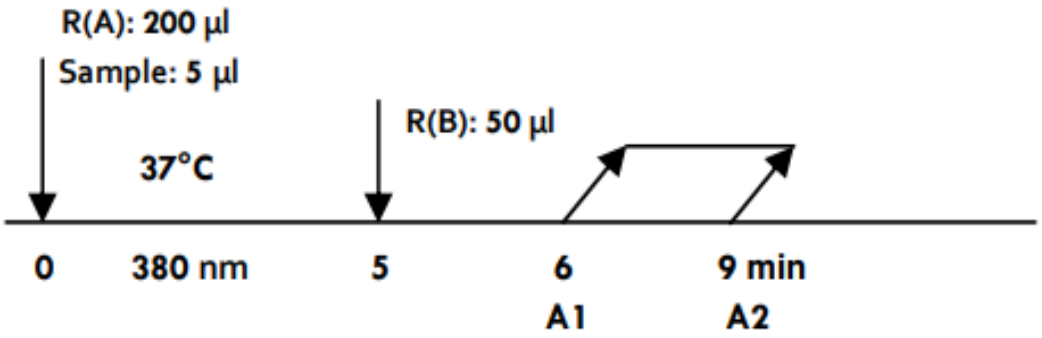
stable for 2 months if contamination is avoided. Keep bottles closed when not in use .

2.11.3. Reagent preparation

Liquid Reagents, ready to use.

2.11.4. Procedure

Diagram of the test to automated chemistry analyzers:



2.11.5. Calibration

For the calibration you must use the standards high and low for potassium supplied. The concentration of the potassium in the sample is determined by a linear calibration curve using the standards high and low.

In the case of analyzers that require a zero calibration, it is possible to use a saline solution for this purpose and use standard high and low potassium provided as calibrators respectively 2 and 3. It is advisable to calibrate weekly.

2.11.6. Reference range

The normal level of potassium in human serum is in a range between 3.5 – 5.1 mmol/L (13.7 - 19.9 mg/dl), Each laboratory should establish appropriate reference intervals related to its population.

2.12 Quantitative measurement of elements (Cr, Cu, Se, Zn) in serum

2.12.1. Principle:

Flameless Atomic absorption spectrophotometer method by the technique of Graphite Furnace (GFAAS) was used to determine the trace element (Magnesium). SHIMADZU AA7000 Atomic Absorption Spectrophotometer was used for determination of these elements. GFAAS is one of the most important of the five techniques of the atomic absorption spectrometry in which has the higher sensitivity which can be reach to the low detection limits (in ppb units). This technique is also called Electro thermal Atomic Absorption Spectrometry (ETAAS) which is a type of spectrometry that uses a graphite furnace tube to vaporize the sample in three stages, drying, ashing, and atomizing. The fact of this technique is based on that free atoms of element absorb light produced from the specific cathode lamp at specific wavelengths characteristic of the interest element. Within certain limits, the amount of light absorbed reflect the concentration of analyst present and can be linearly correlated to this concentration. Most elements can produce free atoms from samples by the application of high temperatures. In GFAAS, very small number of samples (10µL-20µL) is injected in small graphite or paralytic carbon coated graphite tube, which can then be heated by a wide range of temperature to vaporize and atomize the analyst. The atoms absorb the electromagnetic radiation in the ultraviolet or visible region resulting in transitions of electrons to higher electronic energy levels to the excited state and then back to the ground state by emitting its specific characteristic light which can be measured to determine the samples concentrations. The temperature of the Graphite tube increases over a matter of seconds and can reach up to 3000ºC depending on the element being analyzed.

2.12.2 Preparation of Standard Solutions

Original standard solutions were (1000µg/ml in 2% HNO3) for interested elements. Four standard solutions were prepared by dilution from original standard stock solution using general dilution law (C1 V1 = C2 V2). It must prepare a series of concentrations from the highest one reaching the values required for calibration curve performs. Series of concentrations which prepared were as follows: - ϖ (1000µg/ml → 100µg/ml → 10µg/ml → 1µg/ml). The value 1µg/ml (1 ppm) is equal to 1000ng/ml (1000 ppb), so complete preparation from this solution reaching the required concentration of the standard calibration curve as follow: - ϖ (1000ng/ml → 100ng/ml → prepare the needed concentration of any element).

2.12.3. Sample Preparation

Samples were digested by transferring 5ml of whole blood (in EDTA Tube) and then addition of 5 ml of (10% TCA). Both volumes mixed well in vortex for (15 min). Centrifugation for 10 min at 3000 RPM. Digested sample solutions were filtered and then appropriate solution volume of (20µL) was injected into the graphite furnace tube for reading.

2.12.4. Determination of serum Chromium

Four standard solutions of the element were prepared as previously mentioned and described in paragraph. The four standards were (1, 2, 3, 4 ng/ml) for calibration curve as shown in figure (2-1). The concentrations of chromium in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Chromium Determination: Listed in table (2-16). Lamp current 10 mA Wavelength 357.9 nm Slit width 0.5 nm Lighting mode BGC-D2 Sample Size 20 µl Replicates 3

Table (2- 16) ideal condition for chromium determination

Variable	Ideal condition

Lamp current	10 Ma
Wavelength	357.9 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

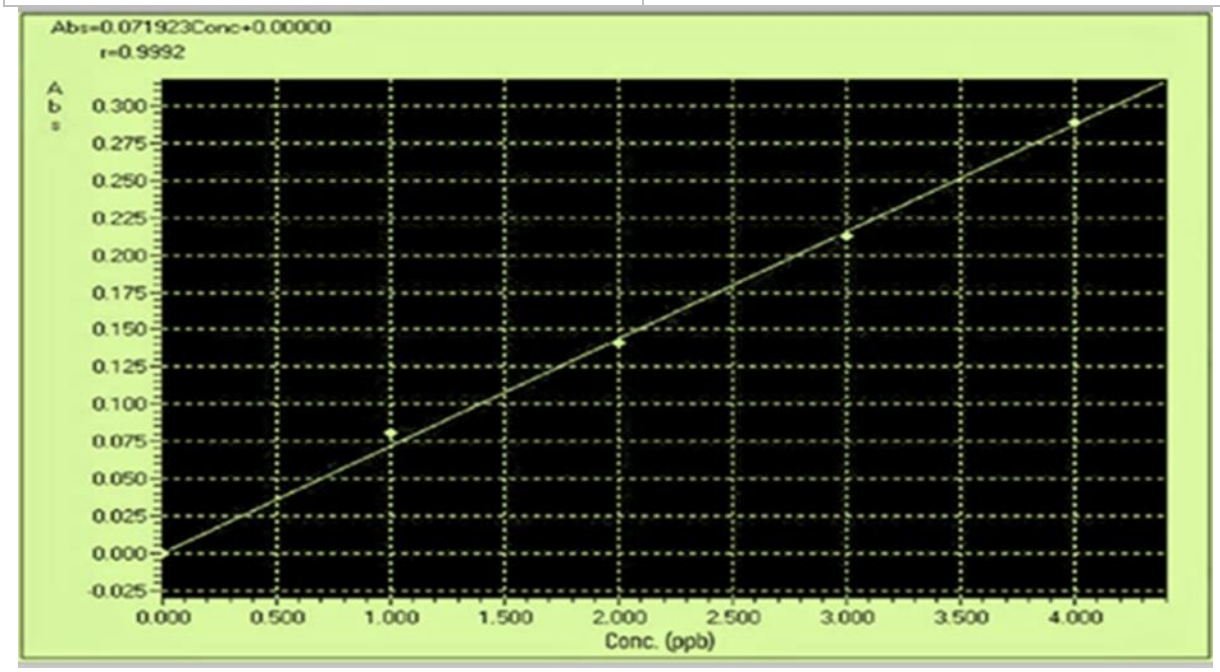


Figure (2- 1) Standard curve for magnesium determination.

2.12.5. Determination of serum Copper:

Four standard solutions of the element were prepared as previously mentioned and described in paragraph (2-2). These standards were (1, 2, 3, 4 ng/ml) for calibration curve as shown in figure (2-2). The concentrations of copper in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Copper Determination [77]: Listed in table (2-17) .

Table (2- 17) Ideal Conditions for Copper Determination.

Variable	Ideal condition

Lamp current	6 mA
Wavelength	324.8 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

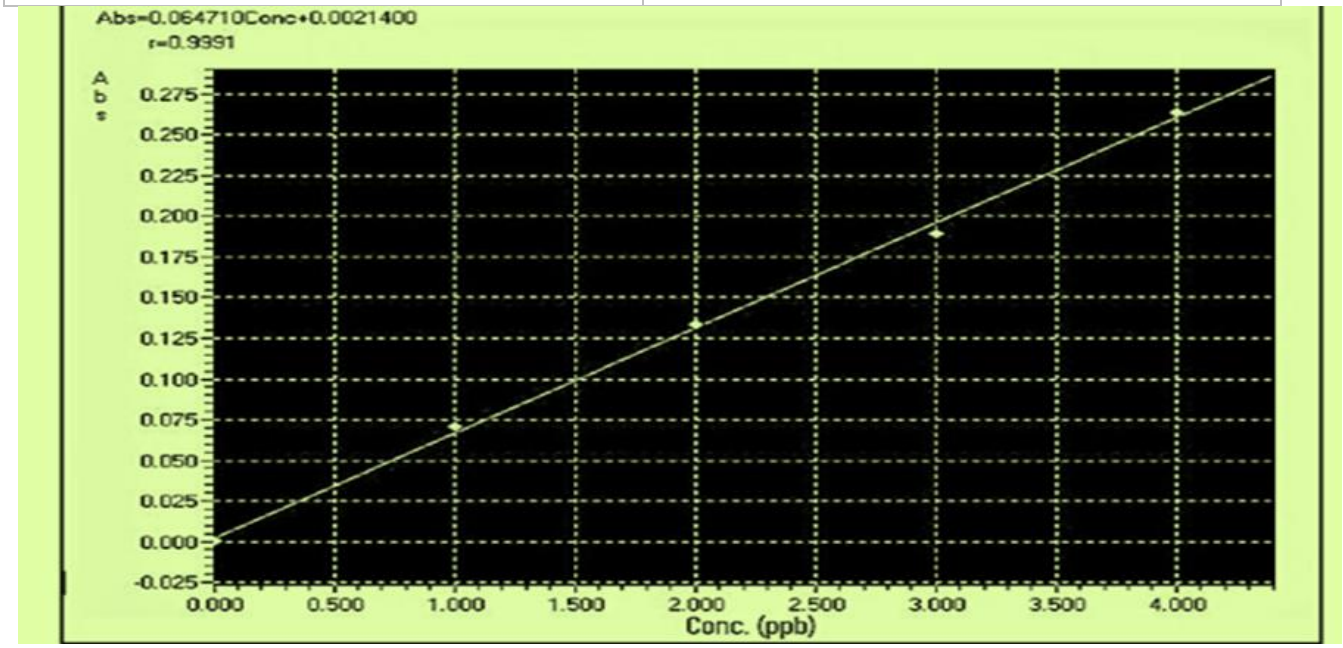


Figure (2- 2) Standard curve for copper determination

2.12.6. Determination of serum Selenium

Four standard solutions of the element were prepared as previously mentioned and described in paragraph (2-3). These standards were (2, 4, 6, 8 ng/ml) for calibration curve as shown in figure (2-3). The concentrations of Selenium in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Selenium Determination [78]: Listed in table (2-18)

Table (2- 18) Ideal Conditions for Selenium Determination.

Variable	Ideal condition
Lamp current	23 mA

Wavelength	196 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

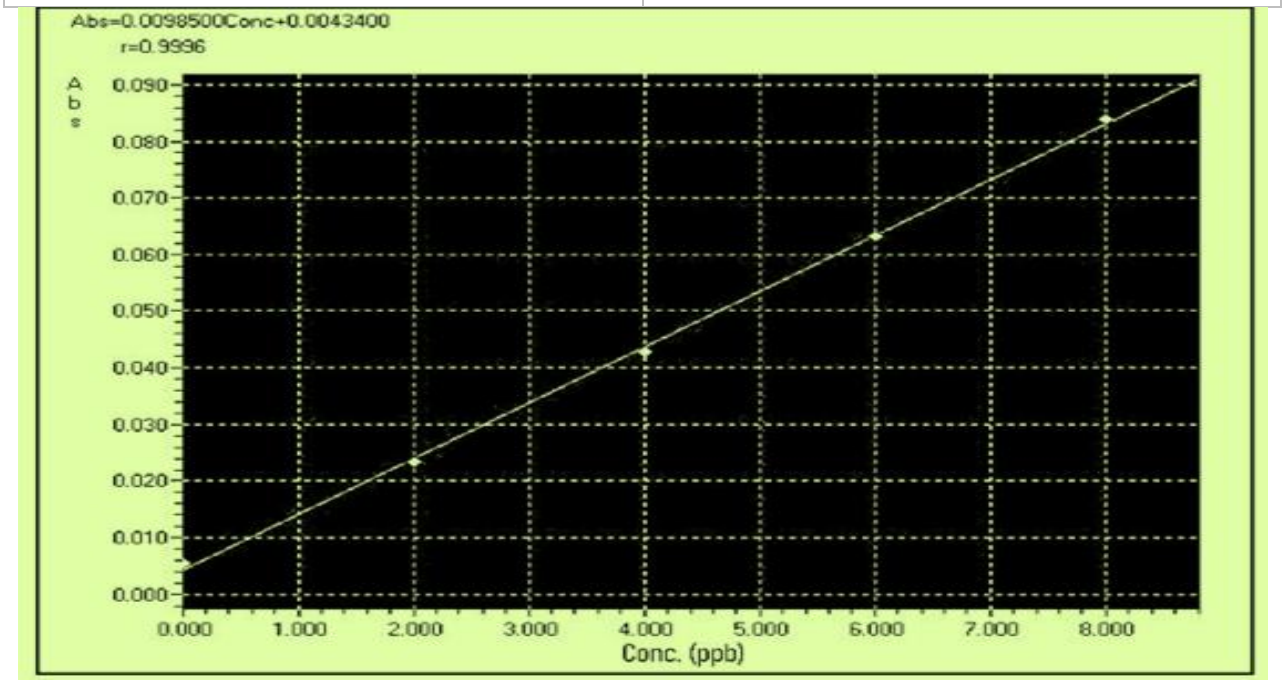


Figure (2- 3) Standard curve for selenium determination

2.12.7. Determination of serum Zinc:

Four standard solutions of the element were prepared as previously mentioned and described in paragraph (2-4). These standards were (0.5, 1, 1.5, 2 ng/ml) for calibration curve as shown in figure (2-4). The concentrations of zinc in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve. Conditions for Zinc Determination [79]: Listed in table (2-19)

Table (2- 19) Ideal Conditions for Zinc Determination.

Variable	Ideal condition
Lamp current	8 mA
Wavelength	213.9 nm

Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

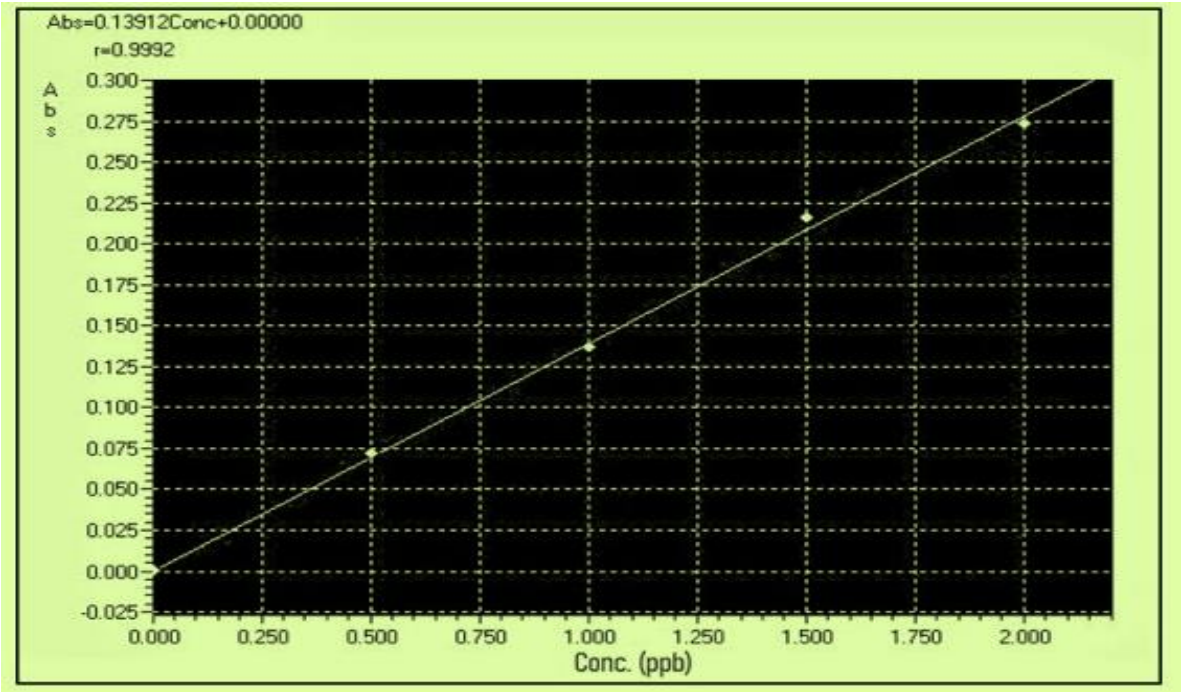


Figure (2- 4) Standard curve for zinc determination

2.12. Statistical Analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. Paired t-test was used to compare means for two paired readings. A p-value of ≤ 0.05 was considered as significant.

Chapter Three

Results and Discussion

3.1 patient information

3.1.1. Age, Sex, and BMI in hyperlipidemia patient

This study was carried out on 100 subjects whose age ranged (20-65) years, 50 healthy subjects as a control group and 50 hyperlipidemic patients.

Table (3-1) and figure (3-1) shows the distribution of patients and controls according to age. The mean age in patients with hyperlipidemia group is (43.28± 13.18) year. The mean age in control group is (42.46.8 ± 14.08) year; the results show no significant differences ($p > 0.05$) in age between control group and patients group as demonstrated in Table 3-1

This matching of age between patients and control is important to eliminate any effects on the results that may arise from the difference in these characteristics.

Table (3- 1)The general characteristics of patients and controls according to study

Study variables	Patients (N=50) Mean ± SD	Controls (N=50) Mean ± SD	P value
Age (range 20-65)	43.28± 13.18	42.46.8 ± 14.08	0.764
BMI (kg/m^2)	26.7± 2.0	23.2± 2.47	0.001

Table(3-1) and figure (3-2) show the deference between patients and controls" according to BMI. Our results showed that patients had mean BMI (26.7± 2.0) and control mean BMI is (23.2± 2.47), this means that hyperlipidemia patient have higher BMI than controls. In accordance with our results, Fentoğlu et al. (2009) evaluated the possible association between periodontal disease and an increased risk for cardiovascular disease among hyperlipidemic patients and reported that BMI were significantly higher in the hyperlipidemic group compared to the control subjects, and suggested a relationship between plasma cholesterol

levels and BMI [80]. Diet and physical exercise are other important factors in lipid metabolism. Therefore, lifestyle changes have been suggested (e.g., diet, physical activity, and smoking status).

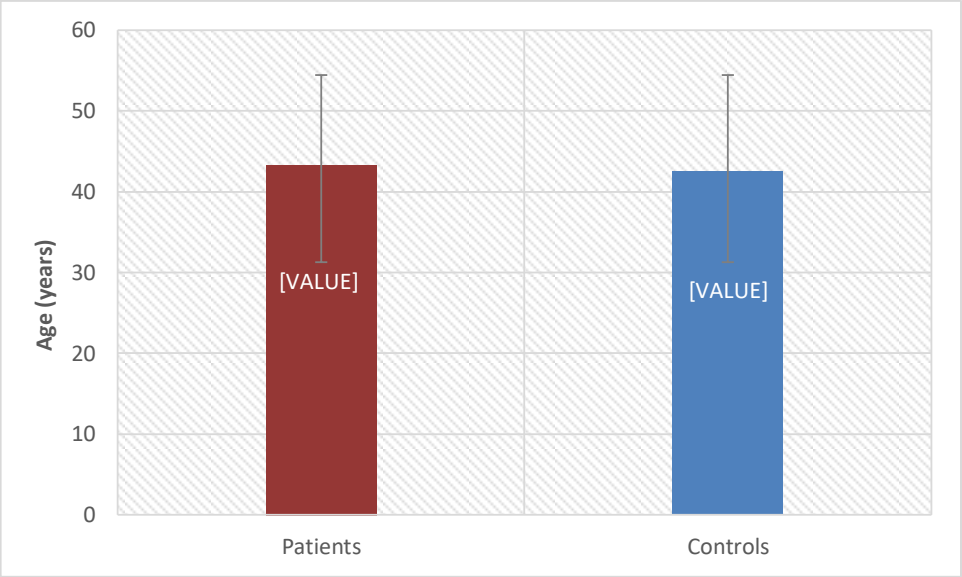


Figure (3- 1) The mean differences of age according to study group (P>0.05)

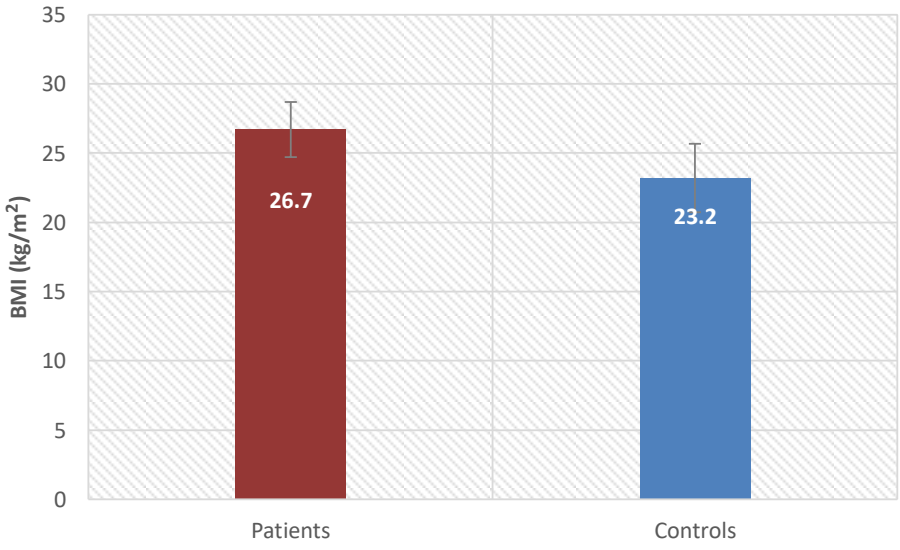


Figure (3- 2) The mean differences of BMI according to study group (P<0.001*)

3.1.2. Sex Distribution in patients and control groups.

Among fifty patients with hyperlipidemia who contributed to this study, there were 23 males and 27 females, and this represents 46% and 54% of patients respectively, as shown in figure (3.3). This means that sex may affects the diagnosis of disease and treatment. Also, there are studies that agree with this, Soriano-Li et al. (2020) [81] revealed that women are more susceptible to disease, but they have less effect on treatment. There are studies that prove that gender has nothing to do with the diagnosis of disease or the effect on treatment [82].

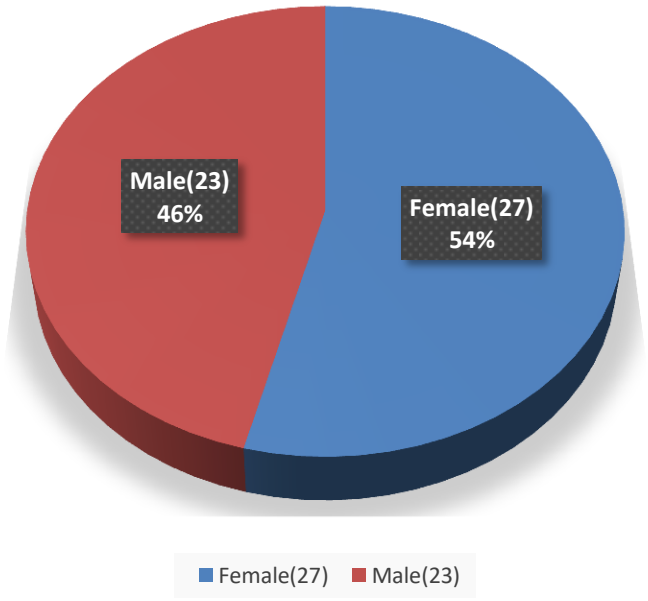


Figure (3- 3)The distribution of gender in study group.

There is worrisome evidence highlighted a disparities in healthcare delivery between women and men. It states that women with the same CVD risk as men may be less likely to receive dyslipidemia diagnosis, treatment, intensified treatment, or recommendations for prevention. These disparities that result from gender inequalities can lead to differences in health outcomes [83].

3.2. Biochemical Parameters.

3.2.1: The mean differences of biochemical parameters according to studied groups

In this study, the lipid profile (cholesterol, triglyceride, HDL, LDL, and VLDL) were carried out in the studied groups. Our results showed that the mean value of total cholesterol is 236.6± 39.3 mg/dl in patients group, and 171.8± 20.0 mg/dl in the control group. The mean value of triglyceride is 217.6 ± 43.3 mg/dl After treatment , and 101.4 ± 36.13 mg/dl in control group. The mean value of HDL is 54.0 ± 12.0 mg/dl After treatment , and 59.1± 10.4 mg/dl in control group. The mean value of LDL is 123.1± 53.6 mg/ After treatment , and 121.7± 28.69 mg/dl in control group. The mean value of VLDL is 43.4 ± 20.9 mg/dl After treatment, and 24.6 ± 6.38 mg/dl in control group. Cholesterol, triglyceride, and VLDL were significantly lowered after administration of atorvastatin, HDL was significantly raised after administration of atorvastatin. While there is no significant difference between the studied groups according to LDL level as shown in table (3-2).

Table (3- 2) The mean differences of biochemical parameters.

Parameter	Group	N	Mean ± SD	t-test	P – value
CHO(mg/dL)	Patients	50	236.6± 39.3	10.5	0.001*
	Control	50	171.8± 20.0		
TG(mg/dL)	Patients	50	217.6 ± 43.3	14.2	0.002*
	Control	50	101.4 ± 36.13		
HDL(mg/dL)	Patients	50	54.0 ± 12.0	- 2.0	0.043
	Control	50	59.1± 10.4		
LDL(mg/dl)	Patients	50	123.1± 53.6	0.19	0.866
	Control	50	121.7± 28.69		
VLDL (mg/dl)	Patients	50	43.4 ± 9.7	10.8	0.001*
	Control	50	24.6 ± 6.38		

*P value ≤ 0.05 was significant

At the end of the three-month intervention period, the study showed that atorvastatin has a promising effect on cholesterol, triglycerides, and VLDL. It could significantly reduce cholesterol by 27 %, triglycerides by 53 %, and VLDL by 43% compared to the baseline group (P-value: 0.001, 0.002, and 0.001 respectively). On the other hand, atorvastatin significantly raised HDL by 9 %. However, atorvastatin didn't show any beneficial effect on LDL in our work.

Our finding is similar to that reported by Shuhaili et al. (2017) who studied the effects of different statins on lipid profile in Asians patients with hypercholesterolemia. This study demonstrated that atorvastatin has a significant effect on the reduction of cholesterol and triglycerides levels. Moreover, the study also showed a significant elevation effect on HDL. However, atorvastatin showed the lowest effect on HDL compared to all other statins [84].

Also, in agreement with our results McCrindle et al. (2003), determined the safety and efficacy of atorvastatin in children and adolescents with severe hypercholesterolemia. According to authors, administration of atorvastatin (10-20 mg/d) for 6 months is effective and safe for 3the treatment of elevated lipid levels and caused a highly significant reduction in both triglycerides (12%; $P = 0.03$) and cholesterol (32% ; $P < .001$), with a significantly greater increase in HDL with atorvastatin (2.8% ; $P = 0.02$) [85].

In contrast to our results, several studies had stated that atorvastatin has a significant effect on reduction of LDL levels. Gupta et al. (2009) evaluated the lipid-modifying effects of statins in patients with CHD. The results showed that atorvastatin 20 mg reduced LDL-C by 43% in both South Asian and by 41% in Caucasian [86].

Statins have become the most widely lipid-lowering drugs in patients with hyperlipidemia. Simvastatin, pravastatin, lovastatin, fluvastatin, and atorvastatin are approved for treatment of hypercholesterolemia in humans: they block the conversion of HMG-CoA to mevalonic acid with consecutive attenuation of the biosynthesis of cholesterol. Indeed, in recent years a substantial quantity of data has accumulated showing that statins exert various effects on multiple targets, which are independent of their plasma cholesterol lowering properties [87]. The effect of statins on lipid profile levels varies from one person to another and depends on the presence of other diseases, such as hypothyroidism or type 2 diabetes, as well as on lifestyle and the percentage of taking the dose of this treatment [88].

3.2.2. The mean differences of electrolyte according to study group including (patients and control group).

Table 3- 3 showed that there is a significant decrease in the sodium and magnesium levels with $P < 0.001$ in patient as compared to control. However, our results didn't show any significant difference between patients and controls as regard to potassium($P = 0.51$) or chlorine ($P= 0.80$).

Table (3- 3) The mean differences of electrolyte according to study group.

Parameter	Group	N	Mean ± SD	t-test	P – value
Na(mmol/L)	Patients	50	136.9 ± 5.3	- 5.06	0.001*
	Control	50	141.3 ± 2.7		
K(mmol/L)	Patients	50	4.2 ±.39	0.66	0.508
	Control	50	4.2 ±0.33		
CL(mmol/L)	Patients	50	100.0 ± 4.6	0.25	0.80
	Control	50	100.2 ± 1.97		
Mg(mmol/L)	Patients	50	1.98 ±.21	- 5.2	0.001*
	Control	50	2.8 ± 1.16		

*P value ≤ 0.05 was significant

The lipid-lowering agents slow the decline in capacity for electrolyte free water clearance, thus reducing the risk of fluid retention and hyponatremia. Several studies paid attention to investigate the effects of atorvastatin on glomerular filtration rate, tubular function, and blood pressure and reported that short-term treatment with atorvastatin has been shown to decrease fractional urinary excretion of sodium in individuals increased tubular reabsorption of sodium [72, 89].

In line with our results, Heydari et al. (2016) evaluated the potential benefit of atorvastatin in electrolytes imbalances in 44 patients and found that the patients' serum calcium an potassium remained unchanged after treatment with atorvastatin ($P>0.05$) [90].

In contrast with our results, Sharma et al. (2017) studied if cholesterol lowering drugs affect electrolyte balance in - hypertensive patients and reported that administration of atorvastatin result in a significant elevated k+ levels and a significant lowered chloride compared to control group ($p<0.01$). However, Sodium levels did not show any significant changes compared to control group [91].

3.2.3. The mean differences of trace elements according to study group including (patients and control group).

There is no enough studies that interested with investigation the effect of either atorvastatin or lipid lowering drugs (statin) on chromium, copper, selenium, and zinc level in hyperlipidemic patients. Our study is the first work that aimed to evaluate the impact of atorvastatin on Cr, Co, Se, and Zn.

Table 3- 4 and figure 4-5-6-7 showed that there is a significant decrease in Cr, Se, and Zn levels in patients group compared to control group with ($P < 0.001$). on the other hand, there is a significant increase in Cu level in patients group compared to control group with ($P < 0.001$). The decrease in chromium, selenium, and zinc levels after atorvastatin administration suggests an impaired effect of statins on these trace elements in the body, it would prevent the absorption of these elements. However, This copper elevation means that the copper is not affected by the treatment, the mechanism still unknown.

Table (3- 4) The mean differences of trace elements according to study.

Parameter	Group	N	Mean ± S.D	t- test	P -value
Cr (mg/dl)	Patients	50	0.31± 0.10	-27.5	0.001*
	Control	50	0.87 ± 0.076		
Cu (mg/dl)	Patients	50	202.5 ± 14.46	37.0	0.001*
	Control	50	101.1 ± 11.99		
Se (mg/dl)	Patients	50	34.9 ± 23.1	- 18.8	0.001*
	Control	50	119.3 ± 21.6		
Zn (mg/dl)	Patients	50	39.3 ± 6.2	- 33.1	0.001*
	Control	50	95.4 ± 8.98		

*P value ≤ 0.05 was significant

Our study showed a decrease in zinc levels in patients' group. In agreement with our result, Al-Sabaawy, (2012) who studied the relationship between serum lipid profile and selected trace elements in 31 hyperlipidemic patients. These results indicate that there was a significant lower level of serum Zn in hyperlipidemic patients compared with the control group [92].

Also, Witwit et al., (2021) agreed with our results, the authors suggested that patients with higher lipid profile are related with lower Zn levels. Decreasing

zinc concentrations may lead to increased lipid peroxidation and increased cholesterol, triglycerides and LDL levels by the releasing of cholesterol stored in lipid droplets of adipose tissue cells during stress, and demonstrated that Zn supplementation significantly reduced cholesterol and triglycerides concentrations, and increased HDL in the bloodstream [93].

According to selenium levels, our study showed a decrease in selenium levels in patients' group. In agreement with our results, Arnaud et al. (2009) studied the effect of fibrates and statins on plasma selenium concentration and in 72 free-living dyslipidemic elderly and stated that , the statin users exhibited lower plasma selenium concentrations. Several hypothesis might explain the statin effect on plasma selenium concentrations, Se decline could be related to the inhibition of selenocystein t RNA by statins. Moreover, coenzyme Q10 depletion during statin therapy might be associated with selenium deficiency. Indeed, it has been reported that the ubiquinol-10 is regenerated by selenoenzyme [94].

Contradictory with our results, Su et al. (2015) study that aimed to determine the association between selenium level and dyslipidemia in elderly Chinese, the rsuts showed that higher selenium levels were significantly associated with higher lipid levels (High-cholesterol, High-LDL and Low-HDL) [95]. However, the associations were inconsistent in populations with low serum selenium concentration.

According to chromium levels, our study showed a decrease in chromium levels in patients' group. Previous findings have shown that chromium deficiency develops lipid metabolism disorders, while chromium supplementation could have favorable effects on lipid profile and prevent metabolic disorders [94]. Furthermore, some studies described associations between dietary intakes of chromium with high free fatty acid levels and the risk of dyslipidemia [96], but the

results remain inconsistent. For example, in line with our finding, Wolide et al. (2017) showed a negative correlation between serum chromium and lipid profile (TGs, CHOL, and LDL) in a cross-sectional study of 214 patients [97]. However, some reviews reported that chromium levels does not affect the lipid profiles [98]. A further systematic review showed that chromium levels has limited effects on lipid profile [99].

Our study showed an increase in copper levels in patients' group, and this increase is proportional to the increase in cholesterol and triglycerides, and this came in line with previous report by Song et al. (2018) who studied the associations of serum copper with lipid concentration and dyslipidemia in 2,678 subjects, and found that the copper-dyslipidemia association was statistically significant, high serum copper was associated with elevated serum concentrations of CHOL and HDL, and was associated with increased risks of dyslipidemia [100].

In contrast to our results, Dhanak et al. (2019) evaluated the correlations of serum concentrations of trace elements including copper with lipid profile parameters. The finding of this study indicated that there was a significant higher level of serum Cu in hyperlipidemic patients compared with the control group, there was a significant negative correlation between serum copper levels and CHOL, TGs, HDL and VLDL levels [101].

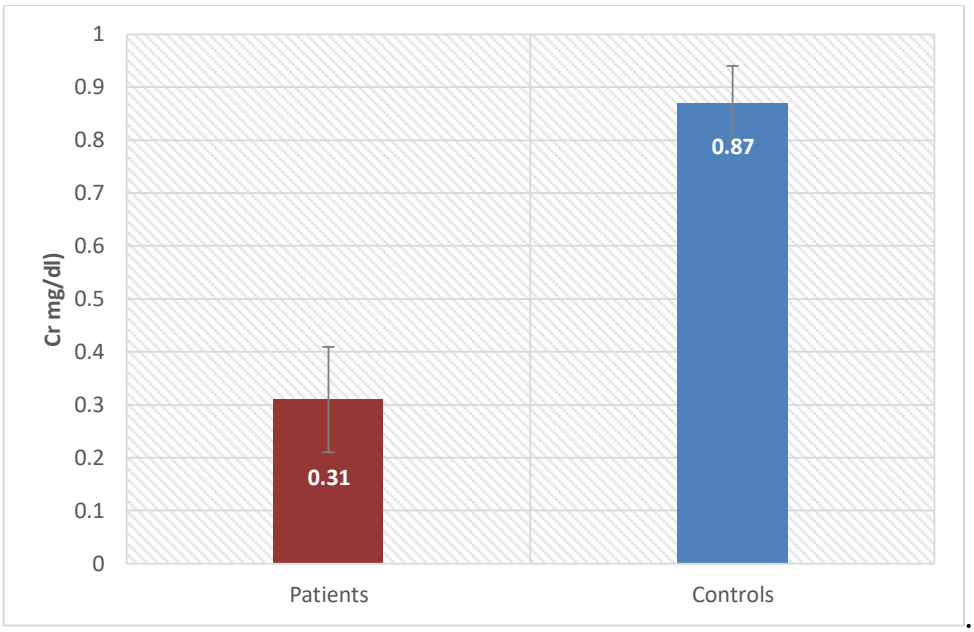


Figure (3- 4) The mean differences of Cr (P= 0.001*).

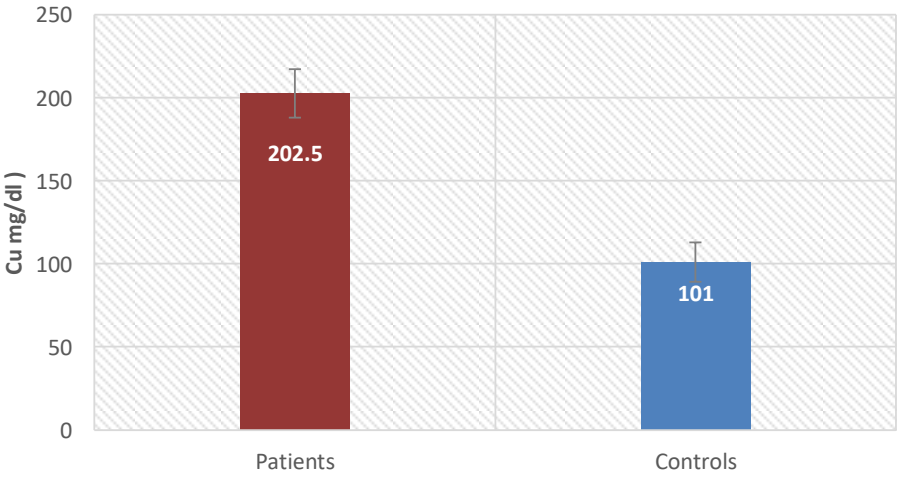


Figure (3- 5) The mean differences of Cu (P=0.00*).

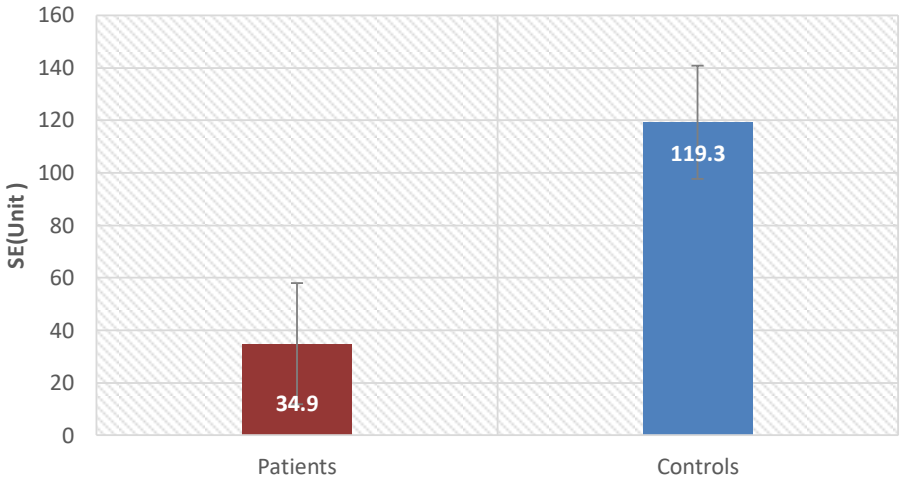


Figure (3- 6) The mean differences of Se (P=0.001).

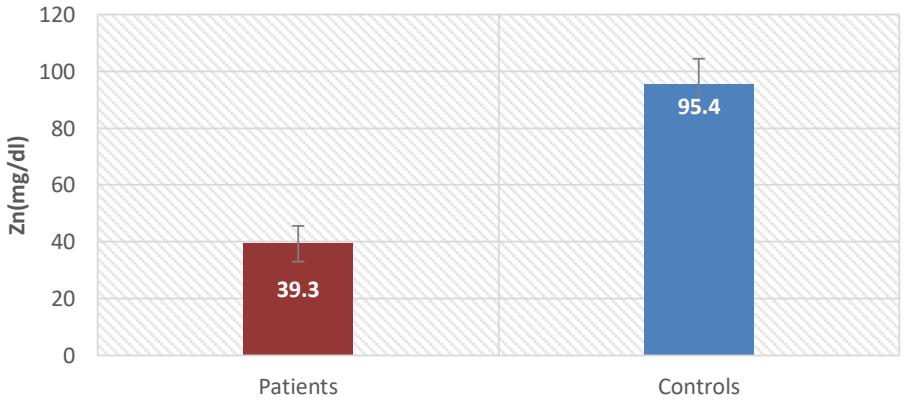


Figure (3- 7) The mean differences of Zn (P=0.001*).

In summary, atorvastatin may have complex effects on trace elements that depend on the population and conditions studied. Atorvastatin effects on zinc, chromium, copper, and selenium levels are still unclear. Atorvastatin may help balance copper and selenium levels in some conditions, especially when they are disrupted by environmental toxicants like cadmium or arsenic. However, conflicting evidence highlight the necessity for more studies utilizing longitudinal data to fully understand how atorvastatin influences trace element homeostasis.

3.3. Correlation

Table (3- 5) correlation between parameters.

		Cho	TG	Na	K	Mg	Cr	Cu	Se	Zn
Cho	r	1	**.639****	**-.466-****	-.074-	-.339-**	**-.692-****	**.732****	**-.624-****	**-.711-****
	p		**.000**	**.000**	.466	.001	**.000**	**.000**	**.000**	**.000**
	N	100	**100**	**100**	100	100	**100**	**100**	**100**	**100**
TG	r	.639**	1	**-.448-****	-.066-	**-.344-****	**-.807-****	**.813****	**-.772-****	**-.815-****
	p	.000		.000	.512	**.000**	**.000**	**.000**	**.000**	**.000**
	N	100	100	100	100	**100**	**100**	**100**	**100**	**100**
Na	r	-.466-**	-.448-**	1	.151	**.268****	**.378****	**-.402-****	**.366****	**.418****
	p	.000	.000		.134	**.007**	**.000**	**.000**	**.000**	**.000**
	N	100	100	100	100	**100**	**100**	**100**	**100**	**100**
K	r	-.074-	-.066-	.151	1	-.004-	.079	-.092-	.058	.044
	p	.466	.512	.134		.967	.433	.362	.566	.665
	N	100	100	100	100	100	100	100	100	100
Mg	r	-.339-**	-.344-**	.268**	-004-	1	**.457****	**-.459-****	**.399****	**.446****
	p	.001	.000	.007	.967		**.000**	**.000**	**.000**	**.000**
	N	100	100	100	100	100	**100**	**100**	**100**	**100**
Cr	r	-.692-**	-.807-**	.378**	.079	.457**	1	**-.959-****	**.935****	**.955****
	p	.000	.000	.000	.433	.000		**.000**	**.000**	**.000**
	N	100	100	100	100	100	100	**100**	**100**	**100**
Cu	r	.732**	.813**	-.402-**	-.092-	-.459-**	-.959-**	1	**-.897-****	**-.963-****
	p	.000	.000	.000	.362	.000	.000		**.000**	**.000**
	N	100	100	100	100	100	100	100	**100**	**100**
Se	r	-.624-**	-.772-**	.366**	.058	.399**	.935**	-.897-**	1	**.885****
	p	.000	.000	.000	.566	.000	.000	.000		**.000**
	N	100	100	100	100	100	100	100	100	**100**
Zn	r	-.711-**	-.815-**	.418**	.044	.446**	.955**	-.963-**	.885**	1
	p	.000	.000	.000	.665	.000	.000	.000	.000	
	N	100	100	100	100	100	100	100	100	100

****. Correlation is significant at the 0.05 level**

Table (3- 6) correlation between parameters with age and BMI.

		GE	MI	CR(mg/dl)	Cu(mg/dl)	Se(mg/dl)	Zn(mg/dl)
Cr(mg/dl)	R	.069-	**508***	1	**-.832-***	**.840***	**.867***
	p-value	633	**000**		**.000**	**.000**	**.000**
	N	50	**50**	50	**50**	**50**	**50**
Cu(mg/dl)	R	020	.266	-.832-*	1	**-.714-***	**-.816-***
	p-value	.890	.061	.000		**.000**	**.000**
	N	0	0	50	50	**50**	**50**
Se(mg/dl)	r	028	314*	.840**	-.714-*	1	**.775***
	p-value	849	026	.000	.000		**.000**
	N	0	0	50	50	50	**50**
Zn(mg/dl)	r	033	309*	.867*	-.816-*	.775*	1
	p-value	821	029	.000	.000	.000	
	N	50	50	50	50	50	50
*. Correlation is significant at the 0.05 level.							

3.3. 1.correlation between Cr, Zn, Se and BMI

Significant positive correlation between Cr, Zn, Se, and BMI as seen in figure (3-7)(3-8)(3- 9) In this relationship, the decrease in chromium, selenium and zinc in hyperlipidemia patients can cause an increase in weight, because this trace element are important elements in the process of metabolism of carbohydrates, fats and protein, and its lack causes weight gain. There are studies that prove that taking chromium as a treatment for weight loss in patients with hyperlipidemia [102].

selenium, which is one of the important elements in the This resulted from a lipolytic impact in adipose tissue concurrent with the accumulation of free fatty acids in the liver and There are studies conducted that prove there is an inverse relationship between selenium and weight [103].

The conclusion that zinc may be a regulator of BW indexes in certain subgroups is supported by the involvement of zinc in the control of appetite and food intake [104, 105]. Zinc is involved in the modulation of leptin, a key regulator of energy balance in the central nervous system.

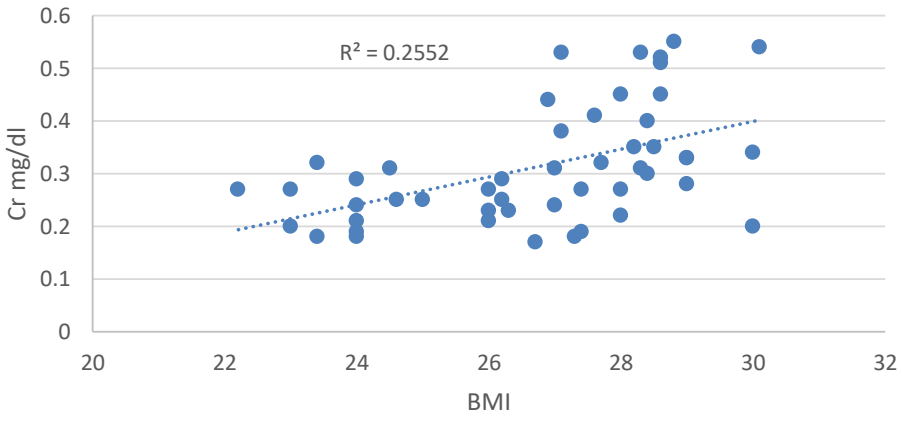


Figure (3- 8) Correlation between BMI and Cr.

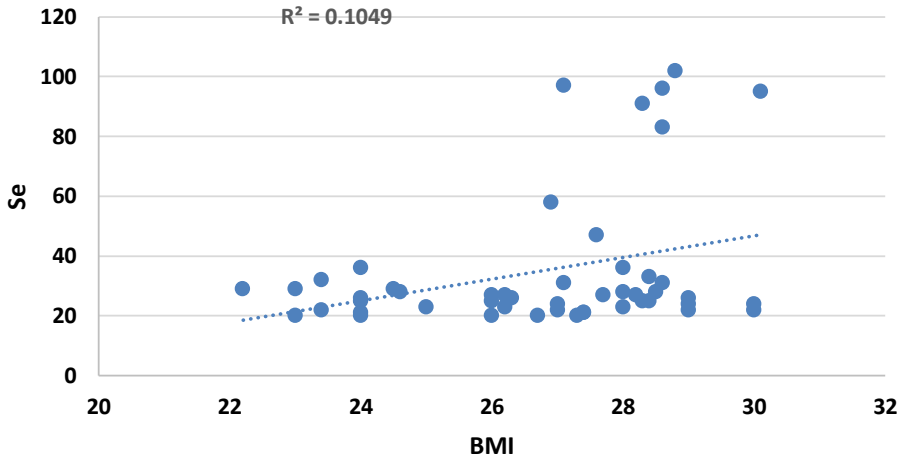


Figure (3- 9) Correlation between BMI and Se.

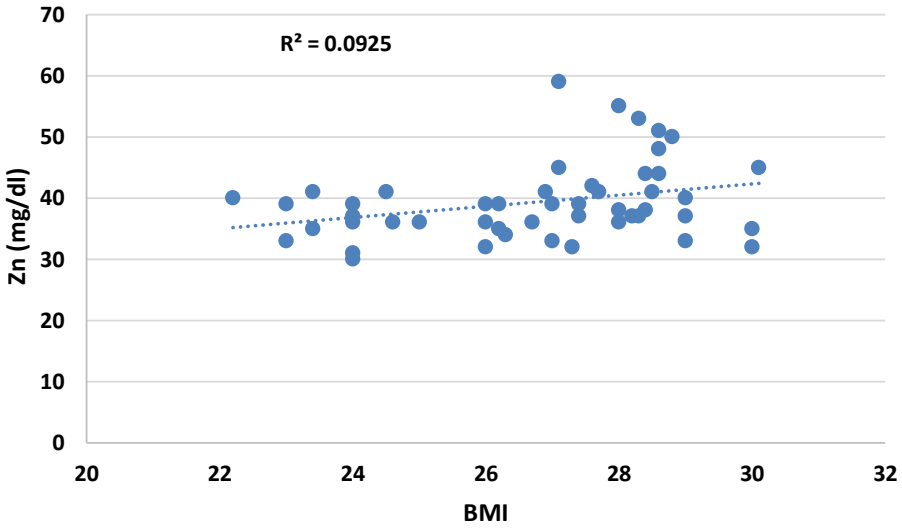


Figure (3- 10) Correlation between BMI and Zn.

3.3.2. Correlation between Cu and Cr , Se, Zn

Ther is a positive significant correlation between selenium and chromium ,This drawing shows in figure(3-10)(3-11(3-12) the inverse relationship between chromium and copper, and copper and selenium where the high copper in patients with hyperlipidemia is directly proportional to the high triglycerides in patients, The reason for this result may be due to the sharing of Cu indirectly in formation of TG in serum of patients by contributing to lipid peroxidation [106], and this is according to recent studies that need more proof.

As for chromium, zinc and selenium, there are inversely proportional to the high lipids. This theory is backed by the finding that even in people with normal diets, Cr shortage causes increased blood sugar, total cholesterol, and triglycerides as well as a decrease in high density lipoproteins (HDL) and insulin sensitivity [107, 108]. A study in European Americans showed that there was a small but significant association between dietary Zn and the TC/HDL-C ratio;

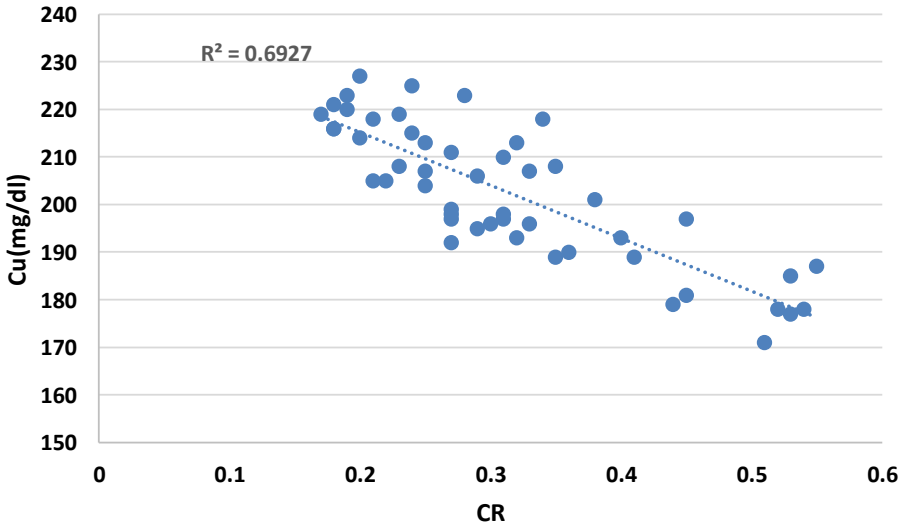


Figure (3- 11) Correlation between Cr and Cu.

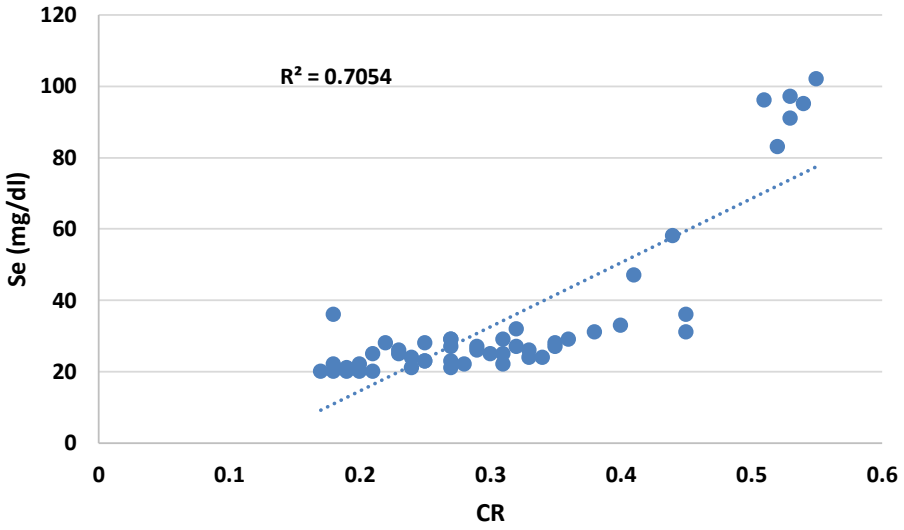


Figure (3- 12) Correlation between Cr and Se.

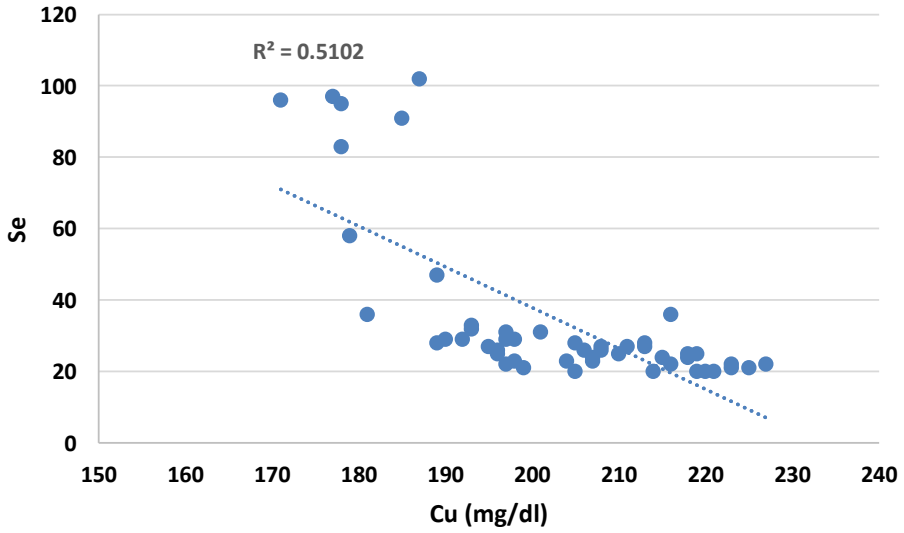


Figure (3- 13) Correlation between Cu and Se.

3.3.3. Correlation between Zn and Cr, Se

Moderate positive significant correlation between Zn and Cr ,Se There is a correlation between these elements, where the decrease of all these elements is inversely proportional to the disease of hyperlipidemia. These elements are all involved, as we mentioned earlier, in the process of metabolism of fats and carbohydrates, so their decrease is very likely with the disease, as triglycerides and cholesterol rise. Some micronutrients have been related to cardiovascular defense, among them chromium, selenium and zinc, antioxidant minerals that can act in the regulation of lipid profile [92, 109].

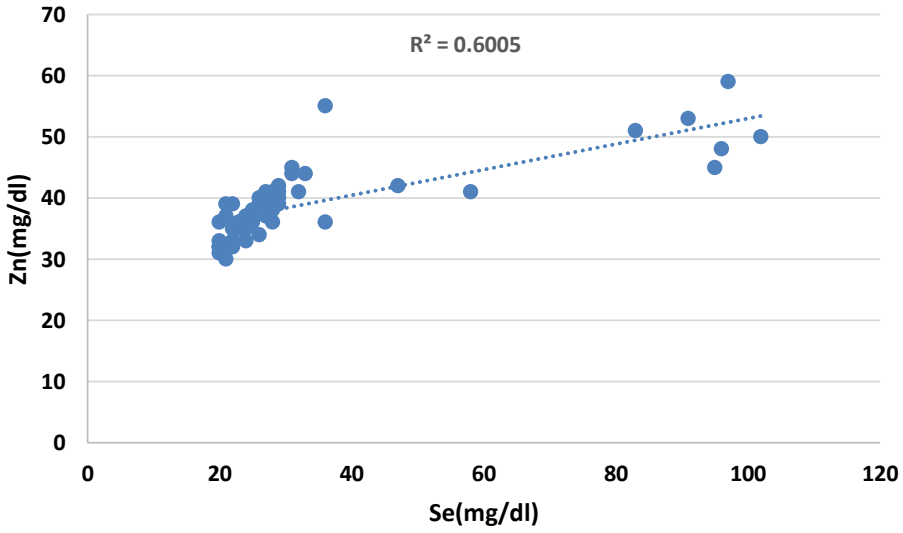


Figure (3- 14) Correlation between Zn and Se.

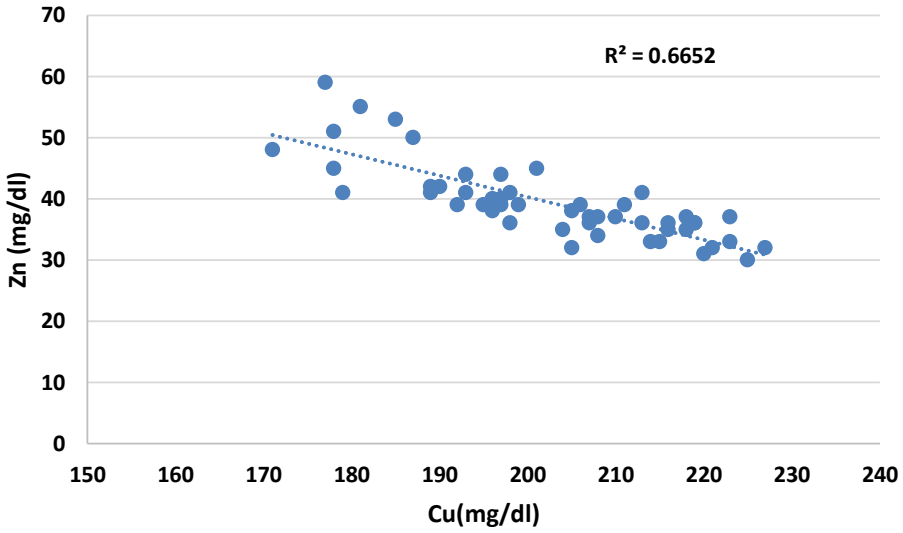


Figure (3- 15) Correlation between Zn and Cu.

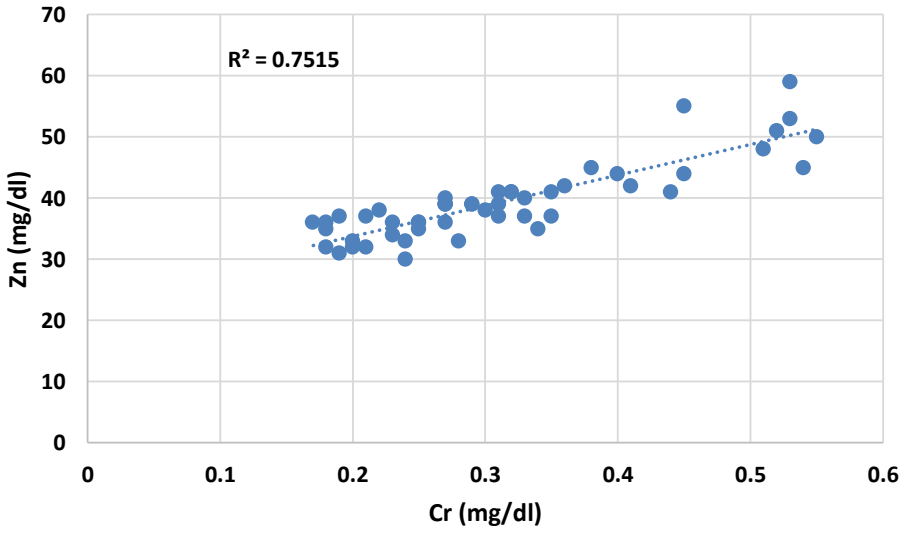


Figure (3- 16) Correlation between Zn and Cr.

3.4.ROC curve

Table (3- 7) ROC curve of Cr, Cu, Se and Zn.

Parameter	Cr	Cu	Se	Zn
Cut-off point	≤ 0.55	≥ 147	≤ 70.0	≥ 71.5
AUC	0.98	0.91	0.98	0.94
Sensitivity	94.0 %	90.0 %	88.0 %	92.0 %
Specificity	96.0 %	92.0 %	92.0 %	90.0 %
P value	0.001	0.00	0.00	0.001

3.4.1. ROC curve for the sensitivity and specificity of Cr for diagnosis of hyperlipidemia

ROC curve for the sensitivity and specificity of Cr (ng/mL) for diagnosis of hyperlipidemia Show in figure 14, (Cut-off point was ≤ 0.55 (ng/mL)), AUC=0.98, P= <0.001*, 95% CI (0.755-0.904), the sensitivity and the specificity was 94.0%, 96.0 % respectively. Plasma chromium levels are also affected by several factors including, stress, diet, exercise Therefore, it is not correct to take it as a routine examination for hyperlipidemia patients.

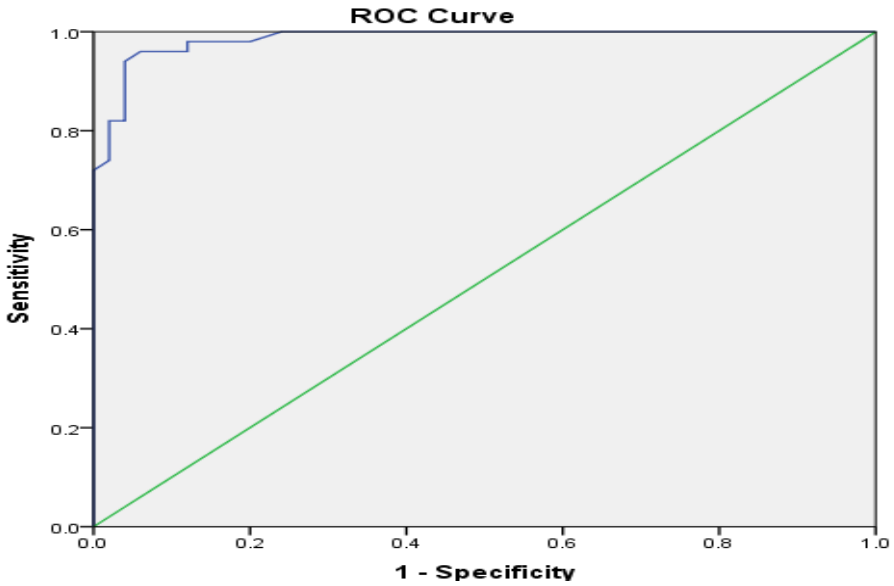


Figure (3- 17) ROC curve for the sensitivity and specificity of Cr for diagnosis of hyper-lipidemia

3.4.2.ROC curve for the sensitivity and specificity of Cu for diagnosis of hyperlipidemia

ROC curve for the sensitivity and specificity of Cu (ng/mL) for diagnosis of hyperlipidemia see in figure 15, (Cut-off point was ≥ 147 (ng/mL)), AUC=0.91, P= <0.00*, 95% the sensitivity and the specificity was 90.0%, 92.0 % respectively.

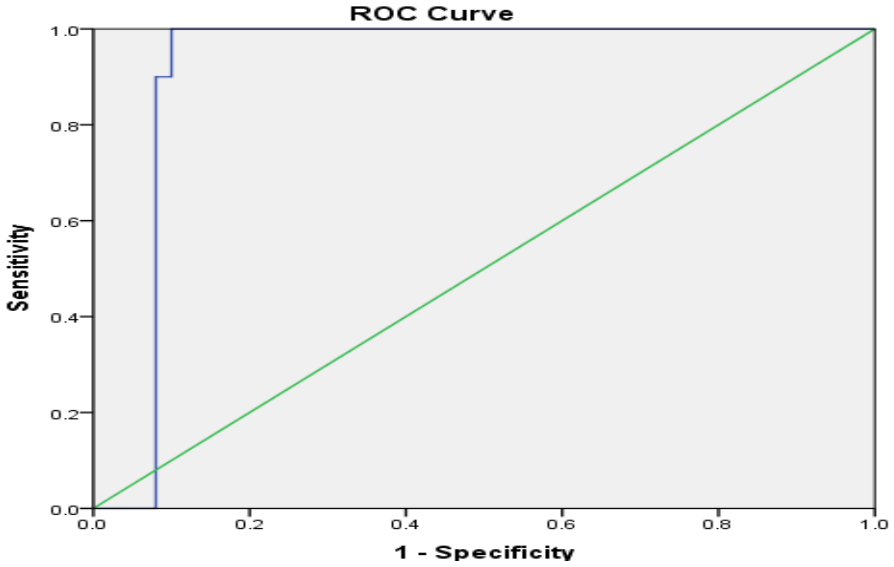


Figure (3- 18) : ROC curve for the sensitivity and specificity of Cu for diagnosis of hyper-lipidemia

3.4.2.ROC curve for the sensitivity and specificity of Se for diagnosis of hyperlipidemia

ROC curve for the sensitivity and specificity of Se (ng/mL) for diagnosis of hyperlipidemia see in figure 16, (Cut-off point was ≤ 70.0 (ng/mL)), AUC=0.98, P= <0.00*, 95% the sensitivity and the specificity was 88.0%, 92.0 % respectively. Important studies have found a high correlation between selenium and high lipids, especially high triglycerides and LDL. ROC

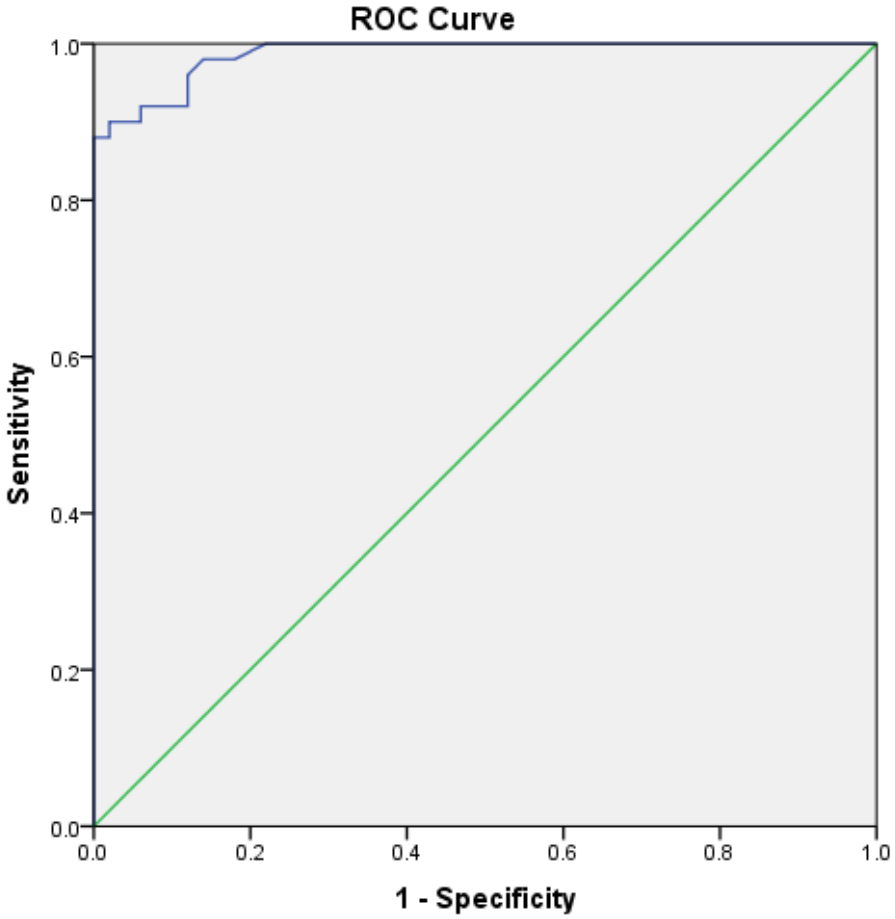


Figure (3- 19) ROC curve for the sensitivity and specificity of Se for diagnosis of hyper-lipidemia

3.4.4.ROC curve for the sensitivity and specificity of Zn for diagnosis of hyperlipidemia

ROC curve for the sensitivity and specificity of Zn(ng/mL) for diagnosis of hyperlipidemia Show in figure 16, (Cut-off point was ≥ 71.5 (ng/mL)) , AUC=0.94, P= <0.001*, 95% CI (0.755-0.904), the sensitivity and the specificity was 92.0%, 90.0 % respectively**.**

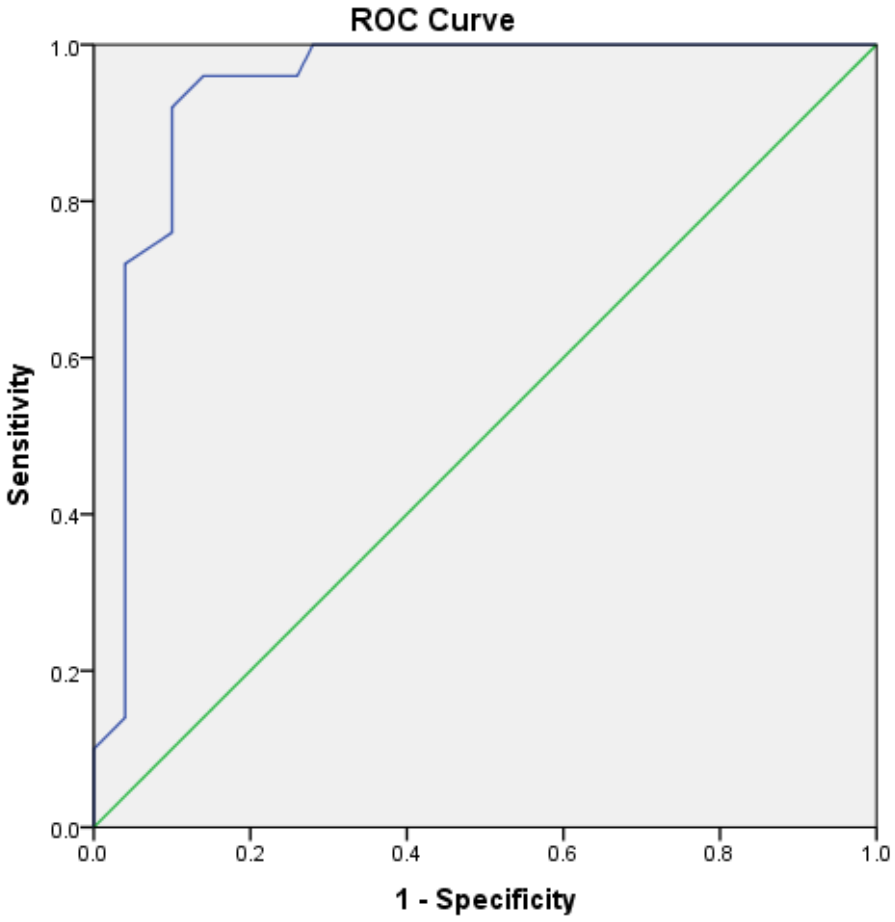


Figure (3- 20) ROC curve for the sensitivity and specificity of Zn for diagnosis of hyper-lipidemia

3.5. Conclusion

1-High triglycerides, cholesterol and VLDL are closely associated with hyperlipidemia

2- Low sodium and magnesium are positively associated with hyperlipidemia patients after taking statin treatment

3- Low selenium and zinc are closely associated with hyperlipidemia patients undergoing statin treatment

4- High copper is associated with hyperlipidemia patients undergoing statin therapy

3.6. Recommendations

1.Conducting tests for patients with high fats, especially trace elements, because they are associated with high fats, so that the patient resorts to taking nutritional supplements.

2. "Correction of trace elements imbalance could be helpful in the managment of hyperlipidemia .

3. Take into account the treatments that the patient is taking that interfere with the work of statins

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الخلاصة:-

فرط شحميات الدم هو مصطلح يشمل العديد من الاضطرابات الوراثية والمكتسبة التي تصف مستويات الدهون المرتفعة داخل الجسم. إنه اضطراب شائع جدًا ، خاصة في نصف الكرة الغربي ، ولكن أيضًا في جميع أنحاء العالم. يتم الكشف عن المرض عن طريق قياس مستويات الدم (كوليسترول الدهون الثلاثية (HDL LDL VLDL ويمكن أن تكون التمارين والتغييرات في النظام الغذائي والأدوية فعالة في المساعدة في العلاج وأهمها الستاتينات.

لا توجد أدلة كافية تشير إلى وجود علاقة بين الدهون العالية والإلكتروليتات والعناصر النزرة. لذا، هدفت هذه الدراسة إلى استخدام طرق مختلفة لقياس وتقدير وتقييم هذه العلاقة، بعد تناول العلاج بالستاتين.

تم إجراء الفحص على 100 شخص ، 50 منهم من مرضى فرط شحميات الدم و 50 كانوا مرضى تحكم. جمعت العينات من أكتوبر إلى ديسمبر 2022 ، وتراوحت أعمارهم بين (20-65) سنة. عند أخذ العينات ، يجب أخذ العديد من الأشياء في الاعتبار ، بما في ذلك التاريخ الطبي ، وفترة العلاج ، والمرضى الذين لا يتناولون بعض العلاجات التي يمكن أن تؤثر على تركيزات العناصر في الجسم ، بما في ذلك مدرات البول ، والعلاجات التي تتداخل مع عمل الستاتينات.

لا توجد فروق ذات دلالة إحصائية بين المجموعات المدروسة حسب العمر. بينما توجد فروق ذات دلالة إحصائية بين الجنس ومؤشر كتلة الجسم بين المجموعات المدروسة.

انخفض مستوى الكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة (VLDL) بشكل ملحوظ بعد تناول أتورفاستاتين، كما ارتفع مستوى HDL بشكل ملحوظ بعد تناول أتورفاستاتين. في حين لا توجد فروق فى مستوى .LDL بعد تناول العلاج.

هناك اتخفاض ذو دلالة إحصائية في مستويات الصوديوم والمغنيسيوم مع $P< 0.001$ في المريض مقارنة بالمجموعة الضابطة. ومع ذلك، لم تظهر نتائجنا أي فرق كبير بين المرضى ومجموعة السيطرة فيما يتعلق بالبوتاسيوم

هناك انخفاض ذو دلالة إحصائية في مستويات الكروم، السيلينيوم، والزنك في مجموعة المرضى مقارنة بالمجموعة الضابطة مع ($P< 0.001$) من ناحية أخرى، هناك ارتفاع ذو دلالة إحصائية في مستوى النحاس في مجموعة المرضى مقارنة بالمجموعة الضابطة مع .($P< 0.001$)

هناك علاقة إيجابية كبيرة بين الكروم والزنك والسيلينيوم ومؤشر كتلة الجسم، حيث أن انخفاض الكروم والسيلينيوم والزنك لدى مرضى فرط شحميات الدم يمكن أن يسبب زيادة في الوزن.

هناك علاقة طردية معنوية بين السيلينيوم والكروم، وهناك علاقة عكسية بين الكروم والنحاس، والنحاس والسيلينيوم.

هناك علاقة عكسية بين الكروم والزنك والسيلينيوم تتناسب مع ارتفاع نسبة الدهون في الجسم.

توجد علاقة ارتباطية موجبة معنوية متوسطة بين الزنك والكروم. وأيضا بين الزنك والسيلينيوم. ومع ذلك، هناك علاقة عكسية معنوية متوسطة بين الزنك والنحاس

أظهر منحنى ROC لـ Cr لتشخيص فرط شحميات الدم (كانت نقطة القطع ≥0.55 (نانوغرام/مل)) AUC=0.98، *P= <0.001، وكانت الحساسية والنوعية 94.0%، 96.0% على التوالي

أظهر منحنى ROC للنحاس لتشخيص فرط شحميات الدم (كانت نقطة القطع ≥ 147 (نانوغرام/مل)) AUC=0.91، *P= <0.00، الحساسية والنوعية كانت 90.0%، 92.0 % على التوالي

أظهر منحنى ROC لـ Se لتشخيص فرط شحميات الدم (كانت نقطة القطع ≥ 70.0 (نانوغرام/مل)) AUC=0.98، *P= <0.00، الحساسية والنوعية 88.0%. 92.0% على التوالي

أظهر منحنى ROC للزنك لتشخيص فرط شحميات الدم (كانت نقطة القطع ≥ 71.5 (نانوغرام/مل) AUC=0.94، *P= <0.001، وكانت الحساسية والنوعية 92.0%، 90.0% على التوالي

يرتبط ارتفاع الدهون الثلاثية والكوليسترول والبروتينات الدهنية منخفضة الكثافة (VLDL) ارتباطًا وثيقًا بفرط شحميات الدم. يرتبط انخفاض الصوديوم والمغنيسيوم بشكل إيجابي مع مرضى فرط شحميات الدم بعد تناول علاج الستاتين. ويرتبط انخفاض السيلينيوم والكروم والزنك ارتباطًا وثيقًا بمرضى فرط شحميات الدم الذين يخضعون لعلاج الستاتين. يرتبط ارتفاع النحاس بمرضى فرط شحميات الدم الذين يخضعون لعلاج الستاتين.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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تقييم مستويات الأملاح وبعض العناصر النادرة بعد إعطاء أدوية الأتورفاستاتين في أمصال المرضى الذين يعانون من فرط شحميات الدم في مدينة الحلة

رسالة

مقدم الى مجلس كلية الطب / جامعة بابل كتحقيق جزئي لمتطلبات درجة الماجستير في العلوم / الكيمياء الحياتيه السريريه

الطالبة

هدى احمد ذهيب خليف

بكالوريوس. في تقنية الطبية ، قسم التحاليل المرضية

2012 - 2013

يشرف عليها

أستاذ الدكتور
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