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Effect of angiotensinogen (M235T) and angiotensin II type 1 receptor (A1166C) genes polymorphisms on response to angiotensin receptor blocker (candesartan) in hypertensive patients of Karbala province

A Thesis Submitted to the Council of College of Pharmacy University of Kerbala as Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology

By:

Zahraa Haider Jasim

Supervised By:

Assistant Professor
Dr. Haidar A N Abood Almuthaffar
PhD. Pharmacology

Assistant Professor
Dr.Suzanne Jubair Abbas
PhD. Biotechnology

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بسم الله الرحمن الرحيم

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صدق الله العلي العظيم

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Supervisor Certification

We certify that this thesis was prepared by (Zahraa Haider Jasim) under our supervision at the Department of Pharmacology and Toxicology in College of Pharmacy / University of Kerbala, as a partial requirement for the degree of Master of Pharmacology and Toxicology

Supervisor

Assistant Professor

Dr. Haidar Abdul Amir Najim

Ph.D. Pharmacology

Supervisor

Assistant Professor

Dr. Suzanne Jubair Abbass

Ph.D. Biochemistry

In the view of the available recommendation, we forward the present thesis for debate by the examining committee.

Assist. Prof. Amal umran mosa

Chairman of Pharmacology and Toxicology Department

University of Kerbala / College of Pharmacy

Committee Certification

We, the examining committee, certify that we have read this thesis; and have examined the student (Zahraa Haider Jasim) in its contents, find it adequate with standing as a thesis for the Degree of Master of Science in Pharmacology and Toxicology

Professor

Dr. Abdulkareem hameed Abd

Ph.D. Pharmacology & Toxicology

Chairman

Assistant Professor
Dr. Kamal Abdul Rasool Al-Yassiry

Ph.D. Pharmacology
Member

Assistant Professor
Dr. Amal umran mosa

MSc. Toxicology
Member

Assistant Professor
Dr. Haidar Abdul Amir Najim

Ph.D. Pharmacology

Assistant Professor
Dr. Suzanne Jubair Abbass

Ph.D. Biochemistry

Approved by

College of Pharmacy / University of Kerbala

As a thesis for the degree of

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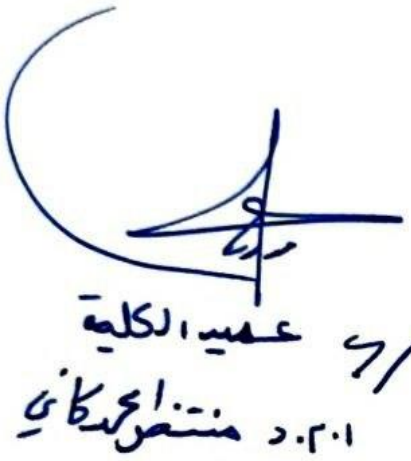

Professor

Dr. Ahmed Salih Sahib

Dean

College of Pharmacy / University of Kerbala

Seal

Higher Studies Registration

College of Pharmacy / University of Kerbala

Dedication

To my soul of the life

To whom I am breathing the air

My family… my father and mother who encourage me and help me in every step in my life

My husband… who has been a consistent source of support and encouragement during graduate school, college and life

And finally, my daughter who patient with me

I dedicate this thesis with love…

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ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
ACE2	Angiotensin converting enzyme 2
ACC	American College of Cardiology
ADBR	Adrenergic beta receptors
AHA	American Heart Association
Arg	Arginine
Asn	Asparagine
AH	Atrial Hypertension
ANOVA	Analysis of variance
AGT	Angiotensinogen
Ang I	Angiotensin I
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
ARBs	Angiotensin receptor blockers
ARMS-PCR	Amplification refractory mutation system-polymerase chain reaction
ATR1	Angiotensin type 1 receptor
ATR2	Angiotensin type 2 receptor
BMI	body mass index
Bp	Blood pressure
BUN	Blood Urea Nitrogen
CKD	Chronic kidney disease
CVD	Cardiovascular disease
CV	Cardiovascular
CCBs	Calcium channel blockers
ECFV	Extracellular fluid volume
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection fraction
ELISA	Enzyme linked immunosorbent assay
EP	Endopeptidases
ESRD	End-stage renal disease
Egfr	Estimated glomerular filtration rate
GPCRs	G protein-coupled receptors
GLDH	Glutamate dehydrogenase
IVSD	Interventricular septum diameter
IL-6	Interleukin-6
JNC	Joint National Committee

LMICs	low and middle-income countries
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVSD	left ventricular systolic dysfunction
LVDD	left ventricular diastolic dysfunction
LVPWD	left ventricular posterior wall diameter
Lys	Lysine
mm Hg	Millimeters of mercury
NAD	nicotinamide adenine dinucleotide
RAAS	Renin angiotensin aldosterone system
NADH	Reduced nicotinamide adenine dinucleotide
PCR	polymerase chain reactions
Phe	Phenylalanine
PS	potassium-sparing
RAAS	Renin angiotensin aldosterone system
SD	Standard Deviation
SNS	sympathetic nervous system
SNP	Single nucleotide polymorphisms
SPSS	Statistical Package for the Social Sciences software
TBE	Tris borate EDTA
TTE	Transthoracic echocardiography
TNF-alpha	Tumor Necrosis Factor Alpha
UTR	Untranslated region
WHO	World health organization
HWE	Hardy-Weinberg Equilibrium

Abstract

Background: Hypertension is one of the most studied subjects in the previous century. Since genetics play a role in hypertension, pharmacogenetic is used to apply the knowledge of genetic predictors on the treatment and the response to blood pressure-lowering drugs in order to ascertain the genetic determinants of drug efficacy and toxicity prior to initiating therapy. **Objective**: To study the effect of angiotensinogen gene polymorphism (M235T) and angiotensin II type1 receptor gene polymorphism (A1166C) on response to angiotensin receptor blocker (candesartan) in hypertensive patients of Karbala province.

Patients and Methods: Ninety-two patients with essential hypertension taking 8 mg/day candesartan for not less than six months were recruited for the investigation from Imam Hussein medical city and from private clinic in Karbala province, Iraq. Blood pressure, heart rate and echocardiography reports were recorded. The kidney function tests (serum creatinine and urea) and other biochemical parameters such as angiotensinogen, angiotensin II levels and some electrolytes were analyzed. The tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for genotyping of the single nucleotide polymorphisms (M235T and A1166C).

Results: The distribution of M235T SNP among hypertensive patients was 39.13% AA, 21.74% AG and 39.13% GG. While the distribution of A1166C SNP was 54.35% AA, 40.22% AC and 5.43% CC. Regarding echocardiogram (Echo) parameters, the M235T SNP and left ventricular hypertrophy (LVH) had a statistically significant association ($p<0.05$). Patients with GG genotype had significantly higher proportion (29.34 %) among the patients who have LVH, while patients with AA and AG genotypes had the proportion of 26.08% and 15.21% respectively. On the other hand, there was no statistical association between A1166C and echocardiogram parameters

or blood pressure (Bp) parameters (P>0.05) except heart rate; patients with AA genotype have higher heart rate (88.32±10.64, P=0.005) than patients with AC and CC genotype. There was no statistically significant association (P>0.05) between M235T or A1166C SNPs and the kidney function and other biochemical parameters.

Conclusion: Hypertensive patients who have the homozygous mutant type (GG) of M235T in angiotensinogen gene have higher incidence of having hypertension complications LVH, while AG genotype may have protective effect against LVH. The A1166C SNP has neither an effect on hypertension complications nor has an effect on the patients' response to candesartan in Iraqi hypertensive patients.

1. Introduction

1.1. Hypertension

Hypertension is defined as an abnormally high level of systemic arterial blood pressure that persists over time. Hypertension becomes more common as people get older.(1) The vascular and heart undergo significant pathological alterations as a result of persistent elevated arterial blood pressure. Blood pressure (BP) of ≥140/90 mmHg is a criteria for hypertension-related cardiovascular disease (CVD) that requires immediate medical attention.(2) Hypertension is one of the most studied subjects in the previous century, and it was one of the most important comorbidities contributing to stroke, myocardial infarction, heart failure, and renal failure.(3) Hypertension has been called the "silent killer" because most individuals with hypertension have no symptoms or have vague or non-specific symptoms. As a result, hypertension is underdiagnosed, and even when it is diagnosed, adherence and compliance to management strategies remains a problem.(4). Recently, the development of a new class of antihypertensive medications has permitted not only proper BP management, but also the selection of preferred therapies depending on each patient's condition, resulting in a reduction in the rate of CVD in hypertensive patients.(5) According to clinical research, ARBs such as candesartan-based therapy successfully decreases left ventricular hypertrophy (LVH) measured by echocardiography in hypertension patients.(6)

1.2. Types of hypertension

Hypertension is classified into two major types: primary and secondary hypertension.(7)

1.2.1 Primary Hypertension:

Primary hypertension, also known as essential hypertension, is a heterogeneous condition in which different causative factors cause high blood pressure in different patients. Essential hypertension accounts for 95% of all hypertension patients. It could be due to environmental factors, genetic factors, interactions between these factors, or due to other unknown causes of hypertension. (8)

1.2.2 Secondary Hypertension:

It accounts for only a small percentage of hypertensive patients (5 -10%). Secondary hypertension can be caused by a variety of conditions, including renal parenchymal diseases, as well as pheochromocytoma, Cushing's syndrome, primary hyperaldosteronism, myxoedema, and renal vascular diseases. [9]

1.3. Epidemiology of hypertension

Hypertension is the most preventable cause of CVD and mortality in the world.(9) Global mean blood pressure has remained constant or reduced slightly during the last four decades due to widespread usage of antihypertensive drugs, on the other hand, prelevance of hypertension has become more common, particularly in low- and middle-income countries (LMICs).(10) The prevalence of hypertension in adults was more common in LMICs (31.5%, 1.04 billion people) than in high-income countries (28.5 %, 349 million people), owing to a inadquate access to healthcare and awareness of the disease.(11) Some of the regional heterogeneity in hypertension prevalence may be explained by variations in the levels of risk factors for

hypertension, such as high salt intake, low potassium intake, obesity, alcohol consumption, physical inactivity, and a poor diet.(12) The prevalence of hypertension is significant in Middle Eastern countries. According to a survey on chronic noncommunicable disease risk factors conducted in Iraq in 2006, the prelevence of hypertension was found 40.4% in both sexes in the age group (25-65) years.(13) As reported by WHO (world heath organzation) Eastern Mediterranean Region health statistics published in 2008, the prevalence of hypertension in Iraq for both sexes was 29.4%.(14) Despite stable global age-standardised prevalence, the number of people aged 30–79 years with hypertension doubled from 1990 to 2019, from 331 million women and 317 million men in 1990 to 626 million women and 652 million men in 2019. (15) By the end of 2025, the percentage of hypertensive patients is expected to reach up to 60% of the total population.(16)

1.4. Etiology of hypertension

In a small percentage of cases, hypertension has a definite cause, but in the vast majority of cases (≈90%), its etiology is unknown; thus, the term essential hypertension is used. Essential hypertension is now recognized as a multifactorial disease caused by the interaction of numerous genetic and environmental factors.(17) Given the multifactorial nature of Bp homeostasis, any variation in Bp is likely to be compensated by complementary action in an attempt to restore normal blood pressure. Essential hypertension occurs when the balance between the factors that tend to raise blood pressure and those that try to lower it is sufficiently disrupted, and the compensatory mechanisms fail to compensate.(18) The link between dietary salt (sodium chloride), renal sodium processing, and Bp has been studied in humans and animals for over a century. An evidence suggests a link between chronically high salt intake and the development of hypertension, which occurs when the kidneys are unable to excrete the ingested sodium.(19) In conjunction with this primary causal

factor, a number of adjunctive factors, such as obesity, diabetes, aging, emotional stress, sedentary lifestyle, alcohol intake, and inadequate potassium intake, may raise the risk of developing hypertension. As a result, some people get hypertension while others do not, and the chance of developing hypertension is determined by the individuals weight of the hypertension s adjunctive factors.(20)

1.5. Regulation of blood pressure.

Blood pressure is the force of blood pushing against the walls of peripheral arteries. Millimeters of mercury are unit that used to measure it (mm Hg). (21) The normal average Bp is 120/80, with 120 being systolic and 80 being diastolic pressure, but this range varies in cases of stress, hypertension, and other health conditions. (22) The regulation of Bp is a complex physiologic function that depends on a number of actions of the cardiovascular, brain, renal, and endocrine systems. Controlling Bp is the process of regulating blood flow to a certain tissue in response to its metabolic demands. Vasoconstriction and vasodilation are local mechanisms that control blood flow, they occur in response to acute and chronic changes in the number and diameter of blood vessels supplying a tissue. Vasoconstriction and vasodilation are controlled by endothelial autocrine secretions. In addition to local control of blood flow, systemic control of blood flow is mediated by the autonomic nervous system, regulates changes in cardiac output and arterial blood pressure primarily via the sympathetic nervous system (SNS), whereas the parasympathetic nervous system primarily regulates cardiac function.(23) The SNS and the renin–angiotensin–aldosterone system (RAAS) controls short-term changes in Bp, while the kidney control long-term BP control. The most important stimulus to renin release in the juxtaglomerular apparatus is through renal sympathetic nerves, hence sympathetic regulation plays a role in long-term BP regulation as well.(24)

1.5.1 Regulation of Blood Pressure by nervous system

Baroreceptors are a type of mechanoreceptor that allows the autonomic nervous system to transfer information obtained from blood pressure. There are two types of baroreceptors: high-pressure arterial baroreceptors and low-pressure volume receptors, which are both stimulated by stretching of the vessel wall. Arterial baroreceptors are located within the carotid sinuses and the aortic arch. Low-pressure volume receptors, or cardiopulmonary receptors, are located within the atria, ventricles, and pulmonary vasculature.(25) Arterial baroreceptors function to inform the autonomic nervous system of beat-to-beat changes in blood pressure within the arterial system. Rapid decreases in blood pressure, such as in orthostatic hypotension, resulted in decreased stretching of the artery wall and decreased action potential frequency, ultimately resulting in increased cardiac output and vasoconstriction resulting in increased blood pressure. The opposite is found to be true of increased blood pressure. Cardiopulmonary receptors are function to inform the autonomic nervous system of the blood volume within the system. In low volume states, circulatory and renal changes result in increased salt and water resorption within the kidneys, increased salt and water oral intake, and slower, longer-term mean pressure changes.(26)

1.5.2 Regulation of Blood Pressure by renal mechanism

Long-term mechanisms regulating arterial pressure are linked to the regulation of sodium balance and extracellular fluid volume (ECFV). ECFV excesses or deficiencies are strongly linked to blood volume, and so can be detected by a variety of cardiovascular receptor mechanisms. Signals are, then, sent to the kidneys, causing changes in renal sodium and water excretion and so correction of the ECFV imbalance.(27) Renal perfusion pressure and sodium excretion have a direct relationship. Natriuresis is caused by a sudden increase in renal perfusion pressure.

The setup of the pressure-natriuresis mechanism is influenced by several important neurohumoral systems, resulting in a much more sensitive and complex long-term relationship between arterial pressure and sodium excretion. Neurohumoral mechanisms allow rapid changes in sodium excretion with small changes in arterial pressure by resetting the pressure-natriuresis Relationship. Under normal circumstances, an increase in blood volume triggers a series of coordinated physiologic reactions that enhance the pressure-natriuresis relationship. In contrast, the slope of the pressure natriuresis relationship is considerably lowered after sodium depletion or reduced blood volume.(28)

1.5.3 Regulation of Blood Pressure by endocrine system

The renin-angiotensin-aldosterone system (RAAS) is the most essential endocrine system in controlling hemodynamic stability by regulating Bp, fluid volume, and sodium-potassium balance, as a result, any change in the molecules that makes up RAAS leads to the development of atrial hypertension (AH).(29) Renin is produced as an inactive form in the kidneys and released into the bloodstream in response to low intratubular sodium levels, hypotension in the afferent arterioles of the renal glomerulus, and sympathetic activity. Proteolytic and nonproteolytic processes in the bloodstream activate pro-renin to produce the active form.(30) Angiotensin I (Ang I) is produced when active renin catalyzes the cleavage of the glycoprotein angiotensinogen. The angiotensin-converting enzyme (ACE) cleaves angiotensin I to produce angiotensin II (Ang II) and breaks down bradykinin which is a vasodilator substance, while neutral endopeptidases (EP) cleave angiotensin I to produce angiotensin-(1-7), another active peptide of this system that typically counteracts the effects of Ang II. The angiotensin type 1 receptor (ATR1) is responsible for the majority of Ang II's known actions, but it can also bind to the Ang II type 2 receptor (ATR2), causing the opposite effects to the ATR1 .(31) Angiotensin-(1-7) can also be

produced by cleaving angiotensin II by angiotensin-converting enzyme 2 (ACE2), which lowers the concentration of Ang II and induces vasodilation in cardiac and vascular tissues.(32) Aldosterone is another RAAS effector which is synthesis and release by Ang II via the ATR1 in the adrenal cortex. Aldosterone stimulates sodium reabsorption, water retention, and potassium and magnesium loss through specialized actions on the distal nephron of the kidney, consequently altering extracellular fluid volume and blood pressure.(33)

1.6. Pathophysiological mechanisms of essential hypertension

Various risk factors such as genetics, sympathetic over activity, salt sensitivity, increase renin secretion, obesity, obstructive sleep apnea, insulin resistance and others all have been linked to high Bp. Despite awareness of various risk factors, the pathophysiology of hypertension remains a mystery.(34)

1.6.1 Genetics

Parents with a history of hypertension are more likely to have hypertension in their children, especially if both parents are hypertensive. Primary hypertension originates from a combination of genetic and environmental factors, the heritability of high BP is 30% to 50%.(35) The major determinants of cardiac output and total peripheral resistance are controlled by a complex network of interacting pathways involving renal, neuronal, endocrine, vascular, and other mechanisms. Multiple genes contribute to the specialized functions that regulate blood pressure in each of these systems.(36) Over 100 single nucleotide polymorphisms related with BP phenotypes have been found using genome-wide association, as well as putative novel routes of BP regulation and potential therapeutic targets. Genes that exhibit allelic variations may result in elevation or reduction in Bp. According to the standard model, the

effects of these multiple alleles are cumulative, with each genetic variant adding a small increase or decrease in Bp.(37)

1.6.2 Sympathetic nervous system over activity

The sympathetic nervous system plays an important role in the pathogenesis of primary hypertension and in certain secondary forms of hypertension. (38) This has been confirmed by a relevant number of studies, which assessed adrenergic drive either indirectly, by measuring circulating blood levels of the adrenergic neurotransmitters epinephrine and norepinephrine or directly, by quantifying efferent postganglionic muscle sympathetic nerve traffic in peripheral nerves as well as regional norepinephrine release and reuptake by adrenergic nerves via the norepinephrine radiolabelled technique.(39, 40) Overt increase in sympathetic activity is associated with increased heart rate, cardiac output, peripheral resistance. Stress promotes sympatho-adrenal activity and raises Bp. (41)

1.6.3 Renal mechanisms: hypertension and salt sensitivity

The kidney plays a pivotal role in the pathophysiology of hypertension and in salt sensitivity, increased salt consumption leads to an increase in extracellular fluid volume and cardiac output hence the pulse volume. When the kidney, which is the basic fluid volume regulator with the classic sodium-pressure mechanism, loses its ability to eliminate excess salt and fluid volume, the pulse volume rises, and Bp rises with it.(42) Salt-sensitive individuals have an abnormal renal reaction to salt consumption; the kidneys retain the majority of the salt due to an excessive over-reactivity of the sympathetic nervous system and a blunted suppression of the renin–angiotensin axis. Furthermore, instead of having lower peripheral vascular resistance, salt-sensitive people have higher vascular resistance, owing to decreased nitric oxide generation in the endothelium. It is reported that salt sensitivity concerns 30–50% of the hypertensive subjects.(43)

1.6.4 Hormonal mechanisms: The renin-angiotensin aldosterone system (RAAS)

Hypertensive patients have elevated renin levels, which are thought to cause hypertension. Angiotensinogen is broken down by renin into angiotensin I. Angiotensin converting enzyme converts Ang I to Ang II, the most vasoactive peptide and a powerful blood vessel constrictor. It affects arterial musculature, increases peripheral resistance, and raises Bp.(44) Angiotensin II has direct sodium-retaining effects because it increases the activity of the Na+/H+ exchanger and Na+/K+ ATPase in the proximal tubule, the Na+/K+/2Cl transport in the loop of Henley, and various ion transporters in the distal nephron and collecting tubules. Angiotensin II also causes the adrenal glands to release aldosterone, which stimulates the epithelial cells of the kidneys to increase salt and water reabsorption, resulting in increased blood volume and Bp.(45) Angiotensin II has a direct hypertrophic effect on cardiomyocytes where the Overstimulation of RAAS in hypertension is considered to be the primary mechanism of development of LVH. (46)

1.7. Complication of hypertension

1.7.1 Hypertensive vasculopathy

Hypertensive vasculopathy is characterized by endothelial dysfunction and remodeling of the small and large arteries, this results in a decreased ability of the high resistance vasculature to dilate, which manifests clinically as angina due to a decrease in coronary reserve, plaque formation, and stenoses and aneurysms, particularly in the aorta. (47)

1.7.2. Hypertensive heart disease

This term refers to a multitude of functional and anatomical changes in the heart, with left ventricular hypertrophy being particularly important. (48) Left ventricular

hypertrophy is a structural remodeling of the heart that occurs as a result of excessive volume and/or pressure. In hypertensive individuals, an increase in left ventricular mass (LVM) identified by echocardiography has been found to be a strong independent predictor of adverse cardiovascular events such as sudden cardiac death, coronary artery disease, stroke, and heart failure. A number of other factors, including age, gender, ethnicity, and genetic factors, as well as comorbidities such as obesity, diabetes, and chronic renal disease, have been discovered to influence LVM, in addition to the severity and duration of hypertension. (49) In hypertensive patients, LVH has been associated with various genetic components. (50) Left ventricle hypertrophy occurs in three stages: adaptive, compensatory, and pathological. The first two phases of contractile dysfunction are reversible, while the third is irreversible. LVH develops in 15-20% of patients with mild arterial hypertension and 50% of patients with severe hypertension. The pathophysiology of LVH include Cardiomyocyte hypertrophy, interstitial and perivascular fibrosis, coronary microangiopathy, and macroangiopathy. When compared to patients without LVH, those with LVH have a 2-4 times higher risk of having a CV event.(51) Hypertensive heart disease is usually asymptomatic at first, but develops into angina pectoris, dyspnea, and arrhythmia as it progresses. Reduced coronary reserve, impaired systolic and diastolic left ventricular function, atrial fibrillation, and ventricular arrhythmia are all causes of these symptoms. (52)

1.7.3 Hypertensive cerebrovascular damage

Arterial hypertension is the leading cause of stroke, which occurs in 80% of cases as a result of an underlying ischemic infarction. Early hypertensive micro angiopathic complications include lacunar infarctions, micro hemorrhages, and focal or diffuse white matter lesions. Untreated or inadequately treated hypertension is also leading factor in the development of vascular dementia.(53)

1.7.4 Hypertensive nephropathy

Hypertension is the second most common cause of end-stage renal disease (ESRD), after a diabetes mellitus.(54) The majority of hypertensive patients develop mild-to-moderate hypertensive nephropathy, which progresses to ESRD in a very small percentage. Nonetheless, the percentage of patients developing ESRD increases dramatically when BP values remain uncontrolled for an extended period of time or if preexisting kidney disease exists. (55) Early symptoms of hypertensive nephropathy include the presence of mild albuminuria and a reduced estimated glomerular filtration rate (eGFR), both of which are easily measured parameters. A more recent Italian study found that using albuminuria and eGFR as renal parameters for hypertensive end organ damage resulted in a significant change in overall cardiovascular risk estimation .(56)

1.7.5 Hypertensive retinopathy

Hypertensive retinopathy plays a role in assessing cardiovascular risk in hypertensive patients, as poorly controlled systemic hypertension damages the retinal microcirculation.(57) The use of an ophthalmoscope to check for hypertensive retinopathy has long been considered standard practice when evaluating patients with hypertension.(58) The Joint National Committee (JNC) on Prevention, Detection, Evaluation, and treatment of High Blood Pressure lists retinopathy as one of several indicators of target-organ damage in hypertension. According to the JNC, even in people with stage 1 hypertension (blood pressure 140-159/90-99 mm Hg) who have no other signs of target-organ damage, the presence of retinopathy may be an indication for the start of antihypertensive treatment.(59)

1.8 Clinical Manifestations of hypertension.

Many of the symptoms commonly associated with uncomplicated hypertension, such as headache, tinnitus, dizziness, nausea and fainting, are likely to be psychogenic in origin. (60) They could be the result of hyperventilation brought on by fear of being diagnosed with a life-threatening disease that threatens one's well-being and survival. Recent data, however, show that, surprisingly, a person's general sense of well-being often improves during the initiation of hypertension medical treatment. These new findings suggest that hypertension isn't as asymptomatic as previously thought. Because overt symptoms and signs usually coincide with the onset of target organ damage, hypertension can go unnoticed for years, even if it is not completely asymptomatic. As a result, proper Bp measurement technique is the cornerstone of hypertension detection.(61)

Secondary hypertension is symptomatic and many of the symptoms often attributed to underlying cause of secondary hypertension such as Primary hyperaldosteronism cause fatigue, hypokalemia, metabolic alkalosis, hypernatremia. Pheochromocytoma cause Interval episodes of hypertension, with flushing, diaphoresis, headaches. Cushing syndrome cause obesity, abnormal menstrual cycle, abdominal striae, hyperglycemia, muscle weakness.(62)

1.9. Diagnosis of hypertension

It is possible to reduce the risk of heart attack, heart failure, stroke, and kidney failure if hypertension is detected early and treated appropriately. Detecting high Bp, unlike the diagnosis of other noncommunicable diseases like diabetes, is relatively simple, cost-effective, and feasible in low-resource settings, as well as all adults can do by themselves, especially where measurement devices are affordable and accessible.(63) Blood pressure can be measured with three different types of devices: mercury, aneroid, and electronic. WHO recommends using low-cost, dependable

electronic devices with the ability to select manual readings. Digital blood pressure measurement machines allow Bp readings to be taken outside of a clinic setting, which is often a more convenient and accurate way to obtain a reading. This is due to the fact that some people become anxious in medical settings, causing their blood pressure to rise a condition known as "white coat hypertension."(64) Some people, on the other hand, have normal blood pressure readings in the doctor's office but have elevated blood pressure at other times of the day or in different settings, a condition known as "masked hypertension." (65)

1.9.1 History

It should include:

- Duration of high blood pressure.
- Any other medical conditions, such as heart failure, peripheral vascular disease, renal disease, cerebrovascular disease, and diabetes
- Symptoms that point to secondary hypertension causes.
- Lifestyle factors such as dietary fat, sodium, and alcohol intake, smoking quantification, and physical activity. (66)

1.9.2 Physical Examination

• Measure the weight and height and find the body mass index (BMI).

• Pulses: It's crucial to check the peripheral pulses; if they're weak or absent, it could mean individuals have peripheral artery disease.

• Blood pressure measurement(67)

Blood pressure must be measured according to standard operating procedure. Before the measurements, the study participants were seated with their legs uncrossed, their backs supported, and their arms supported at heart level for at least 10–15 minutes.

Cuffs of the proper size were worn. Each participant's blood pressure measured twice at 2 minute intervals with a mercury sphygmomanometer, and the average of the two readings was recorded in a data entry form.(68)

Mercury sphygmomanometers, first developed over a century ago and largely unchanged since then, are used to measure Bp in both hospital and ambulatory settings. They are the 'gold standard' Bp measuring device, and treatment guidelines are based on them.(69) Because of pre-hypertension, and hypertension prevalence in the world, Bp measurement and control are critical in preventing the devastating cardiovascular effects of chronic hypertension. In the hospital, Bp readings are also used to represent the cardiovascular and volume status of critically ill patients and those undergoing surgery. As a result, accurate readings are critical to providing high-quality patient care.(70) Some special care is required for this device to accurately measure a person's Bp while minimizing measurement errors. (71)

1.10 Long term assesment of hypertension

1.10.1. Clinical Assessment

The endothelium is recognized recently as a major determinant of vascular physiology and pathophysiology of hypertension. The clinical significance of diagnosing endothelial dysfunction in patients with essential hypertension remains under investigation. Although a number of vascular and non-vascular markers of endothelial dysfunction have been proposed, there is an ongoing quest for a marker in the clinical setting that is optimal, inexpensive, and reproducible. In addition, endothelial dysfunction emerges as a promising therapeutic target of agents that are readily available in clinical practice.(72)

1.10.2. Investigation

1.10.2.1 Echocardiography

Although echocardiography is the second-line test for hypertensive patients, it can reveal a number of clues that point to a poor prognosis, such as increased LVM, decreased left ventricular systolic function, impaired left ventricular diastolic function, and increased left atrial size and function.(73) Echocardiography is useful diagnostic tool for determining overall cardiovascular risk and determining the best antihypertensive therapy.(74)

- **Evaluation of left ventricular hypertrophy**

In hypertensive patients, LVH is the most common target organ damage. (75) LVH increases the risk of cardiovascular complications, but its resolution, as confirmed by an echocardiogram or an electrocardiogram, improves the prognosis. When detecting LVH, echocardiography is more sensitive than electrocardiography. (76) Left ventricular hypertrophy is diagnosed by precise measurement of LVM which is measured by measuring the interventricular septum diameter (IVSd) and left ventricular posterior wall diameter (LVPWd), as well as the diameter of the interventricular cavity.(77). Mild hypertrophy is defined as a measurement of 1.1-1.3 cm, moderate hypertrophy is defined as 1.4-1.6 cm, and severe hypertrophy is defined as a measurement of 1.7 cm or more. (78)

- **Evaluation of systolic function**

Arterial hypertension is one of the most common factors that predisposes to the development of left ventricular systolic dysfunction (LVSD), which can occur even when coronary artery disease is not present. However, most hypertensive patients, particularly those with LVH, may also have a problem with left ventricular relaxation

and filling, which is known as left ventricular diastolic dysfunction (LVDD).(79) Although overt systolic dysfunction is closely associated with LVDD, a large proportion of hypertensive subjects with preserved systolic function can be developed LVDD. The latter is commonly referred to as "isolated diastolic dysfunction". It's worth noting that in patients with "isolated diastolic dysfunction", a normal systolic function is usually defined by the presence of a preserved ejection fraction (EF).(80)Ejection fraction is the most widely used and accepted echocardiographic parameter in evaluation left ventricular systolic function. Left ventricular volumes are measured in both planes at end diastole and end systole and used in the equation to calculate the EF.(81) Reference values (82):

- Normal LV function - >55%
- Mild LV dysfunction - 45%–54%
- Moderate LV dysfunction - 30%–44%
- Severe LV dysfunction - <30%.

1.10.2.2. Laboratory investigations

- **Kidney functions**

Uncontrolled hypertension has been associated with progressive decline in renal function. This decline varies widely depending on comorbidities and BP control.(83) Urea and creatinine are good indicators of a normal functioning kidney and increase in the serum are indications of kidney dysfunction. Blood Urea Nitrogen (BUN) test is an indirect measurement of renal function that measures the amount of urea nitrogen (end product of protein and amino acid catabolism) in blood and is directly related to excretory function of kidney. Creatinine tests diagnose impaired renal function by measurement the amount of creatinine phosphate (breakdown product of creatine phosphate in muscle) in blood. (84)

1.11 Treatment of hypertension

For more than 40 years, guidelines for the treatment of hypertension have been published. Australia (2016), the United States (2017), Canada (2018), Europe (2018), and the United Kingdom (2018) are the most recent years for guideline updates (2019). These guidelines have taken two approaches to defining hypertension: One is based on a treatment threshold, and the other is based on the Bp above which the risk of events increases. (85) By classifying Bp above the cut-off point, these guidelines also emphasize the range of severity of hypertension.(86) The American College of Cardiology (ACC) and the American Heart Association (AHA) defined hypertension stages in 2017 as follows (87):

- Normal BP: systolic BP is less than 120, and diastolic BP is less than 80.
- Elevated BP: systolic BP 120 to 130 and diastolic BP is less than 80.
- Stage 1 hypertension: systolic BP 130 to 139 or diastolic BP 80 to 89.
- Stage 2 hypertension: systolic BP at least 140 or diastolic at least 90.
- Hypertensive crises: systolic BP over 180 and/or diastolic BP over 120.

Goals of therapy

The ultimate goal of hypertensive patient treatment is to reduce the total risk of cardiovascular morbidity and mortality as much as possible over the long term. This requires the treatment of all identified reversible risk factors, such as smoking, dyslipidemia, and diabetes mellitus, as well as the appropriate management of associated clinical conditions like congestive heart failure, coronary artery disease, peripheral vascular disease, and transient ischemic attacks.(88)

Lifestyle and pharmacological modifications are two strategies for achieving therapeutic goals.(89)

1.11.1 Lifestyle modifications

Individuals' adoption of healthy lifestyles is critical in the prevention of high Bp and is an essential component of hypertension management. Lifestyle modifications cause lower Bp, improve the efficacy of antihypertensive drugs, and lower cardiovascular risk.(90) Most adults newly diagnosed with stage 1 hypertension (130 to 139/80 to 89 mm Hg) should make lifestyle changes alone, while those with CVD or increased CVD risk should make lifestyle changes plus drug therapy. The following are the lifestyle modifications: 1) salt restriction, 2) alcohol cessation consumption, 3) increased consumption of vegetables, fruits, and low-fat dairy products, 4) weight loss, 5) regular physical activity, 6) smoking cessation.(91)

1.11.2 Pharmacological therapy

The presence or absence of compelling indications determines initial drug therapy. According to the 2017 American College of Cardiology (ACC) and American Heart Association (AHA) guidelines, patients without compelling indications Initial drug selection are thiazide diuretics, calcium-channel blockers, angiotensin-converting enzyme inhibitors, or angiotensin-receptor blockers. while Initial drug selection for patients with compelling indications is based on favorable outcome data (from clinical trials) for specific antihypertensive drugs in the treatment of specific patient groups.(92)

1.11.2.1. Diuretics

Guidelines throughout the world list diuretics as one of the first-line treatments for patients with essential hypertension. Diuretics counter the extracellular volume expansion and the salt retention associated with hypertension and reduce morbidity and mortality. For most patients, the risk of a clinically meaningful change in laboratory parameters is rather low, whereas the clinical benefits of diuretics are high.(93) diuretics are more commonly used in combination with other classes rather

than alone as a first-line therapy. In fact, the emphasis of guidelines on combination treatments and single-pill combinations continues to increase (94) Diuretic drugs are typically classified first according to their predominant site of action along the nephron and second by the mechanism by which they inhibit transport. The loop diuretics such as furosemide inhibit the Na-K-2Cl cotransporter along the thick ascending limb and macula densa by acting from the lumen.(95) Thiazides and thiazide-like diuretics block the electroneutral Na+–Cl– cotransporter on the apical membrane of the distal convoluted tubule's early segment. (96) The use of thiazide diuretics to treat hypertension has been linked to metabolic side effects, which can be reduced by combining thiazides with potassium-sparing (PS) diuretics. (97)PS block apical sodium channels (amiloride and triamterene) and antagonize mineralocorticoid receptors (spironolactone and eplerenone). Mineralocorticoid blockers, as well as possibly ethacrynic acid, a more toxic loop diuretic, act within cells and do not require tubule lumen secretion.(98)

1.11.2.2 Beta blockers drug

Beta-blockers are one of the basic antihypertensive medications generally prescribed to patients with symptomatic angina and post-myocardial infarction.(94) β-Blockers antagonize catecholamines at β-adrenoceptors. These receptors are Gs type G-protein-coupled receptors classified as β1, present mainly within the heart and kidneys; and β2, present throughout the body in lungs, blood vessels, and muscle. The reduction in Bp achieved by β-blockers is attributable to their effects upon multiple pathways. Block of β1 receptors in the sinoatrial node reduces heart rate and block of myocardial receptors reduces contractility. They also reduce sympathetic nervous system activity, while block of receptors in the juxtaglomerular apparatus reduces renin secretion.(99) β-Blockers are divided into two groups based on selectivity on the heart. Treatment with the beta-blocker metoprolol, the esmolol, and the

atenolol has greater affinity for β1 receptors than β2 (selectivity is reduced at higher doses). This contrasts with non-cardio selective drugs such as propranolol and sotalol. In addition to their antihypertensive effects, β-blockers improve the myocardial oxygen supply: demand ratio and help reduce myocardial ischaemia by prolonging the period of diastole.(100)

1.11.2.3. Calcium channel blockers

There are two types of calcium channel blockers (CCBs): dihydopyridine and non-dihydropyridine. (101) Inhibiting L-type calcium channels and reducing calcium flux to cells are two of the antihypertensive effects of CCBs. Some dihydopyridine, however, are antagonistic to N-type calcium channels. (102) CCBs are one of the most commonly used antihypertensive agents, both as monotherapy and in combination therapy, over the past 20 years. (103) although high dose monotherapy is effective at lowering blood pressure, it can cause side effects like rash and edema. Combination therapy, on the other hand, is preferable to high dose monotherapy. The combination of CCBs and ACEIs/ARBs reduces the frequency of adverse events and cardiovascular events, but has the same antihypertensive effect as the other combination therapies (104)

1.11.2.4. Angiotensin converting enzyme inhibitors

The European and ACC/AHA hypertension guidelines do not recommend a specific antihypertensive drug class as a first-line treatment. NICE, on the other hand, advocates for an age-based algorithm in which an ACE inhibitor or ARB is recommended as a first-line treatment for those under 55 years old. Two crossover studies found that people under the age of 55 are more responsive to ACE inhibitors, ARBs and β-blockers than calcium channel blockers and diuretics, which led to this age-based approach.(105) additionally, they are indicated in the treatment of heart failure, myocardial infarction, diabetic nephropathy, and chronic kidney disease. The

protective effects of ACEIs on the kidneys and heart are greater than those expected from arterial pressure control alone.(106) ACE is a metallopeptidase enzyme which occurs mainly within the pulmonary vasculature. The inhibition of ACE reduces the cleavage of the peptide hormone angiotensin I to angiotensin II and reduces metabolism of the peptide bradykinin to inactive substances. The reduction in angiotensin II is responsible for most of the therapeutic effects. The accumulation of bradykinin has some therapeutic advantage through vasodilatation, but is also responsible for a dry cough in susceptible individuals. (107) ACEIs can also precipitate renal dysfunction by decreasing renal efferent arteriolar tone, thereby decreasing effective renal perfusion pressure, a particular risk in renal artery stenosis. Other side-effects include hyperkalaemia due to reduced aldosterone secretion, agranulocytosis, skin rashes, and taste disturbance. A rare idiosyncratic reaction to ACEIs can cause angioedema.(108)

1.11.2.5. Angiotensin receptor blockers

Angiotensin receptor blockers (ARBs) are often used as first-line therapy for the treatment of systemic hypertension because of their perceived efficacy and relatively low incidence of adverse effects. ARBs decrease mortality in patients with chronic kidney disease who are not on dialysis and also reduce CV mortality and new-onset diabetes in patients who cannot receive ACEIs. (109) ARBs have some advantages like small extent of adverse effects (like angioedema) and minimum first dose hypotension compared with ACEIs and direct Renin inhibitors (Aliskiren).(110)

- **Mechanism of action of ARBs**

Angiotensin receptor blockers are the AT1 receptor antagonists which bind with different pockets of receptor due to slight variations in their chemical structure (But majority have biphenyl-tetrazol and imidazole groups). The more specific approach by inhibiting the AT1-receptor with specific AT1-antagonists leaves the AT2-receptor unopposed and open for stimulation by alternatively formed Ang II. Compared to ACE-inhibition, there might be a more pronounced agonistic effect on AT2-receptors following application of AT1-antagonists. Combination of AT1-antagonists and ACE-inhibitors might produce a more complete inhibition of the system and enhance bradykinin accumulation resulting in increased endothelial NO production.(111)

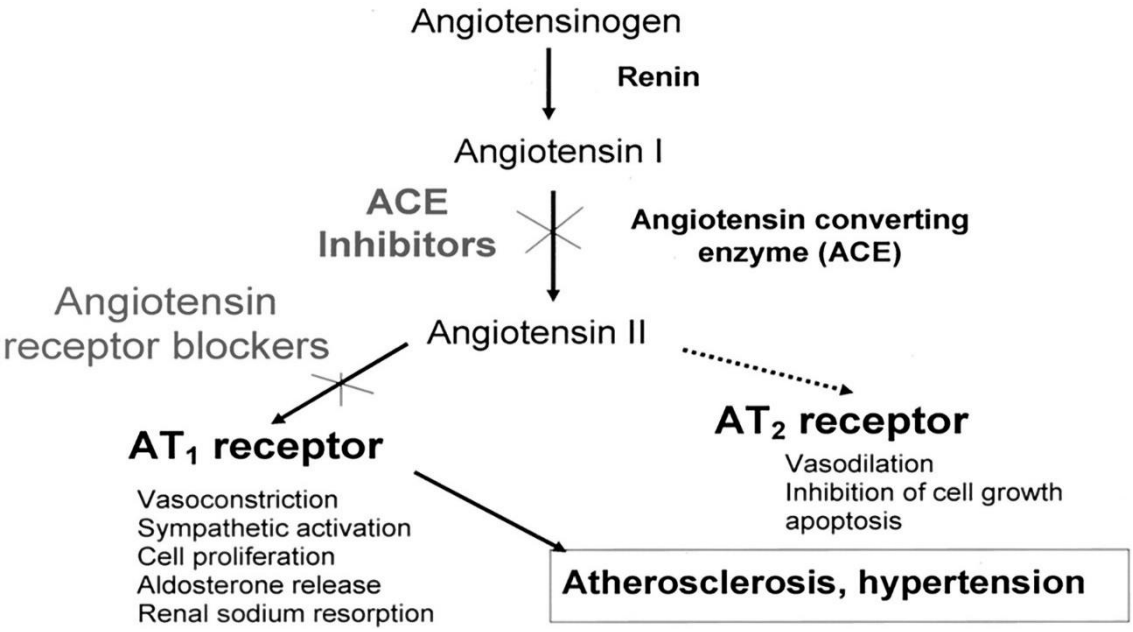

Figure 1-1. Mechanism of action of ARBs and ACE inhibitors.(112)

- **Angiotensin II receptors**

Angiotensin II binds to two subtypes of G protein-coupled receptors (GPCRs), AT1 and AT2 receptors .(113) The activation of AT1 receptors by ATII results in an increase in blood pressure due to smooth muscle contraction, an increase in systemic vascular resistance, an increase in sympathetic activity, sodium and water retention. On the other hand, ATII binding to AT2 receptors results in vasodilation via increased nitrous oxide and bradykinin production. Additionally, activation of AT2 receptors results in sodium excretion from the kidneys. (114) Ang II is an octapeptide that binds to AT1 receptor, which contains 359 amino acids and has a molecular mass of 4 kDa, by four main unique interactions. Two salt bridges, one between the Ang II side-chain Arg2 and the AT1 residue Asp281 and the other between Ang II a-COOH group of Phe8 and the AT1 residue Lys199, may be important for docking the hormone to the receptor. These salt-bridge interactions do not play a role in AT1 receptor activation. In addition, the two important interactions, one between Phe8 of Ang II and His256 in AT1 receptor and the other between Ang II Tyr4 and Asn111, are necessary to activate the receptor.(115) The majority of ARBs share a common molecular structure and exhibit 'class effects'. But recent clinical studies have found that not all ARBs exert the same effects and that some of ARBs benefits may be due to 'molecular effects' rather than class effects, because minor differences in the molecular structure of each ARB. However, ARBs have molecular effects in a clinical setting is controversial.(116)

- **Candesartan**

Candesartan is one of ARBs. Candesartan is selective and competitive binding to the AT(1) receptor prevents angiotensin II, a critical mediator of the renin-angiotensin system, from binding.(117) candesartan is used to treat all grade of hypertension and also used to treat heart failure, myocardial infarction, and diabetic nephropathy. Clinical studies showed that a candesartan-based treatment effectively reduces LVH across the hypertensive patients, regardless of gender or diabetes or metabolic syndrome.(118)

- **Structure and synthesis**

Candesartan is a derivative of the tetrazole class of drugs (five-membered heterocyclic ring with 4 nitrogen atoms). It is used in clinical practice as an ester prodrug called Candesartan cilexetil. Candesartan cilexetil is a nonpeptide compound with the chemical formula (±)-1-Hydroxyethyl2-ethoxy. -1-[p-(o-1Htetrazol-5-ylphenyl) benzyl] cyclohexyl carbonate, -7-benzimidazolecarboxylate (ester). Its empirical formula is C33H34N6O6, and its molecular weight is 610.67 g/mol and its structural formula is: (119)

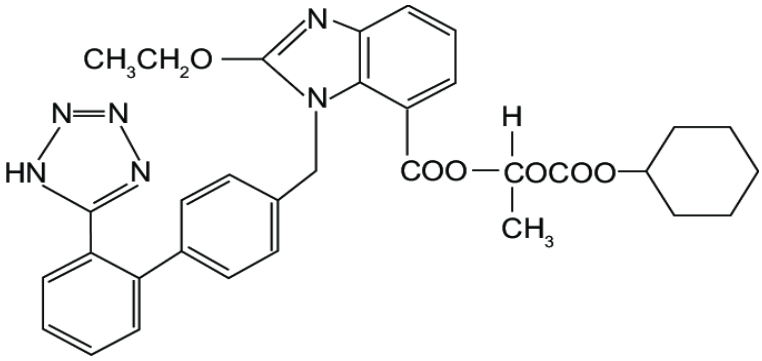

Figure1-2: chemical structure of candesartan cilexetil. (120)

- **Pharmacokinetic of candesartan**

Candesartan is taken orally as a prodrug, candesartan cilexetil which is rapidly and completely hydrolyzed in the gastrointestinal tract by carboxylesterase to form the active metabolite candesartan. Candesartan has a bioavailability of 15%. Due to its high affinity for plasma proteins, this metabolite has a very small volume of distribution (0.13 L/kg). Candesartan undergoes minor hepatic metabolism (CYP2C9) and is mainly excreted unchanged through the urinary and biliary tracts.(121)

- **Pharmacodynamics of candesartan**

Candesartan inhibits the pressor effects of angiotensin II infusion in a dose-dependent manner. After 1 week of once-daily dosing with 8 mg of Candesartan cilexetil, the pressor effect was inhibited by approximately 90% at peak with approximately 50% inhibition persisting for 24 h.(122) Plasma concentrations of angiotensin I and angiotensin II, and plasma renin activity, increased in a dose dependent manner after single and repeated administration of Candesartan cilexetil to healthy subjects and hypertensive patients. ACE activity was not altered in healthy subjects after repeated Candesartan cilexetil administration. The once-daily administration of up to 16 mg of Candesartan Cilexetil to healthy subjects did not influence plasma aldosterone concentrations, but a decrease in the plasma concentration of aldosterone was observed when 32 mg of Candesartan cilexetil was administered to hypertensive patients.(123)

- **Adverse Effects**

Candesartan most frequently reported adverse effects are symptomatic hypotension, abnormal renal function, and hyperkalemia, these occurred at an incidence of 18.8 %, 12.5 %, and 6.3 %, respectively, in the CHARM program.

Hypotension is most frequently encountered in patients who are volume- or salt-depleted as a result of dietary restrictions, dialysis, diarrhea, emesis, or diuretic use.(124) Hyperkalemia is common in the first week after starting an ARB. A few randomized controlled trials on ARB with a three-year follow-up period have found that the risk of hyperkaliemia increases,(125) while other studies have found no link, As a result long-term follow-up is required to determine the risk of hyperkalemia in patients.(126)Serum potassium in mild hyperkalemia is (5.5–6 mmol/L), moderate/serious hyperkalemia is(>6–6.9 mmol/L), and in severe hyperkalemia is (>7 mmol/L) they all can cause life-threatening complications, but they can also go unnoticed with few symptoms before cardiac arrest.(127) Additional side effect of candesartan are headache, back pain, angioedema, and upper respiratory tract infections have been reported, but these are extremely rare clinically.(128)

- **Toxicity**

Acute toxicity studies with single oral doses of up to 2000 mg/kg of Candesartan cilexetil in mice, rats, and dogs revealed no lethality. The minimum lethal dose was greater than 1000 mg/kg but less than 2000 mg/kg in mice given single oral doses of the primary metabolite, Candesartan. (129)

Candesartan toxicity is most likely to present as symptomatic hypotension, dizziness, and reflexive tachycardia. Vital signs should be monitored in patients who develop symptomatic hypotension. Patients should be instructed to lie supine with their legs raised. If these measures are insufficient, clinicians may initiate fluid resuscitation and/or supportive pharmacotherapy to raise blood pressure.(130)

1.11.2.6. Second Line Treatment

When treating resistant hypertension, second line antihypertensive agents like alpha adrenergic receptor blockers, vasodilators, and centrally acting agents can be

used. They may be used in the case of allergic conditions with 1st line antihypertensive agents.(131)

- **Alpha adrenergic receptor blockers**

α_1-Adrenergic receptors have a high affinity for drugs such as prazosin, doxazosin, and terazosin, which act to reduce blood pressure by selective blocking of the receptor. alpha-blockers, work in the peripheral vasculature and inhibit the uptake of catecholamines in smooth muscle cells resulting in vasodilation and blood pressure lowering. (132)

- **Central Acting drug**

Methyldopa, in hypertensive pregnant women is recommended as a first-line therapy for blood pressure control. Methyldopa has minor side effects such as fatigue and dizziness.(133) Clonidine is not a first line antihypertensive drug but like spironolactone it also controls BP in resistant hypertension when added to triple regimen (ACEIs, ARBs and CCBs).(134) Reserpine is a naturally occurring alkaloid derived from Rauwolfia serpentina. When administered at a low dose, it is effective for hypertension. It has comparable activity to other first-line antihypertensive agents in controlling systolic blood pressure, but additional clinical studies are required to determine the dose.(135)

- **Vasodilators**

Hydralazine is an intravenous medication that is effective for treating acute hypertension during pregnancy. it also helps to maintain blood pressure control in hospitalized children, but it can cause hypotension. (136) Minoxidil is a reserve antihypertensive medication for resistant and unregulated hypertension.(137)

1.12. Pharmacogenetics of hypertension

Pharmacogenetics is defined as the study of the genetic basis of drug response. It is based on the idea that an individual's genetic endowment, as expressed phenotypically in protein structure, configuration, and concentration, may affect drug action in a variety of ways. As a background, Genetic polymorphism is an alteration in DNA sequence found in the general population at a frequency greater than 1%.(138) Polymorphism may be associated with a single nucleotide change, known as a single-nucleotide polymorphism (SNP), or with variation in a number of repetitive DNA sequences called length polymorphism. SNP is present either in the coding regions or non-coding regions affecting exon splicing or transcription.(139) Allele is a variant form of a gene, some alleles may affect the quantity or the function of the protein coded by the gene. Therefore, some alleles may be of functional relevance, as they may affect the amount and/or activity of the gene products in the cells, and may change the pharmacokinetics or the pharmacodynamics of the drug depending on the individual genotype.(140) The clinical pharmacology has defined multiple mechanisms underlying genetic variants may alter response to drugs, and divided it into two broad classes. One mechanism is pharmacokinetic: Genetic variants associated with altered uptake, distribution, or metabolism of the agent administered. The other mechanism is pharmacodynamic: Genetic variation in the drug target or a component of the drug pathway leading to altered drug efficacy.(141)

The objectives of pharmacogenetics in hypertension are to apply the knowledge of genetic predictors of treatment response to blood pressure-lowering drugs in order to ascertain genetic determinants of drug efficacy and toxicity prior to initiating therapy. Genetic variation is widespread, with approximately 3,000,000 SNPs varying between individuals. Numerous polymorphisms in blood pressure-lowering drug receptors (e.g., beta-adrenergic receptors (ADBR) and RAAS) have been found to be predictive of

blood pressure-lowering treatment responses.(142) While awareness of the importance of pharmacogenetics in hypertension is growing, progress in the field has been affected in part by hypertension's multifactorial nature. Hypertension is a complex genetic disease with multiple genetic and environmental factors affecting blood pressure regulation (e.g., diet, obesity, and stress). Despite repeated observations across multiple populations that genetic factors account for up to 50% of the variation in blood pressure, major genes responsible for a significant proportion of the variation in blood pressure in the population have yet to be identified. Additionally, evidence suggests that the blood pressure response to antihypertensive medications is genetic in nature.(143) Three RAAS gene polymorphisms have been extensively studied: the AGT M235T, the ACE insertion/deletion (I/D), and the AT1R A1166C polymorphisms.(144)

1.12.1. Polymorphism of human AGT gene

Angiotensinogen (AGT) is a prohormone and the unique substrate of the renin-angiotensin aldosterone system, an enzyme-linked hormonal cascade that regulates blood pressure and fluid homeostasis.(145) Through sequential cleavages by classic or alternative pathways, AGT gives rise to a spectrum of angiotensin peptides, with angiotensin II being the major vasoconstrictive hormone. Circulating AGT is mainly produced in the liver by hepatocytes. AGT expression in other organs such as the brain, heart, kidneys, adrenal gland, ovaries, and placenta as well as the vascular and adipose tissues were found to express its mRNA.(146) Angiotensinogen is one of the plasma proteins that is located in the α2-globulin region in a glycosylated form and consists of 452 amino acids. Non-glycosylated AGT has a molecular weight of 53 kDa and reaches up to 75 kDa based on the extent of glycosylation. (147) The gene that encodes angiotensinogen is found on chromosome 1q42 to 43consists of 5 exons and 4 introns spanning 13 kb. AGT gene polymorphism occur when the methionine-to-threonine amino acid substituted at codon 235 (M235T) by a thymine-

to-cytosine transition at nucleotide 704 in exon 2 at the AGT locus. The change from a nonpolar amino acid (methionine) into a polar amino acid (threonine), is probably damaging/not tolerated, which could result in the abnormal functioning of AGT (Figure 1-3). Among candidate genes for essential hypertension, AGT was the first and remains perhaps "the most scrutinized" gene linked to the disease.(148) Taking into consideration the strong correlation between plasma AGT levels and BP (149), decreased blood pressure with administration of anti-AGT antibodies, increased blood pressure with injection of AGT.(150) The M235T polymorphism of the human AGT gene has been recognized as being associated with a variation of serum AGT concentration, with 235TT homozygotes having between 10% and 20% more plasma AGT than 235MM individuals.(151) However, in subsequent studies the M235T as well as other AGT polymorphisms were not consistently associated with hypertension or other clinical conditions.(152)

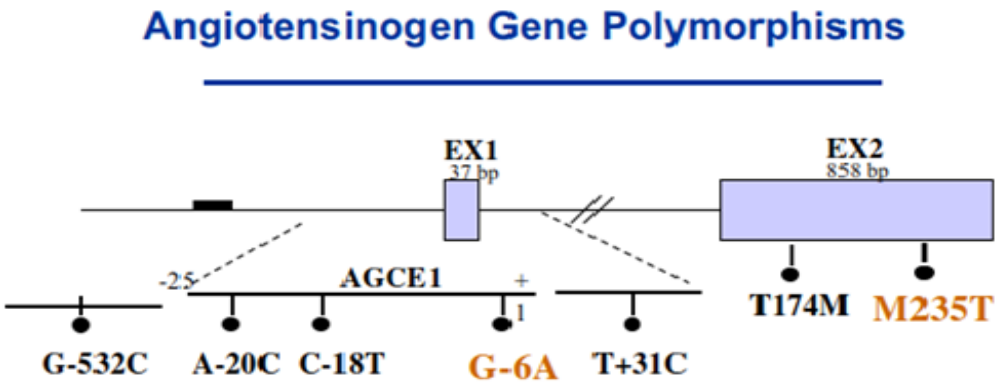

Figure 1-3: Main polymorphisms of the human AGT gene. (153)

1.12.2. Polymorphism of human AT1R gene

The angiotensin II type 1 receptor is a 359-amino acid receptor with seven transmembrane domains that belongs to the G-protein coupled receptor family. It is involved in a variety of signaling pathways, including the activation of phospholipase A or C, production of inositol phosphates, opening of calcium channels, and activation of a variety of serine/threonine- and tyrosine-kinases.(154) The AT1R gene in humans is located on chromosome 3q21-25 and measures 55 kb in length. There are five exons and four introns in this gene. The A1166C polymorphism in the 3′ untranslated region of this gene (Figure 1-4) has been described as AT1R A1166C (SNP ID: rs5186), with either an adenine (A) or a cytosine (C) base (A/C transversion) at the 1166 position.

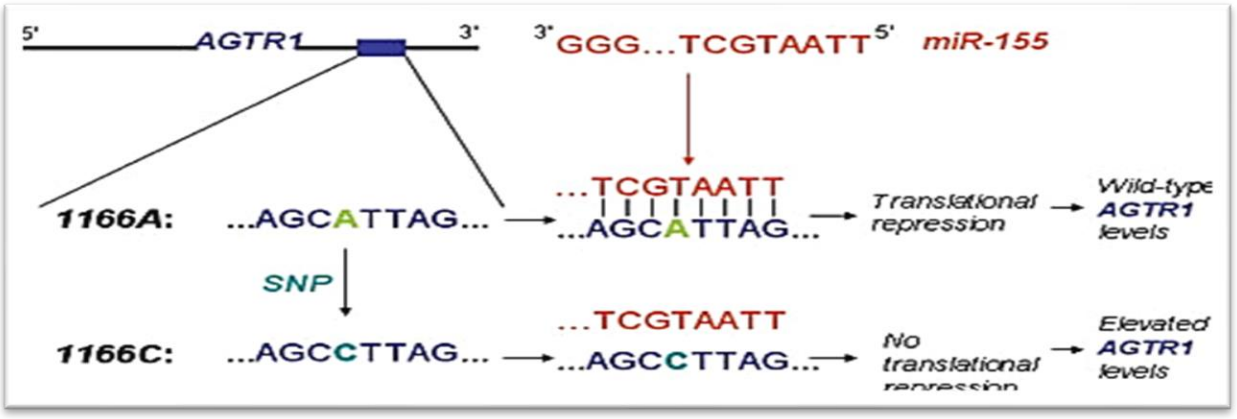

Figure 1-4 Model for molecular mechanism of 1166C association with hypertension(155)

ATR1 polymorphism was extensively studied and found to be associated with essential hypertension. Moreover, ATR1 gene polymorphisms were found to be associated with higher AT1R responsiveness, defects in mRNA processing, and receptor expression .(156)

The physiological significance of the polymorphism is uncertain because data on the function of the AT1R polymorphism is limited. Thus, the mechanism responsible for the association of hypertension status with A1166C polymorphism has remained

largely unknown, and the amino acid sequence of the receptor is not altered. It is however, may affect mRNA stability and transcription and is in linkage disequilibrium with some other polymorphism. The A1166C polymorphism is associated with prevalent hypertension, increased aortic stiffness. This polymorphism has also been associated with other diseases such as left ventricular hypertrophy, pregnancy induced hypertension; early coronary disease and excessive vasoconstriction.(157)

In patients with hypertensive heart disease, the AT1R +1166A/C polymorphism is associated with collagen type I synthesis and myocardial stiffness. Osterop et al. (158) examined the effect of the ACE I/D and A1166C polymorphisms on left ventricular hypertrophy in patients with hypertrophic cardiomyopathy and concluded that the AT1R C allele modifies the hypertrophy phenotype. Takami et al. (159)also found a correlation between the C allele and left ventricular hyperatrophy, but only in subjects with normal blood pressure and no hypertrophic cardiomyopathy. While these findings contradict Andersson et al. (160)who discovered that patients with ACE DD and AT1R CC or AC genotypes tended to have a lower ejection fraction and increased left ventricular mass. Additionally, Hamon et al. (161) observed that subjects homozygous for the AT1R CC genotype had a significantly lower ejection fraction than subjects carrying the A allele. Thus, in the absence of a clear association between the AT1R+1166A/C polymorphism and arterial stiffness and cardiac hypertrophy, the C allele remains a candidate for the association of vascular and cardiac phenotypes with genetic variation in RAAS component genes.(162)

Aim of the Study

To study Effect of angiotensinogen (M235T) and angiotensin II type 1 receptor (A1166C) genes polymorphisms on response to angiotensin receptor blocker (candesartan) and on cardiac and renal functions in hypertensive patients of Karbala province.

2. Materials, Patients and Methods

2.1. Materials

2.1.1. Chemicals

In this study, specific chemicals were used; their manufactures and origins are listed in table 2-1.

Table 2-1: Chemicals used in the study

Chemicals	Manufacture	Origin
Agarose powder	Bio basic	Canada
Absolute Ethanol	Hayman kimia	UK
DNA Ladder 100-1500 bp	Intron	Korea
Ethidium Bromide solution	Intron	Korea
Nuclease free water	Promega	USA
Primers	Bioneer	Korea
Tris borate EDTA (TBE) Buffer 10x	Intron	Korea

2.1.2. Kits

Kits were used are illustrated in table 2-2 with their manufactures and origins.

Table 2-2: Kits used in the study

Kit	Manufacture	Origin
Creatinine2	Abbott	Germany
GoTaq® G2 Green Master Mix PCR Master Mix Kit	Promega	USA
G-spin ™ Total DNA extraction kit	Intron	Korea

Human Angiotensinogen ELISA Kit	Bioassay technology laboratory	China
Human Angiotensin 2 ELISA Kit	Bioassay technology laboratory	China
ICT (Na+, K+, Cl-) Sample Diluent	Abbott	Germany
Urea nitrogen2	Abbott	Germany

2.1.3. Instruments

The Instruments that had been used are listed alphabetically in table 2-3 with their manufactures and origins.

Table 2-3: Instruments used in the study.

Equipment and Instrument	Manufacture (Origin)
Centrifuge	Hettich (Germany)
Conical Flasks (different sizes)	China
Cylinder (different sizes)	China
DNA samples box	BIOBASIC (Canada)
Disposable Gloves	China
Digital Camera	Canon (China)
EDTA Tube	China
Eppendorf tube (1.5,2) ml	China
Echo device	Ecube (India)
Freezer	Panasonic (Korea)
Gel Electrophoresis system	Techne Me (England)
Gel tube	China
Hotplate Stirrer	Labtech (Korea)
Incubator	Binder (Germany)
Micropipettes (different sizes)	Slamsed (Japan)

Micro tip sterile rack	BIOBASIC (Canada)
Mercury Sphygmomanometer	MDF (USA)
Nano pac 500power supplier for electrophoresis	Cleaver (UK)
Pulse Oximeter	Beurer (UK)
PCR- thermocycler	Thermo Fischer (Germany)
Racks Eppendorf	BIOBASIC (Canada)
Sensitive Balance	A&D (Taiwan)
Stethoscope	MDF (USA)
The ARCHITECTc4000SR chemistry	Abbott (Germany)
Tips (Yellow, Blue and white)	China
U.V Trans illuminator	Syngene (England)
Vortex mixer	Human Twist (Germany)

2.1.4. Drugs

Drug used is Candesartan 8 mg tablet (AstraZeneca, UK)

2.2. Study design

This is a cross sectional study conducted on patients with essential hypertension to investigate the role of the genetic polymorphisms in AGT and ATIR on the patients' response to candesartan. The study period was from the first of September 2020 to the end of march 2021.

2.3. Patients selection

Hypertensive patients recruited randomly from the out patients clinic of medical city Teaching Hospital. All precautions were taken in clinical settings to prevent transmission of COVID-19 like wearing mask and gloves and then every patient eligible for the study had been interviewed.

Inclusion criteria were applied for all patients:

- Patients with essential hypertension treated with candesartan 8 mg /day for at least six months
- Age > 25 years
- Karbala province

Exclusion criteria:

- Patients with ischemic heart disease, heart failure and chronic kidney disease
- Patients with metabolic or endocrine disorder
- Patient on other antihypertensive drugs.
- Pregnant women

In this study of 148 enrolled patients, 39 patients were taking other hypertensive drug plus candesartan, and 12 patients were taking 16 mg/day of candesartan and 5 women refused to take echocardiography or give blood sample. Only 92 patients completed the study.

2.4. Study protocol

Hypertensive patients taking candesartan 8mg/ day for at least six months had been included for this study and interviewed with specific questioner specifically designed for this study after taken verbal consent from them. Blood pressure was measured using a mercury sphygmomanometer after the patient had rested for at least 10 min in a sitting position. Heart rate was also measured by using Pulse Oximeter. Echocardiography was performed by cardiologist. A blood sample (5ml) was obtained from each patient for DNA extraction and for biochemical analysis. The flow chart of study protocol is summarized in figure 2-1.

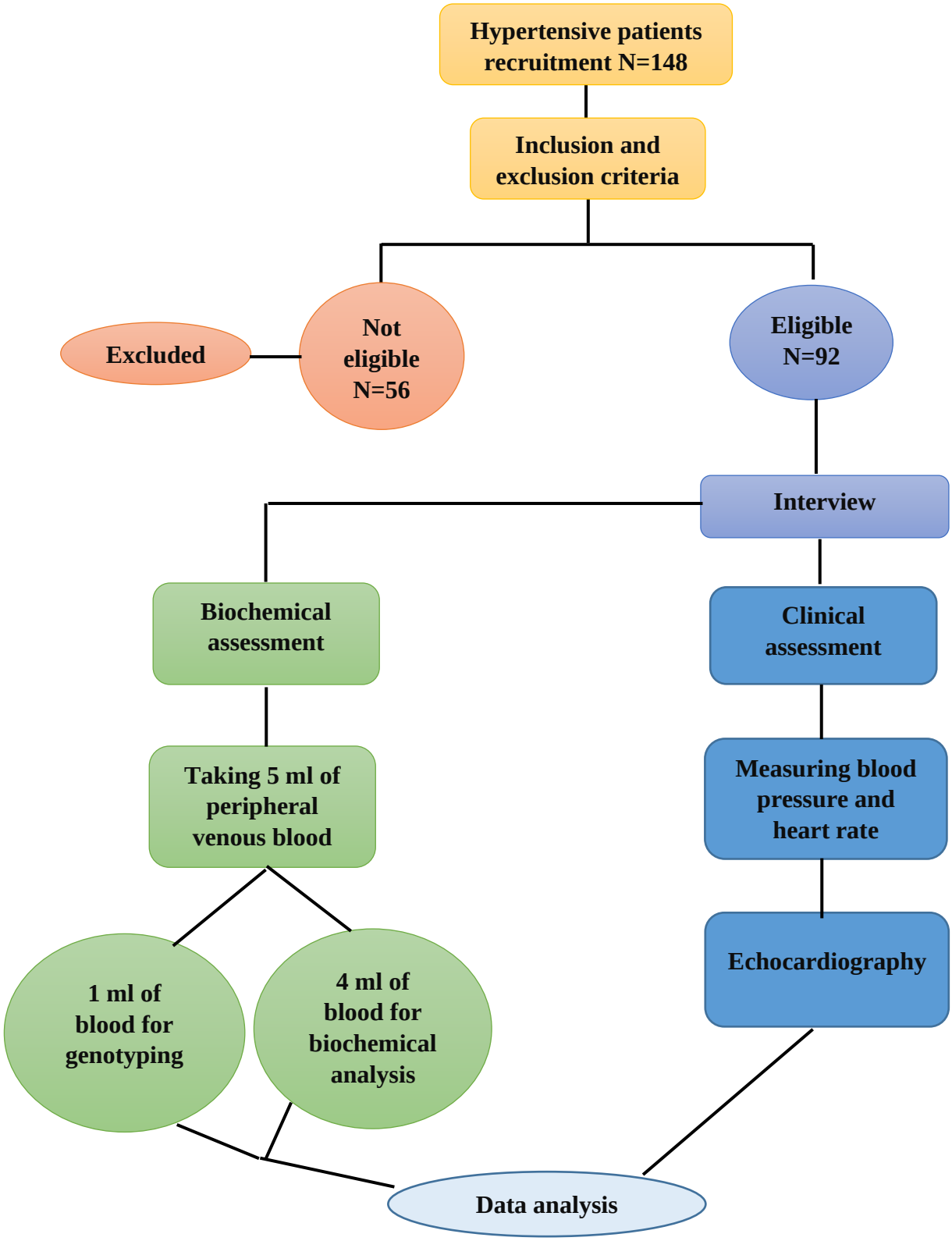

Figure 2-1: The flow chart of study protocol

2.5. Ethical Approval

The protocol of this study was approved by the ethical research and scientific committee of College of Pharmacy, University of Kerbala. Approval was also taken from Karbala Health Directorate and Administration of Imam Hussein medical city. In addition, consent was taken from patient after explaining the nature and purpose of study to them.

2.6. Methods

2.6.1. Samples Collection

Blood samples (5ml of venous blood) were obtained by a disposable plastic syringe from all patients in this study ,one milliliter of venous blood of it placed in EDTA-tube for genetic testing and the remaining four milliliter of venous blood placed in gel tube for biochemical and Elisa analysis.

2.6.2. Assessment of hypertension

Assessment of hypertension was done by Mercury Sphygmomanometers and transthoracic echocardiography (TTE). Specific parameters like: Age, gender, height, weight, body mass index were checked for each patient and recorded with other information like name, smoking, diet, duration of hypertension, duration of candesartan taking, systolic and diastolic blood pressure, mean atrial index and allergy for candesartan.

2.6.3. Biochemical assay

2.6.3.1.1 Principle of Human angiotensinogen assay

The sandwich enzyme linked immunosorbent assay (ELISA) kit was used to detect human angiotensinogen in serum quantitatively. The plate has been pre-coated with an antibody specific for human AGT. AGT present in the sample is added and reacts with antibodies coated on the wells. Then a biotinylated Human AGT Antibody is added, which recognizes the AGT present in the sample and bind to it. Streptavidin-HRP is then added, which reacts with the biotinylated AGT antibody. Following incubation, unbound Streptavidin-HRP is removed by a washing step. After adding the substrate solution, the color develops in proportion to the amount of Human AGT. The reaction is ended by adding an acidic stop solution, and the absorbance at 450 nm was measured.

2.6.3.1.2 Assay Procedure of Human Angiotensinogen

1) Following the manufacturer instructions, all reagents, standard solutions, and samples were prepared. All reagents were brought to room temperature before using.

2) The numbers of needed strips for the assay were determined and placed in the frames. The unused strips were kept at a temperature of 2-8°C.

3) A 50µl of the standard were added to standard well.

4) A 40µl of the sample were added to sample wells followed by addition of 10µl anti-AGT antibody to sample wells, then 50µl of the streptavidin-HRP were add to sample wells and standard wells (Not the blank control well). Then it mixed well and the plate Covered with a sealer and incubated for 60 minutes at 37°C.

5) The sealer was removed and the plate was washed 5 times with wash buffer. Wells was soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each

wash. All wells aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer for automated washing. Then paper towels were used.

6) A 50µl of the substrate solution A were add to each well and then 50µl of the substrate solution B were add to each well. The plate was covered with new sealer and incubated for 10 minutes at 37°C in the dark.

7) A 50µl of the stop solution were added to each well, the blue color was changed into yellow immediately.

8) The optical density (OD) value of each well was determine immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.6.3.2.1 Principle of Human angiotensin II assay

The ELISA sandwich kit was used to detect Human Angiotensin II in serum quantitatively. The plate has been pre-coated with an antibody specific for Human Ang-II. The sample's Ang-II is introduced and binds to the antibodies coated on the wells. Following that, a biotinylated Human Ang-II Antibody is added, which recognizes Ang-II in the sample. Streptavidin-HRP is then added, which reacts with the biotinylated Ang-II antibody. Following incubation, unbound Streptavidin-HRP is removed by a washing procedure. After adding the substrate solution, the color develops in accordance to the amount of Human Ang-II. The reaction is ended by adding an acidic stop solution, and the absorbance at 450 nm is measured.

2.6.3.2.2 Assay Procedure of Human Angiotensin II

1) Following the manufacturer instructions, all reagents, standard solutions, and samples were prepared. All reagents brought to room temperature before using.

2) The numbers of needed strips for the assay were determined and placed in the frames. The unused strips were kept at a temperature of 2-8°C.

3) A 50µl of the standard were added to standard well.

4) A 40µl of the sample were added to sample wells followed by addition of 10µl anti-AGT antibody to sample wells, then 50µl of the streptavidin-HRP were add to sample wells and standard wells (Not the blank control well). Then it mixed well and the plate Covered with a sealer and incubated for 60 minutes at 37°C.

5) The sealer was removed and the plate was washed 5 times with wash buffer. Wells was soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. All wells aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer for automated washing. Then paper towels were used.

6) A 50µl of the substrate solution A were add to each well and then 50µl of the substrate solution B were add to each well. The plate was covered with new sealer and incubated for 10 minutes at 37°C in the dark.

7) A 50µl of the stop solution were added to each well, the blue color was changed into yellow immediately.

8) The optical density (OD) value of each well was determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.6.3.3. Principle of creatinine assay (163)

Creatinine in the sample reacts with picrate to form a creatinine-picrate complex at an alkaline pH. The rate at which the absorbance at 500 nm increases as a result of the formation of this complex is directly proportional to the creatinine concentration in the sample. Creatinine is a metabolic product of creatine phosphate in muscles, which is necessary for muscle tissue energy production.(164)

2.6.3.4 Principle of urea assay (165)

The test is carried out in the form of a kinetic assay in which the initial rate of the reaction is linear for a specified time period. Urease hydrolyzes the urea in the sample to form ammonia and carbon dioxide. The second process, performed by glutamate dehydrogenase (GLDH), transforms ammonia and α-ketoglutarate to glutamate and water, with the concurrent oxidizing the reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). For every mole of urea present, two moles of NADH are oxidized. The rate at which the absorbance at 340 nm decreases initially is proportional to the urea concentration in the sample.

2.6.3.5 Principle of electrolyte assay (Na, K and Cl)

Ion-selective electrodes for sodium, potassium, and chloride make use of membranes that are ion-selective. According to the Nernst equation, an electrical potential (voltage) is created across the membranes connecting the reference and measuring electrodes. The voltage is translated to ion concentration by comparing it to previously measured calibrator voltages.

2.6.3.6 Assay Procedure of serum creatinine, urea and electrolyte

Procedure was done by putting 100 microliters of separated serum in cuvate to be analyzed. The concentration (urea, creatinine and electrolytes) measured automatically by using architect Abbott c4000 chemistry analyzer.

2.6.4. Extraction of Genomic DNA from Blood Sample

Genomic DNA was extracted from a sample of peripheral venous blood using the procedure of (G-spin TM Total DNA extraction kit made in Korea, intron). The following steps were used to carry out the DNA extraction:

1) A total of 200 µL of whole blood was pipetted into a 1.5 ml micro centrifuge tube.

2) A 20 µL of proteinase K was added into the sample tube, and then mixed by pulse vortex for seconds in order to ensure proper mixing.

3) A total of 200 µL of buffer BL was added into the upper sample tube and mixed thoroughly. In order to ensure efficient lysis, it was important that the blood sample and buffer BL were mixed thoroughly to yield a lysis solution.

4) The mixture placed at room temperature for 2 minutes.

5) The lysate was incubated at 56°C for 10 min, for complete lysis, the lysate was mixed 3- or 4-times during incubation by inverting tube, the red color of lysate became dark green when its lysis perfectly.

6) A 1.5 ml tube was briefly centrifuged to remove drops from the inside of the lid.

7) A total of 200 µL of absolute ethanol was added into the lysate, and then mixed well by pulse vortex, after mixing it was briefly centrifuged the 1.5 ml tube in order to remove drops from inside of the lid. This step was an equilibration step for binding genomic DNA to column membrane. It was important to assure proper mixing after adding the ethanol, until not showing 2 phases which was not mixed. Also, this step conduces to pass efficiently cell lysate through a column.

8) The mixture from step 7 was carefully applied to the spin column (in a 2 ml collection tube) without wetting the rim, the cap was closed, and centrifuged at 13,000 rpm for 1 min. the filtrate was discarded and placed the spin column in a new 2 ml collection tube. Each spin column was closed in order to avoid aerosol formation during centrifugation and any solid materials were not transfer.

9) A total of 700 µL of Buffer WA was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. The flow was discarded through and reused the collection tube.

10) A total of 700 µL of Buffer WB was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. The flow-through was discarded and placed the column into a new 2.0 ml collection tube. Then again centrifuged for additional 1 min to dry the membrane the flow-through discarded and collection tube altogether. It was very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, the spin column was removed carefully from the collection tube without contacting with the flow through, since this will result in carryover of ethanol.

11) The spin column was placed into a new 1.5 ml tube, and 80 µL of Buffer CE was added directly onto the membrane. Incubated for 1 min at room temperature and then centrifuged for 1 min at 13,000 rpm to elute. A new 1.5 ml tube was used for the second elution step to prevent dilution of the first eluent.

2.6.5. Polymerase Chain Reactions.

A thermocycler was used to run polymerase chain reactions (PCR), which amplified a desired portion of the genome. The desired target sequence concentration rises from one molecule to several million copies. Any PCR cycled 25-35 times has three steps:

- **Denaturation**: This process involves unwinding dsDNA into two single strands at 94-95°C. Generally, a 2-minute initial denaturation step at 95°C is enough. The duration of subsequent denaturation steps will range from 30 seconds to 1 minute.
- **Annealing**: This step takes place between 55 and 65 °C. The reaction is started by annealing a pair of short (17-26) oligonucleotide sequences (primers) to the ends of the template strands of DNA. Optimize the annealing conditions by starting the reaction at around 5°C below the calculated melting temperature of the primers and gradually increasing the temperature to the annealing temperature in 1°C increments. The annealing time is usually between 30 second and 1 minute.

- **Extension**: At 72-74 °C, the primers are extended to generate a new strand that is complementary to the template strand. This happens in the presence of Taq DNA polymerase, a DNA polymerase obtained from the bacterium *Thermus aquaticus*, which can withstand high temperatures without denaturation.

2.6.5.1. Tetra arm Polymerase Chain Reaction.

Polymorphisms in the AGT and ATR1 were delineated using tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Tetra ARMS-PCR was performed to assess polymorphisms of AGT which result in changes in the encoded amino acids at positions 235 from methionine to threonine (M235T) and polymorphism of ATR1 at nucleic acid 1166 which result in changing adenine to cytosine (A1166C).

2.6.5.2 Primers

The primers were designed using primer1 program and prepared by Bioneer Company (Korea). Primers were made in a lyophilized state. A mass in picomoles is the unit of measurement for a lyophilized primer. The following steps were taken to reconstitute and dilute the primers: Before decamping, the tube was centrifuged at 10000 x g for 5-10 minutes. The manufacturer's recommended volume of nuclease free water was added to give a primary concentration of 100 pmole/µL, therefore lyophilized primers were dissolved in nuclease free water to give a concentration of 100 pmole/µL (stock solution).

For working solution: The primers were re-mixed by suitable vortexing, and 10 µL of stock solution was diluted with 90 µL of nuclease free water in a 0.5 mL Eppendorf tube to achieve 10 pmole/µl as a final concentration. Both the stock and working solution were stored at -20 °C. Tables 2-4 and 2-5 show the AGT and ATR1 forward and reverse sequences of these primers.

Table 2-4: Primer sequence of AGT gene SNP (M235T; rs699)

Primers	Sequence (5'-3')
AGT Inner Forward primer	GCTGTCCACACTGGCTCACA
AGT Inner Reverse primer	GGAAGACTGGCTGCTCCCTTAC
AGT Outer Forward primer	GATACTAAGTCCTAGGGCCAGAGCC
AGT Outer Reverse primer	CACCTGAAGCAGCCGTTTGT

Table 2-5: Primer sequence of ATR1 gene SNP (A1166C; rs5186)

Primers	Sequence (5'-3')
ATR1 Inner Forward primer	AGCACTTCACTACCAAATGAACA
ATR1 Inner Reverse primer	TTCAATTCTGAAAAGTAGCTGAG
ATR1 Outer Forward primer	TGAGCACGCTTTCCTACCGC
ATR1 Outer Reverse primer	CCTTTGGAAACTGGACAGAAC

2.6.5.3. PCR procedure

GoTaq ® Green Master Mix is a premixed ready-to-use solution, containing bacterially derived Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. GoTaq® Green Master Mix contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis.

2.6.5.3.1 Optimization of PCR Conditions

After several trials, the polymerase chain reaction was optimized, and PCR was made using:

• Different volumes of primer.

• Different volumes of DNA template.

• Different temperatures for annealing.

The preferred conditions which provided the best results for AGT gene M235T were by adding volumes of PCR mixture as shown in table 2-6.

Table 2-6: the PCR mixture of AGT gene

Materials	Volumes (µL)
DNA sample	5
outer forward primer	1.25
outer reverse primer	1.25
inner forward primer	1.25
inner reverse primer	1.25
nuclease free water	7.5
master mix	12.5

A total reaction volume of 30 µl was mixed in a PCR tube for a single reaction then centrifuged for 10 seconds at 2000 x g in a micro centrifuge to mix the sample tubes before placing them in the thermocycler. The best PCR program reaction was carried out as following program in table 2-7:

Table 2-7: Optimization conditions of AGT gene SNP (M235T; rs699) polymorphism

Steps	Temperature	Time	Cycles
Initial Denaturation	94	2 min	1
Denaturation	94	35 sec	30
Annealing	56	45 sec	
Extension	72	55 sec	
Final extension	72	5 min	1

Total time is 2 hours

The preferred conditions which provided the best results for ATR1 gene A1166C were by adding volumes of PCR mixture as shown in table 2-8.

A. Table 2-8: PCR mixture of ATR1 gene

Materials	Volumes (µL)
DNA sample	4
outer forward primer	1
outer reverse primer	1
inner forward primer	1
inner reverse primer	1
nuclease free water	4.5
master mix	12.5

A total reaction volume of 25 µl was mixed in a PCR tube for a single reaction, and then centrifuged for 10 seconds at 2000 x g in a micro centrifuge to mix the sample tubes before placing them in the thermocycler. The best PCR program reaction was carried out as following program in table 2-9:

Table 2-9 Optimization conditions for ATR1 SNP (A1166C; rs5186)

Steps	Temperature	Time	Cycles
Initial Denaturation	94	2 min	1
Denaturation	94	30 sec	30
Annealing	65	45 sec	
Extension	72	55 sec	
Final Extension	72	5 min	1

Total time is 1 hours and 45 minutes.

2.6.5.3.2 Agarose Gel Electrophoresis

Electrophoresis is a common method for separating, purifying, and distinguishing DNA fragments of various sizes ranging from 100 bp to 1500 bp on agarose gel. The existence of PCR amplification was confirmed using agarose gel electrophoresis. The rate of DNA migration through agarose gel electrophoresis is affected by the following factors: -

- The type and concentration of agarose powder.
- The size of the DNA (larger size will travel slower).
- The applied voltage.

- **Agarose Gel electrophoresis procedure (166)**

1) Diluting one volume of 10x tris borate EDTA(TBE) buffer with 9 volumes of deionized water: 1:10 dilution to prepare 1x TBE.
2) 100 ml of 1x TBE was taken in a beaker and1.5 gm of 1.5%agarose powder was weighed and added to it.
3) The solution was heated by using hot plate until all the gel particles were dissolved and became clear solution. The solution was cooled down at 50 c◦.
4) When the gel solution became warm 2µLof ethidium bromide (10mg/ml) was added.
5) The comb was fixed at one end of the tray for making wells used for loading the samples, then the agarose solution was poured in to the tray and the gel allowed to solidify at room temperature for approximately 30 minutes.
6) The comb was carefully removed and the gel was placed in a horizontal gel electrophoresis tank and filled with 1X TBE until the buffer filled over the surface of the gel.
7) 5 µL of PCR products were directly loaded to the wells with great precaution to prevent damages of the wells and cross contamination of neighboring wells. Also 5 µL of DNA ladder (Intron, Korea) directly loaded to one well without addition of loading dye, the band size of ladder was 100-1500 bp.
8) The negative pole was linked to the negative side of the unit and the positive pole to another. Electrical power was turned on at 100 volt/50 Amp for 90 min while waiting for dye indicators traveled to the appropriate distance according to the size of DNA fragment. DNA moved from cathode (negative pole) to plus Anode (positive poles). The bands that stained with ethidium bromide in gel were visualized by using electrophoresis System.
9) To visualize the DNA bands, the agarose gel was placed in the UV transilluminator device and exposed to UV light and the photos were captured by digital camera.

2.7. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software version 20 (SPSS, San Diego, California, USA). The information was represented as mean and standard deviation. Fisher's exact was used to investigate differences in data expressed as percentages when the excel cell count was <5. The Chi square test was used to determine examine differences in genotype groups expressed as a percent when the excel cell count was >5. To look for differences between genotype groups, the analysis of variance (ANOVA) test was used. All statistical procedures and tests were carried out with a significance level $P<0.05$.

Hardy-Weinberg Equilibrium (HWE) is a method for estimating allele frequency in non-evolving populations. HWE is a basic principle of population genetics that states that "genotype frequencies in a population stay constant between generations in the absence of external factors." The predicted genotype frequencies can be estimated to see how far a population deviates from HWE, which can be determined by comparing observed genotypes to expected values using the chi squared test. If the P value is less than 0.05, it indicates a deviation from the HWE. One of the reasons for divergence from HWE is a small population size.

3. Results

3.1. The demographic features of the study subjects

The selection of our patients regarding gender was random; the distribution of gender was 50% for both male and female. The ages of patients was mainly ranged between 40 to 65 years with mean ± SD of 52.39±10.67. Smoking was a habit among 14.1% of the patients, 85.9% were non-smokers. The other demographic properties of participant showed mean BMI was 31.04±5.45, and mean duration of hypertension was 7.26±6.76, the demographic features are shown in Table 3-1.

Table 3-1: The Demographic features of hypertensive patients

Demographic features (N = 92)			
Data represented by Mean ± SD		Data represented by percentage	
Age (years)	52.39±10.67	Male Patients	46 (50%)
BMI (kg\m²)	31.04±5.45	Female patients	46 (50%)
Duration of hypertension (years)	7.26± 6.76	Smokers	13 (14.1%)
Duration of treatment (years)	4.21±3.65		

SD: Standard Deviation, BMI: Body mass index, N: Number of the patients.

3.2 Genotyping

3.2.1. Genotyping of M235T SNP of AGT gene

The wild type (AA) showed two DNA fragments; 250 bp and 172 bp, the heterozygous mutant type (AG) showed three DNA fragments; 250, 172 bp and 120 bp and the homozygous mutant type (GG) showed two DNA fragments; 250 bp and 120 bp (Figure 3-1). The size of PCR amplicon was determined by comparing with DNA ladder 100 - 1500 bp.

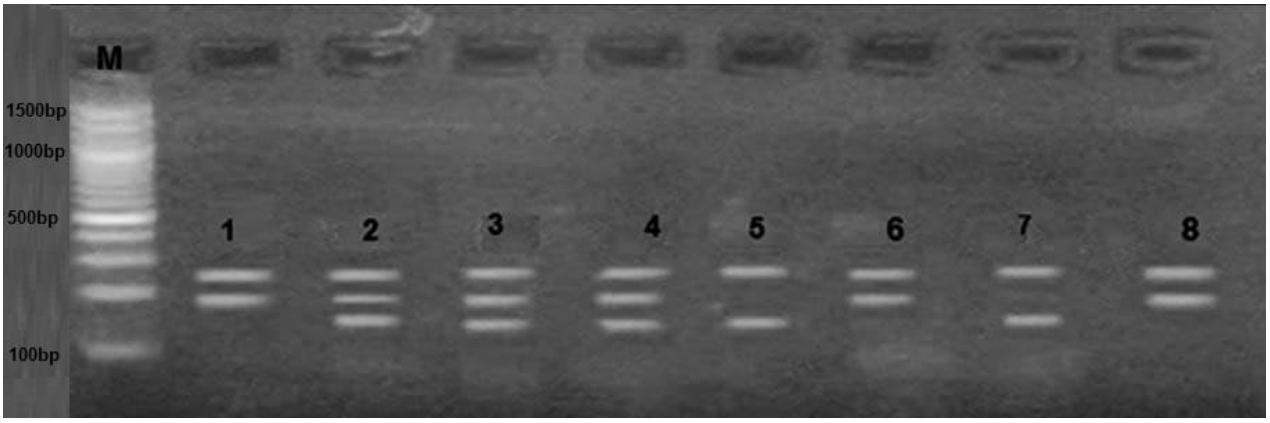

Figure 3-1: Agarose gel 1.5% (w/v) electrophoresis for the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) product of AGT SNP (M235T) pre-stained with 5 µL ethidium bromide. M: 100-1500 bp DNA ladder, lanes 1, 6, 8 show the wild type (AA), lanes 2, 3 and 4 show heterozygous mutant type (AG) and lane 5 and 7 show homozygous mutant type (GG).

The distribution of M235T genotypes in hypertensive patients is illustrated in Table 3-2 and figure 3-2. The result of comparison between observed and anticipated values for AGT M235T SNP in the tested population was shown in figure (3-3). The distribution and percentage of individuals having AGT genotypes differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: A (36, 23); G (36, 23); AG (20, 46) (goodness-of-fit $\chi 2$ for AGT = 29.391, P = < 0.001} and therefore it was statistically significant.

Table 3-2: The distribution of M235T genotypes in hypertensive patients

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			A	G	
Symbol	Frequency	%	0.5	0.5	29.391 P< 0.001
AA	36	39.1			
AG	20	21.74			
GG	36	39.1			
Total	92	100			

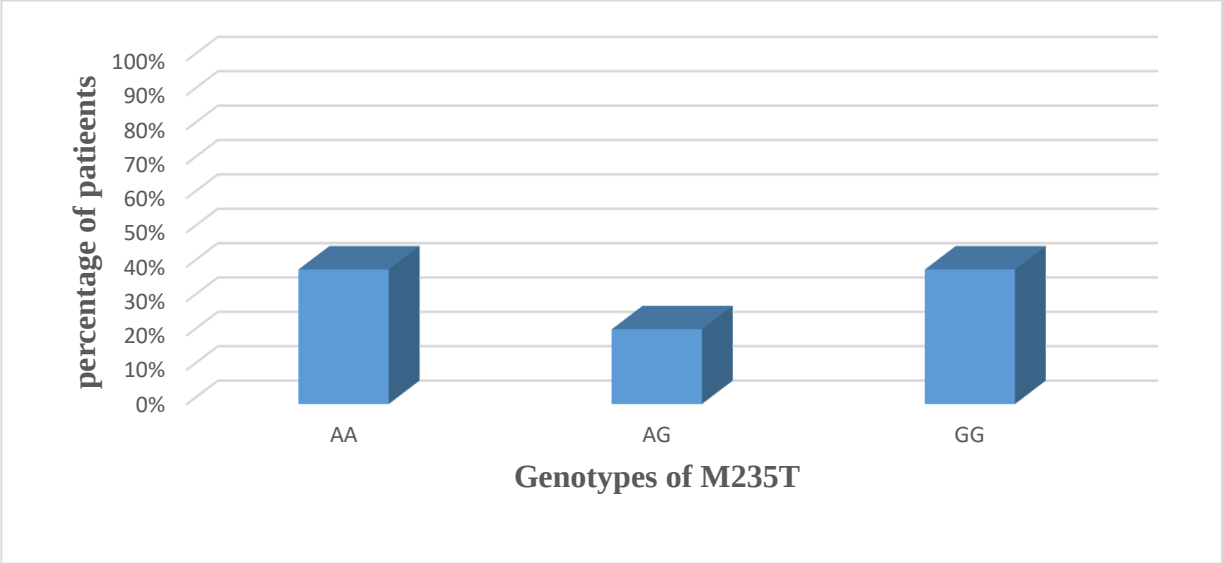

Figure 3-2: The distribution of M235T genotypes in hypertensive patients

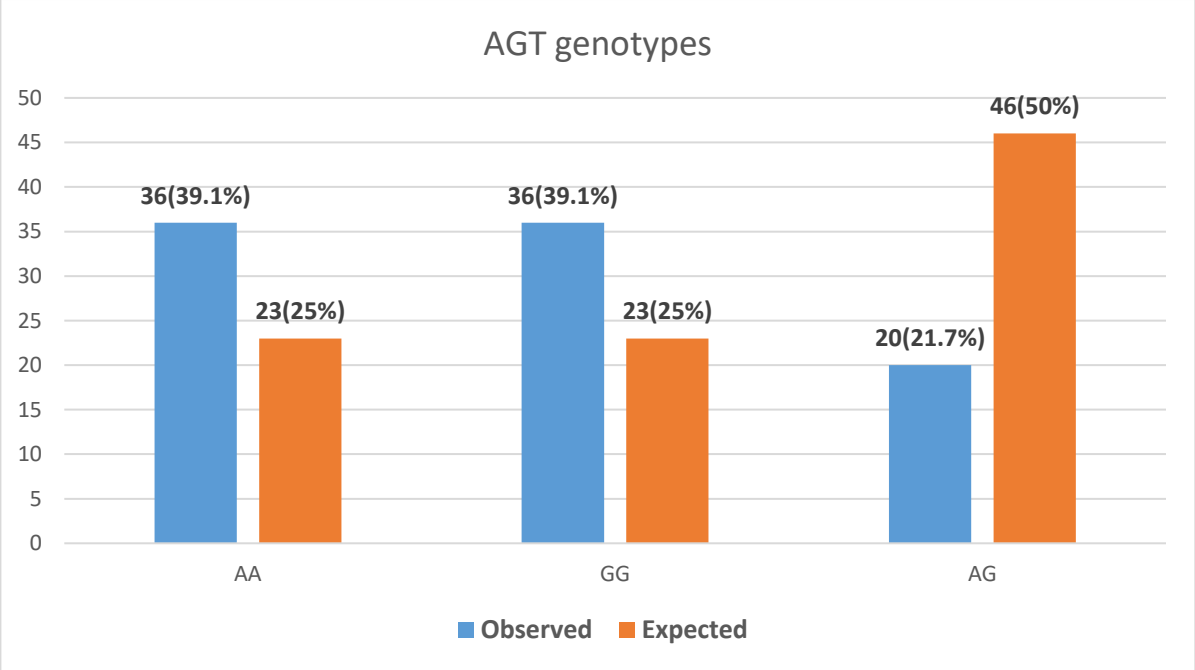

Figure (3-3): Observed (Obs.) vs expected (Exp.) genotype frequencies % of AGT among individuals' sample

3.2.2 Genotyping of A1166C SNP ATR1 gene

The wild type (AA) showed two DNA fragments; 497 bp and 354 bp, the heterozygous mutant type (AC) showed three DNA fragments; 497bp, 354 bp and 188 bp and the homozygous mutant type (CC) showed two DNA fragments; 497 bp and 188 bp (Figure 3-4). The size of PCR amplicon was determined by comparing with DNA ladder 100 - 1500 bp.

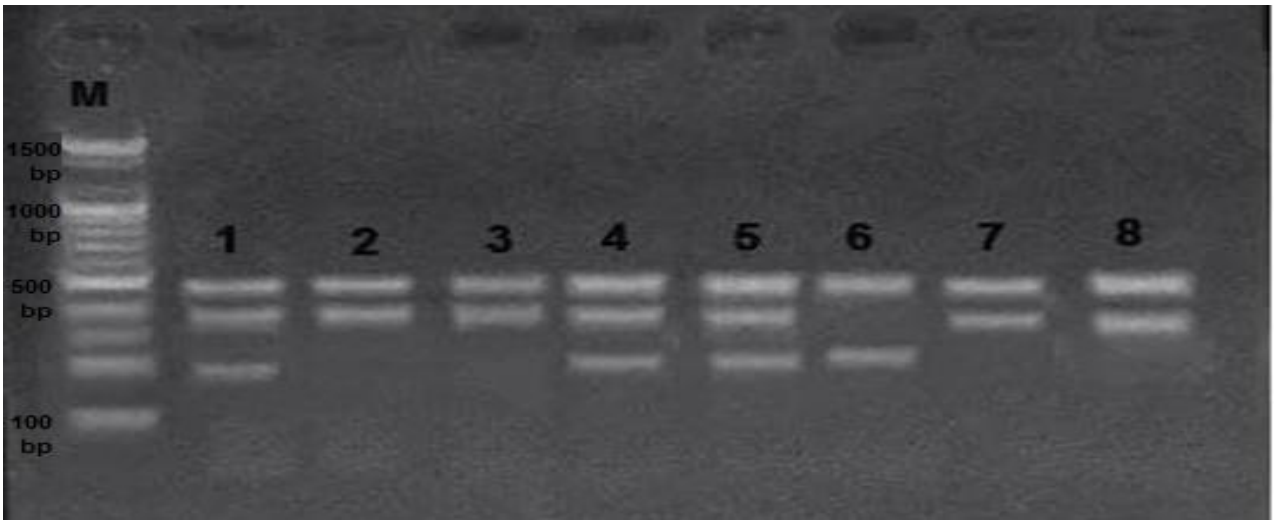

Figure 3-4: Agarose gel 1.5% (w/v) electrophoresis for the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) products of ATR1 SNP (A1166C) pre stained with 5 μL ethidium bromide. M: 100-1500 bp DNA ladder, lanes 1, 4, 5 show heterozygous mutant type (AC), Lanes 2, 3, 7 and 8 show the wild type (AA), lanes 6 show homozygous mutant type (CC).

The distribution of A1166C genotypes in the hypertensive patients is illustrated in Table 3-3 and figure 3-5. The result of comparison between observed and anticipated values for ATR1 A1166C SNP in the tested population was shown in figure (3-6). The distribution and percentage of individuals having ATR1 genotypes so close to those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: A (50, 51); C (5, 6); AC (37, 35) (goodness-of-fit $\chi 2$ for ATR1 = 0.302, P = 0.86} and therefore it was statistically not significant.

Table 3-3: Distribution of A1166C SNP in the hypertensive patients

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			A	C	
Symbol	Frequency	%	0.745	0.255	0.302 P= 0.86
AA	50	54.3			
AC	37	21.7			
CC	5	5.4			
Total	92	100			

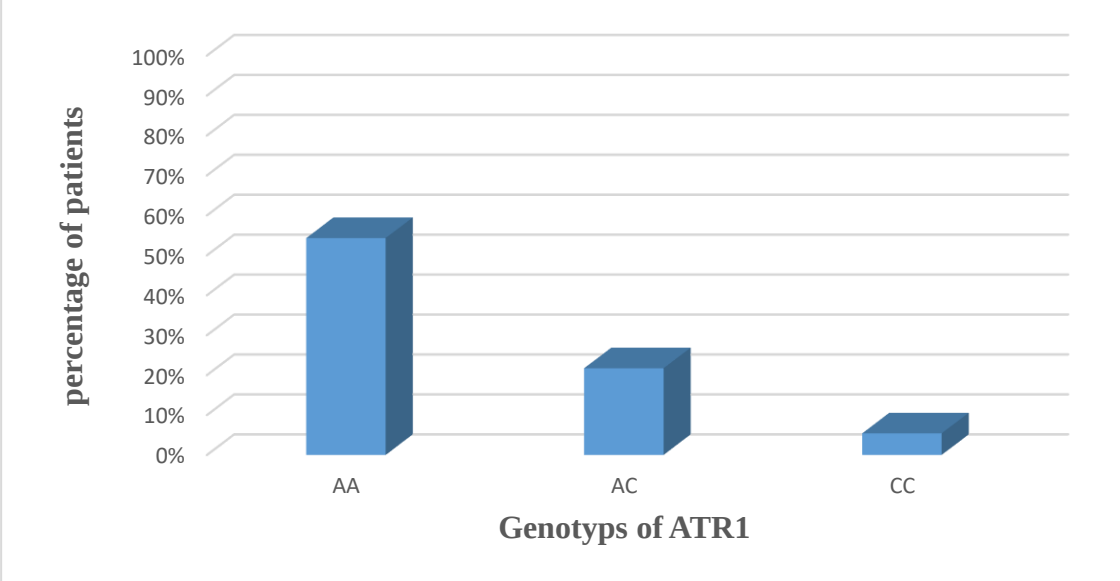

Figure 3-5: Distribution of A1166C SNP in the hypertensive patients

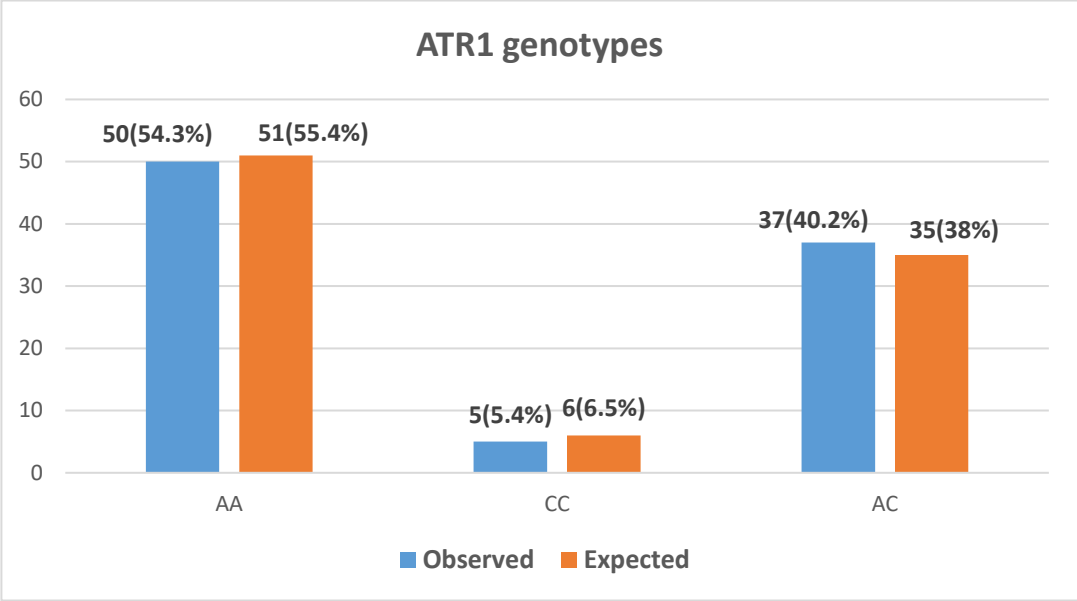

Figure (3-6): Observed (Obs.) vs expected (Exp.) genotype frequencies % of ATR1 among individuals' sample

3.3. Association between gene polymorphisms and demographic characteristics

3.3.1. Association between AGT M235T SNP and demographic characteristics

There was no statically significant association between AGT M235T SNP and demographic characteristics ($p>0.05$) in hypertensive patients as shown in table 3-4.

Table 3-4 Association between demographic characteristic and AGT M235T SNP

Demographic Characteristic	Patient Genotype (N=92)			P value
	AA (N=36)	GG (N=36)	AG (N=20)	
Age (years)	52.31±11.17	54.44±10.71	48.85±9.07	0.171
BMI ($kg/m^{2)}$	31.56±6.06	30.59±5.67	30.91±3.78	0.755
Duration Of Hypertension(years)	6.94±5.34	8.82±8.64	5.03±4.34	0.124

Duration Of treatment (years)		4.05±3.42	5.07±4.35	2.94±2.03	0.105
Gender	Male	19(41.3%)	16(34.8%)	11(23.9%)	0.685
	Female	17(37%)	20(43.5%)	9(19.6%)	
Smoking	Yes	5(38.5%)	3(23.1%)	5(38.5%)	0.27
	No	31(39.2%)	33(41.8%)	15(19%)	

Data is represented as mean ± standard deviation and percentage, N denoting the number of subjects, BMI denoting body mass index

3.3.2. Association between ATR1 A1166C SNP and demographic characteristics

There was no statically significant association between ATR1 A1166C SNP and demographic characteristics ($p>0.05$) in hypertensive patients as shown in table 3-5.

Table 3-5: Association of demographic characteristic between different genotype of ATR1 A1166C SNP

Demographic parameters		Patient Genotype (N=92)			P value
		AA (N=50)	CC (N=5)	AC (N=37)	
Age (years)		53.82±11.18	49.8±12.22	50.81±9.72	0.371
BMI ($kg/m^{2)}$		30.25±5.77	31.57±5.55	32.04±4.93	0.31
Duration Of Hypertension (years)		7.48±7.47	6±5.15	7.14±6.03	0.89
Duration Of treatment (years)		4.39±4.17	2.66±1.98	4.18±3.03	0.605
Gender	Male	23(50%)	2(4.3%)	21(45.7%)	0.609
	Female	27(58.7%)	3(6.5%)	16(34.8%)	
Smoking	Yes	7(53.8%)	0(0%)	6(46.2%)	0.895
	No	43(54.4%)	5(6.3%)	31(39.2%)	

Data is represented as mean ± standard deviation and percentage, N denoting the number of subjects, BMI denoting body mass index

3.4.1. Effect of treatment of Candesartan on blood pressure parameters in hypertensive patients having different genotypes of AGT M235T SNP.

There was no statically significant association between M235T SNP and blood pressure parameters ($p>0.05$) in hypertensive patients as shown in table 3-6.

Table 3-6: The association between M235T SNP and blood pressure parameters.

Blood pressure parameters	Patient Genotype (N=92)			P value
	AA (N=36)	GG (N=36)	AG (N=20)	
Systolic blood pressure (mmHg)	138±23.76	137.14±22.18	133.25±13.21	0.451
Diastolic blood pressure (mmHg)	82.92±14.41	81.53±10.88	82.5±9.53	0.886
Mean arterial pressure (mmHg)	101.97±16.35	101.06±13.48	99.55±9.99	0.827
Heart rate (beats per minute)	85.06±10.88	84.92±12.98	85±11.29	0.999

Data represented as mean±standard deviation, N denoting the number of the study subjects.

3.4.2. Effect of treatment with Candesartan on blood pressure parameters in hypertensive patients having different genotypes of ATR1 A1166C SNP.

There was no statistical association between A1166C SNP and BP parameters except heart rate (P=0.005) as shown in Table 3-7.

Table 3-7: The association between A1166C SNP and blood pressure parameters

Blood pressure Parameters	Patient Genotype (N=92)			P value
	AA (N=50)	CC (N=5)	AC (N=37)	
Systolic blood pressure (mmHg)	139.2±19.7	126±20.75	139.46±23.24	0.399
diastolic blood pressure (mmHg)	82.7±11.66	70±7.07	83.38±12.36	0.06
Mean arterial pressure (mmHg)	101.56±13.23	88.8±11.28	102.11±14.72	0.126
Heart rate (beats per minute)	88.32±10.64	75±7.81	81.84±12.08	0.005

Data represented as mean±standard deviation, N denoting the number of the study subjects.

3.5.1. Effect of treatment with Candesartan on Echo finding in hypertensive patients with different genotypes of AGT M235T SNP.

There was statistically significant association (p=0.012) between M235T SNP and LVH Table (3-8). Patients with GG genotype had significantly higher proportion (41.5%) among the hypertensive patients with LVH, while patients with AA and AG genotypes had the proportion 36.9% and 21.5% respectively. No statistically significant association between M235T SNP and ejection fraction was obtained (P> 0.05) as shown in Table 3-8.

Table 3-8: The association between M235T SNP and echocardiogram findings.

Echocardiogram finding		Patient Genotype (N=92)			P value
		AA (N=36)	GG (N=36)	AG (N=20)	
Ejection Fraction		66.75±7.28	65.03±6.84	68.7±4.07	0.132
LVH	Normal	12(44.4%)	9(33.3%)	6(22.2%)	0.012
	Mild	22(40%)	19(34.5%)	14(25.5%)	
	Moderate	0(0%)	8(100%)	0(0%)	
	Severe	2(100%)	0(0%)	0(0%)	

Data is represented as mean ± standard deviation, N denoting the subjects' number, LVH: Left ventricular hypertrophy, $p<0.05$ considered significantly different.

3.5.2. Effect of treatment with Candesartan on Echo finding in hypertensive patients with different genotypes of ATR1 A1166C SNP

No statistical association was found between A1166C SNP and Echo ($P> 0.05$), as shown in Table 3-9.

Table 3-9: The association between A1166C SNP and Echo parameters.

Echocardiogram parameters		Patient Genotype (N=92)			P value
		AA (N=50)	CC (N=5)	AC (N=37)	
Ejection Fraction		66.6±5.77	67.8±7.22	66.19±7.69	0.869
LVH	Normal	12(44.4%)	2(7.4%)	13(48.1%)	0.29
	Mild	33(60%)	2(3.6%)	20(36.4%)	
	Moderate	5(62.5%)	1(12.5%)	2(25%)	
	Severe	0(0%)	0(0%)	2(100%)	

Data is represented as mean ± standard deviation, N denoting the subjects' number, LVH: Left ventricular hypertrophy, $p<0.05$ considered significantly different.

3.6.1. Effect of treatment of Candesartan on biochemical parameters in hypertensive patients having different genotypes of AGT M235T SNP.

There was no statically significant association ($p>0.05$) between M235T SNP and biochemical parameters in hypertensive patients as shown in table 3-10.

Table 3-10: The association between M235T SNP and some biochemical parameters.

Biochemical parameters	Patient Genotype (N=92)			P value
	AA (N=36)	GG (N=36)	AG (N=20)	
Blood Urea (mg/dL)	30.09±8	31.28±11.01	27.8±9.25	0.428
Serum Creatinine (mg/dL)	0.767±0.25	0.778±0.29	0.713±0.15	0.639
Potassium (mmol/l)	4.18±0.39	4.23±0.45	4.12±0.48	0.619
Sodium (mmol/l)	138.48±4.32	138.65±3.62	137.83±4.12	0.791
Chloride (mmol/L)	100.24±6.28	101.12±6.11	99.61±5.14	0.702
Angiotensinogen Level (ng/L)	82.21±44.79	86..85±79.21	85.58±67.6	0.954
Angiotensin 2 Level (ng/L)	29±44.9	21.48±34.53	22.52±31.21	0.681

Data represented as mean±standard deviation, N denoting the number of the study subjects.

3.6.2. Effect of treatment with Candesartan on biochemical parameters in hypertensive patients having different genotypes of ATR1 A1166C SNP

There was no statically significant association ($p>0.05$) between A1166C SNP and biochemical parameters in hypertensive patients as shown in table 3-11.

Table 3-11: The association between A1166C SNP and the biochemical parameters

Biochemical parameters	Patient Genotype (N=92)			P value
	AA (N=50)	CC (N=5)	AC (N=37)	
Blood Urea (mg/dl)	30.36±10.14	24.8±8.58	30.32±8.83	0.452
Serum Creatinine (mg/dl)	0.74±0.22	0.64±0.2	0.79±0.28	0.322
Potassium (mmol/l)	4.2±0.46	4.18±0.37	4.16±0.42	0.907
Sodium (mmol/l)	137.74±4.1	139.5±1.92	139.2±3.94	0.275
Chloride (mmol/l)	99.45±5.97	105.75±2.06	101.07±5.79	0.093
Angiotensinogen Level (ng/L)	81.43±51.08	70.84±10.22	91.38±83.43	0.694
Angiotensin 2 Level (ng/L)	22.34±28.46	17.7±13.01	28.7±50.13	0.686

Data represented as mean±standard deviation, N denoting the number of the study subjects.

4. Discussion

4.1 Analysis of RAAS genes polymorphism (AGT M235T and ATR1 A1166C)

Up to our knowledge, this is the first genetic study investigated the AGT gene SNP (M235T) and ATR1 gene SNP (A1166C) effect on EH in a sample of Iraqi hypertensive patients who are on candesartan treatment. Having knowledge of the role of this genetic variation might help to predict Bp response to ACEIs and ARBs therapy in each individual patient. (167)

The AGT gene is a logical candidate for hypertension. When methionine amino acid substituted by threonine amino acid at codon 235; adenine is substituted by guanine at the nucleotide 704 in the exon 2, the AGT gene polymorphism (M235T) occurs. The transition from the nonpolar amino acid methionine to the polar amino acid threonine, is probably damaging/not tolerated, which could result in the abnormal functioning of AGT.(168)

The A1166C polymorphism which consists of an AC nucleotide transversion at position 1166, is present in the specific sequence of 3′ UTR (untranslated region) of ATR1 gene, overlapping with specific microRNA (miR-155). MicroRNAs (miRNAs) are single-stranded, non-protein-coding RNA molecules that control gene expression. (169) According to laboratory study, the A-allele regulate the expression of the ATR1 gene through the 3′ UTR's overlap with the miR-155 target. In the presence of the C allele, however, miR-155's capacity to overlap with the 3′ UTR sequence target is diminished, resulting in unusually high levels of ATR1(Figure 1-5). As a result ,this is one possible explanation for the association between 1166C and cardiovascular disease such as hypertension.(155)

4.1.1 The distribution of M235T SNP in hypertensive patients

The distribution of M235T SNP among the patients was 39.13% wild genotype (AA), 21.74% heterozygous mutant genotype (AG) and 39.13% homozygous mutant genotype (GG). This result differ from Saab *et al.* (170) who studied on Lebanese hypertensive patients and founded that genotype distribution of M235T SNP was 27 %AA, 58%AG and 15%GG. Our result also differ from Shamaa *et al*.(171) who studied on Egyptian hypertensive patients and founded that genotype distribution of M235T SNP was 14.5%AA, 21.7% AG and 63.9 % GG. In this study the allele frequencies of M235T SNP was: 0.5 A and 0.5 G, this result is close to Agachan *et al* (172) who studied on Turkish hypertensive patients and founded that allele frequencies of AGT is 0.56 A and 0.44 G. Also our result is close to with Mohana et al (173) who studied on Indian hypertensive patients and founded that allele frequencies of AGT was 0.45 A and 0.55 G.

4.1.2 The distribution of A1166C SNP in hypertensive patients

The distribution of A1166C SNP among the patients was 54.3% wild genotype (AA), 40.22% heterozygous mutant genotype (AC) and 5.4% homozygous mutant genotype (CC). This result differ from Saab *et al.* (170) who studied on Lebanese hypertensive patients and founded that genotype distribution of A1166C SNP was 25% AA, 52% AC and 23%CC. Our result agree with Shamaa *et al*.(174) who studied on Egyptian hypertensive patients and founded that genotype distribution of A1166C SNP was 66.3% AA, 25.3% AC And 8.4%CC.

In this study it was found that the allele frequencies of ATR1 SNP were: 0.74 A and 0.25 C. The allele frequencies in current study subjects was very close to Agachan *et al* (172) who studied on Turkish hypertensive patients and founded that allele frequencies of ATR1 was 0.77 A and 0.22 C, but incompatible to Parchwani *et*

al.(156) who studied on Indian hypertensive patients and founded that allele frequencies of ATR1 was 0.51 A and 0.48 C.

4.2. Association between AGT and ATR1 gene polymorphisms and demographic characteristics

There was no significant association (P>0.05) in both M235T (Table 3-4) and A1166C (Table 3-5) genotypes regarding age, weight, BMI and gender, and other demographic features, this may be due to randomization in selection patients (any patient had the study criteria had been taken in the study). In our study this result is important since the objective of study is to see the effect of AGT and ATR1 genes polymorphisms on response to candesartan, so any differences in the association between genotypes and measured parameters (echo, BP and biochemical) if exist may be attributed to the genetic differences instead of demographic features differences between patients.

4.3. Association between AGT and ATR1 gene polymorphisms and blood pressure parameters

AGT M235T polymorphism has been associated with hypertension in a previous studies such as study done by Fajar *et al*. who had reported that the susceptibility for hypertension was observed in T allele of AGT M235T. While, M allele and MM genotype were found having protective effects against essential hypertension.(175) In current study the blood pressure parameters (systolic BP, diastolic BP and mean atrial pressure)and heart rate showed no significant association (p >0.05) according to the different genotypes of M235T (Table 3-6). The mean of Bp parameters was within the normal range, this indicates that candesartan is a good drug in controlling Bp regardless to the AGT genotypes. In other words, the M235T doesn't make the patient resistant to candesartan regarding the study subjects of Iraqi hypertensive patients.

These results are in line with some earlier studies that indicated that there was no evidence of significant association in BP responses to several antihypertensive drugs and the different genotypes of M235T polymorphism. Suonsyrjä *et al* (176) showed that AGT M235T does not markedly predict BP responses to amlodipine, bisoprolol, HCT, and losartan, in white Finnish hypertensive patients. While Kurland *et al* (177) showed that patients with the AGT polymorphism had the greatest reduction in systolic blood pressure when given atenolol but no such association was found in individuals who were given irbesartan. Finally, Schelleman *et al* (178) who illustrated that the M235T polymorphism did not modify the difference in blood pressure levels among subjects who used diuretics, β-blockers, calcium antagonists, or ACE inhibitors.

ATR1 A1166C polymorphism has been associated with hypertension in a previous studies such as study done by Jiang et al. who had reported that AC genotype is associated with essential hypertension, and the C allele may be a marker for predisposition to hypertension. (179) In current study there was no significant association between the hypertensive patients with AA, AC and CC genotypes of A1166C SNP and the BP parameters (systolic BP, diastolic BP and mean atrial pressure), except the heart rate which was significantly higher in patients with AA genotype (Table 3-7). Since the mean of Bp parameters was within the normal range, candesartan is good drug in controlling Bp regardless to the A1166C genotypes. It can be concluded that A1166C is not responsible for the resistance to candesartan in Iraqi hypertensive patients. These findings agree with Hussain *et al.* (180) who showed that response to ACE inhibitors and ARBs was independent of genetic polymorphisms in RAAS in Faisalabad, Pakistan populations. But they differ from Miller *et al.* (181) who showed that ATR1 gene polymorphism predicts response to losartan, where the C allele showed greater BP response in Caucasian populations.

4.4. Association between AGT and ATR1 gene polymorphisms and Echocardiograph parameters

Regarding Echo findings, there was a significant association (p=0.012) between M235T SNP and LVH. The number of patients with AG genotype that had mild LVH was significantly lower 14(25.5%) than the numbers of the patients with AA 22(40%) and GG 19(34.5%) genotypes. The patients with homozygous mutant type (GG) showed significant higher proportion of the hypertensive patients 8(100%) with moderate LVH, while the number of patients with AA genotype that have sever LVH was 2 (100%) (Table 3-8). After counting the total number of the patients that suffer from LVH, it can be concluded that the number of patients who have GG genotype are significantly greater 27(41.5%) than the patients who have AA 24(36.9%) and AG 14(21.5%) genotypes.

These results could indicate that AG genotype may have protective effect against hypertension complications such as LVH than other M235T genotypes, while GG genotype may be a risk for LVH occurrence in our sample of Iraqi hypertensive patients treated with candesartan. This finding agree with Tran *et al*. (182)who founded that the TT genotype of AGT M23T was associated with greater left ventricular mass in Vietnamese patients diagnosed with essential hypertension. Also this result agree with Jeng (183)who founded that the TT genotype of the AGT gene could be considered a risk factor for the development of LVH in Chinese hypertensive patients. But our results differ from Rasmussen *et al*.(184) who suggested that AGT plays less significant role in simple growth and proliferation of the heart muscle. Our results showed that M235T SNP had no statistically significant association (p>0.05) with the other echo parameter, ejection fraction (Table 3-8). This result agree with Yamada *et al*.(185) who also showed no association between EF and M235T SNP in the Japanese populations .

There was no significant association between A1166C SNP and Echo parameters (Table 3-9). These results agree with Andrikopoulos *et al* (186), who had shown that there is no association between the A1166C SNP and left ventricular hypertrophy in Greek population. And agree with Shlyakhto *et al*.(187)who founded Lack association between the RAAS genes polymorphisms and left ventricular hypertrophy in Florida hypertensive patients. But current results differ from Osterop *et al* (158) who showed that there was a good association between left ventricular hypertrophy occurrence and C allele of the A1166C SNP in Holland hypertensive patients, and also differ from Hamon *et al.* (161) who observed that white subjects having the homozygous AT1R CC genotype had a significantly lower ejection fraction than subjects carrying the A allele, while Baudin (162) discovered that Paris hypertensive patients with ATR1 CC or AC genotypes tended to have a lower ejection fraction and increased left ventricular mass.

4.5. Association between AGT and ATR1 gene polymorphisms and biochemical parameters

- **Renal function tests (serum creatinine and urea)**

Hypertension is one of risk factors for chronic kidney disease (CKD). Renal function gradually decreases in CKD patients, eventually leading to ESRD. CKD is linked to RAAS, when the RAAS is hyperactive, it causes arterial constriction, which raises blood pressure and lowers renal function.(188) The end-product of RAAS activity is Ang II which regulate the synthesis of multiple inflammatory factors associated with CKD such as TNF-alpha and IL-6.(189) As a result, SNPs in RAAS genes may be linked to CKD. A component of the RAAS is AGT. Excess AGT causes an increase in Ang I synthesis that transformed to Ang II, which can cause kidney damage. (190) The current research found no significant association ($p>0.05$) between

M235T SNP and renal function test (serum urea and creatinine), this result agrees with Staessen *et al*. who founded that the T allele encoding angiotensinogen is not associated with atherosclerotic or renal microvascular complications, but it behaves as a marker for hypertension in Caucasians hypertensive patients. But our result differ from the other previous findings by Fabris *et al*. and Thameem *et al*., who founded that there was an association between RAAS gene polymorphism and renal function in north-east Italy and Mexican Americans respectively.(191, 192) Kelly *et al*. (188) founded that the *AGT* gene was consistently associated with risk of renal events in separate analyses in white and black participants.

There was no significant association between A1166C SNP and renal function, there is an agreement between this result and the result of Chang *et al* (193) who found that there is no impact of A1166C SNP on renal function in East Asians. This result agree with Braliou *et al* (194) who founded that neither ATR1 A1166C, nor ATR2 A1332G polymorphisms were significantly associated with the renal diseases, suggesting that they cannot be used as predictive markers in either general or subgroup ethnic populations.

Plasma angiotensinogen and angiotensin II levels

Plasma AGT level is rather stable in one individual, but is under the long-term control of several hormones, such as glucocorticoids and angiotensin II, which are known to induce AGT expression.(195) Beside the hormonal control of AGT expression, a genetic influence on AGT plasma level has been documented since polymorphisms of AGT have been shown to be associated with significant effects on plasma AGT concentration. Interestingly, alleles associated with higher levels of plasma AGT have an increased frequency in hypertensive subjects as compared to normotensive controls, suggesting that AGT variants might be predisposing to essential hypertension.(196)

In this study the M235T SNP had no association ($p>0.05$) with AGT levels (Table 3-10).This result agree with Frossard *et al*.(197) who founded that the M235T polymorphism in the AGT gene had no association with plasma levels of AGT or with essential hypertension occurrence in the Arab gulf population. But this result differ from Jeunemaitre *et al*.(148)who founded that in French subjects, the M235T polymorphism in the AGT gene in the homozygous TT state was associated with an approximate 20% increase in plasma angiotensinogen for hypertension compared with the MM wild type. Sethi *et al.* (198) founded that in white subjects, genotype of AGT was associated with a stepwise increase in plasma angiotensinogen levels of 5% in MT heterozygotes and 11% in TT homozygotes compared with MM individuals while the increment in the plasma angiotensinogen levels in Asian and black subjects were nonsignificant for all comparisons with the MM genotype. Winkelmann *et al*. (199) illustrated that there is significant association observed in German subjects between the AGT M235T variant and the cardiovascular disease phenotypes provide evidence for a possible role of elevated circulating AGT in the pathogenesis of coronary artery disease.

Miller *et al*. (181) hypothesized that people who had the C allele of A1166C SNP had higher renal and systemic angiotensin II activity. This finding is incompatible with our finding that indicates that there are no significant differences between Ang II levels in the patients with AA, AC and CC of A1166C SNP.

According to the findings of this study, the M235T and A1166C SNPs does not influence response to candesartan in hypertensive patients, but the M235T SNP may influence on hypertensive complication such as LVH. Antihypertensive treatment response may still be influenced by the AGT and ATR1 genes despite a lack of association. The incompatibility of our results with the other studies could be due to the small number of participants in our study, genetic heterogeneity among populations or due to differences in sample selection criteria, agents used, design, and methodology.

4.6 Conclusion

1.The heterozygous mutant type (AG) of M235T SNP of angiotensinogen is the least frequent genotypes among our sample of Iraqi hypertensive patients.

2. The homozygous wild type (AA) of A1166C SNP of angiotensin II type 1 receptor is the most frequent genotypes among our sample of Iraqi hypertensive patients.

3. Hypertensive patients who have the homozygous mutant type (GG) of M235T SNP of angiotensinogen have higher incidence of having left ventricular hypertrophy, while AG genotype may have protective effect against left ventricular hypertrophy in Iraqi hypertensive patients treated with candesartan 8mg daily.

4. There was no association between A1166C SNP of angiotensin II type 1 receptor and left ventricular hypertrophy in Iraqi hypertensive patients treated with candesartan 8mg daily.

5. There was no association between of M235T SNP of angiotensinogen and A1166C SNP of angiotensin II type 1 receptor and renal function in Iraqi hypertensive patients treated with candesartan 8mg daily.

6. There was no association between of M235T SNP of angiotensinogen and A1166C SNP of angiotensin II type 1 receptor and angiotensinogen and angiotensin II plasma level in Iraqi hypertensive patients treated with candesartan 8mg daily.

4.7. Recommendation

1) Future prospective studies to investigate the effects of AGT M235T and ATR1 A1166C SNPs on candesartan responsiveness with larger sample size are recommended.

2) Future studies to investigate the effects of other SNPs of AGT and ATR1 on candesartan responsiveness

3) Future studies to investigate the effects of AGT M235T and ATR1 A1166C SNPs on other antihypertensive drugs responsiveness

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الخلاصة

الخلفية: يعد ارتفاع ضغط الدم أحد أكثر الموضوعات التي خضعت للدراسة في القرن الماضي، و حيث أن الجينات تلعب دورًا في مرض ارتفاع ضغط الدم، يستخدام علم الوراثة الدوائية لمعرفة المؤشرات الجينية المحددة للاستجابة لأدوية خفض ضغط الدم الذي من خلاله يتم التأكد من المحددات الجينية المؤثرة على فعالية الدواء وسميته قبل بدء العلاج.الهدف: لدراسة تأثيرتعدد الأشكال الجينيية للأنجيوتنسينوجين (M235T) ومستقبلات النوع الاول للأنجيوتنسين II (A1166C) على الاستجابة لمانع مستقبلات الأنجيوتنسين (كانديسارتان) في مرضى ارتفاع ضغط الدم في محافظة كربلاء.

المرضى والطرق: تم الأستعانة باثنان وتسعون مريضا يعانون من ارتفاع ضغط الدم الأساسي الذين يتعالجون ب 8 ملغ / يوم من الكانديسارتان لمدة لا تقل عن ستة أشهر من مستشفى الإمام الحسين التعليمي ومن عيادة خاصة في محافظة كربلاء ، العراق. تم تسجيل تقارير ضغط الدم ومعدل ضربات القلب وتخطيط صدى القلب وتم تحليل وظائف الكلية (الكرياتينين واليوريا) بالاضافة الى بعض العوامل الكيموحيوية مثل مستويات أنجيوتنسينوجين و الأنجيوتنسين II وبعض الكتروليتات. تم استخدام تفاعل البلمرة المتسلسل- نظام الطفرة الحرارية التضخيم ذات البوادئ الاربعة (tetra ARMS-PCR) من أجل التنميط الجيني لتعدد أشكال النوكليوتيدات الفردي (M235T و A1166C).

النتائج: كان توزيع M235T SNP بين مرضى ارتفاع ضغط الدم 39.13٪ AA و 21.74٪ AG و 39.13٪ GG. بينما كان توزيع A1166C SNP : 54.35٪ AA و 40.22٪ AC و 5.43٪ CC. فيما يتعلق بمعلمات مخطط صدى القلب (Echo) ، فإن M235T SNP وتضخم البطين الأيسر (LVH) لهما علاقة ذات دلالة إحصائية ($P<0.05$). كان المرضى الذين لديهم النمط الجيني GG كانوا يشكلون النسبة الأعلى (29.34٪) بين المرضى الذين يعانون من تضخم البطين الأيسر ، بينما المرضى من الأنماط الجينية AA و AG كانت نسبهم 26.08٪ و 15.21٪ على التوالي. من ناحية أخرى لم يكن هناك ارتباط إحصائي بين A1166C ومعلمات مخطط صدى القلب أو معاملات ضغط الدم ($P>0.05$) باستثناء معدل ضربات القلب. المرضى من النمط الجيني AA كان لديهم معدل ضربات قلب أعلى (88.32 ± 10.64 ، $P = 0.005$) من المرضى من النمط الجيني AC و CC. لم يكن هناك ارتباط ذي دلالة إحصائية ($P>0.05$) بين M235T او A1166C SNPs ووظاءف الكلى والمعلمات البيوكيميائية الاخرى.

الاستنتاج: مرضى ارتفاع ضغط الدم الذين لديهم نوع المتحور متماثل الزيجوت (GG) من M235T في جين الانجيوتينسنوجين لديهم نسبة أعلى للإصابة بمضاعفات ارتفاع ضغط الدم مثل تضخم البطين الأيسر ، بينما قد يكون للنمط الجيني AG تأثير وقائيا ضد تضخم البطين الأيسر. ليس لـ A1166C SNP أي تأثير على مضاعفات ارتفاع ضغط الدم وليس له تأثير على استجابة المرضى للكانديسارتان في مرضى ارتفاع ضغط الدم العراقيين.

جامعة كربلاء

كلية الصيدلة

قسم الادوية والسموم

تأثير تعدد الأشكال الجينية للأنجيوتنسينوجين (M235T) ومستقبلات الأنجيوتنسين II (A1166C) على الاستجابة لمانع مستقبلات الأنجيوتنسين (كانديسارتان) في مرضى ارتفاع ضغط الدم في محافظة كربلاء

رسالة مقدمة لمجلس كلية الصيدلة جامعة كربلاء كجزء من متطلبات الحصول على درجة الماجستير في علم الادوية والسموم

من قبل

زهراء حيدر جاسم

بأشراف

الاستاذ المساعد
د.حيدر عبد الامير نجم المظفر

الاستاذ المساعد
د سوزان جبير عباس