University of AL-Qadisiya

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Study of Biochemical Change Associated with Hypertension

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (١١)

صَدق الله العَلي الْعَظِيمِ

سورة المجادله (١١)

Dedication

To my Mother

To my Father and and my brothers

Supervisor Certification

I certify that this research was carried under my supervision at the College of Pharmacy/ University of AL-Qadisiya, as a partial fulfillment of the requirement for the B.Sc in Pharmacy.

Dr. Ghufran Mohammed Hussien

Supervisor

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Sumayia Ajmi

Samar Safa

Summary

The present study was designed to assess the serum lipid profile and renal functions test in both group patients treated with valsartan and losartan. Valsartan group includes 33 hypertensive patients, the ages of this group ranged between 35-80 year and includes 13 males and 20 female. Losartan group includes 33 hypertensive patients, the ages of this group ranged between (32- 75) year and includes 15 males and 18 females. Serum lipid profile, urea and creatinine measure by spectrophotometric method.

The result of present study shows a non-significant ($p > 0.05$) changes in age, age, duration of disease and duration of treatment in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan. And The study result shows non-significant ($P > 0.05$) change in fasting total cholesterol, HDL-cholesterol, triglyceride, VLDL cholesterol and LDL-cholesterol concentration in hypertensive patients group treated with losartan when compared to valsartan.

Also, the result shows non-significant ($P > 0.05$) change in serum urea and creatinine concentration in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan.

In conclusion, in hypertensive patients group treated with losartan, there are a non-significant changes in serum lipid profile concentrations and in renal functions when compared to those of hypertensive patients group treated with valsartan.

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Chapter One

Introduction and Literature Review

1.1-Hypertension

Hypertension is a heterogeneous disorder resulting from either a specific cause (secondary hypertension) or some underlying pathophysiologic alterations of unknown cause (essential, idiopathic, or primary hypertension) [1].

Hypertension remains a major health problem in most countries because of its impact on the population mortality and morbidity due to insufficient hypertension prevention and control at community level. Indeed, hypertension accounts for more than 5.8% of total deaths, 1.9% of years of life lost and 1.4% disability adjusted life years all over the world. As per WHO by the year 2015, hypertension, one of the commonest diseases of the elderly, is thus likely to pose a considerable burden on developing countries [2].

1.1.1-Classification

The classification of hypertension based on pathophysiology [3] listed in Table 1.1.

Table 1.1: Classification of HTN Based on Pathophysiology [3]

Classification	Systolic mmHg	Diastolic mmHg
Normal	<120	<80
Pre-Hypertension	120-139	80-89
Stage 1 Hypertension	140-159	90-99
Stage 2 Hypertension	>160	>100

1.1.2-Types of Hypertension

1.1.2.1-*Essential Hypertension*

Essential hypertension (EH) represents a multifactorial disorder determined by the interaction of environmental and genetic factors. Oxidative stress, a pathological condition characterized by imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense systems, has been implicated to pathogenesis of essential hypertension (EH) [4].

1.1.2.2-*Secondary Hypertension*

Although most human hypertension arises from unknown causes and is called essential hypertension or primary hypertension, about 5% to 10% of cases arise from a known cause. These are, as a group, called secondary hypertension. These cases can be managed more intelligently and sometimes cured if they are identified [5].

1.1.2.3-*Pseudohypertension*

Pseudo-hypertension is defined as cuff diastolic BP at least 15mm Hg higher than simultaneously measured intra-arterial BP. It is found in elderly patients with calcified and rigid arteries who present with little or no target organ damage despite very high BP readings [6].

1.1.2.4-*White-Coat Hypertension*

White-coat hypertension (WCH) is defined as continued elevation in blood pressure measurements in a medical setting. It is thought to be a conditioned response resulting from a pressor response incited by the clinical setting. Specifically, office blood pressure readings higher than 140/90 and blood pressure measurements out of office less than 135/85 are consistent with WCH [7].

1.1.3-Epidemiology

The age-adjusted prevalence rate of hypertension is around 20-30% in Asian countries, similar to that in developed countries in the Western world. However, the expected increase in prevalence is higher in Asians than in the rest of the world. Between the years 2000 to 2025, there will be a 65.4% increase in prevalence of hypertension in Asia compared with a 51.2% increase in the rest of the world. This change is even more severe in females, with a 81.6% increase in Asia compared to a 54.4% increase in the rest of the world [8].

The latest data from the Global Burden of Disease project show that raised blood pressure (systolic >115 mm Hg) continues to be the biggest single contributor to the global burden of disease and to global mortality, leading to 9·4 million deaths each year [9].

1.1.4-Pathophysiology

The pathogenesis and pathophysiologic consequences of hypertension are myriad and complex. In fact, the 2 are inextricably linked. Clinically, this can create a “chicken versus egg” conundrum [10].

Hypertension is a chronic elevation of blood pressure that, in the long-term, causes end-organ damage and results in increased morbidity and mortality [11].

Blood pressure is the product of cardiac output and systemic vascular resistance. It follows that patients with arterial hypertension may have an increase in cardiac output, an increase in systemic vascular resistance, or both. In the younger age group, the cardiac output is often elevated, while in older patients increased systemic vascular resistance and increased stiffness of the vasculature play a dominant role [11].

Vascular tone may be elevated because of increased a-adrenoceptor stimulation or increased release of peptides such as angiotensin or endothelins. The final pathway is an increase in cytosolic calcium in vascular smooth muscle causing vasoconstriction. Several growth factors, including angiotensin and endothelins, cause an increase in vascular smooth muscle mass termed vascular remodeling [11].

Both an increase in systemic vascular resistance and an increase in vascular stiffness augment the load imposed on the left ventricle; this induces left ventricular hypertrophy and left ventricular diastolic dysfunction [11].

1.1.5-Etiology

The etiology of hypertension reflects a number of factors – genetic predisposition, environmental influences, iatrogenic causes and unknown components [12].

1.1.5.1-*Genetic factors*

It has long been recognized that hyper-tension 'runs in families'. Studies have shown a greater correlation in twin siblings than in non-twin siblings, and siblings are more likely to have hypertension when one or both parents are also known to be hypertensive (though it must be remembered that familial, and apparently genetic, associations may also reflect common environmental influences) [12].

1.1.5.2-*Environmental influences*

Obesity (and physical inactivity) is particularly important; obese individuals are more likely to develop hypertension than non-obese individuals. There is a well-established overlap between hypertension and other clinical features known as metabolic syndrome, insulin resistance or 'syndrome X' [12].

1.1.5.3-*Alcohol*

Regular consumption of large quantities of alcohol is known to have a pressor effect (and is also associated with high calorie intake), such that changing from a high alcohol intake to a lower intake has been shown to reduce blood pressure by about 5–10 mm Hg [12].

1.1.5.4-*Iatrogenic and 'pharmacological'*

Non-steroidal anti-inflammatory drugs acquired by the patient over-the-counter are numerically the most important because they are particularly likely to interfere with the efficacy of antihypertensive drug treatment. Other drugs (e.g. corticosteroids such as prednisolone, combined oral contraceptive pill) are known to predispose to hypertension [12].

1.1.6-Signs and Symptoms

Hypertension is normally asymptomatic, though many patients ascribe symptoms such as epistaxis, headaches, lethargy and dizziness to their raised blood pressure. Hence, clinical assessment should be tailored to answer four key questions [13] :

- Is the patient truly hypertensive?
- Is there evidence that hypertension has caused complications (target organ damage (TOD); major CV events)?
- Is a secondary cause identifiable?
- What is the total CV risk of the patient?

1.1.7- Diagnosis and clinical assessment

Accurate measurement of BP is critical to both diagnosis and management of hypertension [6].

For 2015, 2 new recommendations were added. A revised algorithm for the diagnosis of hypertension is presented. Two major changes are proposed:

- measurement using validated electronic (oscillometric) upper arm devices is preferred over auscultation for accurate office BP measurement;
- If the visit 1 mean BP is increased but (< 180/110) mm Hg, out of office BP measurements using ambulatory BP monitoring (preferably) or home BP monitoring should be performed before visit 2 to rule out white coat hypertension, for which pharmacologic treatment is not recommended [14].

Clinical assessment involves taking a complete clinical history with review of diet (salt intake, liquorice), prescription and over the counter (OTC) drugs (e.g. steroids, non-steroidal anti-inflammatory drugs (NSAID), illicit drug use (amphetamines, cocaine), herbal remedies (ginseng, ma huang), smoking status and performing a focused medical examination to establish the cause, severity and associated risk [6].

1.1.8- Biochemical investigations

Both routine and specialised biochemical investigations are required for the initial evaluation and optimisation of the clinical management of hypertensive patients. Many hypertensive individuals have multiple cardiovascular risk factors at the time of presentation [6].

1.1.8.1- *Routine investigations*

All patients with either prehypertension or hypertension should have blood sampled, preferably while fasting, to measure creatinine and calculate the estimated Glomerular Filtration Rate (eGFR), urea, glucose, glycated hemoglobin A1c (HbA1c), electrolytes, lipid profile (total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL cholesterol), low density lipoprotein cholesterol (LDL cholesterol) , thyroid function tests (free thyroxine (FT4) and thyroxine stimulating hormone (TSH), urate and liver function tests (LFTs: total protein (TProt), albumin, total bilirubin (TBili), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT)) [6].

The measurement of these analyses permits evaluation of kidney, thyroid and liver function, the identification of dyslipidemia, glucose intolerance, metabolic syndrome and cardiovascular risk stratification [6].

1.1.8.2- *Specialised investigations*

Specialised biochemical testing for secondary causes of hypertension are indicated when the probability of an identifiable cause of hypertension is increased: onset of hypertension outside of the normal age for essential hypertension (30-55 years), sudden onset or worsening of hypertension, stage 2 hypertension, family history of secondary hypertension and blood pressure that responds poorly to treatment. However, prior to screening pseudo-hypertension and pseudo-resistance should be excluded [6].

It must be acknowledged that for endocrine hypertension there is variable information regarding prevalence of disease in various clinical contexts that influence the ability to estimate the predicative value of screening tests in all circumstances. Hence, the importance of pretest probability in test interpretation that is dependent on a comprehensive clinical examination and careful recording of a family history cannot be overstated [6].

1.1.9-Complications

The common complications of HTN include manifestations of aneurysms, CKD, cognitive changes include memory loss, losing focus during conversations and difficulty in finding words, eye damage, heart attack, heart failure (HF), peripheral artery disease and stroke [15].

Early reports by the Framingham Heart Study demonstrated that hypertension, SBP, and DBP are risk factors for CHD [16, 17], helping dispel a longstanding belief that the common variety of hypertension was a benign condition essential for adequate perfusion of peripheral tissues. A 36-year follow-up from the Framingham Heart Study showed that hypertension is associated with twofold to fourfold increases in risk for the development of CHD, stroke, peripheral artery disease, cardiac failure, and overall cardiovascular events in both men and women [18].

The Seven Countries Study provided strong ecologic study evidence demonstrating a direct relation of SBP and DBP within 16 communities to CHD death rates, showing a doubling in risk for every increment of 10 mmHg in the population's median SBP [19].

Among middle-aged men screened in the Multiple Risk Factor Intervention Trial (MRFIT), a direct relation of increasing SBP and DBP with CHD mortality over 11.6 years was shown [20].

Moreover, a pooling of results from 418,343 persons initially free of CHD showed CHD mortality to begin increasing at levels of DBP above 73 mm Hg and to increase more than fivefold between levels of 73 and 105 mm Hg [21].

1.1.10-Treatment of hypertension

1.1.10.2- Pharmacological therapy

The 2013 ESH/ESC guidelines reiterating previous indications confirm that five classes of drugs such as: **1)** diuretics, **2)** beta-blockers, **3)** calcium antagonists, **4)** angiotensin converting enzyme (ACE) inhibitors and **5)** angiotensin receptor blockers are all suitable for the initiation and maintenance of antihypertensive treatment, either as monotherapy or in some combinations [22].

1.1.10.2.1- Angiotensin Converting Enzyme (ACE) inhibitors

The use of angiotensin-converting enzyme (ACE) inhibitors is well established as one of the main therapeutic agents for the treatment of hypertension. ACE is a component of the renin–angiotensin–aldosterone system [23].

It plays a key role in the homoeostatic mechanism of mammals, by contributing to the maintenance of normal blood pressure and to the electrolyte balance, and by being involved in the regulation and control of the arterial pressure [23].

ACE inhibitor includes (candesartan, telmisartan, losartan, valsartan....).

1.1.10.2.1.1- *Losartan*

1.1.10.2.1.1.1-*Pharmacology*

Goa and Wagstaff [24] reported that losartan potassium is an orally active, nonpeptide AIIRA. It is the first of a new class of drugs to be introduced for clinical use in hypertension. This novel agent binds competitively and selectively to the AII subtype 1 (AT(1)) receptor, thereby blocking AII induced physiological effects [25].

An active metabolite, E-3174, contributes substantially to its antihypertensive effect, which persists throughout 24 h after once-daily administration. In patients with mild to moderate hypertension, losartan potassium 50–100 mg once daily as monotherapy lowers blood pressure to a similar degree to enalapril, atenolol, and felodipine extended release [25].

1.1.10.2.1.1.2-*Pharmacokinetics*

Losartan is readily absorbed from the gastrointestinal tract after oral doses, but undergoes substantial first pass metabolism resulting in a systemic bioavailability of about 33%.

It is metabolised to an active carboxylic acid metabolite E-3174 (EXP-3174), which has greater pharmacological activity than losartan; some inactive metabolites are also formed. Metabolism is primarily by cytochrome P450 isoenzymes CYP2C9 and CYP3A4. Peak plasma concentrations of losartan and E-3174 occur about 1 hour and 3 to 4 hours, respectively after an oral dose [25].

Both losartan and E-3174 are more than 98% bound to plasma proteins. Losartan is excreted in the urine, and in the faeces via bile, as unchanged drug and metabolites. About 4% of an oral dose is excreted unchanged in urine and about 6% is excreted in urine as the active metabolite. The terminal elimination half-lives of losartan and E-3174 are about1.5 to 2.5 hours and 3 to 9 hours, respectively [25].

1.1.10.2.1.1.3-*Adverse Effects*

Adverse effects of losartan have been reported to be usually mild and transient, and include dizziness, headache, and dose-related orthostatic hypotension [25].

Hypotension may occur particularly in patients with volume depletion (for example those who have received high dose diuretics). Impaired renal function and, rarely, rash, urticaria, pruritus, angioedema, and raised liver enzyme values may occur [25].

Hyperkalaemia, myalgia, and arthralgia have been reported. Losartan appears less likely than ACE inhibitors to cause cough. Other adverse effects that have been reported with angiotensin II receptor antagonists include respiratory-tract disorders, back pain, gastrointestinal disturbances, fatigue, and neutropenia. Rhabdomyolysis has been reported rarely [25].

1.1.10.2.1.2-Valsartan

1.1.10.2.1.2.1- *Pharmacology*

Valsartan is a selective angiotensin type 1 receptor (AT1R) inverse agonist and is one of the most highly prescribed angiotensin receptor blocker (ARB). Valsartan is used to treat hypertension, postmyocardial infarction, heart failure, chronic kidney disease, and diabetic nephropathy. The most severe contraindication is to avoid use in pregnancy, as ARBs are fetal toxic, and drug–drug interactions include hypotension and increased potassium levels [26].

1.1.10.2.1.2.2- *Pharmacokinetics*

Valsartan is rapidly absorbed after oral doses, with a bioavailability of about 23%. Peak plasma concentrations of valsartan occur 2 to 4 hours after an oral dose. It is between 94 and 97% bound to plasma proteins. Valsartan is not significantly metabolised and is excreted mainly via the bile as unchanged drug. The terminal elimination half-life is about 5 to 9 hours. Following an oral dose about 83% is excreted in the faeces and 13% in urine [25].

1.1.10.2.1.2.3- *Adverse Effects*

As for Losartan Potassium, Valsartan should be used with caution in patients with hepatic impairment, cirrhosis, or biliary obstruction [25].

1.1.10.2.2- Beta-blockers

Beta-blockers refer to a mixed group of drugs with diverse pharmacodynamics and pharmacokinetic properties. They have shown long-term beneficial effects on mortality and cardiovascular disease (CVD) when used in people with heart failure or acute myocardial infarction [27].

Beta-blockers were thought to have similar beneficial effects when used as first-line therapy for hypertension. However, the benefit of beta-blockers as first-line therapy for hypertension without compelling indications is controversial [27].

Beta-blocker includes (Atenolol, bisoprolol, carvedilol, metoprolol, propranolol.....).

1.1.10.2.2.1-Bisoprolol

1.1.10.2.2.1.1- *Pharmacology*

Bisoprolol is a highly selective β1-adrenoceptor antagonist that does not have intrinsic sympathomimetic (partial agonist) or membrane stabilising (local anaesthetic) activity [28].

Bisoprolol can increase serum triglycerides and reduce HDL cholesterol [29].

1.1.10.2.2.1.2-*Pharmacokinetics*

Bisoprolol is almost completely absorbed from the gastrointestinal tract and undergoes only minimal firstpass metabolism resulting in an oral bioavailability of about 90% [25].

Peak plasma concentrations are reached 2 to 4 hours after oral doses. Bisoprolol is about 30% bound to plasma proteins. It has a plasma elimination half-life of 10 to 12 hours. Bisoprolol is moderately lipid-soluble. It is metabolised in the liver and excreted in urine, about 50% as unchanged drug and 50% as metabolites [25].

1.1.10.2.2.1.3-*Adverse Effects*

Bisoprolol has been well tolerated by most patients in long term trials (6 to 24 months' duration). 'Giddiness', headache and tiredness were the most commonly reported adverse reactions. In long term trials, side effects necessitated discontinuation of treatment in about 2.7% of patients [29].

In comparative trials the tolerability of bisoprolol 5 to 20 mg/day appeared to be comparable to atenolol 50 to 100 mg/day, metoprolol 100 mg/day and verapamil 240 to 360 mg/day, and superior to nifedipine SR 40 to 80 mg/day or hydrochlorothiazide 50 mg/day plus amiloride 5 mg/day [29].

Adverse reactions tend to occur more frequently during the first few weeks of bisoprolol treatment and then subside with continued therapy [29].

Aims of the study

The main objectives of the present study can be summarized as follows:

1- To assess the serum lipid profile concentration in hypertensive patients group treated with valsartan and hypertensive patients group treated with losartan.

2- To evaluate the renal functions in hypertensive patients group treated with valsartan and hypertensive patients group treated with losartan.

Chapter Two

Materials and Methods

2.1-Materials

2.1.1-Subjects

The study included two groups with hypertension (hypertensive patients treated with valsartan and hypertensive patients treated with losartan). The work was carried out in the biochemistry laboratory in ALdiwaniyah Teaching Hospital. Hypertensive patients were diagnosed by specialist physicians. They were selected from AL-diwaniyah Teaching Hospital. Any subject suffered from the other health problems were excluded from the current study.

2.1.1.1-Hypertensive Patients Group Treated with Valsartan

This group includes 33 hypertensive patients treated with valsartan, the ages of this group ranged between 35-80 year and includes 13 males and 20 females .

2.1.1.2-Hypertensive Patients Group Treated with Losartan

This group includes 33 hypertensive patients treated with losartan, the ages of this group ranged between 32-75 year and includes 15 males and 18 female.

2.1.2-Blood Sampling

Five milliliters of blood were withdrawn from all subjects by vein puncture in fasting status and placed in plain tubes. It was left 10-15 minutes at room temperature for coagulation. Blood was centrifuged for 10-15 minutes at 2000 xg.

Sera were obtained for determination of total cholesterol, HDL-cholesterol, TGs, VLDL-cholesterol, LDL-cholesterol, urea and creatinine concentrations.

2.1.3-Determination of Body Mass Index

Body Mass Index (BMI) was calculated according to the following equation [30]:

$$\textbf{BMI} = \textbf{(weight in kg) / (height in meters)}^2$$

For type 2 diabetic patients and control groups, weight status were categorized according to the level of their BMI as shown in the following [30]:

BMI (kg/m2)	Weight Status
Below 19.5	Underweight
19.5 to 24.9	Healthy weight
25.0 and 29.9	Overweight
30.0 and above	Obese

2.1.4-Chemicals

Chemicals and kits used in this study were tabulated in Table 2-1.

Table 2-1: Chemicals and Kits Used

No.	Chemicals and Kits	Origin
1	Total cholesterol kit	Biolabo SA (France)
2	HDL-cholesterol kit	Biolabo SA (France)
3	Triglyceride kit	Biolabo SA (France)
4	Urea kit	Biolabo SA (France)
5	Creatinine kit	Biolabo SA (France)

2.1.5-Apparatus and Equipment

Apparatus and equipments used in this study were tabulated in Table 2-2.

Table 2-2: Apparatus and Equipments Used

No.	Apparatus and Equipments	Origin
1	Centrifuge	Hettich (Germany)
2	Spectrophotometer	Apel (Japan)
3	Disposable syringes (5 mL)	Medical jet (Syria)
4	Distillator	England
5	Micropipette tips (different size)	Promega (USA)
6	Micropipettes (different size)	Watson Nexty (Japan)

2.2-Methods

2.2.1-Determination of Serum Lipid Profile Concentration

2.2.1.1-Determination of Serum Total Cholesterol

Principle:

Cholesterol concentration was determined enzymatically according to the method described by Allain *et al*. [31], as shown in the following reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten 4 one 3} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

Reagents:

Reagents	Composition	
Reagent 1 (Buffer)	Phosphate buffer Chloro-4-phenol Sodium chloride Triton x 100 Preservative	100 mmol/L 5.0 mmol/L 2.3 mmol/L 1.5 mmol/L
Reagent 2 (Enzymes)	Cholesterol oxidase Cholesterol esterase peroxidase PAP PEG 6000	100 IU/L 170 IU/L 1200 IU/L 0.25 mmol/L 167 µmol/L
Reagent 3 (Standard)	Cholesterol 200 mg/dL or	5.17 mmol/L

Procedure:

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against blank at 500 nm.

Calculation:

$$\text{Total cholesterol (mg/dL)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard con.}$$

2.2.1.2-Determination of Serum HDL-Cholesterol Concentration

Principle:

Phosphotungstic acid and magnesium chloride were used to precipitate LDL, VLDL and Chylomicron from specimens. After centrifugation, HDL-cholesterol was obtained in supernatant and measured with total cholesterol reagent [32] (as described in section 2.2.1.1).

Reagents:

Reagents	Composition
Reagent 1 (precipitant)	Phosphotungstic acid 13.9 mmol/L Magnesium chloride pH 6.2 490 mmol/L
Reagent 2 (Standard)	Cholesterol 100 mg/dl or 2.58 mmol/L

Procedure:

Reagents	Volume
Serum	0.5 mL
Precipitant	50 µL

The tubes were mixed vigorously, then left at room temperature for 10 minutes and centrifuged at 3000 xg for 15 minute. The procedure for measurement of cholesterol in supernatant was used.

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	25 µL	-	-
Standard	-	25 µL	
Supernatant	-	-	25 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against a blank at 500 nm.

Calculation:

$$\textbf{HDL-cholesterol (mg/dL)} = \frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \times \textbf{Standard con.}$$

2.2.1.3-Determination of Serum Triglycerides Concentration

Principle:

Triglycerides concentration was determined enzymatically [33,34], as shown in the following reactions:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Free fatty acids}$$

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

$$\text{Glycerol-3-P + } O_2 \xrightarrow{\text{Glycerol-3- p Oxidase}} \text{Dihydroxyacetone-p + } H_2O_2$$

$$H_2O_2\text{+4-chlorophenol+PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink) + 4 } H_2O$$

The absorbance of the colored complex (quinonimine) is proportional to TG concentration in the sample.

Reagent:

Reagents	Composition	
Reagent 1 (Buffer)	PIPES	100 mmol/L
	Magnesium chloride	9.8 mmol/L
	Chloro-4-phenol	3.5 mmol/L
	Preservative	
Reagent 2 (Enzymes)	lipase	1000 IU/L
	Peroxidase	1700 IU/L
	Glycerol-3-p-oxidase	3000 IU/L
	Glycerol kinase	660 IU/L
	PAP	0.5 mmol/L
	ATP	1.3 mmol/L
Reagent 3 (Standard)	Triglycerides 200 mg/dl or 2.28 mmol/L	

Procedure:

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	
Sample	-	-	10 µL

The tubes were mixed, then left at 37˚C for 5 minutes. The absorbance was recorded against blank at 500 nm.

Calculation:

$$\textbf{Triglyceride (mg/dL)} = \frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \times \textbf{Standard con.}$$

2.2.1.4-Determination of Serum VLDL-Cholesterol Concentration

VLDL-cholesterol concentration was calculated by dividing TGs value obtained in section (2.2.1.3) by 2.22 at which VLDL-cholesterol was measured in mmol/L [35].

$$\textbf{VLDL-cholesterol (mg/dL)} = \frac{\textbf{TG}}{\textbf{5}}$$

2.2.1.5-Determination of Serum LDL-Cholesterol Concentration

The concentration of LDL-cholesterol was calculated by using Friedewald equation [36].

$$\textbf{LDL-cholesterol (mg/dL)} = \textbf{Total-cholesterol} - \textbf{HDL-cholesterol} - \frac{\textbf{TG}}{\textbf{5}}$$

2.2.2-Determination of Serum Urea Concentration

Principle:

Enzymatic and colorimetric method based on the specific action of urease which hydrolysis urea in ammonium ion and carbon dioxide. Ammonium ions then from with chloride and salicylate a blue-green complex. This coloration, proportional to urea concentration in the specimen, is measured at 600 nm [37].

Reagent:

Reagents	Composition	
Reagent 1 (Salicylate)	Salicylate Nitroprussiate	31 mmol/L 1.67 mmol/L
Reagent 2 (Urease)	Urease	≥ 15 KU/L
Reagent 3 (Base)	Sodium hydrochloride Sodium Hydroxide	7 mmol/L 62 mmol/L
Reagent 4 (Standard)	Urea 40 mg/dL (6.66 mmol/L)	

Procedure:

The content of vial reagent 2 (Urease) was added to vial reagent 1 (Salicylate), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Demineralised water	5 µL	-	-
Standard	-	10 µL	
Specimen	-	-	5 µL
Base (Vial R3) diluted 1/4	1 mL	1 mL	1 mL

Calculation:

$$\textbf{Urea (mg/dL)} = \frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \times \textbf{Standard con.}$$

2.2.3-Determination of Serum Creatinine Concentration

Principle:

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pre-treatment step. This reaction has been improved (specificity, speed and adaptability) by the development of an initial-rate method [38,39].

Reagent:

Reagents	Composition	
Reagent 1 (Base)	Disodium phosphate Sodium hydroxide	6.4 mmol/L 150 mmol/L
Reagent 2 (Dye)	Sodium dodecyl sulfate Picric acid Ph 4.0	0.75 mmol/L 4.0 mmol/L
Reagent 3 (Standard)	177 µmol/L (2mg/dL)	

Procedure:

The content of vial reagent 2 (Dye) was added to vial reagent 1 (Base), then mixed gently until complete dissolution (2 minutes) to prepare work reagent. The procedure was carried out as in the following:

Reagents	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Demineralised water	100 µL	-	-
Standard	-	100 µL	
Supernatant	-	-	100 µL

Calculation: [40]

$$\textbf{Creatinine (mg/dL)} = \frac{\textbf{Sample absorbance}}{\textbf{Standard absorbance}} \times \textbf{Standard con.}$$

2.3. Statistical Analysis

The results were expressed as mean ± SD. Student's t-test was used for the evaluation of data. P-value of < 0.05 was considered to be statistically significant.

Chapter Three

Results and Discussion

3.1-General Characteristics of Study Groups

The result of present study shows a non-significant ($p > 0.05$) changes in age, age, duration of disease and duration of treatment in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan (Table 3-1).

Table 3-1: General Characteristics of Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

Characteristics	Group	Mean ± SD	P value
No.	Patient (Valsartan)	33	
	Patient (Losartan)	33	
Sex M/F	Patient (Valsartan)	13/20	
	Patient (Losartan)	15/18	
Age (y)	Patient (Valsartan)	60.24 ± 11.13	> 0.05
	Patient (Losartan)	53.74 ± 16.63	
Duration of disease (y)	Patient (Valsartan)	5.12 ± 3.60	> 0.05
	Patient (Losartan)	4.75 ± 2.81	
Duration of treatment (y)	Patient (Valsartan)	2.66 ± 0.92	> 0.05
	Patient (Losartan)	2.93 ± 1.95	

Also this study show a non-significant ($p > 0.05$) changes in weight, height and BMI in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan (Table 3-2).

Table 3-2: General Characteristics of Patients Hypertensive Group Treated with Valsartan and hypertensive Patients Group Treated with Losartan

Characteristics	Group	Mean ± SD	P value
Weight (kg)	Patient (Valsartan)	79.07 ± 13.53	> 0.05
	Patient (Losartan)	76.78 ± 14.4	
Height (m)	Patient (Valsartan)	1.68 ± 0.09	> 0.05
	Patient (Losartan)	1.69 ± 0.08	
BMI (kg/m^2)	Patient (Valsartan)	28.03 ± 4.09	> 0.05
	Patient (Losartan)	27.68 ± 3.42	

3.2-Serum Lipid Profile Concentration in Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

Fasting total cholesterol, HDL-cholesterol, triglyceride, VLDL-cholesterol and LDL-cholesterol concentration were found to be non-significantly ($p > 0.05$) changed in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan (Table 3-3).

Table 3-3: Mean Fasting Serum Total Cholesterol, HDL-Cholesterol, Triglyceride, VLDL-Cholesterol and LDL-Cholesterol Concentration in Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

Parameter	Group	Mean ± SD	P-value
Total Cholesterol (mg/dL)	Patient (Valsartan)	187.11 ± 71.35	> 0.05
	Patient (Losartan)	195.45 ± 63.11	
HDL Cholesterol (mg/dL)	Patient (Valsartan)	45.79 ± 15.97	> 0.05
	Patient (Losartan)	47.89 ± 15.59	
Triglyceride (mg/dL)	Patient (Valsartan)	175.23 ± 100.9	> 0.05
	Patient (Losartan)	180.54 ± 88.76	
VLDL Cholesterol (mg/dL)	Patient (Valsartan)	32.51 ± 18.99	> 0.05
	Patient (Losartan)	27.66 ± 14.42	
LDL Cholesterol (mg/dL)	Patient (Valsartan)	99.19 ± 51.78	> 0.05
	Patient (Losartan)	116.53 ± 64.07	

3.3-Serum Urea and Creatinine Concentration in Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

The result of present study show non-significant ($p > 0.05$) changes in serum urea (Figure 3-1) and creatinine (Figure 3-2) concentration in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan.

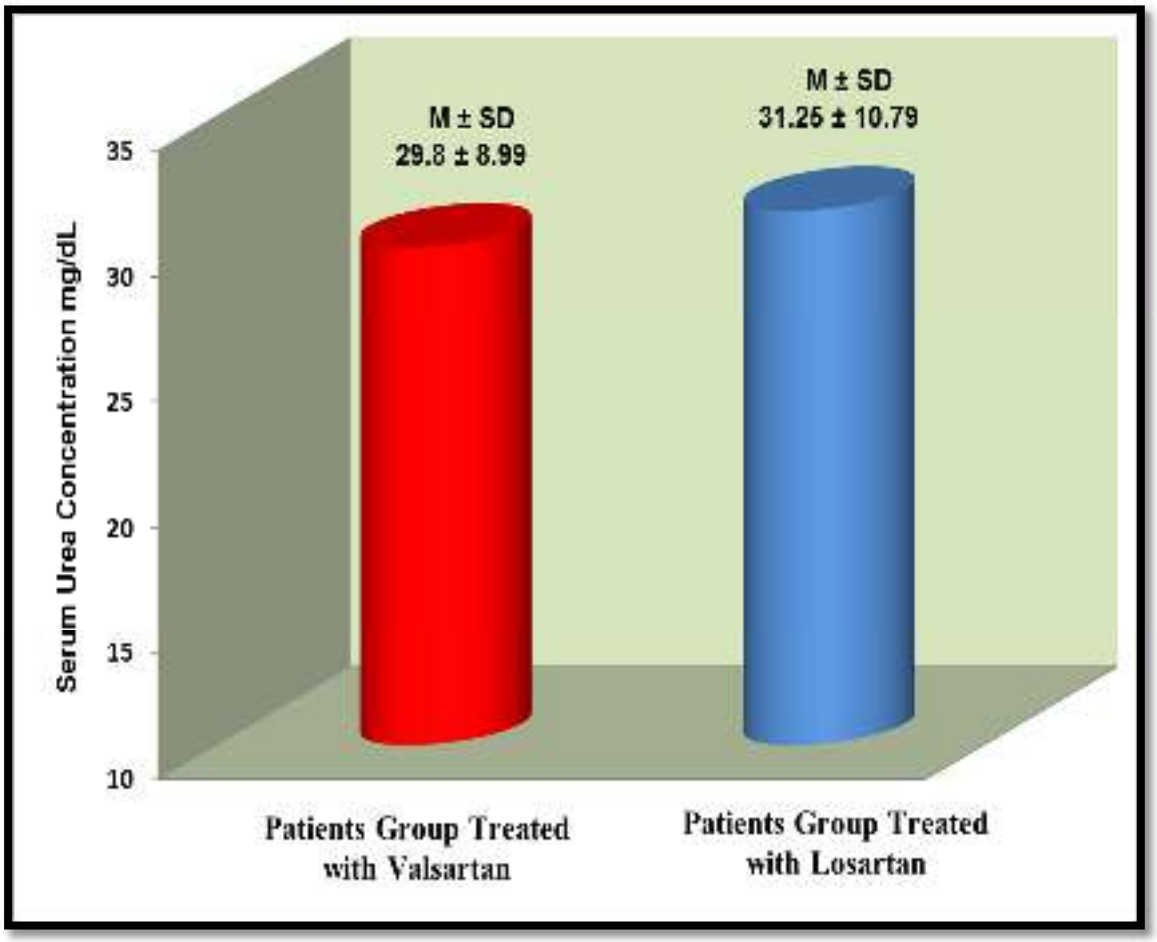

Figure 3-1: Mean Serum Urea Concentration in Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

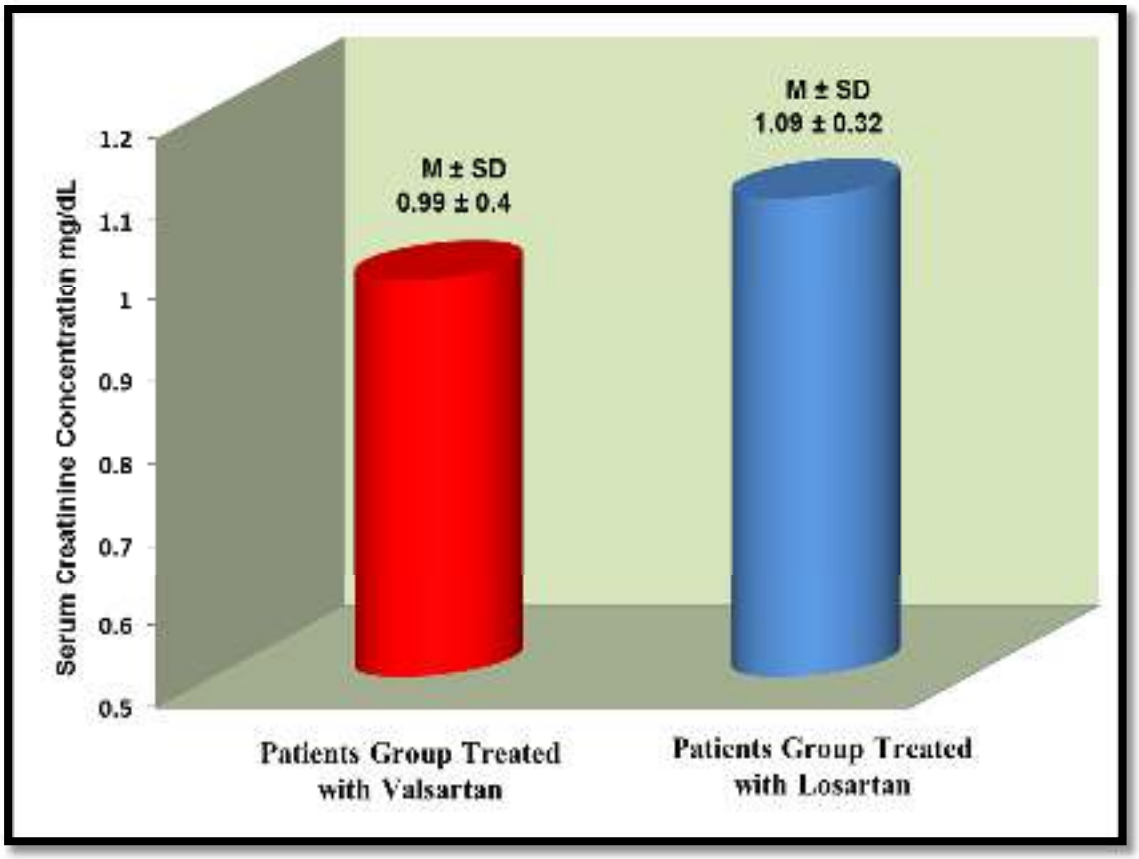

Figure 3-2: Mean Serum Creatinine Concentration in Hypertensive Patients Group Treated with Valsartan and Hypertensive Patients Group Treated with Losartan

The results of this study demonstrate that hypertensive patients group treated with losartan, there are a non-significant changes in serum lipid profile concentrations when compared to those of hypertensive patients group treated with valsartan, the result of this study is identical to Elliott et al.[41] and Hedner et al study [42].

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In contrast we find that this study is not compatible with Fogari, Mugellini, et al. [43] and Fogari, Zoppi, et al. study[44] that appear hypertensive patients group treated with losartan, there are a significant changes in serum lipid profile concentrations when compared to those of hypertensive patients group treated with valsartan; this differential effect may be due to differences in the pharmacologic properties of these agents [43].

Clinical presentation negate the difference between valsartan and losartan, We should consider the differences between losartan and valsartan, such as inverse agonism, their mode of binding to AT1 receptor (valsartan is an insurmountable type, whereas losartan is a surmountable type), area under the drug concentration time curve, AT1 receptor selectivity, half-life in blood etc. [45] but, The results of this study demonstrate that losartan and Valsartan similarly effective on serum lipid profile may be attributed to the valsartan and losartan own almost the

same bioavailability ~26.5%, and the both drug have highly affinity bound to plasma proteins, losartan 98% and valsartan 94-97% [46].

Conclusions

1. In hypertensive patients group treated with losartan, there are a non-significant changes in serum lipid profile concentrations when compared to those of hypertensive patients group treated with valsartan.

2. There are a non-significant changes in renal functions in hypertensive patients group treated with losartan when compared to those of hypertensive patients group treated with valsartan.

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الخلاصة

صُممت الدراسة الحالية لتقييم مستوى الدهون في الدم واختبار وظائف الكلى في كل من مجموعة المرضى الذين عولجوا مع (فالسارتان) والمرضى المعالجين ب(اللوسارتان). تضم مجموعة (فالسارتان) 33 مريضًا مصابين بارتفاع ضغط الدم ، تراوحت أعمار هذه المجموعة بين 35-80 عامًا وتضم 13 ذكرًا و 20 أنثى. و تضم مجموعة (لوسارتان) 33 مريضًا مصابين بارتفاع ضغط الدم ، تراوحت أعمار هذه المجموعة بين 32-75 عامًا وتضم 15 ذكرًا و 18 أنثى. السيرم لبيد، اليوريا والكرياتينين قيس بواسطة الطريقة (الأنزيمية الطيفية).

أظهرت نتائج الدراسة الحالية تغيرات غير ملحوظه ($p > 0.05$) في العمر، مدة المرض ومدة العلاج في مجموعة مرضى ارتفاع ضغط الدم التي عولجت ب (لوزارتان) بالمقارنة مع مجموعة مرضى ارتفاع ضغط الدم التي عولجت ب(الفالسارتان). وتظهر نتائج الدراسة تغيرات غير ملحوظه ($P > 0.05$) في إجمالي كوليسترول الصائم، كولسترول عالي الكثافة، الدهون الثلاثية، البروتينات ذات الكثافة المنخفضة و البروتينات ذات الكثافة العالية في مجموعة المرضى المصابين بارتفاع ضغط الدم المعالج ب(الوسارتان) بالمقارنة مع (فالسارتان).

أيضا ، تظهر النتيجة تغيرات غير ملحوظه ($P > 0.05$) في مصل اليوريا وتركيز الكرياتينين في مجموعة مرضى ارتفاع ضغط الدم التي عولجت ب(لوزارتان) بالمقارنة مع تلك المجموعة من مرضى ارتفاع ضغط الدم التي عولجت ب(فالسارتان).

في الختام ، في مجموعة مرضى ارتفاع ضغط الدم عولجت ب(لوسارتان)، هناك تغييرات طفيفه في تركيزات الدهون في الدم، المصل وفي وظائف الكلى عند مقارنتها مع تلك المجموعة من مرضى ارتفاع ضغط الدم التي عولجت ب(فالسارتان).

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (١١)

صَدق الله العَلي الْعَظِيمِ

سورة المجادله (١١)

جامعة القادسية
كلية الصيدلة

دراسة التغيرات البيوكيميائية المرتبطة بارتفاع ضغط الدم

سميه عجمي عسكر

سمر صفاء شاكر

إشراف

د.غفران محمد حسين

١٤٣٩هـ ٢٠١٨م