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Association of Angiotensin-Converting Enzyme Gene Polymorphism with the Progression of End-Stage Renal Disease in Dialysis Palestinian Patients

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Thesis Approval

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Jerusalem-Palestine

1446/2025

Dedication

I dedicate this work to my mother and father,

to my dear husband Nael Salem,

to my Lovely Kids; Alwaleed, Alyamamah and Shahem.

to my sisters, brother, teachers and friends.

Marwa Mahmoud Ahmad Al-Salahat

Declaration

I certify that this thesis submitted for the degree of Master, is the result of my own research, except there otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signature: 

Marwa Mahmoud Ahmad Al-Salahat

Date: 05.01.2025

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Abstract

End-Stage Renal Disease (ESRD) is a significant public health issue globally. The progression of chronic kidney disease (CKD) to ESRD necessitating dialysis presents a considerable healthcare challenge. ESRD is a complex phenotypic structure of renal diseases affected by different etiologies. Although ethnic, social and environmental factors play a part in the development of the disease, to a large extent the cause of the disease is determined by genetic factors. One of these genes is *ACE* gene which is responsible for converting angiotensin I to angiotensin II, a potent vasoconstrictor. *ACE* polymorphisms appear to have significant impact on the progression of ESRD.

The study is a cross sectional that was done in the period from June to September 2024. A total of 215 participants, including 110 patients diagnosed with end-stage renal disease (ESRD) and 105 healthy controls, were recruited from Beit Jala hospital. Clinical data were collected through medical records and questionnaires, while blood samples were obtained for molecular analysis. DNA extraction, quantification, amplification, and genotyping were conducted to investigate the relationship between ACE polymorphisms and clinical outcomes.

Our results showed that the frequency of *D* allele was the highest (63%) and *DI* genotype was present in all subjects with a frequency of 58.1%. whereas, the *DD* genotype was present in approximately 34% of all subjects with slightly more frequency in ESRD patients (34.5%) compare to controls (33.3%). Moreover, the *DI* genotype is significantly more common in ESRD patients (63.6%) compared to controls (52.4%), with a *p*-value of 0.003. Significant differences were observed in ESRD patients with high systolic blood pressure (SBP) (163.7 ± 17.8 mmHg) compared to controls (126.1 ± 21.1 mmHg) , especially among those with the *DD* genotype There was no significant

differences in diastolic blood pressure (DBP) or creatinine levels between genotypes. The mean age at first dialysis for ESRD patients with the *DD* genotype was 46.3 years, *DI* was 46.9 years, and *II* was 54.2 years, with no statistically significant differences (*p*-value = 0.126). The mean duration of dialysis was longest for *DD* (4.3 years) and shortest for *II* genotype (3.1 years).

In conclusion, association between *ACE I/D* gene polymorphisms and progression to ESRD is varied among different ethnic groups. Our study showed a significant association between the *DD* genotype and increased risk of ESRD. Further research should be conducted using larger sample size to confirm the study results.

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List of abbreviations

Abbreviation	Term
ESRD	End Stage Renal Disease
CKD	Chronic Kidney Disease
ACE	Angiotensin Converting Enzyme
I/D	Insertion/ Deletion Polymorphism
RAAS	Renin-Angiotensin-Aldosterone System
DN	Diabetic Nephropathy
GFR	Glomerular Filtration Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
BUN	Blood Urea Nitrogen
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
AGT	Angiotensinogen
AGTR1	Angiotensin II receptor type 1
ARBs	Angiotensin II Receptor Blockers
BMI	Body Mass Index
PCR	polymerase Chain Reaction
DNA	Deoxyribonucleic acid
EDTA	Ethylene diaminetetraacetic acid
FBS	Fasting blood sugar
Hb	Hemoglobin
SPSS	Statistical product and service solutions

Chapter One

1. Introduction

1.1 Function Of Normal Kidney

The kidneys are vital organs with multiple essential functions that contribute to maintaining the body's internal environment. Their primary role is to filter metabolic wastes from the bloodstream, such as urea, creatinine, and other byproducts, which are then excreted in the urine (Brenner & Rector, 2011). They also regulate the body's water and electrolyte balance by adjusting the levels of sodium, potassium, calcium, and other ions, which is crucial for proper cell and organ function. The kidneys help in the excretion of bioactive substances, including hormones, drugs, and toxins, thereby protecting the body from harmful substances (Davidson, et al 2005). They play a key role in regulating arterial blood pressure through the renin-angiotensin-aldosterone system and by controlling blood volume based on sodium and water excretion. Furthermore, the kidneys regulate red blood cell production by producing erythropoietin, a hormone that stimulates the bone marrow to generate red blood cells. They also convert vitamin D into its active form; calcitriol, which is necessary for calcium absorption and maintaining bone health. Lastly, the kidneys participate in gluconeogenesis, producing glucose from non-carbohydrate sources, which helps sustain blood sugar levels, especially during periods of fasting or low glucose intake (Brenner & Rector, 2011). Figure 1.1 shows the anatomy and major functions of the kidney.

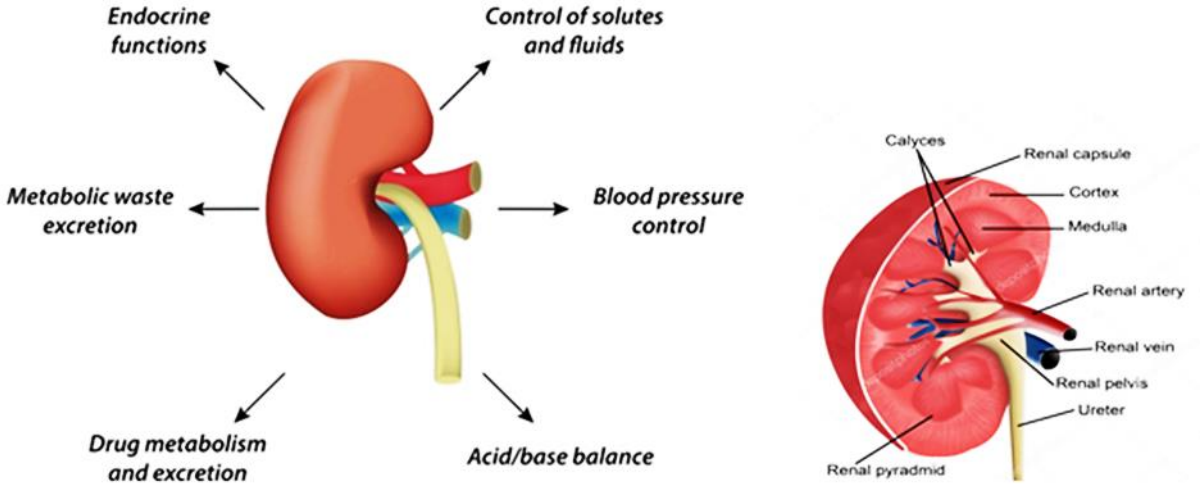

Figure 1.1: Anatomy and function of normal kidney; the figure on the right shows the major anatomic parts of the kidney, and on the left summary of the basic kidney functions.

1.2 Chronic Kidney Disease

Chronic kidney disease (CKD) is a clinical syndrome that results in a definitive change in the function and /or the structure of the kidneys and is characterized by its irreversibility, and slow and progressive evolution. Another important aspect is that its pathology represents a higher risk of development of further health complications and mortality, especially among cardiovascular patients (Himmelfarb & Sayegh, 2010).

An adult patient is identified with CKD when presenting a period equal to or greater than three months of lower glomerular filtration rate (GFR) of 60 ml/min/1.73 m2, or GFR greater than 60 ml/min/1.73 m2, but with evidence of injury of the renal structure (Kimmel, et al. 2014). Some indicators of renal injury include albuminuria, changes in renal imaging, hematuria/pyuria, persistent hydro electrolytic disorders and histological changes in the kidneys (Himmelfarb & Sayegh, 2010).

1.2.1 Causes of Chronic kidney Disease

Chronic kidney disease can arise from various underlying conditions, with diabetes mellitus being the leading cause due to diabetic nephropathy, followed by hypertension, which contributes to hypertensive nephrosclerosis (Webster, et al. 2017). Other causes include glomerulonephritis; an inflammatory condition affecting the glomeruli, and polycystic kidney disease; a genetic disorder that leads to the formation of multiple cysts in the kidneys. Additionally, obstructive uropathy, which results from blockages in the urinary tract, and recurrent kidney infections such as chronic pyelonephritis, can also lead to CKD (Harris and Ismail. 2015) .

1.2.2 Stages of Chronic kidney disease

CKD is categorized into five stages, according to the GFR, and in three stages, according to the albuminuria, as shown in the tables below (Levey & Inker. 2015).

Table 1.1: CKD Stages according to the GFR

Stages	GFR value ml/min/1.73m2	Classification
I	>90	Normal or High
II	60-89	Slightly decreased
III A	45-59	Mild to moderately decreased
III B	30-44	Moderately to severely decreased
IV	15-29	Severely decreased
V	<15	Kidney failure

Table 1.2: CKD Stages according to the albuminuria

Category	24-Hour Albuminuria mg/24 h	A/C Ratio Mg/g	Classification
A1	<30	<30	Normal to discrete
A2	30-300	30-300	Moderate
A3	>300	>300	Severe

A/C Ratio = Albumin/Creatinin ratio in isolated urine samples.

1.2.3 Symptoms and Complications of CKD

CKD often progresses silently, with symptoms appearing in the later stages. But as it advances, common symptoms can include fatigue, weakness, swelling in the legs, ankles, and feet (edema), shortness of breath, nausea, vomiting, persistent itching, high blood pressure, and changes in urination. (Levin and Stevens. 2014). CKD can also lead to several complications, such as cardiovascular disease, anemia, bone disease, and electrolyte imbalance like hyperkalemia and metabolic acidosis (Wouters, et al. 2015).

1.3 End-Stage Renal Disease (ESRD)

End-Stage Renal Disease (ESRD) is the final stage of CKD, where kidneys can no longer sustain the body's needs, requiring renal replacement therapy. It can lead to severe health problems and is associated with high morbidity and mortality rate (Foley and Collins. 2007). Diagnosing ESRD typically involves a physician reviewing the patient's medical history and conducting a physical examination. A patient with a history of CKD that has progressed may be suspected of having ESRD. The physical examination includes tests to

determine the advancement of the kidney disease and often includes measuring the patient's blood pressure. Additional tests may include the following (Harris and Ismail. 2015):

1. Blood tests: These assess serum creatinine, blood urea nitrogen (BUN), and other waste products to indicate the kidneys' filtration capacity and health.
2. Glomerular filtration rate (GFR): This indirect measurement of kidney function traditionally requires an injection of a substance followed by a 24-hour urine sample analysis. Now, GFR can be estimated based on blood test results. A GFR of less than 15 milliliters per minute indicates ESRD.
3. Microalbuminuria test: This urine test detects small amounts of protein and is used to identify early kidney disease.
4. Urinalysis: This standard test detects larger amounts of protein in the urine (proteinuria), indicating severe kidney disease.
5. Imaging tests: Such as ultrasound, CT scan, MRI, or intravenous pyelography (IVP) help identify possible blockages in the urinary tract.
6. Kidney biopsy: Sometimes performed to examine the health of the kidney tissue.

1.3.1 Causes of ESRD

ESRD can result from various causes, with key risk factors for chronic kidney disease including aging, type II diabetes mellitus, and hypertension. Common causes include uncontrolled hypertension, which can damage the kidneys over time, and glomerulonephritis, where inflammation and damage to the kidney's filtration system can lead to kidney failure. Polycystic kidney disease, a hereditary condition causing multiple cysts in the kidneys, and long-term use of certain medications, which can result in analgesic nephropathy, are also significant contributors (Wouk, 2021). Additionally, atherosclerosis can cause ischemic nephropathy, while urinary tract obstructions from stones or cancer may lead to kidney damage. Diabetes mellitus, both type I and II, is a leading cause of ESRD, particularly in the UK, and obesity significantly increases the risk of kidney failure (Molitch, et al. 2010).

1.3.2 Treatment of ESRD

The primary treatment options for ESRD are hemodialysis, peritoneal dialysis, and kidney transplantation.

Kidney Transplantation: Kidney transplantation involves surgically placing a functioning kidney into a person with ESRD. This elective procedure is performed after careful preoperative assessment and preparation. The transplanted kidney may come from a deceased donor or a living donor (Hariharan, et al. 2021).

Dialysis: Dialysis is the most common treatment for end-stage kidney failure, replacing the impaired filtering ability of the kidneys. Most patients with end-stage kidney failure eventually require a kidney transplant. Dialysis involves removing waste substances and fluid from the blood that the kidneys would normally eliminate. It is also used for individuals exposed to or ingesting toxic substances to prevent renal failure (Mehrotra, et al. 2011).

Peritoneal Dialysis: Peritoneal dialysis involves surgically placing a soft, hollow tube into the lower abdomen near the umbilicus. A special solution, called dialysis, is instilled into the peritoneal cavity. This fluid absorbs waste products and toxins through the peritoneum (Jaar, et al. 2012).

Hemodialysis: It involves creating an arteriovenous (AV) fistula, joining an artery and vein together, or using an external intravenous (IV) catheter (Vachharajani, et al. 2021). A dialysis machine as shown in figure 1.2 filters extra fluid and wastes from the blood, and then the filtered blood returned to the body. Medication is given to prevent blood clotting.

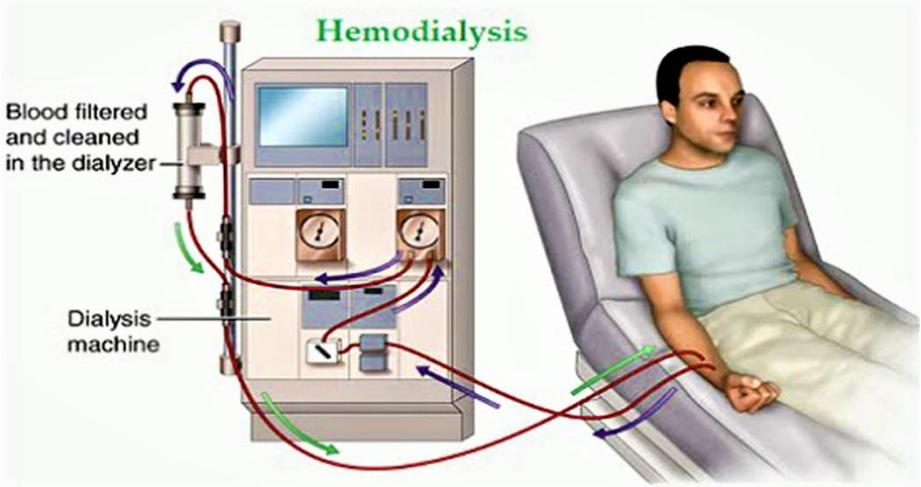

Figure 1.2: Hemodialysis machine

1.3.3 Complications of ESRD

1.3.3.1 Systemic Complications

As kidney function deteriorates, loss of excretory, regulatory, and endocrine functions occurs, leading to clinical complications in almost every organ system (Parera, et al. 2005). These complications include the followings:

1. Gastrointestinal complications: Anorexia, nausea, vomiting, and hiccups are common. Peptic ulcer disease and symptomatic diverticular disease may develop.
2. Cardiovascular complications: Cardiovascular disease is the leading cause of death in ESRD patients. Volume overload can cause congestive heart failure (CHF) and pulmonary edema. Hypertension and dyslipidemia are common complications.
3. Hematological complications: Anemia due to loss of erythropoietin production, increased susceptibility to infection, and easy bruising.
4. Bone disease: Abnormal calcium and phosphorus metabolism leading to bone disease.

1.3.3.2 Electrolyte Disturbances

ESRD) disrupts electrolyte levels, water balance, and acid-base equilibrium, leading to metabolic acidosis, sodium imbalance, potassium abnormalities, and water imbalance. In metabolic acidosis, the kidneys' reduced capacity to excrete hydrogen ions results in

a drop in serum bicarbonate concentration. Sodium balance is generally maintained until late in ESRD, at which point the kidneys lose the ability to handle significant variations in salt intake. Potassium imbalances also occur; hyperkalemia may arise due to dietary factors or acidosis, while hypokalemia can result from gastrointestinal losses or excessive use of sodium polystyrene sulfonate. Furthermore, the kidneys' impaired ability to concentrate or dilute urine heightens susceptibility to hypernatremia. (Hasslacher, et al. 1999).

1.3.4 The Economic Challenge of ESRD

The economic costs of ESRD are significant and multifaceted, impacting patients, healthcare systems, and society at large. These costs include direct medical expenses, indirect costs, and intangible costs. In the United States, for instance, Medicare covers the majority of dialysis costs, which places a substantial financial load on the federal healthcare system. According to the United States Renal Data System (USRDS), the annual cost of care for a dialysis patient is significantly higher compared to the average Medicare beneficiary. As of 2023, more than 800,000 Americans are living with ESRD, requiring dialysis therapy and/or transplantation. In 2020, Medicare spending on ESRD care was approximately $49 billion (United States Renal Data System, 2022). This represents a significant increase from the $11 billion reported in 1999.

1.3.5 Epidemiology of ESRD in Palestine

The incidence and prevalence of ESRD vary significantly across different regions and countries, influenced by factors such as healthcare access, socioeconomic conditions, and the prevalence of risk factors like diabetes and hypertension.

The prevalence of ESRD is a significant public health concern, influenced by the high rates of diabetes and hypertension, as well as socio-economic and political factors. According to recent data, the prevalence of CKD and ESRD in Palestine is rising, based on the Palestinian Ministry of Health reports, there is an increasing numbers of patients requiring dialysis each year. The prevalence of ESRD in Palestinian populations is estimated to be comparable to or slightly higher than global averages, reflecting the high burden of risk factors such as diabetes, hypertension, and genetic predispositions. In 2021, the number of patients receiving hemodialysis services in the hospitals on a regular basis in West Bank was 1,641 patients in addition to 1,034 patients In Gaza Strip (Ministry of health report, 2021).

1.4 Genetic Factors Influencing Renal Disease Progression

CKD and its progression to ESRD are influenced by a complex interplay of genetic, environmental, and lifestyle factors. Among these, genetic factors play a critical role in determining individual susceptibility to kidney disease, the rate of disease progression, and response to treatment (Barlow, et al. 2023). Understanding these genetic influences is crucial for developing personalized medical approaches and improving patient outcomes. Several genes have been identified that influence kidney function and susceptibility to CKD and ESRD. These genes can affect various biological pathways, including those involved in blood pressure regulation, glomerular filtration, and inflammatory responses (Faller & Roberts, 2023).

Key Genetic Factors

APOL1 Gene: Variants of the APOL1 gene, primarily found in individuals of African descent, are strongly associated with an increased risk of developing CKD and ESRD. These variants are thought to confer protection against *Trypanosoma brucei* (the cause of African sleeping sickness) but increase the risk of kidney disease (Genovese, et al. 2010; Tzur, et al. 2010).

UMOD Gene: Mutations in the UMOD gene, which encodes uromodulin (a protein produced in the kidney), are linked to familial juvenile hyperuricemic nephropathy and other forms of hereditary CKD. Abnormal uromodulin can lead to the buildup of proteins in the kidneys, causing damage (Hart, et al. 2002).

PKD1 and PKD2 Genes: Mutations in these genes cause autosomal dominant polycystic kidney disease (ADPKD), a common genetic disorder characterized by the development of numerous cysts in the kidneys, leading to progressive kidney failure (Torres, et al. 2007).

***ACE* Gene Polymorphism**: The angiotensin-converting enzyme (*ACE*) gene polymorphism, particularly the insertion/deletion (*I/D*) variant, has been widely studied for its role in renal disease progression. The *D* allele is associated with higher *ACE* levels, increased angiotensin *II* production, and greater susceptibility to hypertension and kidney damage (Brkovic,, et al. 2019).

1.4.1 Mechanisms by Which Genetic Factors Influence Renal Disease

Genetic factors can influence renal disease progression through various mechanisms:

1. Blood Pressure Regulation: Genes involved in the Renin-Angiotensin-Aldosterone System (RAAS), such as ACE, AGT (angiotensinogen), and AGTR1 (angiotensin II receptor type 1), play a critical role in regulating blood pressure. Variants in these genes can lead to hypertension, a major risk factor for CKD and ESRD (Samani, et al. 1996; Delles, et al. 2008).

2. Glomerular Filtration and Structure: Genetic mutations affecting glomerular structure and function can lead to glomerulopathies, contributing to CKD. For example, mutations in genes encoding collagen (COL4A3, COL4A4, COL4A5) can cause Alport syndrome, a hereditary nephritis (Kashtan, 2000).

3. Inflammation and Immune Response: Genetic variants influencing immune system function can affect the inflammatory response in the kidneys. For example, polymorphisms in genes related to the complement system (CFH, CFI) can lead to atypical hemolytic uremic syndrome, a condition associated with renal failure (Noris & Remuzzi, 2009).

4. Metabolic Pathways: Genes involved in metabolic processes, such as SLC12A1 and KCNJ1, which are associated with Bartter syndrome, affect electrolyte balance and kidney function, contributing to CKD progression (Schaefer, et al. 2008).

1.4.2 The Role of Angiotensin-Converting Enzyme in Renal Physiology

The Renin-Angiotensin-Aldosterone System (RAAS) is a critical regulatory system that maintains blood pressure, fluid, and electrolyte balance, as well as systemic vascular resistance (Bernstein, et al. 2012). It plays a significant role in renal physiology and the

pathophysiology of renal diseases. The Components of RAAS is represented in table 1.4 (Oparil & Haber, 1974), and the mechanism is represented in figure 1.4.

Table 1.3: The Components of the Renin-Angiotensin-Aldosterone System

Component	Description
Renin	An enzyme secreted by the juxtaglomerular cells of the kidneys in response to decreased blood pressure, low sodium levels, or sympathetic nervous system stimulation. Renin catalyzes the conversion of angiotensinogen, a plasma protein produced by the liver, to angiotensin I
Angiotensin I	A decapeptide that is relatively inactive. It serves as a precursor to angiotensin II.
Angiotensin-Converting Enzyme (ACE)	A key enzyme primarily located in the lungs' endothelial cells, although it is also found in the kidneys and other tissues. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor.
Angiotensin II	The primary effector molecule of the RAAS. It exerts multiple physiological effects, including vasoconstriction, aldosterone secretion, and stimulation of the release of antidiuretic hormone (ADH).
Aldosterone	A steroid hormone produced by the adrenal cortex in response to angiotensin II. It promotes sodium and water reabsorption in the distal tubules and collecting ducts of the kidneys, increasing blood volume and pressure.

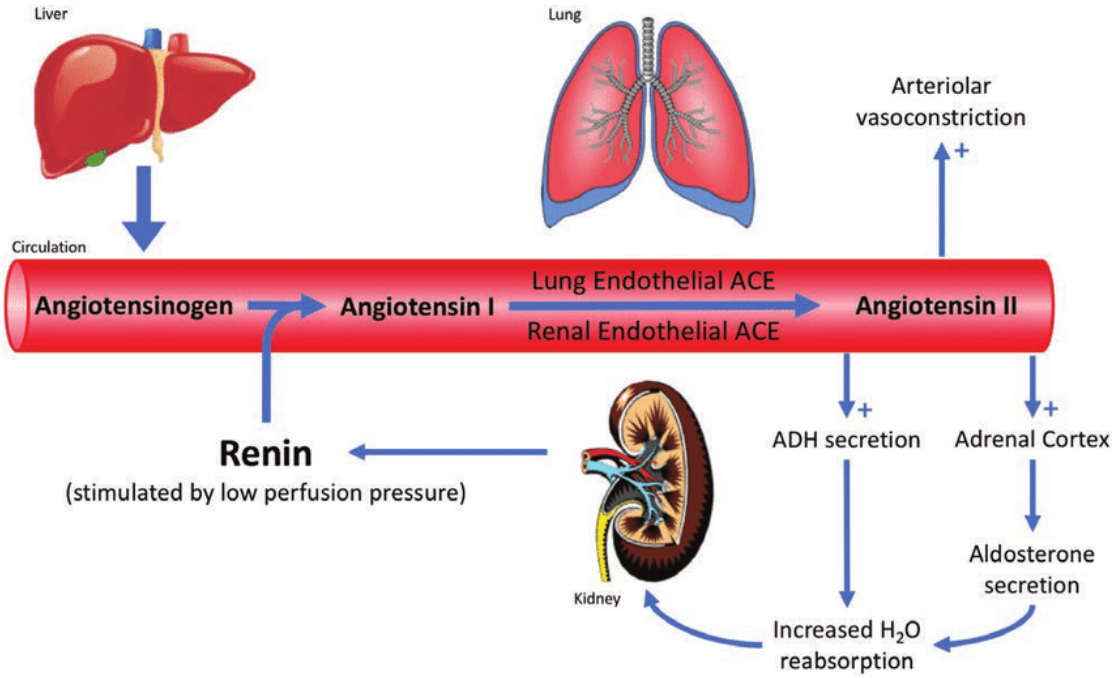

Figure 1.3: Schematic representation of the renin-angiotensin-aldosterone system. Low renal perfusion pressure causes renin to stimulate the conversion of angiotensinogen to angiotensin I. Angiotensin I is hydrolyzed by *ACE* in the lung and renal endothelium to produce angiotensin II, leading to increased sympathetic tone, vasoconstriction, ADH secretion, and aldosterone secretion. *ACE* indicates angiotensin-converting enzyme; ADH, antidiuretic hormone (chow et al., 2018).

1.4.3 Activation and Regulation of RAAS:

Renin secretion is triggered by various factors, including low blood pressure, a reduced sodium chloride concentration in the distal tubule, and activation of the sympathetic nervous system. Conversely, negative feedback mechanisms such as high blood pressure and increased sodium chloride levels work to inhibit renin release, maintaining homeostasis in blood pressure regulation and fluid balance (Crowley & Coffman, 2012).

1.4.4 Impact of ACE Activity on Renal Health

ACE activity has significant implications for renal health through its influence on blood pressure, renal hemodynamics, and sodium and water balance:

1. Blood Pressure Regulation: Angiotensin II, produced by the action of ACE, causes vasoconstriction of the arterioles, leading to an increase in systemic blood pressure. Sustained high blood pressure (hypertension) is a major risk factor for the development and progression of CKD and ESRD (Lely, et al. 2004).

2. Renal Hemodynamics: Angiotensin II preferentially constricts the efferent arterioles more than the afferent arterioles in the glomerulus, leading to increased glomerular capillary pressure and filtration rate. While this mechanism helps maintain GFR during periods of low renal perfusion, chronic elevation of angiotensin II can cause glomerular hypertension and damage, contributing to CKD progression (Hall, et al. 1990).

3. Sodium and Water Reabsorption: Angiotensin II stimulates the adrenal cortex to secrete aldosterone, which increases sodium and water reabsorption in the kidneys. This leads to increased blood volume and pressure. Excessive sodium retention and fluid overload are common in CKD and can exacerbate hypertension and cardiovascular complications (Chevalier, et al. 2011).

1.4.5 Therapeutic Implications

Understanding the role of ACE in RAAS and its impact on renal health has led to the development of therapeutic interventions aimed at modulating this system to protect renal function:

ACE Inhibitors: Medications such as enalapril, lisinopril, and ramipril inhibit ACE activity, reducing the production of angiotensin II. This leads to vasodilation, decreased blood pressure, and reduced glomerular pressure (Bakris & Weir, 2000).

Angiotensin II Receptor Blockers (ARBs): ARBs like losartan and valsartan block the effects of angiotensin II at its receptor sites. They provide similar benefits to ACE inhibitors and are often used in patients who are intolerant to ACE inhibitors (McMurray, et al. 2010).

1.5 Angiotensin-Converting Enzyme Gene

The ACE gene is responsible for encoding the ACE protein. The ACE gene is located on the long arm of chromosome 17, specifically at position 17q23. This gene spans approximately 21 kilobases and consists of 26 exons and 25 introns (Rigat, et al. 1990). Figure 1.5 shows the localization of angiotensin-converting enzyme gene.

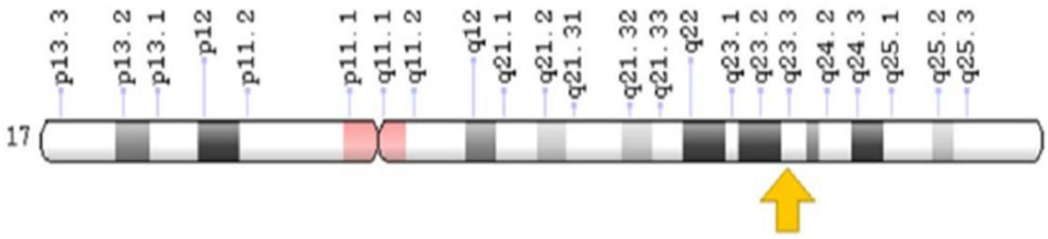

Figure 1.4: Localization of the angiotensin converting enzyme (ACE) gene (Silene, et al. 2018).

1.5.1 Angiotensin-Converting Enzyme Gene Polymorphism

One of the most studied polymorphisms of the *ACE* gene is the Insertion/Deletion (*I/D*) polymorphism. This polymorphism is characterized by the presence (insertion, *I*) or absence (deletion, *D*) of a 287 base pair sequence in intron 16 of the *ACE* gene (Rigat, et al. 1990). Figure 1.6 below shows the position of the frequently investigated insertion/deletion polymorphism.

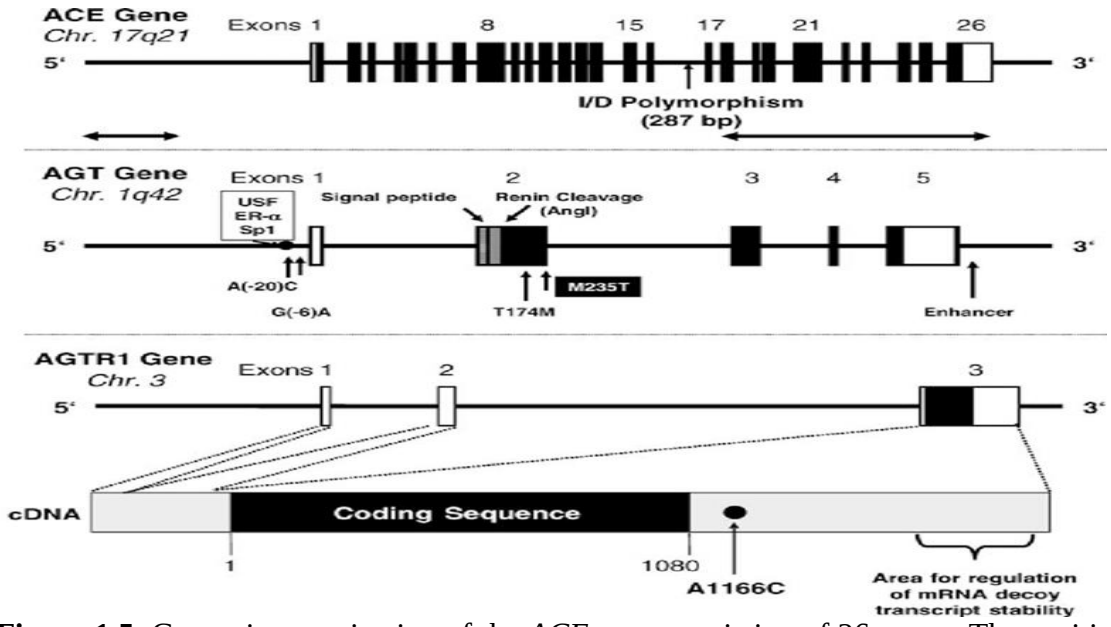

Figure 1.5: Genomic organization of the *ACE* gene consisting of 26 exons. The position of the frequently investigated insertion/deletion polymorphism in intron 16 is depicted. The *D* allele is associated with enhanced *ACE* levels (Rosskopf, et al. 2007).

The *I/D* polymorphism results in three possible genotypes:

- Genotype *II* (Insertion/Insertion): Homozygous for the insertion allele.
- Genotype *ID* (Insertion/Deletion): Heterozygous, carrying one insertion and one deletion allele.
- Genotype *DD* (Deletion/Deletion): Homozygous for the deletion allele.

1.5.2 Clinical Implications of ACE Gene Polymorphism

The *ACE I/D* polymorphism has been extensively studied for its association with various diseases, particularly those involving the cardiovascular and renal systems. The *DD* genotype, with its higher *ACE* activity, has been linked to an increased risk of several conditions:

Hypertension: Higher *ACE* activity results in elevated levels of angiotensin II, a potent vasoconstrictor, contributing to increased blood pressure and the risk of hypertension (Abhishek, et al. 2014).

Cardiovascular Diseases: The *DD* genotype has been associated with a higher risk of myocardial infarction, stroke, and left ventricular hypertrophy due to its impact on blood pressure regulation and vascular health (Reddy & Pande, 2015).

Chronic Kidney Disease: Increased *ACE* activity and angiotensin *II* levels contribute to glomerular hypertension, inflammation, and fibrosis, accelerating the progression of CKD to ESRD (Bzdega, et al. 2011).

Diabetic Nephropathy: Diabetic patients with the *DD* genotype are at a higher risk of developing diabetic nephropathy, a leading cause of CKD and ESRD, due to the

combined effects of hyperglycemia and elevated angiotensin *II* levels (Carey, et al. 2003).

1.6 Literature Review : Genetic Studies On ACE Gene I/D Polymorphism

Numerous studies worldwide have investigated the association between *ACE* gene *I/D* polymorphism and renal outcomes, with varying results across different populations. For example, the research conducted by El Sayed et al. (2023) analyzed *ACE* polymorphism in Egyptian patients with type 2 diabetes, finding a higher frequency of the *D* allele in those with diabetics nephropathy (DN) compared to diabetics without renal complications. The *D* allele's presence correlated with an increased risk of DN, reinforcing its role in progressive renal impairment in diabetic patients. Similarly, El-Baz et al. (2018) demonstrated the association of the *DD* genotype with DN in Egyptian diabetics, indicating that individuals with the *DD* genotype were more susceptible to developing kidney complications. In Jordanian adults, Al-Eitan et al. (2024) noted a heightened hypertension susceptibility in *D* allele carriers, further linking this polymorphism to risk factors that contribute to renal disease.

Pereira et al. (2006) reviewed *ACE I/D*'s influence on renal failure in polycystic kidney disease, confirming that the *D* allele exacerbates renal deterioration, though its exact impact may vary. Yu et al. (2012) conducted a meta-analysis of Asian populations and found that the *D* allele increased susceptibility to ESRD in diabetic patients. This analysis revealed ethnic variability in *ACE* polymorphism effects, with Asian individuals showing a stronger association between the *D* allele and ESRD compared to Caucasian populations. Golmohamadi et al. (2006) investigated Iranian patients, confirming a positive association between the *D* allele and DN, suggesting this polymorphism is a significant genetic marker for nephropathy risk.

In a systematic review, Shen et al. (2019) highlighted that the *D* allele contributes to an elevated risk of diabetes-related ESRD, which is particularly relevant in populations with a high prevalence of hypertension and diabetes. Zhou et al. (2014) demonstrated that the *ACE D* allele could be a genetic predictor for ESRD. These findings were confirmed by Lin et al. (2014), who focused on hypertensive Asians, supporting that the *D* allele's association with CKD risk is higher among those with diabetes.

Shanmuganathan et al. (2015) analyzed the ACE polymorphism in South Indian populations, observing that individuals with the *DD* genotype had a higher risk of CKD, particularly when combined with hypertension. This study, like others, underscores the interplay between *ACE* polymorphism and hypertension in advancing kidney disease. Furthermore, a recent study by Ud Din et al. (2023) in Pakistan revealed that the *D* allele was prevalent among diabetics with DN, suggesting that this genetic factor could be critical in South Asian populations with elevated diabetes rates.

Al-Radeef et al. (2019) focused on Iraqi patients and found that the *DD* genotype was significantly more frequent among ESRD patients compared to healthy controls. The study also observed that the *ID* genotype had a moderate association with ESRD, while the *II* genotype appeared to be protective. The *DD* genotype increased the risk of ESRD, reinforcing the role of the *ACE I/D* polymorphism in Middle Eastern populations. Susilo et al. (2022) examined the relationship between *ACE* gene polymorphisms and renal failure in an Indonesian population. It found that individuals

with the *DD* genotype had a higher risk of ESRD, particularly in patients with underlying hypertension, a key risk factor for kidney disease. The *DD* genotype was significantly associated with ESRD risk in hypertensive patients, emphasizing the role of *ACE* polymorphisms in kidney disease progression.

In a cohort of dialysis patients, van der Sman-de Beer et al. (2005) reported that the *D* allele correlates with higher mortality, highlighting its potential as a risk marker in renal disease prognosis. Further supporting this, Abouleka et al. (2021) associated the *D* allele with adverse kidney outcomes and all-cause mortality in type 1 diabetes.

Zeng et al. (2022) addressed conflicting results in *ACE* polymorphism's impact on diabetic kidney disease, emphasizing the need for more population-specific data. Similarly, Al-Jebouri and Al-Alwani (2014) highlighted variability in chronic renal failure outcomes related to the *ACE* genotype, reflecting differences in genetic and environmental interactions.

1.7 Study Objectives

This study aims to investigate the association between ACE polymorphism and the progression of ESRD in dialysis Palestinian patients. It addresses the high prevalence of ESRD among Palestinians and aims to fill existing research gaps regarding the role of ACE polymorphism in this population. By exploring genetic factors that may influence ESRD progression, the study aims to provide a deeper understanding of the disease's underlying mechanisms in Palestinian patients. Therefore, the following specific objectives are addressed in this study:

1. To investigate the prevalence of ACE gene polymorphisms among Palestinian patients with CKD undergoing dialysis.
2. To evaluate the association between ACE gene polymorphism and the development and progression of renal disease, including CKD stages and progression to ESRD.
3. To assess the relationship between ACE polymorphism and clinical outcomes in ESRD patients.

Chapter Two

2. Materials and Methods

2.1 Study design

Cross-section study was designed to assess the prevalence of ACE gene polymorphisms and their association with renal disease progression.

2.1.1 Study participants

A total of 215 subjects were recruited from Beit Jala hospital during the period from June to September 2024. Among them, 110 patients were diagnosed with ESRD and 105 were healthy control subjects. The ratio of male to female in ESRD patients group is 73/37 while it is 67/38 in healthy individuals.

2.1.2 Data collection

Clinical data, including demographic information, medical history, laboratory results, and dialysis duration and treatment parameters, were collected from medical records and through the use of a baseline questionnaire. (Appendix1). The data collected included age, sex, demographic information, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP), medical history, medication status, biochemical parameters and laboratory investigations.

2.1.3 Sample collection and preparation

A total of 5 ml of blood samples were collected into two sterile tubes: one containing 0.5 ml of ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant for molecular assay, and the other a plain tube to obtain serum for biochemical tests. All control subjects were recruited based on the absence of any history of kidney disease and the presence of a normal serum creatinine level. A negative control group of healthy individuals (n=105) was sampled for blood and serum to be used in the comparison arm of the study.

2.1.4 Ethical approval

The study was approved by Al-Quds University ethics committee 71/REC/2019 (Appendix 2 and Appendix 3). Written informed consent was obtained from all subjects before participation.

2.2 Molecular diagnosis of ACE gene polymorphism

2.2.1 DNA extraction

Genomic DNA was extracted from blood samples using Epicenter Blood kit. Extraction steps were performed following the manufacturer's instruction. Briefly, Blood cells were lysed using a lysis buffer to release DNA, Incubation was done at 37°C for 30 min. Then followed by the removal of proteins and cellular debris through a protein precipitation solution and centrifugation. The DNA-containing supernatant was transferred to a new tube, and DNA was precipitated using isopropanol . The DNA pellet was then washed with ethanol to remove contaminants and suspended in a TE buffer, the DNA samples in the tubes were stored at -20°C until further analysis. For more details, see appendix 4.

2.2.2 DNA Quantification

The concentration of DNA samples was measured using Nano drop 1000 and the 260/280 and 260/230 were determined. One microliter (1µl) of DNA sample was loaded in the correct place after blanking the device with water. Ratios of 260/280 and 260/230 were recorded for DNA quality.

2.2.3 DNA Amplification

The ACE gene polymorphism was identified using polymerase chain reaction (PCR) based on the PCR primers (Rigat, et al. 1990)**:**

Forward 5'-CTGGAGACCACTCCCATCCTTTCT-3'

Reverse 5'-GATGTGGCCATCACATTCGTCAGAT-3'.

The PCR amplification was carried out using 3 µl of the extracted DNA in a final volume of 25 µl, which was contained 12.5 µl PCRBIO HS *Taq* Mix Red (the mix contains *Taq* DNA polymerase, deoxynucleotide triphosphates (dNTPs), $MgCl_2$, and a specialized buffer system), 8.5 µl double distilled and purified water (ddH2O), and 0.5 µl of each primer (10 pmol/µl). The PCR amplification program: annealing temperature at 62°C, an initial denaturation at 95℃ for 5 min followed by 35 cycles of 95℃ for 30 s, 62℃ for 30 s, and 72℃ for 40 s and final extension at 72℃ for 6 min.

2.2.4 Gel electrophoresis

The gel was prepared by addition of 2g agarose to 100ml TAE buffer, boiling the mixture and adding 2µl(10ug/ml) ethidium bromide. The mixture is then poured into agarose gel casting system (Bio-Rad, SUB-CELL®GT). After that, solidified gel was placed into electrophoresis chamber and covered by TAE buffer and 8µl of PCR products were loaded into the wells, alongside with 100 bp DNA size marker. Finally, samples were electrophoresed /run at voltage of 120 volts for 45min. The PCR product bands were seen using gel documentation system (GelDoc).

2.2.5 Genotyping based on banding pattern

The genotypes were determined based on the banding patterns of the digested PCR products.

The absence or presence of a 287-bp Alu repeated sequence represents the II, ID and DD genotypes.

- **(DD) homozygous** genotype was identified by the presence of one band of (190 bp).
- **(II) homozygous** genotype was identified by the presence of one band of (490 bp).
- **(ID) heterozygous** genotype was identified by the presence of two bands of (190 and 490 bp).

2.3 Statistical Analysis

The data were analyzed using SPSS software, version 23. Statistical tests, including the T-test, and Chi-square (χ^2) test, were employed to evaluate the independence between two variables when comparing uncorrelated and independent groups. The frequency of ACE polymorphisms was calculated using the gene counting method. Allelic frequencies were derived from the genotype distribution among cases and controls. ANOVA was performed to evaluate the association between ACE genotypes and continuous variables. Multivariate Analysis was performed to adjust for potential confounders such as age, gender, hypertension, and diabetes. A *P*-value less than 0.05 was considered statistically significant.

Chapter Three

3. Results

3.1 Demographic characteristic

Table 3.1 compares demographic characteristics between ESRD patients and controls, showing no significant differences in age or gender distribution. ESRD patients have a slightly higher average age (53.18 years) compared to controls (43.85 years), but this difference is not statistically significant (P = 0.605). Gender proportions are similar, with males making up about two-thirds of each group and females one-third, also showing no significant difference (P = 0.463). This indicates that the groups are well-matched demographically.

. **Table 3.1:** Demographic characteristics of study subjects.

Variable	ESRD patients	Controls	*P-value
Age, years (mean±SD)	53.1 ± 17.1	43.8 ±16.6	0.605
Male, n (%)	73 66.4%	67 %63.8	0.463
Female, n (%)	37 33.6%	38 %36.2	
Total, n	110	105	

*P-value<0.05 is considered significant.

3.2 Clinical and Biochemical Characteristics of the Study Subjects

The ESRD patients exhibit significantly higher Systolic Blood Pressure (SBP) (158.0 ± 22.9) compared to the control group (122.2 ± 17.1), with a *p*-value of 0.038. Despite the elevation in SBP, there is no significant difference in diastolic blood pressure (DBP) between ESRD patients (80.6 ± 11.76) and controls (74.2 ± 10.9), as indicated by the non-significant *p*-value (0.958). ESRD patients have lower hemoglobin levels (10.3 ± 1.8) than controls (13.4 ± 1.9), but the difference is not statistically significant (*p*-value 0.878). Both creatinine (8.3 ± 3.3 in ESRD patients vs. 0.8 ± 0.65 in controls) and blood urea nitrogen (BUN) levels (48.4 ± 19.7 vs. 12.3 ± 5.0) show highly significant differences, with *p*-values of < 0.001 for both. Triglyceride levels show no significant difference between ESRD patients and controls (*p*-value 0.090), and similarly, total cholesterol levels are not significantly different (*p*-value 0.984). Although ESRD patients have lower HDL (32.4 ± 12.1) and LDL (87.4 ± 34.3) levels compared to controls (43.8 ± 9.1 for HDL and 124.2 ± 36.2 for LDL), these differences are not statistically significant (*p*-values 0.318 and 0.396, respectively). FBS is significantly elevated in ESRD patients (119.6 ± 56.7) compared to controls (108.33 ± 39.15), with a *p*-value of 0.004. Sodium (142.1 ± 3.1) and potassium (4.6 ± 0.8) levels are significantly higher in ESRD patients compared to controls (139.6 ± 2.3 for sodium and 4.1 ± 0.5 for potassium), with *p*-values of 0.003 and < 0.001, respectively (Table 3.2).

Table 3.2: Clinical and biochemical analysis between ESRD patients and healthy control participants.

Variable	ESRD Patients (n=110)	Controls (n=105)	*P*-Value
SBP (mmHg)	158.1 ± 22.9	122.2 ± 17.1	**0.038**
DBP (mmHg)	80.6 ± 11.7	74.2 ± 10.9	0.958
Hb (g/dL)	10.3 ± 1.8	13.4 ± 1.9	0.878
Creatinine (mg/dL)	8.3 ± 3.3	0.819 ± 0.6	**0.000**
BUN (mg/dL)	48.4± 19.7	12.3 ± 5.0	**0.000**
Triglycerides (mg/dL)	152.3 ± 90.7	147.2 ± 64.7	0.090
Total Cholesterol (mg/dL)	149.6 ± 42.0	184.1 ± 40.0	0.984
HDL-cholesterol (mg/dL)	32.4± 12.1	43.8 ± 9.1	0.318
LDL-cholesterol (mg/dL)	87.4 ± 34.3	124.2 ± 36.2	0.396

FBS (mg/dl)	119.6 ± 56.7	108.3 ± 39.1	0.004
Sodium (mmol/L)	142.1 ± 3.1	139.6 ± 2.3	0.003
Potassium (mmol/L)	4.68 ± 0.8	4.1 ± 0.5	0.000

*P-value<0.05 is considered significant.

3.3 Molecular Diagnosis of ACE polymorphism

3.3.1 DNA Concentration and Purity Measurement

The concentration and purity of DNA in the samples were measured using the Nanodrop 1000. DNA concentration was reported in ng/µl, while purity was assessed based on the 260/280 and 260/230 ratios. For a sample to be suitable for PCR analysis, the DNA concentration needed to exceed 10 ng/µl. The concentrations of the samples in this study ranged between 10-1400 ng/µl, indicating successful DNA extraction.

3.3.2. PCR amplification

The PCR amplification of the *ACE* gene produced two bands at 190 bp and 490 bp, corresponding to the targeted *I/D* polymorphisms. The gel electrophoresis results, shown in Figure 3.1, confirmed the successful amplification of these PCR products.

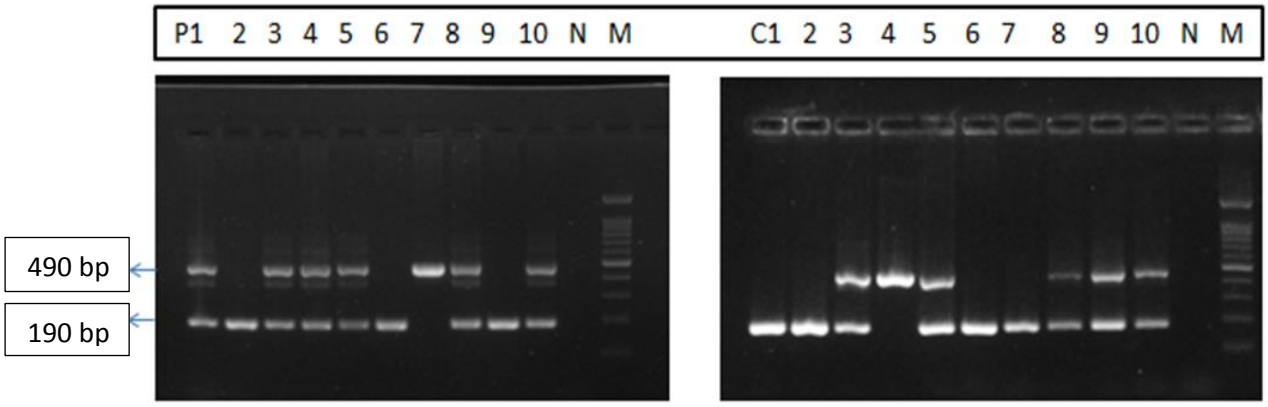

Figure 3.1: PCR products of *ACE* gene *I/D* polymorphisms analyzed by agarose gel electrophoresis. Line M refers to DNA ladder (100 bp); N: negative control; P1-10: PCR products of ESRD patient samples. C1-10: PCR products of control group samples.

3.4 *ACE* genotype and alleles frequency

Table 3.3 shows the distribution of *ACE* genotypes and alleles between control subjects and ESRD patients, with corresponding *p*-values indicating the statistical significance of differences.

Table 3.3: ACE genotype and allele frequencies in ESRD patients (110) versus control subjects (105).

Genotype/allele	ESRD patients (n=110; %)	Controls (n=1; %05)	*P*-value*
DD	38 34.5%	35 33.3%	**0.003**
DI	70 63.6%	55 52.4%	
II	2 1.8%	15 14.3%	
***D* allele**	0.664 66.4%	0.595 59.5%	**0.004**
***I* allele**	0.336 33.6%	0.405 40.5%	**0.004**

* Chi-square (χ^2) test, significant test; P-value <0.05 is considered significant.

The proportion of individuals with the *DD* genotype is similar between controls (33.3%) and ESRD patients (34.5%). The *DI* genotype is more common in ESRD patients (63.6%) than in controls (52.4%). The *II* genotype is significantly less frequent in ESRD patients (1.8%) compared to controls (14.3%), These differences is statistically significant, with a *p*-value of 0.003. The *D* allele is more frequent in ESRD patients (66.4%) than in controls (59.5%), while the *I* allele is slightly more frequent in controls (40.5%) compared to ESRD patients (33.6%). Both allele distributions show statistical significance, with a *p*-value of 0.004.

3.5 Comparison of clinical parameters between ESRD patients and controls subjects based on *ACE* genotypes (*DD, DI, II*)

Table 3.4 shows a comparison of various clinical parameters between ESRD patients and control subjects, stratified by *ACE* genotypes, along with their corresponding *p*-values.

Table 3.4: Comparison of clinical parameters between ESRD patients and control subjects based on *ACE* genotypes

Variable	ESRD patients(n=110)	Controls(n=105)

	DD (n=38)	*DI (n=70)*	*II (n=2)*	***P*-value**	*DD (n=35)*	*DI (n=55)*	*II (n=15)*	***P*-value**
SBP (mmHg)	163.7 ±17.8	156.9 ±21.8	91 ±43.8	**0.001**	126.1 ±21.1	119.7 ±13.8	122 ±16.2	0.234
DBP (mmHg)	81.9 ±10.9	80.5 ±12.2	62 ±1.4	0.065	76.6 ±12.3	72.9 ±10.6	73.9 ±8.2	0.323
Hb g/dL	9.9 ±1.5	10.5 ±2	11.2 ±3.5	0.203	12.9 ±1.8	13.7 ±1.9	13.7 ±1.8	0.124
Creatinine mg/dL	7.72 ±3.2	8.68 ±3.34	9.91 ±0.98	0.289	0.72 ±0.14	0.81 ±0.2	1.14 ±1.8	0.140
BUN mg/dL	49.9 ±21.2	47.2 ±18.6	66 ±32.5	0.354	11.3 ±3.7	12.9 ±5.8	13.4 ±4.5	0.286
FBS mg/dL	117 ±48.2	122 ±61.4	87.5 ±41.7	0.662	116.9 ±46.7	106.3 ±36.6	92.3 ±14.3	0.207
Triglyceride mg/dL	125.4 ±76.4	164.2 ±94.2	185 ±117	0.113	139.2 ±61	150.4 ±70	162.3 ±55.6	0.782
Total. Cholesterol mg/dL	147.7 ±46.9	149.8 ±40.2	175 ±31.1	0.674	190.9 ±47	175.4 ±33.8	204.8 ±39	0.285
HDL-cholesterol mg/dL	35.4 ±15	31.1 ±10.3	36 ±16.9	0.253	45.1 ±9.7	43.3 ±9.5	41.3 ±2.3	0.751
LDL-cholesterol mg/dL	85.3 ±34	87.9 ±34.6	100 ±29.7	0.818	132.4 ±40.8	114.5 ±29.3	147.8 ±44.9	0.118
Sodium mmol/L	142.9 ±3.3	141.6 ±2.9	145.5 ±2.1	**0.026**	140 ±2.3	139.5 ±2.4	139.1 ±1.8	0.454
Potassium mmol/L	4.6 ±0.7	4.7 ±0.9	4.6 ±0.4	0.646	4.1 ±0.6	4.2 ±0.4	4.1 ±0.5	0.677

*Data presented as mean+SD, ANOVA analysis, *P*-value<0.05 is considered significant.

ESRD patients show significantly higher systolic blood pressure across all genotypes, especially in the *DD* genotype (163.7 ± 17.8 mmHg) compared to controls (126.1 ± 21.1 mmHg for *DD*). This difference is statistically significant, with a *p*-value of < 0.001 for ESRD patients, indicating a strong association between higher SBP and the presence of the *DD* genotype in ESRD patients. There is no significant difference in controls (*p*-value = 0.234).

There is no statistically significant difference in DBP between genotypes in both ESRD patients (*p*-value = 0.065) and controls (*p*-value = 0.323), DBP tends to be higher in ESRD patients, particularly in those with the *DD* genotype.

ESRD patients tend to have lower hemoglobin levels across all genotypes compared to controls, but the differences are not statistically significant for either group (*p*-values: 0.203 for ESRD, 0.124 for controls).

Creatinine and BUN levels are markedly elevated in ESRD patients compared to controls across all genotypes. However, there is no statistically significant difference between genotypes within each group (*p*-values: 0.289 for creatinine and 0.354 for BUN in ESRD patients).

FBS shows no significant difference between genotypes in both ESRD patients and controls (*p*-values: 0.662 and 0.207, respectively). While lipid levels (triglycerides, total cholesterol, HDL, LDL) vary slightly between genotypes, none of these differences are statistically significant for either ESRD patients or controls. The higher triglyceride levels observed in ESRD patients with the *II* genotype (185 ± 117 mg/dl) and controls with the *II* genotype (162.3 ± 55.6 mg/dl) do not reach significance level.

Sodium levels are significantly higher in ESRD patients with the II genotype (145.5 ± 2.1 mmol/L) compared to those with other genotypes (*p*-value = 0.026). However, potassium levels show no significant differences between genotypes in either ESRD patients or controls (*p*-values: 0.646 and 0.677, respectively).

3.6 Analysis of age at first dialysis and dialysis duration across different *ACE* genotypes (*DD, DI, II*)

Table 3.5 shows comparison of two variables; age at first dialysis and duration of dialysis, across different *ACE* genotypes (*DD*, *DI*, and *II*) in ESRD patients, along with corresponding *p*-values to assess statistical significance.

Table 3.5: Age at first dialysis and dialysis duration across different ACE genotypes of ESRD patients.

variable	**DD (n=38)**	**DI (n=70)**	**II (n=2)**	***P*-value**
Age at first dialysis (years), mean±SD	46.3±31.4	46.9±17.4	54.2±17.7	0.126
Duration of dialysis (years)	4.3±2.4	4.0±2.6	3.1±2.5	0.275

**P*-value<0.05 is considered significant.

The average age at which patients started dialysis varies slightly between genotypes, with the *II* genotype having the highest average age (54.2 ± 17.7 years), followed by *DI* (46.9 ± 17.4 years), and *DD* (46.3 ± 31.4 years).

The duration of dialysis appears to be shortest for the *II* genotype (3.1 ± 2.5 years) and longest for the *DD* genotype (4.3 ± 2.4 years), with DI patients falling in between (4 ± 2.6 years).

However, the *p*-value of 0.275 indicates no statistically significant difference in the duration of dialysis between the genotypes.

Chapter Four

4.1 Discussion

ESRD is the final stage of CKD, where the kidneys are no longer able to function adequately, leading to the need for dialysis or kidney transplantation. By identifying genetic markers (such as the *DD* genotype in *ACE* polymorphisms) linked to faster progression to ESRD, healthcare providers can offer targeted treatments to delay disease progression, reduce complications, and improve patient outcomes (Wang, et al. 2022).

To our knowledge, this is the first study that evaluates the association of *ACE* gene *I/D* polymorphisms with the progression to ESRD in Palestine. Our results revealed that the frequency of *D* allele was the highest 63% and *DI* genotype was also found to be highest in all subjects with a frequency of 58.1%. Whereas , the *DD* genotype was present in approximately 34% of all subjects with slightly more frequent in ESRD patients (34.5%) than in controls (33.3%). Moreover, the *DI* genotype is significantly more common in ESRD patients (63.6%) compared to controls (52.4%). Similar results were reported from a study done in Egypt showed a higher frequency of the *D* allele in those with DN compared to diabetics without renal complications (El Sayed, et al. 2023) , (El-Baz, et al. 2018). Compared to study on an Iranian population showed a high frequency of *D* allele in DN patients and suggesting this polymorphism as a genetic marker for nephropathy risk (Golmohamadi, et al. 2006). While our study found that the DD genotype was slightly more common in ESRD patients than in controls with a significant association. So both studies observed a high frequency of the D allele, suggesting a similar risk factor in Middle Eastern populations. In British and German populations, similar frequencies have been reported, with the *D* allele being common, suggesting that European populations generally have a higher risk of developing cardiovascular and renal diseases associated with the *DD* genotype (Brkovic, et al. 2019). Middle Eastern populations often show a relatively high prevalence of the *D* allele. In an Iraqi study, Al-Radeef, et al (2019) a similar proportion of the *DD* genotype (32-34%) and a low frequency of the II genotype (~12-15%) among ESRD patients, consistent with our findings (*DD* = 34.5%, II = 1.8% in ESRD patients). They also observed that the *II* genotype appeared to offer a protective effect, correlating with

lower susceptibility to ESRD. Our study similarly found that the *II* genotype was less frequent in ESRD patients, indicating possible protective effects. On the other hand, the study by Trevisano et al. (2024) analyzed the frequency of the *ACE I/D* polymorphism in South American populations through a systematic review and meta-analysis. It found considerable variability in *ACE I/D* allele distribution across the region, with unique population-specific frequencies. The results indicated that the *D* allele frequency could be linked to higher risks of cardiovascular and renal diseases in some South American subgroups. While our study, focusing on Palestinian ESRD patients highlights the need for regional genetic studies, as shown by the high *D* allele frequency and the significant association between the *DI* genotype and ESRD progression.

4.1.1 Association between *ACE* gene *I/D* polymorphism and the progression to ESRD

In this study, statistical analysis adjusted for age, gender and BMI showed association between *ACE* genotypes and renal outcomes, The findings revealed that the *DD* genotype is slightly more frequent in ESRD patients (34.5%) than in controls (33.3%), though this difference is not statistically significant. Whereas, the *DI* genotype is significantly more common in ESRD patients (63.6%) compared to controls (52.4%), with a *p*-value of 0.003, indicating a significant association between the *DI* genotype and the progression of CKD to ESRD. The *II* genotype, on the other hand, is much less frequent in ESRD patients (1.8%) compared to controls (14.3%), with a *p*-value of 0.004, suggesting that individuals with the *II* genotype are less likely to develop ESRD. At the level of allele frequency, the *D* allele is more frequent in ESRD patients (66.4) compared to controls (59.5), while the *I* allele is slightly more common in controls (33.6% vs. 40.5%). Both allele frequencies show statistical significance with a *p*-value of 0.004, indicating that the *D* allele may contribute to the progression of kidney disease, while the *I* allele may have a protective effect. The study findings confirms the previously reported association between the *DD* genotype of the *ACE* gene and the progression to ESRD.

4.1.2 Association between *ACE* Genotypes and clinical parameters among ESRD patients and controls subjects

In our study, significant differences were observed in systolic blood pressure, with ESRD patients exhibiting higher SBP, especially those with the *DD* genotype (163.7 ± 17.8 mmHg) compared to controls (126.1 ± 21.1 mmHg). There was no significant difference in diastolic blood pressure (DBP) or creatinine levels between genotypes (Table 3.4). Elevated SBP and creatinine are strong indicators of deteriorating kidney function, as shown by numerous studies. This aligns with the hypothesis that the *DD* genotype leads to increased angiotensin *II* production, contributing to higher blood pressure and lead to kidney damage. The study findings corroborate those of Ahmad et al. (2022) which found that the *DD* genotype was linked to elevated SBP in ESRD patients compared to controls. This results were also observed in studies from El Sayed, et al. (2023), where ESRD patients with the *DD* genotype had worse renal function compared to other genotypes, including higher creatinine levels and faster progression to ESRD. El-Baz, et al. (2018) Observed that *DD* genotype carriers had a faster decline in kidney function in diabetic nephropathy patients.

4.1.3 Association between ACE Genotypes and dialysis duration among ESRD patients

In our study, the mean age at first dialysis for ESRD patients with the *DD* genotype was 46.3 years, *DI* was 46.9 years, and *II* was 54.2 years, with no statistically significant difference (*p*-value = 0.126). The mean of duration of dialysis was longest for *DD* (4.3 years), shorter for *DI* (4 years), and shortest for *II* genotype (3.1 years) (Table 3.5).

Similar results were observed in Park, et al. (2005), where *DD* genotype patients started dialysis earlier and had longer durations of dialysis compared to other genotypes. Conversely Al-Jebouri, et al. (2014) found that *DD* carriers had earlier onset of ESRD and required dialysis sooner, especially in diabetic patients. Our findings, shows relatively consistent results but with non-significant differences between genotypes. This could indicate that genotypes might influence the timing of dialysis initiation, together with other confounding factors such as health conditions (e.g., hypertension, diabetes), medication use, and lifestyle (diet and physical activity) which may be influence renal outcomes.

Our study is limited by its single-region sample, as it only includes patients from Beit Jala Hospital. This regional focus may restrict the generalizability of findings across other Palestinian populations or broader demographic groups. The relatively small sample size may also limit the statistical power to detect associations, especially in subgroup analyses. Additionally, potential confounding variables, such as lifestyle factors, were not fully addressed, which may impact the study's conclusions regarding the relationship between *ACE* polymorphism and ESRD progression.

4.2 Conclusions

The genotype *DI* was dominant genotype in the Palestinian population. There was a significant association between the *DD* genotype and increased risk of ESRD. Patients with the *DD* genotype showed higher systolic blood pressure and earlier onset of dialysis, although the differences in dialysis duration were not statistically significant. This supports global findings that genetic predisposition, particularly related to the ACE gene, plays a critical role in renal disease progression, especially in populations with prevalent risk factors such as hypertension and diabetes.

4.3 Recommendations

1. Future research should focus on larger, multi-center studies that explore the impact of *ACE* gene polymorphisms on ESRD progression in diverse populations, particularly in those with varying genetic backgrounds.
2. Investigating gene-environment interactions, such as the influence of lifestyle factors, medication, would provide deeper insights into ESRD risk. Additionally, exploring the potential for personalized medicine, where treatments are tailored based on genetic predispositions, could lead to more effective interventions in delaying CKD progression to ESRD.
3. longitudinal studies assessing the long-term impact of *ACE* polymorphisms on patient outcomes, including dialysis duration and survival rates, would offer valuable data for developing targeted therapies.

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Appendices

Appendix 1: Study questionnaire

Al-Quds University

Faculty of Graduate Studies
Biochemistry and Molecular biology

Study Questionnaire

About

"Association of Angiotensin-Converting Enzyme Gene Polymorphism with Renal Disease Progression to End-Stage Renal Disease in Dialyzed Palestinian Patients"

Researcher's name: Marwa Mahmoud Salahat.

Supervisor's name: Omar Hamarshah

جامعة القدس

كلية الدراسات العليا

نموذج موافقة

عزيزي/عزيزتي/المريض/ة

تحية طيبة وبعد:

ارجو التكرم بالإجابة على اسئلة الاستبيان الذي تم اعداده لجمع المعلومات اللازمة لدراسة احتمالية وجود علاقة بين الطراز الجيني لجين الإنزيم المحول للأنجيوتنسين مع تطور مرض الكلى إلى المرحلة النهائية لدى مرضى غسيل الكلى الفلسطينيين .

يتم هذا البحث بالتنسيق مع وزارة الصحة الفلسطينية وجامعة القدس كمتطلب تخرج لإنهاء درجة الماجستير برنامج الكيمياء الحيوية والاحياء الجزيئية- كلية الطب.

إن تعاونكم وموافقتكم على المشاركة والاجابة على اسئلة الاستبيان سيكون له اهمية بالغة لإنجاز هذا البحث علماً بأن المعلومات الخاصة بكم وبنتائج البحث ستكون في سرية تامة.

شاكرين تعاونكم

- **تعبئة هذه الاستمارة تعني قبول المشاركة في هذا البحث**

لقد قرأت التوضيح بخصوص الدراسة . و أعطيت الفرصة لمناقشة اسالتي و استفساراتي و قد تمت الإجابة عليها جميعا . وبناء عليه فأنني أوافق على أن أشترك في هذه الدراسة و كما أدرك أن اشتراكي في هذه الدراسة هو إرادي و لي الحق في الانسحاب من الدراسة متى أردت.

الاسم المشترك .....

التاريخ .....

التوقيع .....

الباحثة: مروة محمود الصلاحات

جامعة القدس- كلية الدراسات العليا

شاكرين لكم مشاركتم في هذه الدراسة

A questionnaire about the "Association of Angiotensin-Converting Enzyme Gene Polymorphism with the Progression of End-Stage Renal Disease in Dialysis Palestinian Patients"

This study was designed to determine the ACE gene polymorphism that associated with renal disease progression to ESRD in dialysis Palestinian patients, with the knowledge that this information will be used for the purposes of scientific research and will be treated strictly confidential, so your cooperation with us in completing this questionnaire will be appreciated.

Serial number: _____ Date: _____

Mobile number: _____ Place of residence: _____

Dear participant :

Please tick ✓ inside the ☐ in sections 1,2,3,4 and 5, which is consistent with your situation:

Section 1: Patient Demographics

1.Gender : ☐ Male. ☐ Female.

2.Age: ☐ Below 18 years old. ☐ 18-30 years old.

☐ 30-45 years old. ☐ 46 years or older.

3.Place of residence: ☐ Rural. ☐ Urban.

4.Occupation: _____

5.Height: _____

6.Wieght: _____

Section 2: Medical History

1. Diagnosis of Renal Disease:

☐ Chronic Kidney Disease (CKD) ☐ Diabetic Nephropathy

☐ Hypertensive Nephropathy ☐ Glomerulonephritis

☐ Other (please specify) _____

2. Duration of Renal Disease (in years): _____

3. History of Hypertension: ☐ Yes ☐ No

(If the answer is yes) Please answer this: The date of hypertension onset

_____

4. The date of first diagnosis with CKD: _____

5. The date of first dialysis: _____

6. History of Diabetes: : ☐ Yes ☐ No

7. Family History of Renal Disease: : ☐ Yes ☐ No

Section 3: Treatment and Dialysis Details

1. Type of Dialysis: ☐ Hemodialysis ☐ Peritoneal Dialysis

2. Duration of Dialysis (in years): _____

3. Frequency of Dialysis Sessions per Week: _____

6. Cardiovascular Events:

☐ Myocardial Infarction ☐ Stroke

☐ Heart Failure ☐ Other (please specify) _____

Section 4: Medication History

1. Angiotensin-Converting Enzyme Inhibitors (ACEIs): ☐ Yes ☐ No

2. Angiotensin Receptor Blockers (ARBs):): ☐ Yes ☐ No

3. Other Antihypertensive Medications (please specify):_____

4. Metformin ☐ Yes ☐ No.

5. Glimepiride ☐ Yes ☐ No.

6. Insulin ☐ Yes ☐ No.

7. Antilipidemic medication ☐ Yes ☐ No.

8. Others (please specify) _____

Section 5: Lifestyle Factors

1. Smoking Status:

   ☐ Current Smoker

   ☐ Former Smoker

   ☐ Never Smoked

2. Alcohol Consumption: ☐ Yes ☐ No

3. Physical Activity Level (hours per week): _____

Additional Comments

Please use this space to provide any additional information or comments related to the study.

_____

Thank you for your cooperation

Section 6: Clinical Parameters and Laboratory Investigations

**This Section is filled in by the researcher

1. Blood Pressure (mmHg):

   - Systolic: _____
   - Diastolic: _____

2. Angiotensin-Converting Enzyme (ACE) Gene Polymorphism Analysis:

ACE I/D genotype (Insertion/Deletion)

☐ II ☐ ID ☐ DD

**(Data collected from patient's medical record)

1. Serum Creatinine Level (mg/dL): _____

2. Estimated Glomerular Filtration Rate (eGFR) (mL/min/1.73 m^2):

_____

3. Serum Potassium Level (mq/L): _____

4. Serum Calcium Level (mg/dL): _____

5. Serum Phosphate Level (mg/dL): _____

6. Serum Albumin Level (g/dL): _____

7. Hemoglobin Level (g/dL): _____

8. Serum Parathyroid Hormone (PTH) Level (pg/mL): _____

Appendix 2: Research approval by research ethics committee at Al-Quds University.

Al-Quds University
Faculty of Medicine
Abu-Dies, Jerusalem

جامعة القدس
كلية الطب
أبو ديس – القدس

Research Ethics subcommittee of Faculty of medicine

Letter of Ethical approval

Date:12/5/2024

Ref#: Re3-13-24

Dear Applicants: Prof. Omar Hamarsheh and Miss. Marwa Salem

Biochemistry and Molecular Biology master program

The Research Ethics subcommittee of faculty of medicine has recently reviewed your proposal entitled "Association of Angiotensin-Converting Enzyme Gene Polymorphism with Renal Disease Progression to End-Stage Renal Disease in Dialyzed Palestinian Patients."

Your proposal is deemed to meet the requirements of research ethics subcommittee at Al-Quds University. This approval does not substitute for any administrative or other approvals that may be necessary.

Note: This letter is valid for 2 years and can be used to apply for the central Al-Quds University research ethics committee if needed

Best of luck,

Dr. Suheir Ereqat

Head of research ethics subcommittee

Biochemistry and Molecular Biology master program

Faculty of Medicine-Al-Quds University

Appendix 3: Letter to facilitate the task

State of Palestine
Ministry of Health
Education in Health and Scientific
Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي
والبحث العلمي

Ref.: .....
Date:.....

الرقم: ٢٠٢٤/١٧٩٥/٢٠٢٤
التاريخ: ٢٠٢٤/٨/١٣

الأخ مدير عام الادارة العامة للمستشفيات المحترم،،،

الاخت ق. أ. مدير عام الادارة العامة لتكنولوجيا المعلومات المحترمة،،،

تحية واحترام،

الموضـــوع: تسهيل مهمة بحث

يرجى تسهيل مهمة طالبة الماجستير: مروة محمود صلاحات – كيمياء حيوية واحياء جزيئية / جامعة القدس، وبإشراف أ. د. عمر حمارشه، في عمل بحث بعنوان:

العلاقة بين تعدد أشكال جين الانزيم المحول للأنجيوتنسين ACE وتطور مرض الكلى الى المرحلة النهائية لدى مرضى غسيل الكلى للفلسطينيين

من خلال السماح للطالبة بجمع معلومات عن طريق استبانة ومراجعة ملفات المرضى وأخذ عينة دم لاجراء الفحص، وذلك في:

– مستشفى عالية – مستشفى بيت جالا

على ان يتم الالتزام بأساليب واخلاقيات البحث العلمي، وعدم التعرض للمعلومات التعريفية للمرضى.

على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة الوزارة على نتائج البحث.

مع التحية،

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: عميد كلية الطب المحترم – جامعة القدس

Appendix 4: DNA extraction protocol.

The procedure was done as followed:

1. 600 µL of Red Cell Lysis solution were added to 200 µL blood in a 1.5 mL microcentrifuge tube.
2. Samples' mixtures were incubated at room temperature for 5 minutes and then vortexed for 10-15 seconds. Another incubation at room temperature for 5 minutes was done and followed again by brief vortex mixing.
3. Centrifugation was done at 11,000 x g for 25 seconds.
4. The supernatant was removed, leaving approximately 25 µl of liquid, then vortexed.
5. 300 µl of Tissue and Cell Lysis solution were added to each sample.
6. 1 µl of RNase A were added to each sample.
7. Samples' mixtures were incubated at 37 °C for 30 minutes.
8. Samples then placed on ice for 3-5 min.
9. 175 µl of MPC Protein Precipitation Reagent were added to 300 µl of lysed sample followed with vortex and centrifugation at 4°C for 10 min.
10. The resulted mixture was transferred carefully to a clean microcentrifuge tube and the pellet was discarded.
11. 500 µl of Isopropanol were added to the recovered supernatant. Centrifugation was done at 4°C for 10 min.
12. Carefully the isopropanol were poured off without dislodging the DNA pellet.
13. The samples were rinsed twice with 70% Ethanol, finally resuspended in 35 µl of TE buffer.

علاقة تعدد اشكال جين المحول للانجيوتينسين مع تقدم المرحلة النهائية لأمراض الكلى لدى مرضى غسيل الكلى في فلسطين

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ملخص

تُعتبر المرحلة النهائية من مرض الكلى مشكلة صحية عامة هامة على مستوى العالم. تقدم مرض الكلى المزمن إلى المرحلة النهائية من مرض الكلى الذي يتطلب الغسيل الكلوي، يُمثل تحديًا كبيرًا في الرعاية الصحية. يُعتبر المرحلة النهائية من مرض الكلى هيكلًا ظاهريًا معقدًا من أمراض الكلى المتأثرة بمسببات مختلفة. على الرغم من أن العوامل العرقية والاجتماعية والبيئية تلعب دورًا في تطور المرض، فإن السبب يعود في المقام الأول إلى العوامل الجينية. أحد هذه الجينات هو جين *ACE* المسؤول عن تحويل الأنجيوتنسين I إلى الأنجيوتنسينII ، وهو مسبب قوي لتضيق الأوعية الدموية. تظهر تعدد أشكال جين *ACE* تأثيرًا كبيرًا على تقدم مرض الكلى الى ماحله النهائية.

أُجريت الدراسة في الفترة من يونيو إلى سبتمبر 2024، حيث تم استقطاب 215 مشاركًا، بما في ذلك 110 مرضى تم تشخيصهم بـمرض الكلى بمراحله الاخيرة ويخضعون للغسيل الكلوي و105 أشخاص أصحاء، من مستشفى بيت جالا. تم جمع البيانات السريرية من السجلات الطبية والاستبيانات، بينما تم أخذ عينات دم للتحليل الجزيئي لدراسة العلاقة بين تعدد أشكال جين *ACE* والنتائج السريرية.

أظهرت نتائجنا أن تكرار الأليل *D* كان الأعلى بنسبة 63%، كما وُجد أن النمط الجيني *DI* كان الأكثر شيوعًا بين جميع المشاركين بنسبة 58.1%. بينما كان النمط الجيني *DD* موجودًا تقريبًا في 34% من جميع المشاركين، مع تكرار طفيف أعلى لدى مرضى الغسيل الكلوي مقارنةً بالمجموعة المقابلة (33.3%). علاوة على ذلك، كان النمط الجيني *DI* أكثر شيوعًا بشكل ملحوظ لدى مرضى الغسيل الكلوي (63.6%) مقارنةً بالاصحاء (52.4%)، مع قيمة *p* تساوي 0.003. لوحظت اختلافات ملحوظة في ضغط الدم الانقباضي، حيث أظهر مرضى الغسيل الكلوي ضغط دم انقباضي أعلى، خاصةً أولئك الذين لديهم النمط الجيني *DD* (163.7 ± 17.8 مقارنةً بالمجموعة المقابلة (126.1 ± 21.1 مم زئبقي). لم تكن هناك اختلافات ذات دلالة إحصائية في ضغط الدم الانبساطي أو مستويات الكرياتينين بين الأنماط الجينية. كان متوسط العمر عند بدء الغسيل الكلوي للمرضى الذين لديهم النمط الجيني *DD* هو 46.3 عامًا، و(*DI*) كان 46.9 عامًا، و (*II*)كان 54.2 عامًا، مع عدم وجود فرق ذات دلالة إحصائية.

في الختام، تختلف العلاقة بين تعدد أشكال جين *ACE* وتقدم مرض الكلى الى مراحله الاخيرة بين مجموعات عرقية مختلفة. أظهرت دراستنا وجود ارتباط ملحوظ بين النمط الجيني *DD*

وزيادة خطر الإصابة بـالمرحلة النهائية من مرض الكلى. يجب إجراء دراسات إضافية على عينة أكبر لتأكيد نتائج الدراسة.