Republic of Iraq

Ministry of Higher Education &

Scientific Research

University of Karbala

College of Medicine

Department of Clinical Biochemistry

Evaluation of the Chymase and Angiotensin ll in the development of Type 2 Diabetic Nephropathy

A Thesis

Submitted to the Council of the College of Medicine/ University of Karbala in Partial Fulfillment of the Requirements for the Degree of Master in Clinical Biochemistry

By

Sattar Gaber Hassan

B.Sc. Chemistry 2001-University of Babylon

Supervised by

Assist. Prof. Dr. Shaymaa Z. Nada

Prof. Dr. Firas Shawqi Algburi

2022 A. D 1443 A. H

بسم الله الرحمن الرحيم

﴿ قَالُواْ سُبۡحَٰنَكَ لَا عِلۡمَ لَنَآ إِلَّا مَا عَلَّمۡتَنَآۖ إِنَّكَ أَنتَ ٱلۡعَلِيمُ ٱلۡحَكِيمُ ﴾

صدق الله العلي العظيم

سورة البقرة الاية(٣٢)

Supervisors' Certification

We certify that this M.Sc. thesis titled: **Evaluation of the Chymase and Angiotensin II in the development of Type 2 Diabetes Mellitus with Diabetic Nephropathy** was prepared under our supervision in laboratories at the Department of Chemistry and Biochemistry - College of Medicine / University of Kerbala as a partial requirement for the Degree of Master in Clinical Chemistry.

Signature
Asst. Prof.
Dr.Shaymaa Z. Nada
College of Medicine
University of Karbala
Date: / /2022

Signature
Prof.
Dr. Firas Shawqi Algburi
College of Science
University of Tikrit
Date: / /2022

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

Signature:
Dr. Atheer Hameed
Head of Chemistry and Biochemistry Department
College of Medicine - University of Kerbala

Date: / /2022

Examination Committee

We certify that we have read this thesis entitled " **Evaluation of Chymase and Angiotensin II in the development of Type 2 diabetic Nephropathy** " and as examining committee examined the student " **Sattar Gaber Hassan** " in its content and in our opinion it is adequate with "Excellent" as a thesis for the degree of master in science of Clinical Biochemistry.

Signature

Prof. Dr. Halla Ghazi Mahmood

College of medicine

University of Baghdad

(Chairman)

Date: / /2022

Signature

Prof. Dr. Narjis Hadi Al-Saadi

College of Science

University of Kerbala

Member

Date: / /2022

Signature

Asst. Prof. Dr. Wafaa Raji Alfatlawi

College of Applied Science

University of Technology

Member

Date: / /2022

Signature

Asst. Prof. Dr.Shaymaa Z. Nada

College of Medicine

University of Karbala

(Member and Supervisor)

Date: / /2022

Signature

Prof. Dr. Firas Shawqi Algburi

College of Science

University of Tikrit

(Member and Supervisor)

Date: / /2022

Signature

Approved for the College Council

Professor Dr. Riad Dhiyad Mahdi Al-Zbaidi

Dean of Medicine College/ University of Karbala

Date: / /2022

Dedication

To…. the soul of the late, my supervisor, ***Dr. Shaymaa Zahraw Nada****, our gratitude from me for the effort, follow-up and advice you have exerted in order to complete this work, which I hoped would be present between us, but the difference between us and her is death. We belong to God and to Him we shall return. I ask God to open up her pure soul and to enter her into heaven and paradise. The highest is the answerer of prayer*

To .....Whom Allah has sent as a light in darkness and messenger to guide us ***Prophet Mohammed (May Allah pray upon him and his family)***

To her..... Who supported me in her lifetime and planted hope inside me and gave me life secret, and the brush which drew to me the way of success and the cloud which I appeal when I was thirsty then it rained love and yearning

My Mother

To him ... Who planned my life way and surrounded me with his warm heart and my wings which hold me and threw me on success beach and faithfulness and love…

My Father

To... supporter, dignity simple, my strength source, my heart parts, my happy home, and ***my*** *soul pulse…*

My brothers, Sisters

To ... Who believe in me and thus gave me the power to continue in all situations ….

MY family

To... those, who water me the science from rich science seas and put me between their hands faithfully and with patience and love…

My Supervisor

Searcher
Sattar gaber

Acknowledgments

Praise is to our Almighty Allah, the Gracious who gave me the power and motivation to perform and present this work.

Appreciation to late Assist Prof. Dr. Shaymaa Z. Nada, and Prof. Dr.Firas Shawqi Algburi my supervisors, for their support guidance, and advice during the study

Special thanks go to Dr. Atheer (head of the biochemistry department in the college of medicine/ university of Karbala) for his help, support and encouragement to me during my work.

I want to thank the staff of the Department of Biochemistry, College of Medicine, Karbala University, particularly, Prof Dr. fadial toamaa, Dr. Rana, Dr. zohier, Dr. Sattar, and Dr. Sheherazade for their kind, support, attention, cooperation, and notification during the work and their help. For their kind, support, attention, cooperation, and notification during the work and their help

Gratitude should also be expressed to the staff of the Chemical Lab. unit in Al- Hussein General Hospital in Karbala City for their help in getting the specimens and measurement of the parameters.

Gratitude should also be expressed to the staff of the Chemical Lab. unit in the Center for Diabetes and Endocrine in Karbala for their help in getting the specimens and measurement of the parameters.

Summary

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

This study aims to investigate the association between chymase and angiotensin ll levels in type 2 diabetic patients with early and chronic diabetic nephropathy, As well as study the effect of gender, body mass index, duration of disease, and age groups on the values of the variables studied with other variables in patients and healthy people and also researching, are there statistically significant differences between the levels of chymase and angiotensinogen and type 2 diabetes patients with early and chronic diabetic nephropathy

A case-control study of individuals with type 2 diabetes was conducted from November 2021 to May 2022. This study was conducted on 62 patients with type 2 diabetes who attended Imam Hussein Medical City in Karbala. and 28 cases in the control group. They were divided into three groups according to the ratio of their urinary albumin to creatinine ratio (ACR), the first group (Normoalbuminuria) where the ACR was less than 30 and their number was 22 patients, and the second group (Microalbuminuria) was the ACR (30-299) and their number was 20 patients and the group the third (Macroalbuminuria), where the ACR was greater or equal to 300, and their number was 20 patients, as well as they, were divided into four groups according to their body mass index, the first group (BMI less than 18) their number was 4, and the second group (BMI 18-24.9) their number 10 and the group The third group (BMI 25-29.9), numbered 21, and the fourth group (BMI greater or equal to 30), numbered 27 patients, and they were also divided into three groups according to age, the first group was age (35-45) and their number was 11, and the second group (age 46-60 And their number is 26, and the third group (age over 60) and their number is 25. They were also divided into three groups according to the duration of the disease, the first group (the duration of the disease is less than 5 years) and their number was 13, and the second group (the duration of the disease is 5-10 years) and their number is 16

and the third group (The duration of the disease is greater than 10 years) and their number was 33, and they were divided by gender into 35 males and 27 females.

The biochemical study included the patient and control group. Measure levels of fasting sugar (FBS), glycated hemoglobin (HbA_{1c}), blood urea, Serum Creatinine, albumin-creatinine ratio (ACR), Microalbuminuria, cholesterol (CHOL), triglyceride (TG), and high-density lipoprotein (HDL) levels were also measured low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), Chymase and AngiotensinII.

This study showed a highly significant difference ($P \leq 0.01$) in fasting sugar (FBS), glycated hemoglobin (HbA1c), blood urea, Serum Creatinine, Microalbuminuria, albumin-creatinine ratio (ACR), cholesterol (CHOL), triglyceride (TG), and high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), High-density lipoprotein (HDL), Chymase and AngiotensinII, Between diabetic patients and the control groups.

This study also showed according to the effect of age in (FBS, blood urea, HbA1c, CHOL, LDL, VLDL, AngiotensinII Microalbuminuria, albumin-creatinine ratio (ACR)) there were statistically significant differences between the first and second group, as well as between the first and third groups. As for (TG, serum creatinine, HDL, and Chymase), there were statistically significant differences between the first and third groups.

This study also showed the effect of body mass index, In (TG, VLDL, and Duration of the disease) there were statistically significant differences between each of the first and fourth groups, the first and third, and the second and fourth, as well as we noticed in the (blood urea, Microalbuminuria, albumin-creatinine ratio (ACR), serum creatinine and Angiotensin II) significant differences between the first and fourth group, and also in (HDL, LDL, FBS, CHOL, HbA1c) there were statistically significant differences between the first and fourth group, and the second and fourth, and in the(Chymase) there were statistically significant differences in the first and fourth group, the second and fourth group, and the third and fourth groups.

This study also showed according to the effect of disease duration, On (HDL, LDL, FBS, CHOL, HbA1c, Microalbuminuria, albumin-creatinine ratio (ACR), TG, and VLDL), there were statistically significant differences between all groups, as well

as we noticed in (blood urea, serum creatinine, AngiotensinII, and Chymase) statistically significant differences between the first and third, and second and third groups.

The study of the Comparison between type 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters showed In(HDL, LDL, FBS, CHOL, HbA1c, Macroalbuminuria, TG, VLDL, blood urea, serum creatinine, and Angiotensin II), there were statistically significant differences between all groups, as well as in the Chymase it was statistically significant differences between the first and third and second and third groups.

The results showed that there was a significant positive correlation between the blood levels of HbA1c, FBS, blood urea, serum creatinine, Microalbuminuria, CHOL, TG, LDL, VLDL Opposite Chymase, and ANGII levels of the patients, The study also showed a negative linear regression between Chymase and ANGII with HDL.

List of contents

List of Figures

List of Tables

List of abbreviation

Abbreviation	*Description*
4-AA	4 – Aminoantipyrine
AA	amino acids
ACE	angiotensin-converting-enzyme
ACEI	angiotensin-converting enzyme inhibitors
ACR	albumin/creatinine ratio
ANG I	Angiotensin I
ANG II	Angiotensin II
AT1R	angiotensin II type 1 receptor
AT2R	angiotensin II type 2 receptor
CKD	Chronic kidney disease
CMA1	Chymase 1
CPA	carboxypeptidase A
CTGF	connective tissue growth factor
CTMC	connective tissue mast cell
DAG	diacylglycerol
DCCT	Diabetes Control and Complications Trial
DM	Diabetes Mellitus
DNP	Diabetic Nephropathy
DPPI	Dipeptidyl Peptidase I
ECM	extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ESRD	End-stage renal disease
FPG	fasting plasma glucose
GDM	Gestational Diabetes Mellitus
GFR	glomerular filtration rate
GPCR	G-protein-coupled receptor

HbA_1c	hemoglobin A_1c
HDAOS	N-(2-hydroxy-3-sulfopropyl)-3,5 dimethoxyaniline
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein Cholesterol
HLA	human leukocyte antigen
IDDM	insulin-dependent diabetes mellitus
IP3	inositol triphosphate
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
MC	Mast Cell
MCP-1	monocyte chemoattractant protein - 1
mMCP-4	mast cell protease 4
MMP-9	metalloprotease Pro
MMPs	matrix metalloproteases
NGSP	National Glycohemoglobin Standardization Program
NIDM	non-insulin-dependent diabetes mellitus
NO	nitric oxide
OGTT	Oral glucose tolerance test
PEG	Poly Ethylene Glycol
RAS	Renin-Angiotensin system
ROS	oxygen species
SD	standard deviation
SMC	Smooth Muscle Cell
T1DM	Type1 Diabetes Mellitus
T2DM	Type2 Diabetes Mellitus
TG	Triglyceride
TGF	Transforming growth factor

TGF-B	transforming growth factor-B
TNFα	Tumor necrosis factor α
UAE	urinary albumin excretion
VEGF	Vascular endothelial growth factor
VEGF-A	vascular endothelial growth factor-A
VLDL	Very low-density lipoprotein
VSMC	vascular smooth muscle cells
WAT	white adipose tissue
WHO	World Health Organization

Chapter One Introduction and Literature Revie

1. Introduction

Diabetes mellitus(DM) is a metabolic disease with the mean characteristic is hyperglycemia resulting from defects in insulin action, insulin secretion, or insulin resistance, it is always jointed with long-term harm, dysfunction, and failure of organs, particularly the heart, nerves, eyes, kidneys, and blood vessels (American Diabetes, 2011) Diabetes mellitus is a chronic metabolic disease with a world prevalence of 8.4% (Cho *et al*., 2018). The prevalence of DM in Iraq is very high and approaching 20% which is comparable to that in some other Middle Eastern countries (Mansour *et al*., 2014) In 2017, the mortality rate due to diabetes reached 10.7% in patients (20-79years) Around 1.4 million of Iraqis have diabetes reported T2DM prevalence in Iraq ranges from 8.5% to 13.9% (Abusaib *et al*., 2020).

Uncontrolled hyperglycemia is associated with the development of DM microvascular (neuropathy, retinopathy, and nephropathy) and macrovascular (stroke, ischemic heart disease, and peripheral vascular disease) complications (Mikhael *et al*., 2021).

Type 2 Diabetes Mellitus: accounts for 90- 95 % of diabetes mellitus also previously referred to as non-insulin-dependent diabetes or adult-onset diabetes. Mostly present in adulthood, although it can be diagnosed in teens and young adults due to the high prevalence of obesity(Olokoba *et al*., 2012)

Diabetic nephropathy accounts for 40 % of new cases of end-stage renal disease (Molitch et al., 2004) Is an increment in protein excretion in urine with Microalbuminuria considered as an early stage of DNP in which a small increase in urinary albumin excretion (UAE) also called incipient DNP. As the disease advanced with the presence of Macroalbuminuria with overt DNP(Edelman *et al*., 2004).

Mast cells (MCs), fibroblasts, and vascular endothelial cells are the main sources of Chymase. Inflammatory signals, tissue damage, and cellular stress cause MC Chymase to be released into the extracellular. Chymase is a significant extravascular generator of angiotensin II (ANG II) (Ahmad and Ferrario, 2018) Inflammation and fibrosis are typical symptoms of CKD, regardless of the cause. Excessive innate and adaptive immune responses, as

well as infiltration of inflammatory cells and the production of cytokines, characterize inflammation. Extracellular matrix (ECM) proteins eventually replace normal tissue architecture in fibrosis, preventing the normal functioning of specialized kidney cells such as tubular epithelial cells, podocyte, and mesangial cells in the glomeruli, and vascular endothelial Cells (Owens *et al*., 2019)

Renal fibrosis is characterized by tubulointerstitial fibrosis and glomerulosclerosis and is frequent in chronic kidney disease (CKD) that progresses to end-stage renal disease (ESRD). Renal fibro genesis involves the activation of inflammatory cells inside the glomerulus or renal interstitium, causing them to generate fibrogenic and inflammatory cytokines, promote cellular phenotypic change, and create extracellular matrix components (Liu, 2006). Renal damage that lasts for a long time results in a fibrous scar and kidney failure. In renal fibrosis, TGF- is an established cytokine. (Schnaper *et al*., 2003) Extracellular matrix (ECM), which is largely made up of collagen, accumulates in the kidneys, causing fibrosis. MMPs are proteins that break down collagen and ECM proteins and are generated in a variety of organs, including the kidney. MMPs are thought to have both anti- and pro-fibrotic properties. The majority of MMPs are secreted as pro-MMPs that must be cleaved to be activated (Visse and Nagase, 2003).

Literature Review

1.1. Diabetes Mellitus

1.1.1. Definition:

The term "diabetes mellitus" refers to often used to refer to a type of metabolic illness characterized by chronic hyperglycemia as its primary symptom. The reason is either a disturbance in insulin secretion or a disturbance in insulin effect or both in most cases (van Netten *et al*., 2020) it has been shown that diabetes' characteristic hyperglycemia may cause long-term damage to and dysfunction of some systems. These organs include the eyes, the heart, the kidneys, the nerves, and the blood vessels (Xue *et al*., 2020). Worldwide, diabetes is growing more common. Disease results in serious consequences such as cardiovascular and chronic renal disease, which significantly increase the chance of mortality over time (Tatsumi and Ohkubo, 2017)

1.1.2. Classification of DM

The Expert Committee on Diabetes of the World Health Organization (WHO) has created numerous classification systems for diabetes mellitus. Co-operation between the (WHO) and the National Diabetes Data Group led to the present WHO categorization system (USA). It used to be common practice to use the names the phrases "insulin-dependent diabetes mellitus" (IDDM) and "non-insulin-dependent diabetes mellitus" (NIDM) are used to describe two kinds of diabetes. The names "Type 1" and "Type 2" diabetes are now utilized. Type 2 Diabetes mellitus is the most common kind (Petersmann *et al*., 2019).

This classification is dependent on American Diabetes Association, 2020 (care, 2020)

1.1.2.1. Type 1 Diabetes mellitus

Insulin deficiency is usually caused by:

A - Immune-Mediated Diabetes

An auto-immune disorder, type1 is characterized by insulin insufficiency and hyperglycemia as the outcome (DiMeglio *et al*., 2018)

Autoimmune damage of pancreatic beta cells, where the immune system produces large amounts of antibodies as a consequence of some immunological diseases, and beta cells in the pancreas are among the body's most vulnerable cells to stress. Oxidative stress, as a result of its insufficient antioxidant levels, As a result, antioxidants might be directly damaged by these antibodies and free radicals (Katsarou *et al.*, 2017) could be accompanied by ketone bodies and their high quantities in patients' blood and urine. 90% of Type 1 diabetics have this(Ranjan et al., 2017).

B – Idiopathic Diabetes

Type 1 diabetes can have many different causes, some of which are unknown. This makes them difficult to diagnose. The patients in this group have persistent insulinopenia and are at risk of ketoacidosis, but there is no indication of autoimmunity in any of the individuals. Individuals suffering from this kind of diabetes have bouts of ketoacidosis that are accompanied by between episodes, and varying degrees of insulin deficiency. This kind of diabetes is highly hereditary, is not linked to any HLA genes or antigens, and does not reveal immunological evidence of -cell autoimmunity (Catarino *et al*., 2020).

1.1.2.2. Type 2 Diabetes mellitus

Diabetes that is not insulin-dependent and adult-onset diabetes were previously included in this category of diabetes mellitus. It is a word that refers to those who have a relative (as opposed to absolute) insulin insufficiency. Individuals with this kind of diabetes commonly develop resistance to insulin activity (Kumar *et al*., 2020). Often, these people do not need insulin medication to survive throughout their lives. Because hyperglycemia is typically not high enough to generate apparent symptoms of diabetes many people with type 2 diabetes go years without receiving treatment. Despite this, individuals with this type of diabetes are at an increased chance of developing macrovascular and microvascular diseases (Climie *et al*., 2019). Obesity is common in adults with this kind of diabetes, and obesity may cause or

worsen insulin resistance in certain diabetics. Many people who may not meet the usual definition of obesity based on body weight may have a higher amount of body fat that is concentrated mostly in the abdominal area (Rutter, 2018). Insulin levels may look normal or increased in diabetic individuals; however, if these diabetic individuals' beta-cell function was normal, their elevated blood glucose levels should result in even greater insulin levels (Wang *et al*., 2018b). This results in inefficient and insufficient insulin production to compensate for insulin resistance. However, some people have normal insulin action but significantly reduced insulin production. Weight loss, greater physical activity, and/or pharmaceutical therapy of hyperglycemia may all improve insulin sensitivity, but it never returns to baseline (Jung *et al*., 2018)

1.1.2.3. Other Special Types of Diabetes

A - β-Cell Genetic Defects

Diabetic symptoms may be caused by a single genetic abnormality in -β-cell function. Hyperglycemia can be a symptom of many different types of diabetes at a young age (generally before age 25 years). Mature-onset diabetes of the young (MODY) occurs when insulin production is restricted and insulin resistance is mild or absent (Yahaya and Ufuoma, 2020).

B - Insulin Action Genetic Defects

There are a few rare types of diabetes that are caused by genetically set insulin function defects, These are referred to as atypical diabetes. The metabolic issues caused by insulin receptor mutations might range from hyperinsulinemia to moderate hyperglycemia to severe diabetes. Women who have been virilized may experience enlarged, cystic ovaries (Moghetti and Tosi, 2021).

C- Exocrine Pancreas Diseases

Diabetes can be caused by any procedure that causes the pancreas to be injured diffusely. Acquired processes in the pancreas include pancreatitis, infection, trauma, pancreatic cancer, and pancreatectomy(Cervantes *et al*., 2019).

D – Endocrinopathies

Insulin and many other hormones (such as growth hormone, glucagon, adrenalin and cortisol) compete with one other. Diabetes can be caused by high levels of these hormones. People who have had past insulin production irregularities are more likely to suffer from hyperglycemia (Mancuso, 2021).

E - Diabetes Caused By Drugs or Chemicals

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance, Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells(Masoudi, 2021). Many drugs and hormones can impair insulin action, Examples include nicotinic acid and glucocorticoids (Dodia and Sahoo, 2021).

F- Infections

Certain viruses have been linked to apoptosis (cell death), and congenital rubella patients acquire diabetes even though the vast majority of them have HLA and immunological markers associated with type 1 diabetes. (Mishto *et al*., 2021)

G- Immune-Mediated Diabetes in Uncommon Forms

An autoimmune disorder of the central nervous system known as stiff-person syndrome causes muscles to become excruciatingly spasmodic. (Lee *et al*., 2020)

H - Other Genetic Syndromes Linked To Diabetes

Diabetes mellitus is associated with several hereditary disorders. These include Klinefelter's syndrome is defined as the presence of one or more extra “X” chromosomes in a male patient (Deebel *et al*., 2020), Turner's syndrome is a sex chromosome disorder characterized by partial or complete loss of an X chromosome (Fuchs *et al*., 2019), and Down's syndrome occurs when a person has three, rather than two copies of chromosome 21 (Alldred *et al*., 2017). It's an autosomal recessive condition with symptoms that include insulin-deficient diabetes and the absence of postmortem β -cells (Shi *et al*., 2021).

1.1.2.4. Gestational Diabetes

Gestational Diabetes Mellitus (GDM) is one of the most metabolic disorders, common during pregnancy and affects between (2-5%) of pregnant women, and its causes are similar to type 2 diabetes There is a relationship between pregnancy and an increase in resistance of cells to insulin, and the glucose level often returns In the blood, it returns to its normal levels after birth, and the newborn has a greater weight than normal (Rosik *et al*., 2020).

1.1.3. Diagnosis of Diabetes Mellitus

Diabetes Mellitus is diagnosed and classified as follows (Petersmann *et al*., 2019):

1. HbA1C value of ≥ 6.5 percent. The examination needs to be conducted in the laboratory with a National Glycohemoglobin Standardization Program (NGSP) a certified technique that is To the Diabetes Control and Complications Trial (DCCT) test standard (Serdar *et al*., 2020).
2. The concentration of fasting plasma glucose (FPG) is ≥ 126 mg/dl (7.0 mmol/ liter). The definition of fasting is at least eight hours of absence from eating (Tian *et al*., 2020).
3. The level of blood glucose must be ≥ 200 milligrams per deciliter (11.1 milli moles per liter) after two hours When an OGTT test is completed According to WHO criteria, a glucose load of 75 grams of anhydrous glucose dissolved (Molina-Vega *et al*., 2021)
4. It was a patient with typical hyperglycemic symptoms Individuals, at random, had amounts of glucose in the blood of ≥ 200 mg/dl (11.1 mmol/ liter) (Franklin *et al*., 2020).

1.1.4. Complications of Diabetes Mellitus

Patients with diabetes who have long-term vascular issues are at higher risk of morbidity and mortality than those who do not (Andrén *et al*., 2018) chronic hyperglycemia may lead to a reduction in blood flow to several parts of the body, including the retina and renal glomeruli, as well as microvessels that nourish peripheral and autonomic nerve fibers (causing diabetic neuropathy). The macro vasculature, in particular the brain, has been hypothesized to go through a similar process, speeding up atherosclerosis. (Iba *et al*., 2019) High levels of glucose in the

blood can affect almost every organ in the body The complications that occur in diabetic patients can be classified into two types of complications: acute Chronic complications, and chronic complications (Pih *et al*., 2018)

1.1.4.1. Acute Complications

Symptoms of acute complications appear suddenly and are relatively severe, and the patient returns to his condition The usual treatment when the patient receives the appropriate treatment and may develop into chronic complications and if not treated, it may lead to the loss of life These complications are : (Hulkoti *et al*., 2021)

A - Hypoglycemia

Low blood sugar below the normal level is one of the most serious complications of diabetes the most common of which is caused by taking an excessive dose of insulin, or it may be the result of taking insulin without eating food, and the patient feels, Dizziness, Sweating, Headache The case may arrive Loss of consciousness, coma, and sudden death may occur (Yale *et al*., 2018)

B - Diabetic Ketoacidosis

This condition occurs as a result of a loss of insulin, which helps glucose enter the bloodstream cell for energy production, and as a result of this loss the body depends on fats and proteins present, Therefore, the concentration of lipid metabolites increases in the blood (de Moraes and Surani, 2019), which is the so-called, Ketone bodies leading to the stimulation of Gluconeogenesis in the liver, and thus to the occurrence of Hyperglycemia, glycosuria, and an imbalance in the acidity function of the blood (acid/base balance) (Metabolic acidosis) (Jung *et al*., 2019)

1.1.4.2. Chronic Complications

Chronic complications occur gradually, and are it continue with the patient for life and occur as a result of the continuous rise in the level of glucose in the blood they include:

A - Retinopathy

Retinopathy is one of the most common complications of diabetes It affects more than 80% of diabetic patients, as it results from damage to the retinal microvasculature, or may result from the clouding of the lens of the eye (Opacification) which is called a cataract and this condition is related to age, As black water (Glaucoma) is defined as the loss of vision due to high eye pressure(Praidou et al., 2017)

B - Diabetic Neuropathy

The incidence of neuropathy is between 60% - 70% of patients with diabetes, where occurs Inflammation and degeneration in these nerves, as a result of elevated Blood glucose levels and decreased blood flow in the capillary blood vessels (Mayeda *et al*., 2020)

1.2 .Diabetic Nephropathy (DNP)

An increase in protein excretion in the urine associated with Microalbuminuria is considered an early stage of DNP, in which a slight rise in urinary albumin excretion (UAE) is referred to as incipient DNP As the illness progressed, Macroalbuminuria with overt DNP became more common (Alzahrani *et al*., 2019) Diabetes most important End_ stage renal disease (ESRD) is the leading cause of kidney failure in the US and Europe (Kumar *et al*., 2020) These are attributed due to:

• Increased prevalence of diabetes mellitus, particularly type 2 diabetes

• Increase the lifespan of patients with diabetes

Diabetic nephropathy is responsible for 40% of new instances of end _stage renal disease in the US. (Wang *et al*., 2018a) As of end_ stage renal disease(ESRD) which means Renal failure that requires dialysis or transplantation is an expensive condition and disabling condition with a high monthly rate (Kirchhoff, 2018) Nephropathy may occur in 20-30% of diabetics, whether they have type 1 or type 2 but with an increasing prevalence of type 2 diabetes constitutes Dialysis patients with diabetes make up more than half of all dialysis patients. (Zhang *et al*., 2020) Several studies have shown that numerous therapies may significantly alter the incidence and course of diabetic nephropathy if implemented at an early stage of the disease's development.

(Selvarajah *et al*., 2019) Diabetic nephropathy is related to increased cardiovascular mortality and considers the chief cause of chronic renal disease in patients (Warren et al., 2019).

1.2.1. Stages of Diabetic Nephropathy

Diabetic nephropathy is classified according to UAE value into 3 stages, depending on cutoff values adapted by American Diabetes Association As shown in Table 1-1 (Zelmanovitz *et al*., 2009).

Table 1.1 Stages of diabetic nephropathy based on urinary albumin excretion(Zelmanovitz et al., 2009).

Stage	Urine with marked time (µg/min)	24- hour urine (mg/24 h)	Random urine sample	
			Albumin concentration (mg/l)	Albumin/creatinine ratio (mg/g)
Normoalbuminuria	**< 20**	**< 30**	**< 17**	**< 30**
Microalbuminuria	**20-199**	**30-299**	**17-173**	**30-299**
Macroalbuminuria	**≥ 200**	**≥ 300**	**≥ 174**	**≥ 300**

Although the development of Microalbuminuria is considered a risk factor for the development of Macroalbuminuria, it had been found that not all patients underwent progression to that stage, and some patients may be returned to Normoalbuminuria(Moriya *et al*., 2019) Recent studies had shown that 30 – 45 % of Microalbuminuria that had been followed for 10 years will progress to overt proteinuria(Caramori *et al*., 2000) In addition to progression factor for overt proteinuria, Microalbuminuria reflect a condition of generalized endothelial dysfunction and consider as a factor associated with an increased risk of cardiovascular and mortality (Provenzano *et al*., 2019).

1.2.2. Diabetic Nephropathy Screening and Diagnosis

The first stage of screening for DNP is to determine albumin content in fresh urine samples, which may be reported as albumin level (mg/l) or albumin/creatinine ratio(ACR)(Yan *et al*., 2021) Diabetic Nephropathy screening should be done at the time of diagnosis of DM with Type 2. Since some patients had the silent form of DM in time earlier periods. In type 1 diabetes mellitus screening should be performed after 5 years of onset of disease or earlier in poorly controlled patients and repeated annually (Samsu, 2021) Although Microalbuminuria is an essential compartment in the diagnosis of DNP, deterioration of glomerular filtration rate (GFR) might be present with normal UAE. Based on these facts national kidney foundation's categorization may be used to stage these individuals' chronic kidney disease (Table 1-2) GFR is calculated using serum creatinine levels, and the following variables: age, gender, weight, and race(Association, 2009).

Table 1.2 Chronic Kidney Disease Stage (Zelmanovitz et al., 2009)

Stage	Description	GFR(ml\min\1.73m2)
1	**Renal damage with GFR N or over**	**≥90**
2	**Renal damage with GFR slightly**	**60—89**
3	**GFR moderately**	**30—59**
4	**GFR severely**	**15—29**
5	**End stage chronic renal failure**	**<15 or dialysis**

1.2.3. General Risk Factors For Diabetic Nephropathy

1.2.3.1. Race

DNP is more prevalent and severe among blacks, Mexican Americans, Pima Indians, and Hispanics than in Caucasians (Gheith *et al*., 2016) Even after correcting for confounding variables such as poorer socioeconomic position and a higher prevalence of hypertension in blacks, blacks continue to have a 4.8 fold increase risk of ESRD compared to Caucasians(Cañadas-Garre *et al*., 2019).

1.2.3.2. Genetic Predisposition

The fact is that a diabetic sibling of a patient with diabetes mellitus has a threefold increased risk of developing nephropathy than a diabetic sibling of a diabetic without nephropathy suggests a genetic predisposition to DNP. According to Seaquist et al., 83 percent of type-2 diabetic siblings of probands with DNP had signs of renal illness, but only 17 percent of siblings of probands without nephropathy have the renal disease(Loon, 2003).

1.2.3.3. Elevated Blood Pressure

Diabetes patients have a 1.5–3 folds prevalence of hypertension as non-diabetics. (Homoud *et al*., 2020) In prospective investigations, a connection has been shown between greater systemic pressures and the development of nephropathy, especially if the pressures are in the hypertensive range. (Patel *et al*., 2020) Another finding is that albuminuria is closely associated with an aberrant circadian blood pressure profile and that this abnormal blood pressure profile serves as a predictive of Type 2 diabetes patients are more likely to have renal and cardiovascular problems. (Tankeu *et al*., 2017)

1.2.3.4. Glycemic Control

Patients with poor glycemic control are more prone to develop diabetic nephropathy (DNP) (Fan et al., 2021) This is supported by studies, which showed that Improved glycemic controls might significantly minimize the risk of developing and progressing albuminuria. (Mohsen *et al*., 2021) When blood sugar levels are out of control, diabetic nephropathy is more common. Renal disease is substantially less common in patients with type 1 diabetes whose HbA1c levels are kept below 8.1 percent(Kaneto *et al*., 2020) Randomized clinical studies have validated the predictive usefulness of poor control when compared to excellent control in evaluating the likelihood of nephropathy and retinopathy in patients with diabetes. A study of individuals with type 2 diabetes conducted in the United Kingdom discovered that fewer patients treated with intensive vs. standard treatment had progression of Microalbuminuria (27 % against 39 %) and proteinuria (7 % versus 13 %) (Lancent,1998)

1.2.3.5. Smoking

Smoking increases the incidence of both microvascular and macrovascular disorders in people with diabetes significantly. The use of tobacco products is an independent risk factor for the development of DNP and is related to a rapid loss of renal function, an increased chance of developing an end-stage renal disease, and a reduced chance of survival after starting dialysis (Campagna *et al*., 2019).

1.2.3.6. Male Gender

The development of nephropathy in diabetes has been linked to males in some studies. Researchers showed that men with type-2 diabetes were 2.5 times more likely than women to develop incipient or overt nephropathy (Shahwan *et al*., 2019)

1.2.3.7. Dyslipidemia

In Microalbuminuria individuals with type-2 diabetes, higher cholesterol levels are associated with higher urine albumin excretion, according to many observational studies. These results show that lipids may contribute to the development and progression of glomerular damage (Bacci *et al*., 2021).

1.2.4. Prognosis of Diabetic Nephropathy

Diabetic kidney disease is a major contributor to illness and mortality in diabetics. When detected and treated early, kidney damage may be delayed once increased levels of protein are detected in the urine, and kidney damage will gradually worsen. Frequently, this will necessitate a kidney transplant or dialysis. High blood pressure, heart disease, and eye problems are all common complications for diabetic kidney disease patients. (Campion *et al*., 2017)

1.2.5. Pathophysiological Mechanisms of Diabetic Nephropathy

1.2.5.1. Hemodynamic factor

Diabetic Nephropathy is characterized in its early stage by glomerular hyperfiltration attributed to reducing resistance to both glomerular arterioles efferent (Zhao *et al*., 2017) Endothelin1(ET-1) a vasoconstrictor peptide was associated with urinary albumin excretion (UAE), its level increases progressively with an increment in degree of DNP (Coelho *et al*., 2018) Early disturbance in renal perfusion

autoregulation leads to the leak of albumin from capillaries to glomerulus with a compensatory increase in the mesangial matrix, thickened damage to the glomerular basement membrane, and podocyte, This has been aggravated by the stimulation of mechanisms of inflammatory reaction by tubular cells (Murray and Paolini, 2020). Additionally, renal hyper perfusion-induced mechanical stress results in the production of growth factors (Vascular endothelial growth factor (VEGF), Transforming growth factor (TGF-1)), some cytokines Tumor necrosis factor α (TNFα), triglycerides, and cholesterol. which induce protein accumulation from the extracellular matrix, with subsequent mesangial expansion and glomularsclesosis The progression of DNP and preservation of glomerular morphology can be maintained by reduction of TGF-β1 which block renin-angiotensin-aldosterone systems (Alexandru *et al*., 2017)

1.2.5.2. Hyperglycemia and advanced products of non-enzymatic glycosylation

A strong risk factor for DNP is the presence of persistent hyperglycemia that causes the thickening of the basement membrane and increases the proliferation of mesangial cells and their matrix. It had been found that the expression of a growth factor called (VEGF) in podocytes increases their vascular permeability(Park *et al*., 2019). Hyperglycemia also increases the formation of the advanced glycation end product of protein in different parts through activation of protein kinase C and the aldol reeducates pathway. The final non–enzymatic glycation of glomerular basement membrane protein and collagen makes that barrier more permeable to protein transport with the subsequent rise of UAE (Ma *et al*., 2021).

1.2.5.3. Cytokines

Diabetic Nephropathy characterizes by the production of a series of inflammatory markers such as C reactive protein and interleukin1, 6, 18, and TNF- α which was correlated with albumin secretion and progression to ESRD. In addition, other stimuli such as hyperglycemia, TGF-β1, and ANG II lead to the secretion of (VEGF) causing increased nitric oxide generation in the endothelium, with subsequent vasodilatation and glomerular hyperfiltration (Bahreini *et al*., 2021). Hyperglycemia through oxidative stress increases the production of angiotensin II,

collagen, TGF-β, and fibronectin which leads to progressive glomerulosclerosis (Samadi-Noshahr *et al*., 2021). In an animal model, it had been found that inflammatory markers also being involved in the development of tubulointerstitial lesions and accumulation of macrophages in the tubular interstice. Macrophages through the production of free radicals, inflammatory cytokines, and proteases lead to more tubular damage. With the presence of hyperfiltration and increased UAE, the glomerular and renal cells will be exposed more to more inflammatory markers that intensify the process (Lu *et al*., 2018).

1.3. Mast Cell Proteases

The granule content of mast cell (MC) is thought to have a role in a variety of physiological and pathological functions. MC degranulation may be triggered by a variety of events, including Fc receptor activation in allergic responses (Elieh Ali Komi *et al*., 2020). Once degranulated, MC releases several mediators such as histamine (known role in allergies), proteases, and cytokines. MC granules contain several proteases such as matrix metalloproteases (MMPs) (Pal *et al*., 2020) MC-specific enzymes include Chymase, tryptase, and carboxypeptidase A (MC-CPA). The serine proteases include tryptase and Chymase, whereas MCCPA is a zinc-dependent metalloprotease. The substrate specificities of these proteases vary. Chymase has a Chymotrypsin-like action, whereas Tryptase has a Trypsin-like activity. These MC proteases, particularly the Chymase and tryptase, are thought to be the basis of MC's participation in cardiovascular and metabolic disorders (Sun *et al.,* 2009, Thorpe *et al.,* 2018)

1.3.1. Chymase Synthesis

An enzyme present in mast cells known as Chymase is produced as a preproenzyme. The peptide is guided to the endoplasmic reticulum lumen by a signal on the preproenzyme's N terminus(Pejler *et al*., 2010). This signal peptide is thought to have a length of two amino acids (AA). Cleavage of the preproenzyme yields the proenzyme, which in turn will be cleaved at its N-terminal location to produce the active enzyme, the active enzyme is found in the granules, there are 226 amino acids in the prochymase, after cleavage of the prochymase N-terminal two amino acids, the active Chymase is formed (Huang *et al*., 1991, Serafin *et al*., 1991). The enzyme

responsible for this cleavage is considered to be Dipeptidyl Peptidase I (DPPI) or cathepsin C. Heparin plays a critical function in this cleavage (McEuen *et al*., 1998). When the active Chymase is released from the granules, it is attached to the extracellular matrix (ECM). Endogenous Chymase inhibitors (such as 1-antitrypsin, 2-antichymotrypsin, 2-macroglobulin, and Eglin C) impede the Chymase's action inside the ECM. However, since it is linked to heparin, it becomes immune to endogenous inhibitors and retains its action for many weeks (Lindstedt *et al*., 2001). To present, just one Chymase has been found in humans, the CMA1. Although it is an α-Chymase, its proteolytic activities are comparable to those of the mouse mast cell protease 4 (mMCP-4), a β-Chymase found in murine connective tissue(Andersson *et al*., 2008).

1.3.2. Structural of Chymase

Figure 1.1 shows the basic skeleton structure of the human Chymase which is very different from that of the mouse Chymase, only 50-76 percent identical (Shiota *et al*., 2005). Mature Chymase has a molecular weight of 26000 - 32000 kDa and may be N-glycosylated at one or more sites (Caughey *et al*., 1991). Compared with the majority of serine proteases of another trypsin family, Chymase contains fewer cysteine (seven in the majority of Chymase) and only three disulfide bonds(Zamolodchikova *et al*., 2005) The physical properties, including surface properties affecting solubility and binding to polyanionic proteoglycans, differ strikingly between Chemises, even at the same Mammals (Sali *et al*., 1993).

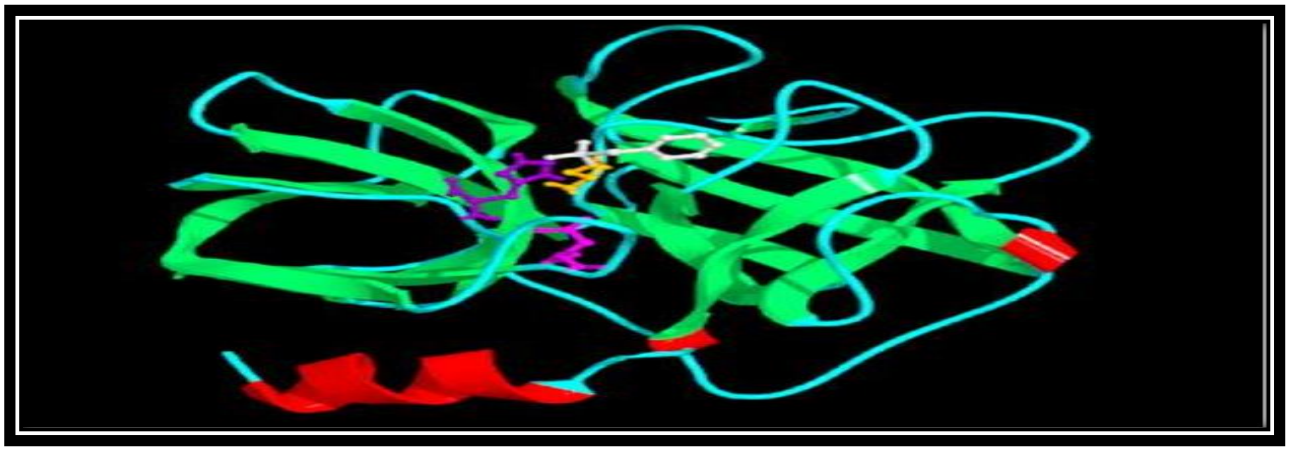

Figure1.1: Structure of Chymase; His66 is displayed in purple, Asp110 is shown in pink, and Ser203 is shown in orange. -Helices are shown in red, whereas -pleated sheets are depicted in green (Schlatter et al., 2012).

1.3.3. Chymase's Physiological and Pathological Importance

1.3.3.1. The role of Chymase in wound healing

The inflammatory phase, the proliferative phase, and the remodeling phase are the three major phases of wound healing(Schilling, 1976). At each step of the process, Chymase plays an important function. One of the most important aspects of wound healing is the modulation of the extracellular matrix (ECM) (Younan et al., 2010). This is consistent with the high concentration of connective tissue mast cell (CTMC) seen in connective tissue(Kawakami and Galli, 2002). Chymase degrades fibronectin and vitronectin, both of which are ECM components (Tchougounova *et al*., 2003). On the other side, Chymase activates fibroblasts during the proliferative and remodeling stages by producing TGF-β1, resulting in ECM deposition (Lindstedt *et al*., 2001). Additionally, it was shown that Chymase contributes to angiogenesis and vascular development in granulation tissue(Norrby *et al.*, 1986). Furthermore, it has been proven that Chymase cleaves precollagen, resulting in the production of fibrils (Atiakshin *et al*., 2020).

1.3.3.2. Chymase in cardiovascular and atherosclerotic disorders

Atherosclerosis, an inflammatory condition of the arteries, is the major cause of cardiovascular disease. It has been shown that Chymase has a role in the development of atherosclerosis (Bot *et al*., 2015). Smooth Muscle Cells (SMC) may be impacted both directly and indirectly by Chymase. Through the stimulation of TGF-1, it affects SMC differentiation, migration, and proliferation indirectly (Leskinen *et al*., 2001) This explains why the aortic wall media thins in atherosclerosis. Chymase may also limit collagen formation and promote endothelial cell death by degrading the Vitronectin and Fibronectin of the ECM (Heikkilä *et al*., 2008) and/or through TGF-1, both of which contribute to endothelial dysfunction. Additionally, Chymase is involved in the activation of the metalloprotease Pro-MMP-9, which has been linked to atherosclerosis (Wågsäter *et al*., 2011) Within the atheroma, Chymase is involved in the proteolysis of LDL, which occurs before the production of foam cells (Kovanen *et al*., 2000) In addition, by degrading ApoE, ApoAI, and HDL3, it limits the outflow of cholesterol from foam cells,the mechanism

that maintains the existence of foam cells in atherosclerotic plaques (Lindstedt *et al*., 1996) Finally, patients who had a myocardial infarction or unstable angina had greater levels of Chymase in their blood than those without coronary artery disease (Xiang *et al*., 2011).

1.3.3.3. Chymase in metabolic disorders and diabetes mellitus

Metabolic illnesses such as Obesity, Diabetes Mellitus (DM), type I and II, and DM complications have been linked to mast cells. Obese people had higher levels of MC in their white adipose tissue (WAT) compared to lean individuals, according to research (Tanaka *et al*., 2011) It was shown that mast cell-deficient mice weighed less, had less adipose tissue inflammation, and had better glucose tolerance (Liu *et al*., 2009) A study of blood samples from individuals with prediabetes and DM II found that Chymase levels were greater than those in normal blood glucose levels (Wang *et al*., 2011) Complications of diabetes mellitus, such as nephropathy and retinopathy, may be debilitating. Although not directly associated with these difficulties, Chymase has been implicated in these processes. In diabetic nephropathy, Chymase levels were increased. These levels were connected with glomerulosclerosis and tubulointerstitial fibrosis, as well as diabetic vascular disorders caused by the production of ANG II (Ritz, 2003). Additionally, pro-MMP-9 is hypothesized to be involved in diabetic nephropathy and retinopathy once activated by the Chymase (Kowluru *et al*., 2012)

1.3.3.4. Chymase's function in converting Angiotensin I to Angiotensin II

The role of Chymase in ANG II synthesis (Roszkowska-Chojecka *et al*., 2021) Chymase was discovered to be capable of cleaving Ang I and forming ANG II. ANG I is a ten-amino acid peptide. "Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8-His9-Leu10", Chymase catalyzes the hydrolysis of the Phe8-His9 link to produce ANG II, an eight amino acid peptide (Urata *et al*., 1990) The Renin-Angiotensin system, which produces ANG II, is well-known (RAS) (Figure1.3). The angiotensin-converting enzyme is used to convert Ang I to ANG II in a typical manner (ACE). When the body detects a drop in kidney blood flow (decreased intrarenal pressure), which predicts a decrease in blood pressure, RAS is activated in normal physiology. Blood pressure will return to normal after the formation of ANG II, which will create

vasoconstriction. This is thought to take place in the body's blood supply the fact that ACE inhibitors were unable to completely stop the synthesis of ANG II suggests that this is not the only route to consider(Padmanabhan *et al*., 1999) The alternate method, using the Chymase, was shown to produce ANG II primarily in tissues (as opposed to the circulation) in tissues As in Figure 1.2 where ANG II plays a significant role in pathophysiology (Miyazaki *et al*., 2006).

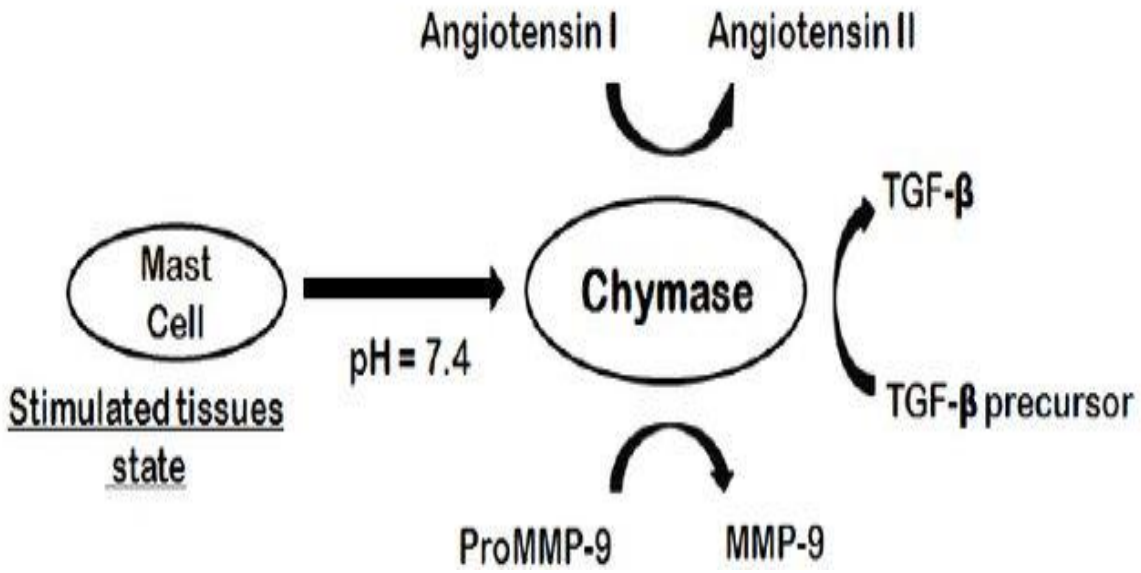

Figure1.2: Chymase's function in converting Angiotensin I to Angiotensin II (Miyazaki *et al*., 2006)

1.4. The Renin-Angiotensin System (RAS)

1.4.1. The Classical RAS

The RAS was discovered in the 1890s by Tigerstedt and Bergmann(Tigerstedt and Bergman, 1898) and was immediately recognized as a critical endocrine system for cardiovascular function regulation (Cat and Touyz, 2011b) The classical RAS is characterized by the release into the bloodstream of a glycoprotein called renin. The renal afferent arteriole's juxtaglomerular cells produce renin(Griendling *et al*., 1993) Preprorenin is converted to renin and then to active renin in juxtaglomerular cells. Renin, once released into the bloodstream, cleaves angiotensinogen produced from the liver to generate angiotensin I (ANG I) (Gomez, 1990) The angiotensin-converting

enzyme (ACE) further cleaves Ang I to generate Angiotensin II (ANG II) (Gomez, 1990) (Figure1.3) Angiotensin II exerts its physiological effects by acting as an agonist on both the angiotensin II type 1 receptor (AT1R) and the angiotensin II type 2 receptor (AT2R), which are members of the G-protein-coupled receptor (GPCR) superfamily with seven trans membrane spanning regions(Verdonk *et al*., 2012) Although angiotensin II binds similarly to the AT1R and AT2R, these two receptors have distinct genetic structures, tissue-specific expression patterns, and functional effects(Paul *et al*., 2006) The AT1 receptor activates numerous heterotrimeric G proteins, resulting in the production of second messengers such as reactive oxygen species (ROS), diacylglycerol (DAG), and inositol triphosphate (IP3) (Higuchi et al., 2007) Vasoconstriction, thirst, renal tubular sodium reabsorption, aldosterone production, sympathetic nervous system activation, chronotropic and cardiac inotropic activities, inflammation, hypertrophy, and fibrosis are all physiological outcomes linked with activation of these pathways(Cat and Touyz, 2011a, Savoia *et al*., 2011) The AT2 receptor is the most abundant receptor in the fetus, sharing just 34% of its amino acid sequence with the AT1 receptor(Grady *et al*., 1991) Notably, the AT2 receptor is expressed in adult vasculature, juxtaglomerular cells, glomeruli, and tubules in order to counterbalance the AT1 receptor's effects. The physiological consequences of activating the AT2 receptor include vasodilation, anti-inflammatory activity, natriuresis, cell growth suppression, and anti-fibrosis(Lemarié and Schiffrin, 2010) These are mediated through the activation of protein tyrosine phosphatase, the production of nitric oxide (NO), and sphingolipid signaling pathways(Lemarié and Schiffrin, 2010).

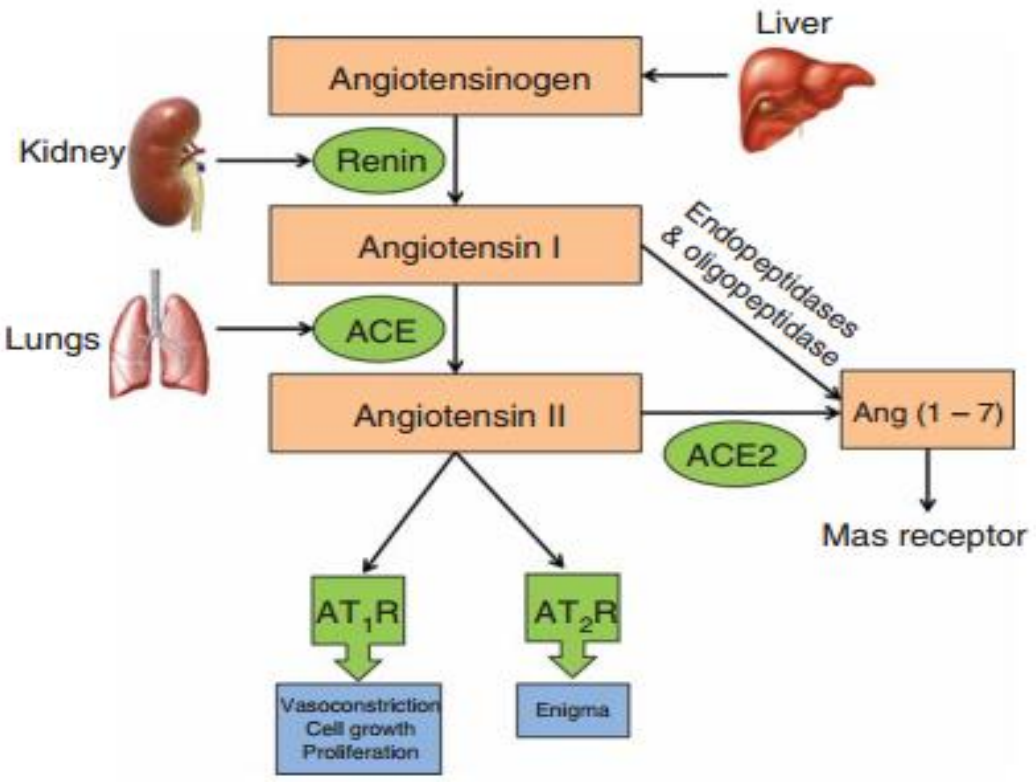

Figure1.3: Schematic overview of the renin-angiotensin system AT1R: Angiotensin II type 1 receptor; AT2R: Angiotensin II type receptor (Smith and Muralidharan, 2015)

1.4.2. Angiotensin-converting enzyme 2, Angiotensin-(1-7), and the Mas receptor

Until, the RAS was seen as a linear mechanism with ANG II as the major effector peptide (Stegbauer *et al*., 2011). The discovery of angiotensin-converting enzyme 2 (ACE2) and the Mas receptor turned the attention to the biochemical, physiological, and pathological significance of the angiotensin metabolite angiotensin-(1-7) (Ang-(1-7)) (Ferrario and Varagic, 2010) ACE2 can synthesize the heptapeptide Ang-(1-7) ACE2 is mostly expressed in the heart, kidney, and testis and has 42% amino acid sequence similarity with ACE but is not inhibited by traditional ACE inhibitors (Donoghue *et al*., 2000) It works as a monocarboxypeptidase, cleaving a single amino acid from the carboxyl terminus of ANG II to generate ANG-(1-7), or cleaving ANG I to form ANG- (1-9) (Ferrario and Varagic, 2010) ACE may further cleave Ang-(1-9) to produce ANG- (1-7) (Donoghue *et al*., 2000) In order for

ANG (1-7) to exert its biological and physiological effects, the G protein-coupled receptor encoded by the Mas proto-oncogene (Santos *et al*., 2003) As a consequence, ANG-(1-7) acts in opposition to ANG II's activities, which include lowering blood pressure, dilation of intrarenal blood vessels, increase in renal blood flow and glomerular filtration rate, inhibition of proximal tubule transport, and induction of diuresis (Ferrario and Varagic, 2010).

1.4.3. Intrarenal RAS

The RAS is now well recognized as a dual vasoactive system that acts on both the circulating endocrine and local tissue paracrine levels (Dzau, 2001) Local RAS has been shown to exist and function in the kidney, adrenal glands, heart, blood arteries, pancreas, liver, brain, and adipose tissues (Baltatu *et al*., 2011) The kidney contains all key components of the RAS, including angiotensinogen, renin, ACE, AT1, and AT2 receptors, ACE2, angiotensin-(1-7) receptor, and the Mas receptor (Carey and Siragy, 2003) Additionally, it has been shown that intracrine/intracellular RAS play critical physiological and pathophysiological functions in the kidneys (Li *et al*., 2011)

1.4.4. Renin-angiotensin system in diabetic nephropathy

The activation of the RAS and the subsequent production of ANG II in the kidney is a risk factor for diabetic nephropathy (Jandeleit-Dahm *et al*., 2006) High glucose levels boost the production of renin and angiotensinogen in mesangial cells, which raises intrarenal ANG II concentrations and eventually results in the formation of different cytokines and glomerular extracellular matrix (ECM) buildup Through a variety of paracrine and autocrine pathways (Singh *et al*., 2003) The upregulation of cytokines such as transforming growth factor-B (TGF-B), connective tissue growth factor (CTGF), interleukin-6, monocyte chemoattractant protein - 1 (MCP-1), and vascular endothelial growth factor-A (VEGF-A), which increase glomerular ECM deposition and decrease glome formation, has been shown to exacerbate hemodynamic injury to the kidney, contribute to hyperplasia and hypertrophy of the renal cells(Hostetter *et al*., 1982, Jandeleit-Dahm *et al*., 2006, Wolf, 2004).

1.4.5. The role of Angiotensin II in physiology and Pathophysiology

ANG II is involved in various systems and has a critical function in physiology and pathophysiology. The cardiovascular system is the key system of interest here, since ANG II may drive cardiac remodeling and hypertrophy, as well as vascular hypertrophy(Mehta and Griendling, 2007) This also induces constriction of resistance arteries, which raises systemic vascular resistance and hence elevates arterial pressure(Humma and Terra, 2002) Additionally, ANG II affects the renal system. It increases salt reabsorption and water retention by stimulating the production of aldosterone in the adrenal cortex; moreover, it increases fluid retention in the body by stimulating the release of Vasopressin or antidiuretic hormone (ADH) (Nishiyama *et al*., 2018) On another level, ANG II affects the nervous system by increasing the release of Norepinephrine and preventing its absorption at sympathetic synapses(Humma and Terra, 2002) ANG II has a direct effect on all cells in the cardiovascular system. Excess ANG II synthesis results in the development, hypertrophy, and migration of vascular smooth muscle cells (VSMC). Additionally, endothelial dysfunction will develop, as will an increase in adhesion molecule expression. Additionally, cardiac remodeling alters electrical conduction(Mehta and Griendling, 2007) These physiological changes associated with increased ANG II synthesis indicate that this peptide plays a critical role in myocardial infarction, arrhythmias, strokes, diabetic vascular disorders, and congestive heart failure (Xue *et al*., 2019)

Aims of the study

1. Find the association between Chymase and angiotensin ll levels and the type 2 Diabetes Mellitus Patients with Early and Chronic Diabetic Nephropathy.

2. Studying the effect of gender, body mass index, duration of disease, and age groups on the values of the studied variables with other parameters in patients and healthy subjects.

3. Are there statistically significant differences between Chymase and angiotensin ll levels and the type 2 Diabetes Mellitus Patients with Early and Chronic Diabetic Nephropathy?

Chapter two
Materials
and
Methods

2.1. Study Design and patients

A case-control study was conducted for individuals with type 2 diabetes from November 2021 to May 2022. This study was conducted on 62 patients with type 2 diabetes who attended Imam Hussein Medical City in Karbala and 28 cases in The control group, and the patients were divided into three groups according to their urinary albumin ratio to creatinine ratio (ACR), as well as they were divided into four groups according to body mass index and also they were divided into three groups according to age, as well as they were divided into three groups according to the duration of the disease.

2.2. Inclusion and Exclusion Criteria

The selection of patients with nephropathy and no nephropathy depend on the level of Microalbuminuria

2.2.1. Inclusion Criteria:

1. T2DM
2. Age (38-75)

2.2.2. Exclusion Criteria

1. Patients with T1DM
2. Patients with a statin drug, or angiotensin-converting enzyme inhibitors(ACEI)
3. Patient T2DM without hyperglycemic drugs
4. Pregnant women
5. Patients with urinary tract infection
6. Patient with chemotherapy
7. Systemic illness

2.3. Control Group

Twenty-eight healthy volunteers were included in this study. They were matched in their sex and age with patient groups. They were collected from the same center in Imam Hussein Medical City hospital, 12 of them were female and 16 were males. Their age ranged from (39-70) years.

2.4. Data Collection

2.4.1 Questionnaire

A questionnaire was designed to obtain information about diabetes. It contained the name, age, sex, weight, height, BMI (weight/ height2), duration of disease, type of treatment and medical history, smoking, and family history.

2.4.2. Examination

• Measurement of weight, height

• Calculate BMI (weight/ height^2)

2.5. Materials

2.5.1 . Instruments and tools &Diagnostic Kits

Table (2.1) Instruments and their origin used in this study

Name	Company	Country
Centrifuge	Hettich	Germany
Automatic Pipette	Eppendrof	England
UV 1650 PC Spectrophotometer	Shimazu COR	JAPAN
Water bath	kottermann-laboratechnic	Germany
ELISA washing machine and ELISA reader	Bio-Rad	U.S.A
Disposable syringe 10 ml and 5ml	Sterile EO	China
Incubator	Mammert	Germany
Timer	Lab Tech	Korea
Pipette tips (Blue & Yellow Tips)	CitoTest	China

Table (2.2) Diagnostic kits and their supplier used in this study

Kit name	Company	Country
Glucose kit	Plasmatic	France
Urea kit	BioSystem	Spain
Creatinine kit	Agappe	United Kingdom
Cholesterol kit	Spinreact	Spain
triglyceride kit	Spinreact	Spain
HDL-Cholesterol kit	Spinreact	Spain
Glycohemoglobin (HbA1c) Kit	STANBIO Laboratory	USA
Microalbumin kit	Humane	Germany
Human Mast Cell Chymase ELISA Kit	BT LAB	China
Human Angiotensin II ELISA Kit	BT LAB	China

2.5.2. Samples Collection

2.5.2.1. Blood specimen

Venous blood samples were obtained from control individuals and the study group five to eight milliliters of blood were obtained from each subject after overnight fasting by use of disposable syringes and divided into two parts:

A. Venous blood (2 ml) from each drawn sample was saved in a tube containing EDTA as an anticoagulant to be used in the measurement of the HbA1c level.

B. the remaining blood specimen were allowed to clot at room temperature and then centrifuged at 3000 Xg for 10 minutes. Fasting blood sugar, blood urea, Lipid profile, and serum creatinine was measured; remaining Sera were transferred carefully and stored at -17ºC until analysis time in suitable serum tubes, Chymase and angiotensin II were measured

2.5.2.2. Urine specimen

A random urine sample was collected from each patient with T2DM in the morning and taken directly to determine the microalbumin and creatinine in urine and then ACR measurement.

2.6. Methods

2.6.1. Determination of fasting blood glucose concentration

- **Principle**

Glucose is oxidized by glucose-oxidase to gluconate and hydrogen peroxide according to the following equation:

$$\text{B-D Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} H_2O_2 + \text{gluconate}$$

$$H_2O_2 + \text{Phenol} + \text{4 Amino –antipyrine} \xrightarrow{\text{Peroxidase}} 2H_2O + \text{Quinonimine}$$

- **Reagents**

Table (2-3): Reagents and their quantities in blood sugar kit.

Reagents	**Composition**	**concentration**
Reagent 1 Buffer solution	Tris buffer pH 7 Phenol	100 mmol/l 0.3 mmol/l
Reagent 2	Glucose oxidase Peroxidase 4-amino-antipyrine	10000 U/l 1000 U/l 2.6 mmol/l
Reagent 3 Standard	Standard glucose	100 mg/dl 5.56 mmol/l

- **Procedure**

Wavelength: 505nm

Cuvette: I cm light path

Temperature: 37 C

Working Reagent R1 + R2 same volume

Tubes were mixed and incubated for ten minutes at 37ºC. Then they were read at a wavelength of about (505 nm) at room temperature (25ºC), by using a cuvette of 1 cm light path.

Table (2-4): Reagent, standard, and sample concentration in each tube.

	Reagent blank	Standard	Sample
Working solution	1 ml	1ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

- **Calculation**

Determine the absorbance change as:

$\Delta A_{Sample} = (A_{sample} - A_{blank})$

$\Delta A_{Standard} = (A_{standard} - A_{blank})$

And used this for the calculation of serum glucose

$$\text{Glucose Concentration} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard Concentration}$$

The concentration of the supplied standard/calibrator R4: (100 mg/dl) or (5.55mmol/l).

- **Reference values**

74-106 mg/dl
4.1- 5.9 mmol/I

2.6.2. Determination of Blood Urea Concentration

- **Principle**

Urease hydrolyzes urea to produce ammonium:

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

In an alkaline medium, the ammonium ions are react with the salicylate and hypochlorite to form a green-colored indophenol (2, 2-dicarboxylindophenol). The reaction is catalyzed by sodium nitroprusside.

$NH4^+$ +salicylate+ hypochlorite ⟶ indophenol

The color intensity is proportional to the urea concentration in the sample.

- **Reagent Composition**

Table (2-5): Reagents and their quantities in blood urea kit.

Reagent 1 Standard	**R1**	**Urea**	**8.3 mmol/l 50 mg/dl**
Reagnt2 Enzymes	R2	Urease	500 U/mI
Reagent 3 Color Reagent	R3	Phosphate buffer Sodium salicylate Sodium nitroprusside	20 mmol/l 62 mmol/l 3.4 mmol/l
Reagent 4 Alkaline Reagent	R4	Sodium hydroxide(NaOH) Sodium hypochlorite(NaCIO)	150 mmol/l 7 mmol/l

- **Procedure**

Wavelength: - 600 nm

Zero adjustments: - reagent blank

Working solution R2+R3

Table (2-6): Reagent, standard, and sample concentration in each tube.

	Reagent blank	**standard**	**sample**
Standard	-	10µl	-
sample	-	-	10 µl
working solution	1 ml	1ml	1ml

Tubes were mixed and incubated for 10 minutes at 16-25 ºC.

Reagent 4	**1 ml**	**1 ml**	**1 ml**

Tubes were mixed and incubated for 10 minutes at 16-25 C

Photometry was performed.

- **Calculation**

$\Delta A_{Sample} = (A_{sample} - A_{blank})$

$\Delta A_{Standard} = (A_{standard} - A_{blank})$

$$\text{Sample concentration} = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Standard}} \times n$$ [n =concentration of standard=50 mg /dl]

- **Reference values**

12.8-42.8 mg/dl

2.6.3. A- Measurement of Serum Creatinine

For the quantitative in vitro determination of creatinine in serum

- **Principle:**

Creatinine in an alkaline solution reacts with picrate to form a colored complex.

Creatinine + Picric acid ⟶ Red addition complex

- **Reagent Composition:**

Table (2-7): Reagents and their quantities in serum creatinine kit.

contents	the initial concentration of Solutions
Creatinine Base Reagent (R1) Sodium hydroxide Sodium phosphate	 300 mmol/l 25 mmol/l
Creatinine Dye Reagent (R2) Picric Acid	 8.73 mmol/l
Creatinine Standard	2 mg/dl

- **Procedure**

Wavelength: 500nm

Cuvette: I cm light path

Temperature: 37 C

Blank: Dl Water

Working Reagent R1 + R2 same volume

The following chemicals were pipetted into test tubes:

Table (2-8): Reagent, standard, and sample concentration in each tube.

	Standard	Sample
Working Reagent	1000 µl	1000 µl
Standard	100 µl	-
Serum	-	100 µl

Mixed and read the optical density (T1) 60 seconds after the sample or standard addition Exactly 60 seconds after the first reading take a second reading (T2)

- **CALCULATION**

$$\text{Creatinine concentration} = \frac{(T2\text{-}T1) \text{ of sample}}{(T2\text{-}T1) \text{ of standard}} \times \text{standard conc. (2mg /dl)}$$

- **Reference values**

Creatinine	Mg/dl
Male	0.7-1.4
Female	0.6-1.2
Urine	0.80-1.80 gm/24hour

2.6.3. B- Measurement of Urinary Creatinine

The same method as plasma creatinine has been used, but the difference is that we dilute the urine sample by 500 µl (490 µl of distilled water + 10 µl of the urine sample) and then multiply the result by 50 The result comes out in units of milligrams per deciliter and by dividing the result by 1000 to convert it to units of grams per deciliter

2.6.4. Determination of Total Cholesterol (TC)

- **Principle**

the cholesterol in the sample originates from a colored complex according to the following reaction :

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4- Cholestenona} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

- **Reagents**

Table (2-9): Reagents and their quantities in the Total Cholesterol kit

Reagents	Composition	concentration
Reagent 1 (Buffer)	Phosphate buffer phenol	90 mmol 26 mmol
Reagent 2 (Enzymes)	Cholesterol esterase Cholesterol oxidase Peroxidase 4-Aminophenazone	300 U/l 300 U/l 1250 U/l 0.4 mmol/l
Reagent 3 (Standard)	Cholesterol	200 mg/dl

- **Procedure**

The content of bottle reagent 2 (Enzymes) was added to vial reagent 1 (buffer), meld gently until complete dissolution (approximately 2 minutes) to prepare work reagents. The procedure was carried out as in the following:

Table (2-10): Reagent, standard, and sample concentration in each tube.

Reagents	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Standard	-	10µl	
Sample	-	-	10µl

The tubes were mixed and then let stand for 5 minutes at 37˚C or10 min at room temperature. Record absorbance at 500 nm (480-520) against the blank, the color is stable for 1 hour.

- **Calculation**

$\Delta A_{Sample} = (A_{sample} - A_{blank})$
$\Delta A_{Standard} = (A_{standard} - A_{blank})$

$$\text{Cholesterol (mg /dl)} = \frac{\Delta \text{ A Sample}}{\Delta \text{ A Standard}} \times \text{n} \quad \text{[n =concentration of standard=200 mg /dl.]}$$

- **Reference values**

Less than 200 mg/dl

2.6.5. Determination of Triglyceride (TG)

- **Principle**

The TG in the sample originates a colored complex according to the following reaction:

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

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

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

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

- **Reagents**

Table (2-11): Reagents and their quantities in the TG kit

Reagents	Composition	concentration
Reagent 1 (Buffer)	Phosphate buffer 4-chlorophenol	50 mmol 2 mmol
Reagent 2 (Enzymes)	Lipoprotein lipase Glycerol Kinase Glycerol-3- phosphate Oxidase Peroxidase 4-Aminophenazone ATP	150000 U/l 500 U/l 2500 U/l 440 U/l 0.1 mmol/l 0.1 mmol/l
Reagent 3 (Standard)	TG	200 mg/dl

- **Procedure**

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

Table (2-12): Reagent, standard, and sample concentration in each tube.

Reagents	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Standard	-	10µl	
Sample	-	-	10µl

The tubes were mixed and then let stand for 5 minutes at 37˚C or 10 min at room temperature. Record absorbance at 500 nm (480-520) against the blank, the color is stable for 1 hour.

- **Calculation**

$\Delta A_{Sample} = (A_{sample} - A_{blank})$

$\Delta A_{Standard} = (A_{standard} - A_{blank})$

$$\text{Cholesterol (mmol/L)} = \frac{\Delta \text{ A Sample}}{\Delta \text{ A Standard}} \times n$$

[n =concentration of standard=200 mg /dl]

- **Reference values**

Men 40 -160 mg/dl Women 35-135 mg/dl

2.6.6. Measurement of High-Density Lipoprotein Cholesterol (HDL-C)

- **Principle**

Directly determination of serum HDL-C (high-density lipoprotein cholesterol) levels the assay takes place in two steps

1- Elimination of lipoprotein no-HDL

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4- Cholestenona} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

2- Measurement of HDL-C

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4- Cholestenona} + H_2O_2$$

$$2H_2O_2 + \text{HDAOS} + \text{4-AA} \xrightarrow{\text{peroxidase}} \text{Quinone pigment} + 4H_2O$$

- **Reagents**

Table (2-13) Reagents and their quantities in the Cholesterol HDL kit

Reagents	Composition	concentration
Reagent 1	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 6,6 N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) Cholesterol Esterase Cholesterol Oxidase Catalase Ascorbic Oxidase	100 mmol/l 0.7 mmol/l 800 U/l 500 U/l 300 U/l 3000U/l
Reagent 2	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 7,0 4 – Aminoantipyrine (4-AA) Peroxidase	1.1mmol/l 100 mmol/l 3500 U/l
HDL Standard	Cholesterol HDL	2.58 mg/dl

- **Procedure**

Table (2-14): Reagent, standard, and sample concentration in each tube.

Reagents	Blank	Standard	Sample
Reagent	300 µl	300 µl	300 µl
Standard	-	3µl	
Sample	-	-	3µl

Mix vigorously. Let stand for 5 minutes at 37 C record absorbance A1at 600 nm against reagent blank

add	Blank	Standard	Sample
Reagent R2	100 µl	100 µl	100 µl

Mix vigorously. Let stand for 5 minutes at 37 C record absorbance A2at 600 nm against reagent blank

- **Calculation**

Δ A Sample = (A 1 – A 2) for Sample

Δ A Standard = (A 1 – A 2) For Standard

$$\text{Cholesterol (mg /dl)} = \frac{\Delta \text{ A Sample}}{\Delta \text{ A Standard}} \times \text{n [n =concentration of standard=2.58 mg /dl.]}$$

• Reference values

Men 35-50 mg/dl women 45-60 mg/dl

2.6.7. Calculation of LDL-Cholesterol

LDL-Cholesterol was appraised indirectly (Friedewald et al., 1972) using a formula as follows:
LDL-C= TC – (HDL-C + VLDL-C) and VLDL-C was
calculated as:
The concentration of VLDL-C (mg/dl)= TG/5

2.6.8. Measurement of Glycohemoglobin (HbA_{1c}) in whole blood

• Principle

In this method, a preparation of hemolyzed whole blood was mixed with a weakly binding cation-exchange resin. The non-glycosylated hemoglobin (HbA_0) binds to the resin, leaving (HbA_1) free to be removed through a resin separator in the supernatant. The percent of HbA_1 was determined by measuring the absorbance values at 415 nm of the HbA_1 fraction and the total Hb fraction, calculating the ratio of absorbance (R), and comparing this ratio to that of a glycohemoglobin standard carried through the same procedure.

Results were expressed as HbA_1 but can be converted or derived as HbA_{1c} by using a conversion factor or when using an HbA_{1c} value for the standard.

• Reagents and materials provided

1. Glycohemoglobin Ion-Exchange Resin: Each tube contains: 3.0 ml cation-exchange resin, 8 mg/dl, buffered at PH 6.9.
2. Glycohemoglobin Lysin Reagent: contains potassium cyanide, 10 mmol/l, and surfactant.
3. Glycohemoglobin Standard (Lyophilized): prepared from packed human erythrocytes.
4. Filter Separators.

• Reagent Preparation

To reconstitute standards, the aluminum seal and rubber stopper were removed carefully to avoid loss of contents. A volumetric pipette was used; 1.0ml distilled/deionized water was added to the vial, the rubber stopper was replaced and allowed to stand for 10 minutes at room temperature, and contents were swirled gently while observing for the presence of undissolved material until the solution was complete. Reconstitution could be done by mechanically shaking the vial gently. The standard was used exactly as you would a patient sample in the procedure.

• Procedure

A- Hemolysate Preparation

1- A volume of 0.1 ml (100 µl) of each well-mixed blood sample was pipetted into an appropriately labeled tube and mixed.

2- For 5 minutes at room temperature (15-30^{o}C); was allowed to stand to complete hemolysis.

3- A volume of 0.5ml (500 µl) Lysing reagent was pipetted into a tube labeled Standard (S), Unknown (U), and Control (C).

B- Glycohemoglobin Separation and Assay

1. Pre-Fil resin tube Standard (S), Unknown (U), and Control (C) were labeled.
2. A volume of 0.1 ml of the prepared hemolysate was pipetted into an appropriately labeled resin tube.
3. A resin separator was positioned in the Pre-Fil tube so the rubber sleeve was approximately 1-2 cm above the liquid level.
4. Tubes on a hematology rocker were mixed for 5 minutes. Alternatively, tubes may be mixed by hand if held above the resin.
5. At the end of the 5-minute mixing, the resin separator was pushed into the tube until the resin was firmly packed in the bottom of the 13 mm tube.
6. Each supernatant was poured directly into separate cuvettes for absorbance measurements.
7. Absorbance (A_{gly}) of Standard, Unknown, and Control versus water were read at 415 nm for 60 minutes.

C- Total Hemoglobin Assay

1. A volume of 5.0 ml of deionized water was pipetted into tubes labeled Standard (S), Unknown (U), and Control (C).
2. A volume of 0.02 ml (20 µl) of hemolysate was pipetted into an appropriately labeled tube. The tube was mixed well and transferred to a cuvette for absorbance reading.
3. Absorbance (A_{tot}) of Standard, Unknown, and Control vs. water were read at 415 nm within 60 minutes.

- ## Calculations

For each standard and unknown, the ratio (R) of the glycohemoglobin absorbance to the hemoglobin absorbance was calculated as follows:

$$R = A_{gly} / A_{total}$$

$$\text{Glycohemoglobin (\%)} = \frac{R_{(Unknown)}}{R_{(Standard)}} \times \text{Concentration of Glycohemoglobin Standard (\%)}$$

• Reference values

Normal ranges: 4.2-6.2 %

Diabetic:

Good control: 5.5-6.8%

Fair control: 6.8-7.6 %

Poor control: above 7.6%

2.6.9. Measurement of Microalbumin

• Principle

Albumin in the standard and standard dilutions with 0.9% NaCl reacts with the anti-albumin antibody in the reagent. The increase in the aggregate causes an increase in the absorbance that could be measured by the turbidimetric endpoint method. The resulting (concentration /absorbance) pair gives a calibration curve from which the concentrations of albumin in known samples are read

• Reagents

Table (2-15) Reagents in Microalbumin kit

Reagents	Composition	concentration
Reagent 1 Buffer	phosphate buffer Poly Ethylene Glycol(PEG) sodium azide	20 mmol/l 6% 150 mmol/l
Reagent 2	phosphate buffer anti-human albumin(Antiserum) sodiumazide	20mmol/l 5% 150 mmol/l

• Procedure

A dilution of the standard was prepared using 0.9 % NaCl as diluents

Table (2-16) preparation of calibrations of microalbumine standard

Calibrator	6	5	4	3	2	1
Dilution		1:2	1:4	1:8	1:16	0
Saline µl	0	100	100	100	100	100
Standard µl	100	100	100	100	100	0
Dilution factor	1	0.5	0.25	0.125	0.0625	0

Then, we multiplied the concentration of the microalbumin standard by the corresponding dilution factor to obtain the microalbumin concentration of the dilution.

Table (2-17): the manual procedure for measurement of microalbumin standard

Pipette in to cuvette	
Dilution of standard	60 µl
Buffer (phosphate buffer +PEG+ sodiumazide)	900 µl

Mixed and the absorbance (A1) was recorded at 340 nm. Then, we added 150 µL of antiserum and incubated it for 5 minutes at room temperature and the Absorbance (A2) was read at 340 nm. Finally, plot ΔA (Y-axis) against the corresponding concentration (X-axis) and the calibration curve was obtained as shown in figure (2-1).

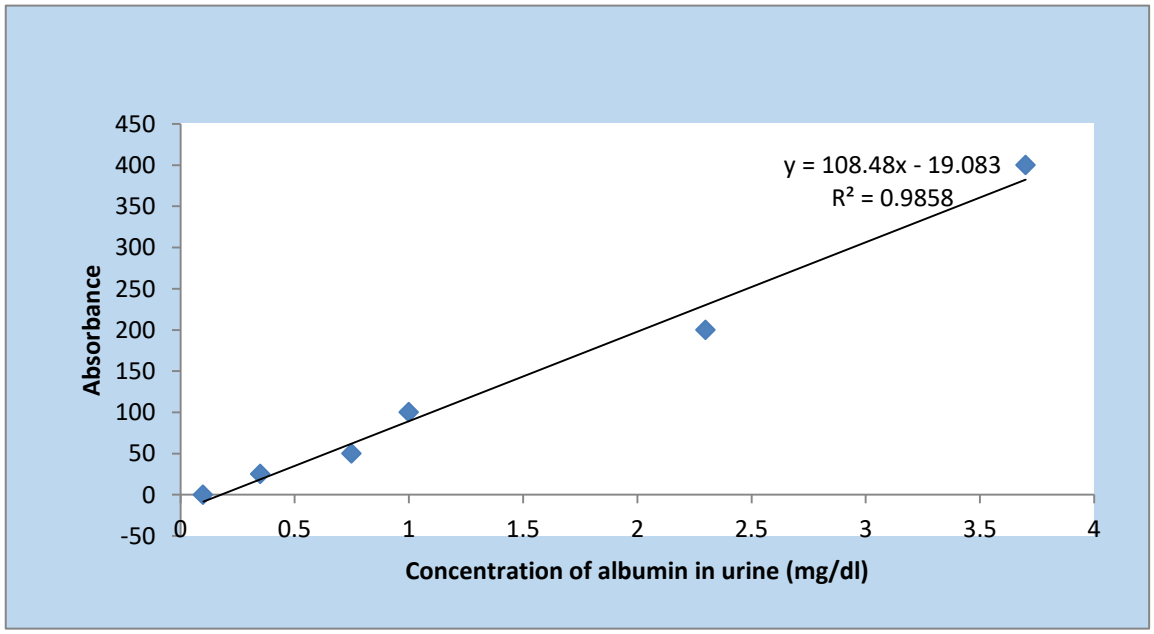

Figure (2-1): the standard curve of microalbumin measurement.

For measurement of microalbumin by turbidimetric test for quantitated assay of microalbumin in urine sample albumin in a sample or standard could react with the anti-albumin antibody. Measurement of microalbumin could be observed by the turbidimetric endpoint method and the absorbance is done at 340 nm.

Table (2-18): the manual procedure for measurement of microalbumin inpatient and control urine samples.

Pipette in to cuvettes	
buffer	900 µl
Sample	60 µl
Mix and insert cuvette into photometer read the absorbance(A1) at 340 nm	
Antiserum	150 µl
Mix and incubate for 5 min at room temperature, and read the absorbance(A2) at 340 nm	

• Calculation

The albumin concentration in the sample is calculated by interpretation of its absorbance from the calibration curve

ΔA= (A2-A1).

ACR (mg/g) = value of Microalbuminuria/ Urinary Creatinine

• Reference values

Table (2.19) Reference value of Microalbuminuria

Urine sample	**Mg/24h**	**µg/min**	**mg/g creatinine**
Normal	<30	<20	<30
Microalbuminuria	30-300	20-200	30-300
Macroalbuminuria	>300	>200	>300

2.6.10. Measurement of Chymase

• Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with the Human Cma1 antibody. Cma1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human Cma1 Antibody is added and binds to Cma1 in the sample. Then StreptavidinHRP is added and binds to the Biotinylated Cma1 antibody. After incubation unbound Streptavidin-HRP has washed away during a washing step. The substrate solution is then added and color develops in proportion to the amount of Human Cma1. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm

• Reagents

Components	**Quantity (96T)**
Standard solution (24ng/ml)	0.5ml
Pre-coated ELISA plate	12 * 8 well strips
Standard diluent	3ml
Streptavidin-HRP	6ml
Stop solution	6ml
Substrate solution A	6ml
Substrate solution B	6ml
Wash buffer Concentrate (25x)	20ml
Biotinylated Human Cma1 antibody	1ml
User instruction	1
Plate sealer	2 pics

• Reagent Preparation

• All reagents should be brought to room temperature before use.

• **Standard** Reconstitute the 120ul of the standard (24ng/ml) with 120ul of standard diluent to generate a 12ng/ml standard stock solution. Allow the standard to sit for 15 minutes with gentle agitation before making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (12ng/ml) 1:2 with standard diluent to

produce 6ng/ml, 3ng/ml, 1.5ng/ml, and 0.75ng/ml solutions. Standard diluent serves as the zero standards (0ng/ml).

Table (2.20) Dilution of Chymase standard solutions

12ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
6ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
3ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
1.5ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
0.75ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

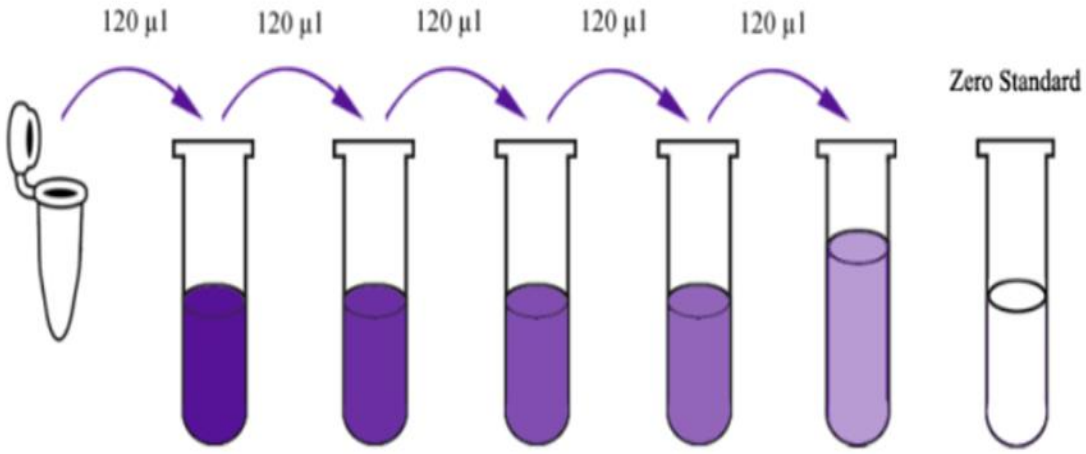

Figure (2-2): the Dilution of Chymase standard solutions

• Wash Buffer

Dilute 20ml of Wash Buffer Concentrate 25X into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

• Assay Procedure

1. All reagents are Prepared, standard solutions, and samples as instructed. all reagents are brought to room temperature before use. The assay is performed at room temperature.
2. The numbers of strips are determined required for the assay. The strips are inserted in the frames for use. The unused strips should be stored at 2-8°C

3. 50ul standard is added to standard well.
4. 40ul sample is added to sample wells and then 10ul Human Cma1 antibody is added to sample wells, and then 50ul streptavidin-HRP is added to sample wells and standard wells. Well mixed. The plate is covered with a sealer. Incubate for 60 minutes at 37°C.
5. The sealer is removed and the plate is washed 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash it 5 times with a wash buffer. Blot the plate onto paper towels or other absorbent material.
6. 50ul substrate solution A is added to each well and then 50ul substrate solution B is added to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50ul Stop Solution is added to each well, the blue color will change into yellow immediately.
8. The optical density (OD value) is determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after the stop solution is added.

• Calculation

A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and I drew the best fit curve through the points on the graph in Figures (2-3).

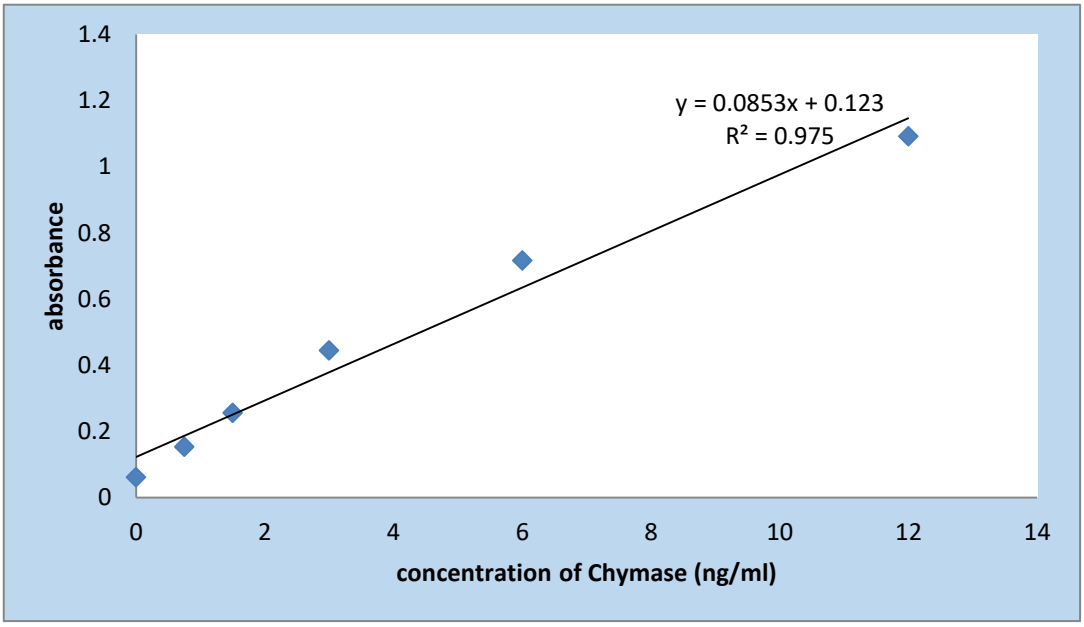

Figure (2-3): the standard curve for the determination of Chymase concentration

2.6.11. Measurement of ANG-II

• Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with a Human ANG-II antibody. ANG-II present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human ANG-II Antibody is added and binds to ANG-II in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated ANG-II antibody. After incubation unbound Streptavidin-HRP has washed away during a washing step. The substrate solution is then added and color develops in proportion to the amount of Human ANG-II. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

• Reagents

Components	Quantity
Standard Solution (400ng/L)	0.5ml
Pre-coated ELISA Plate	12 * 8 well strips
Standard Diluent	3ml
Streptavidin-HRP	6ml
Stop Solution	6ml
Substrate Solution A	6ml
Substrate Solution B	6ml
Wash Buffer Concentrate (25x)	20ml
Biotinylated Human Ang-II Antibody	1ml
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

• Reagent Preparation

• All reagents should be brought to room temperature before use.

• Standard Reconstitute the 120µl of the standard (400ng/l) with 120µl of standard diluent to generate a 200ng/l standard stock solution. Allow the standard to sit for 15 minutes with gentle agitation before making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (200ng/l) 1:2 with standard diluent to

produce 100ng/l, 50ng/l, 25ng/l, and 12.5ng/l solutions. Standard diluent serves as the zero standards 0 (ng/l)

• Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25X into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

• Assay Procedure

1. All reagents are prepared, standard solutions, and samples as instructed. All reagents are brought to room temperature before use. The assay is performed at room temperature.
2. The number of strips is determined required for the assay. The strips are Inserted in the frames for use. The unused strips should be stored at 2-8°C.
3. 50µl standard is added to standard well.
4. 40µl sample is added to sample wells and then 10µl anti-Ang-II antibody is added to sample wells, and then 50µl streptavidin-HRP is added to sample wells and standard wells. Well mixed. The plate is covered with a sealer. Incubate for 60 minutes at 37°C.
5. The sealer is removed and the plate is washed 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. 50µl substrate solution A is added to each well and then 50µl substrate solution B is added to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. 50µl Stop Solution is added to each well, the blue color will change into yellow immediately.
8. The optical density (OD value) is determined of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

• Calculation

A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and I drew the best fit curve through the points on the graph in Figures (2-4).

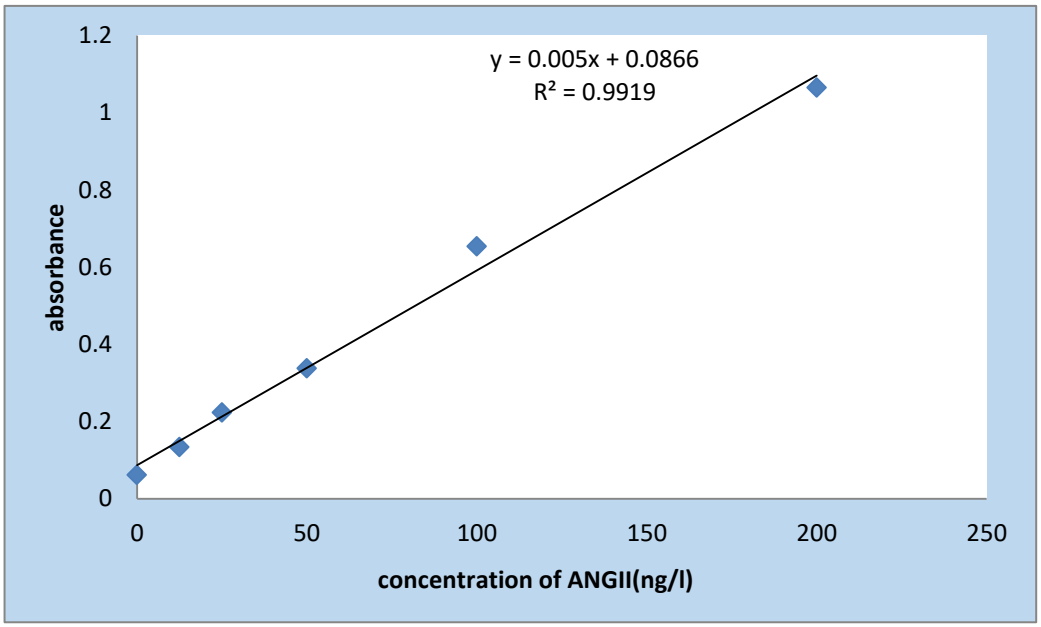

Figure (2-4): the standard curve for the **determination of ANG-II concentration.**

2.7. Statistical Analysis

The Statistical Package for Social Science (SPSS), version 22 was employed for the analysis of different biomarkers in the study. Chi-square analysis was utilized for a comparison between percentage and analysis of variance (ANOVA), the least significant difference and the Duncan test or t-test was employed for a comparison between means. The correlation coefficient between variables in this study also estimated

Chapter three

Results

3.1. The demographic and clinical characteristics of the study

The results of the data analyzed were arranged according to the grouping of subjects enrolled in this study they were classified into two groups: -

3.1.1. Control group

The control group included 28 samples of healthy people of both sexes, the number of females was 12 (42.9%), the number of males was 16 (57.1%), and their ages ranged between (39-70) years, and some biochemical variables were measured, which included fasting sugar (FBS), glycated hemoglobin (HbA_{1c}), blood urea, Serum Creatinine, albumin-creatinine ratio(ACR), Microalbuminuria, cholesterol (CHOL), triglyceride (TG) and high-density lipoprotein (HDL) levels were also measured low-density lipoprotein (LDL), very low-density lipoprotein VLDL, Chymase and AngiotensinII. Table(3-1) shows the demographic characteristics of the control group.

3.1.2. Patients group

The current study included patients having type 2 diabetes, and their fasting blood sugar (FBS) level was measured for them at Al-Hussein Teaching Hospital in Karbala Governorate. The number of 62 patients was 27 (43.5%) and the number of males was 35 (56.5%). Their ages ranged from (38-75) years. The same variables that were measured in the control group were measured .as shown in Table (3.1) and figure (3.1).

The group of patients was divided into three groups according to age, where the first group was (35-45) years and their number was 11 (17.7%), and the second group was (46-60) years and their number was 26 (41.9%) and the third group (>60) years, numbering 25 (40.3% .as shown in Table (3.1) and figure (3.2).

As well as divided according to body mass index (BMI) into four groups: the first group (<18) and its number 4 (6.5%), the second group (18-24.9) and its number 10 (16.1%), the third group (25-29.9) and its number is 21 (33.9%), the fourth group (≥30) and its number is 27 (43.5%. as shown in Table (3.1) and figure (3.3).

As well as it was divided according to the duration of the disease into three groups: the first group (<5 years) and their number 13 (21.0%), the second group (5-10 years), and their number 16 (25.8%), the third group (>10) and their number 33 (53.2%. as shown in Table (3.1) and figure (3.4).

As well as they were divided according to the Ratio of albumin and creatinine (ACR) into three groups: the first group (<30), whose number is 22 (35.5%), the second group (30-299), whose number is 20 (32.3%), and the third group (≥ 300), their number is 20 (32.3%), as shown in Table (3.1) and figure (3.5).

Table (3-1): Demographic characteristics of diabetic nephropathy groups and control groups

Variables	Control group	Diabetic group
Total Number	**28(100%)**	**62(100%)**
Age (years)		
(35-45) years	**9 (32.1%)**	**11 (17.7%)**
(46-60) years	**8 (28.6%)**	**26 (41.9%)**
(>60) years	**11 (39.3%)**	**25 (40.3%)**
Sex		
Male	**16(57.1%)**	**35(56.5%)**
Female	**12(42.9%)**	**27(43.5%)**
Body mass index (BMI) Kg/m^2		
Underweight <18	**1 (3.6%)**	**4 (6.5%)**
Normal weight 18-24.9	**8 (28.6%)**	**10 (16.1%)**
Over weight 25- 29.9	**14 (50.0%)**	**21 (33.9%)**
Obesity ≥30	**5 (17.9%)**	**27 (43.5%)**
Family history		
Present	**0**	**39 (62.9%)**
Absent	**28 (100%)**	**23(37.1%)**
Smoking		
Yes	**0**	**9(14.5%)**
NO	**28 (100%)**	**53(85.5%)**
Hypertension		
Present	**0**	**39 (62.9%)**
Absent	**28 (100%)**	**23 (73.1%)**
Drug Diabetes Mellitus		
Present	**0**	**51 (82.3%)**
Absent	**28 (100%)**	**11 (17.7%)**
Duration of the disease(year)		
<5 year	**0**	**13 (21.0%)**
5-10	**0**	**16 (25.8%)**
>10	**0**	**33(53.2%)**
Albumin-creatinine ratio(µg/mg)		
< 30	**28 (100%)**	**22 (35.5%)**
30-299		**20(32.3%)**
≥ 300		**20(32.3%)**

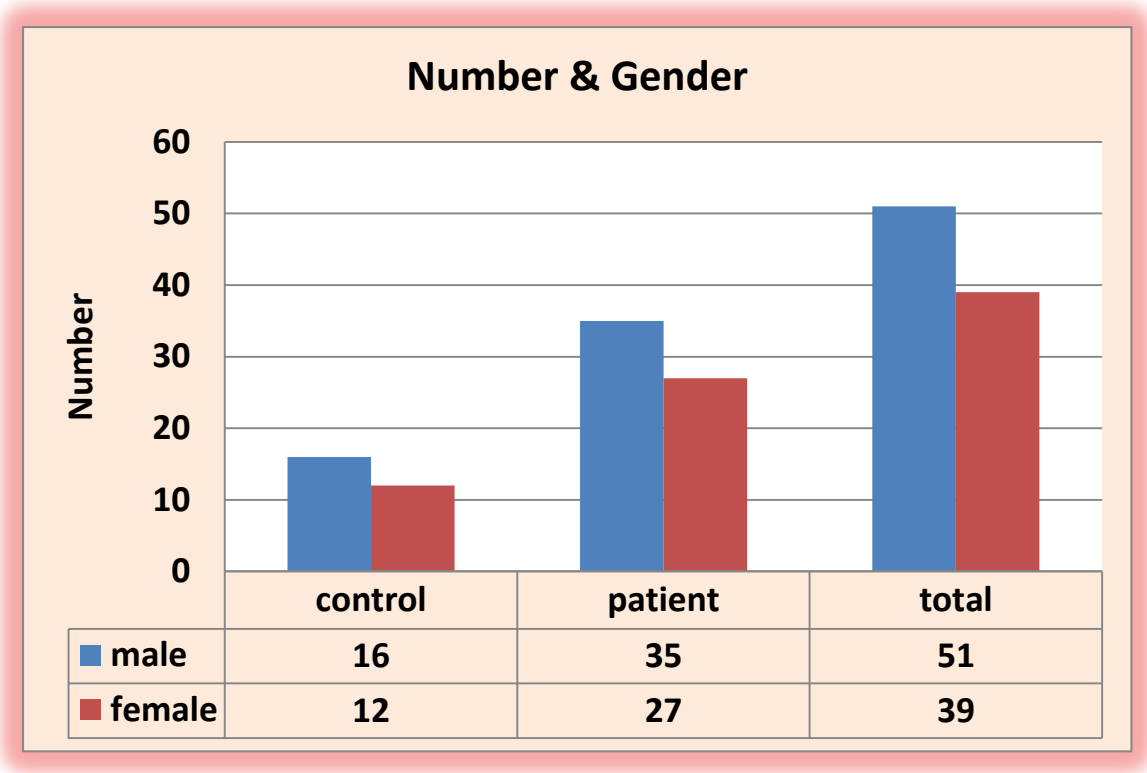

Figure (3.1) Distribution of Gender

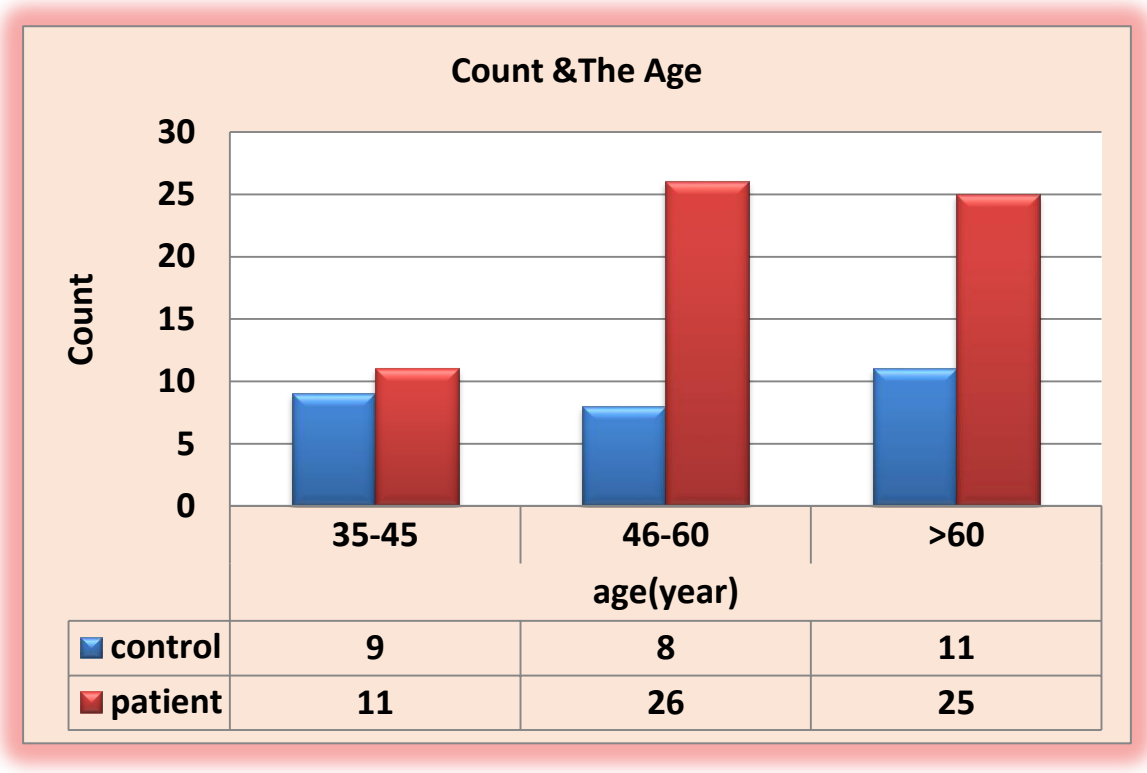

Figure (3.2) Distribution of Age

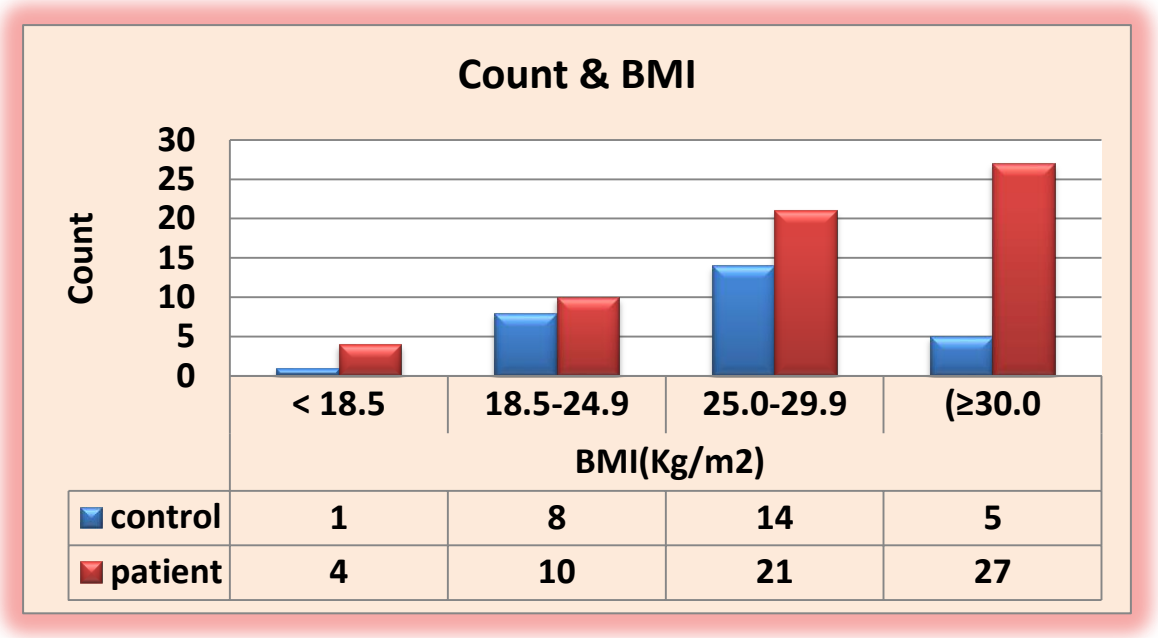

Figure (3.3) Distribution of BMI

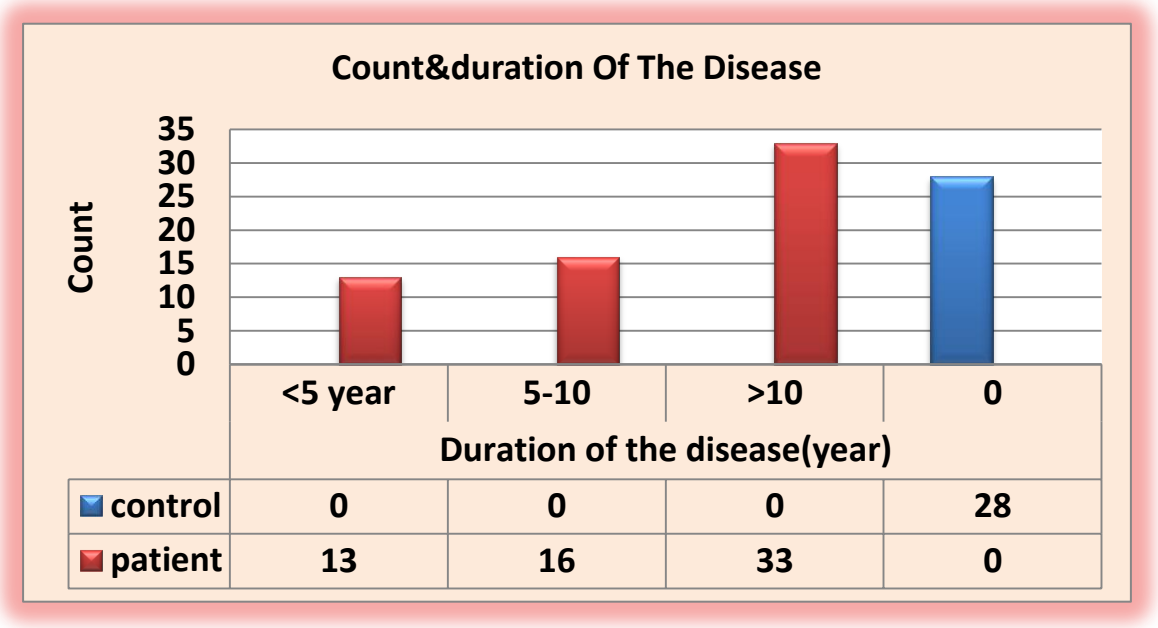

Figure (3.4) Distribution of Duration of the Disease

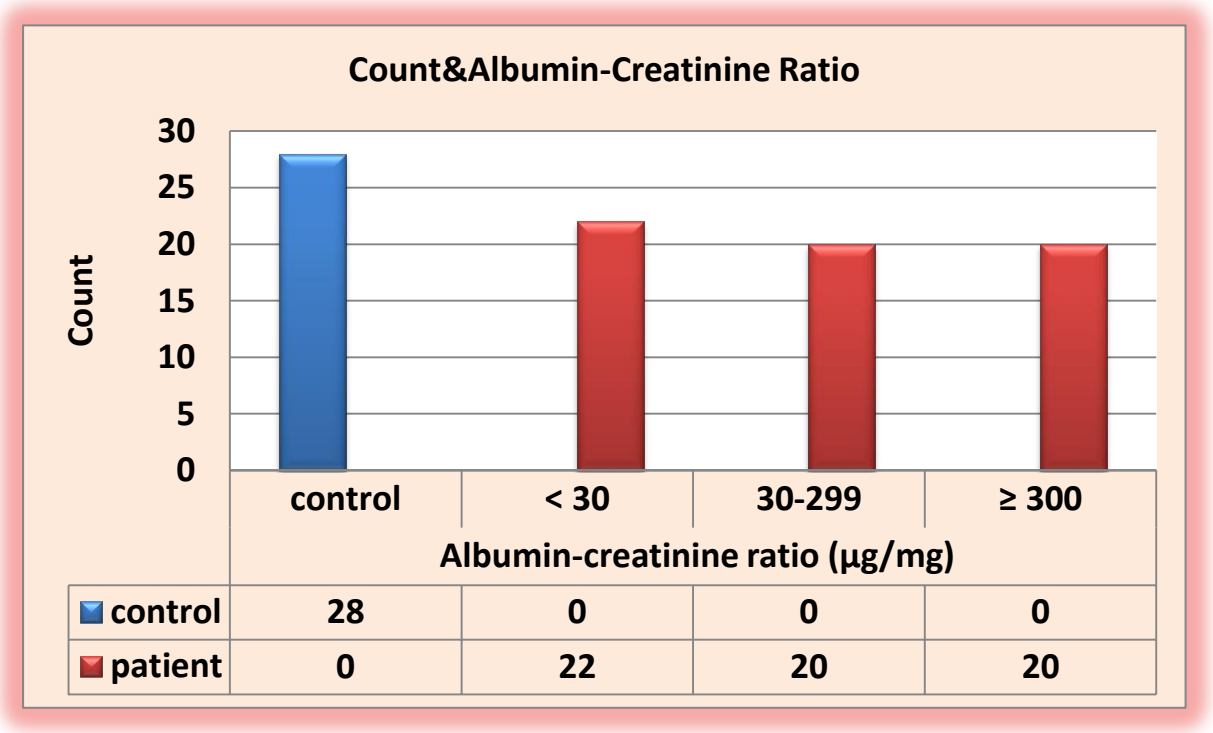

Figure (3.5) Distribution of Albumin-Creatinine Ratio

3.2. Association of study groups by different variables

The ratio between males and females is equal in the group of patients and the control group, and there were no statistically significant differences between them in age and body mass index (BMI). The differences between diabetic patients and control groups were significant (P≤0.01) in the Duration of the disease and Albumin - Creatinine Ratio (ACR) as shown in Table (3-2).

Table (3-2): The association of study groups by different variables

Variables	Control group	Diabetic group	$\chi 2$	p-value
Total Number	**28(100%)**	**62(100%)**		
Age (years)				
(35-45) years	9 (32.1%)	11 (17.7%)	2.71	0.257
(46-60) years	8 (28.6%)	26 (41.9%)		
(>60) years	11 (39.3%)	25 (40.3%)		
Sex				
Male	16(57.1%)	35(56.5%)	0.04	0.951
Female	12(42.9%)	27(43.5%)		
Body mass index (BMI) Kg/m^2				
Underweight (<18)	1 (3.6%)	4 (6.5%)	6.65	0.084
Normal weight (18-24.9)	8 (28.6%)	10 (16.1%)		
Over weight (25- 29.9)	14 (50.0%)	21 (33.9%)		
Obesity (≥30)	5 (17.9%)	27 (43.5%)		
Albumin-creatinine ratio(µg/mg)				
Control	28 (100%)	0	90.00	0.001
< 30	0	22 (35.5%)		
30-299	0	20(32.3%)		
≥ 300	0	20(32.3%)		
Duration of the disease(year)				
Control	28(100%)	0	90.00	0.001
<5 year	0	13 (21.0%)		
5-10	0	16 (25.8%)		
>10	0	33(53.2%)		

Fisher-Exact test: significant ≤ 0.05.

3.3. Comparison of biochemical characteristics between patients group and Control Group

This study showed a highly significant difference (P≤0.01) in fasting sugar (FBS), glycated hemoglobin (HbA1c), blood urea, Serum Creatinine, Microalbuminuria, albumin-creatinine ratio (ACR), cholesterol (CHOL), triglyceride (TG), and high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein

(VLDL), High-density lipoprotein (HDL), Chymase and AngiotensinII. Between diabetic patients and the control groups and there were no significant differences (P>0.05) in age and BMI as shown in Table (3-3).

Table (3-3): Comparison of biochemical characteristics between patients group and control group included in the study

Group / Parameters	CONTROL (Mean ± S.D) N= 28	RANGE (Min-Max)	Diabetic patients (Mean ± S.D) N= 62	RANGE (Min-Max)	p-value
age(year)	55.82 ± 11.70	39.00-70.00	57.96 ± 10.25	38.00-75.00	0. 382
BMI(Kg/m2)	26.58 ± 3.61	16.75-32.50	28.00 ± 4.41	17.17-36.26	0.141
Duration of the Disease(year)	0	0.00	11.27±6.17	2.00-23.00	0. 001
FBS(mg/dl)	94.13± 8.02	82.00-110.00	175.14± 23.75	124.00-211.00	0. 001
Blood urea (mg/dl)	26.84 ± 6.79	18.50-40.00	73.77± 41.79	30.30-175.00	0. 001
S. creatinine (mg/dl)	0.70 ± 0.14	0.45-1.10	2.14± 1.29	0.90-5.50	0. 001
HbA_{1c} (%)	5.45 ± 0.36	4.50-5.80	8.92± 1.83	6.20-12.90	0. 001
Microalbuminuria (mg/l)	13.51± 2.18	10.50-19.00	100.93± 66.11	5.50-205.00	0. 001
Albumin-creatinine ratio (µg/mg)	15.77±2.26	13.00-21.50	142.84 ± 94.41	11.00-320.00	0. 001
CHOL(mg/dl)	160.03 ± 11.28	137.00-185.00	200.23 ± 27.25	148.20-245.00	0. 001
TG(mg/dl)	109.68 ± 11.82	86.50-133.00	176.99± 51.83	76.40-265.30	0.001
HDL(mg/dl)	38.84± 5.11	34.00-53.00	35.13 ± 9.31	24.30-59.50	0. 001
LDL(mg/dl)	99.23± 10.55	79.30-116.90	130.35± 23.75	75.10-172.60	0. 001
VLDL(mg/dl)	21.91± 2.35	17.30-26.60	35.50± 10.21	15.28-53.00	0. 001
Chymase(ng/ml)	1.64 ± 0.39	0.66-2.09	3.25± 1.55	2.04-8.27	0. 001
AngiotensinII(ng/l)	16.98 3.55	8.28-21.68	41.25 ± 17.03	21.88-80.01	0. 001

t-test (normal distribution) and Mann-Whitney Test (abnormal distribution), P≤0.01 highly significant difference, P≤0.05 significant difference, S.D: Standard Deviation.

3.4. Impact of Age Groups on Biochemical Parameters of patients groups

Statistical analysis of biochemical parameters in patients groups according to age, in the duration of the disease, there were statistically significant differences between all groups, but in (FBS, blood urea, HbA1c, CHOL, LDL, VLDL, AngiotensinII Microalbuminuria, albumin-creatinine ratio (ACR)) there were statistically significant differences between the first and second group, as well as between the first and third groups. As for (TG, serum creatinine, HDL, and Chymase), there were statistically significant differences between the first and third groups, and the no statistically significant value for BMI, as shown in Table (3-4).

Table (3-4): The impact of age groups on some biochemical parameters of patients Groups

Age Group (Year) / Parameters	Mean ± S.D		
	35-45 N= 11	**46-60 N= 26**	**> 60 N= 25**
BMI(Kg/m^2)	25.91± 5.46 **a**	27.89 ± 4.78 **a**	29.03 ± 3.17 **a**
Duration of Disease(year)	4.09± 2.25 **a**	9.88 ± 4.11 **b**	15.88 ± 5.42 **c**
FBS(mg/dl)	155.86 ± 19.80 **a**	172.84± 24.23 **b**	186.10 ± 18.84 **b**
Blood urea (mg/dl)	41.87 ± 9.79 **a**	68.30± 35.74 **b**	93.50 ± 46.52 **b**
S. creatinine (mg/dl)	1.19 ± 0.22 **a**	1.84± 0.90 **ab**	2.46± 1.35 **b**
HbA_1c (%)	7.40 ± 0.87 **a**	8.72± 1.73 **b**	9.80 ± 1.78 **b**
Microalbuminuria(mg/l)	36.07 ± 5.86 **a**	96.25± 30.13**b**	134.34 ± 30.92 **b**
Albumin-creatinine ratio (µg/mg)	41.42 ± 8.55 **a**	140.72± 94.57 **b**	189.75± 97.90 **b**
CHOL(mg/dl)	175.49 ± 17.70 **a**	200.53± 24.41**b**	210.82 ± 27.18 **b**
TG(mg/dl)	140.75 ± 39.56 **a**	172.96± 49.03**ab**	197.11 ± 49.50**b**
HDL(mg/dl)	41.07 ± 10.44 **a**	35.98± 10.41 **ab**	31.64 ± 5.69 **b**
LDL(mg/dl)	108.13 ± 15.47**a**	130.36± 23.04 **b**	140.11 ± 20.83 **b**
VLDL(mg/dl)	28.09±7.85 **a**	34.86±9.79 **b**	39.42±9.89 **b**
Chymase(ng/ml)	2.36 ± 0.24 **a**	2.98± 1.14 **ab**	3.92 ± 1.96 **b**
AngiotensinII(ng/l)	28.28 ± 5.01 **a**	39.41± 14.55 **b**	41.25 ± 17.03 **b**

- **One-Way ANOVA Test (normal distribution) and Kruskal-Wallis Test (abnormal distribution), S.D: Standard Deviation.**
- **Different letters in the same raw refer to significant differences ($p \leq 0.05$)**

3.5. Impact of BMI Groups on Biochemical Parameters of patients groups

Statistical analysis of biochemical parameters in patients according to BMI, In (TG, VLDL, Duration of the disease) there were statistically significant differences

between each of the first and fourth groups, the first and third, and the second and fourth, as well as we noticed in the(blood urea, Microalbuminuria, albumin-creatinine ratio (ACR), serum creatinine and AngiotensinII) significant differences between the first and fourth group, and also in (HDL, LDL, FBS, CHOL, HbA1c) there were statistically significant differences between the first and fourth group, and the second and fourth, and in the(Chymase) there were statistically significant differences in the first and fourth group, the second and fourth group, and the third and fourth groups Also the no statistically significant value for age, as shown in Table (3-5).

Table (3-5): The impact of BMI groups on some biochemical parameters in patients groups

BMI Group / Parameters	Mean± S.D			
	< 18 **N= 4**	**18-24.9** **N= 10**	**25-29.9** **N= 21**	**≥ 30** **N=27**
Age (year)	46.50 ± 9.74**a**	55.20 ± 11.95 **a**	58.52± 8.73 **a**	60.25± 9.94 **a**
Duration of Disease(year)	4.75 ± 4.19 **a**	6.90 ± 6.04 **ab**	11.04 ± 5.38 **bc**	14.03 ± 5.53**c**
FBS(mg/dl)	157.75 ± 11.61 **a**	155.60± 26.51 **a**	157.83 ± 21.55**ab**	184.42 ± 20.60 **b**
Blood urea (mg/dl)	45.05 ± 12.57**a**	52.79 ± 39.28 **ab**	71.18± 38.48 **ab**	87.81± 43.27 **b**
S. creatinine (mg/dl)	1.19 ± 0.005**a**	1.49± 0.99 **ab**	1.87 ± 0.97 **ab**	2.35± 1.25 **b**
HbA_1c (%)	7.31 ± 0.62 **a**	7.66± 1.62**a**	8.81 ± 1.59 a**b**	9.71± 1.83 **b**
Microalbuminuria(mg/l)	22.73 ± 5.50 **a**	47.94± 41.06 **ab**	102.11 ± 52.55**bc**	131.28 ± 51.65 **c**
Albumin-creatinine ratio (µg/mg)	26.75 ± 5.66 **a**	61.46± 68.14 **ab**	141.87 ± 97.30 **bc**	190.95± 89.09 **c**
CHOL(mg/dl)	176.17 ± 13.10 **a**	178.11± 29.07**a**	198.14 ± 26.66 **ab**	213.62 ± 20.40 **b**
TG(mg/dl)	116.67 ± 44.49 **a**	137.80± 48.23 **ab**	174.24 ± 51.16 **bc**	202.57 ± 37.68 **c**
HDL(mg/dl)	48.62 ± 12.57 **a**	37.93± 8.49 **a**	37.43 ± 9.19 **ab**	30.13 ± 5.98 **b**
LDL(mg/dl)	106.69 ± 2.90 **a**	113.60± 24.21 **a**	126.45 ± 23.83**ab**	143.08 ± 16.79 **b**
VLDL(mg/dl)	23.33±8.89 **a**	27.57±9.62 **ab**	34.81±10.23 **bc**	40.78± 6.84 **c**
Chymase(ng/ml)	2.32 ± 0.14**a**	2.80± 1.48 **a**	2.89 ± 0.76 **a**	3.83 ± 1.97 **b**
AngiotensinII(ng/l)	27.43 ± 2.65 **a**	32.48± 15.99 **ab**	39.35 ± 13.62 **ab**	48.02± 18.42 **b**

- **One-Way ANOVA Test (normal distribution) and Kruskal-Wallis Test (abnormal distribution), S.D: Standard Deviation.**
- **Different letters in the same raw refer to significant differences ($p \leq 0.05$)**

3.6. Impact of duration of disease Groups on Biochemical Parameters of patients groups

Statistical analysis of biochemical parameters in patients according to the duration of disease, In (HDL, LDL, FBS, CHOL, HbA1c, Microalbuminuria, albumin-creatinine ratio (ACR), TG, VLDL, and Age), there were statistically significant differences between all groups, as well as we noticed in (blood urea, serum creatinine, AngiotensinII, and Chymase) statistically significant differences between the first and third, and second and third groups, and either In BMI, there were statistically significant differences in the first and second groups, and in the first and third groups, as shown in Table (3-6).

Table (3-6): The impact of duration of disease on some biochemical parameters of patients groups

duration of disease (Year) / Parameters	Mean ± S.D		
	<5 N= 13	**5-10 N= 16**	**> 10 N= 33**
Age (year)	46.38± 7.83 **a**	55.52± 9.58 **b**	63.84 ± 6.37 **c**
BMI(Kg/m^2)	24.41± 5.63 **a**	27.62 ± 4.47 **b**	29.60± 2.99 **b**
FBS(mg/dl)	146.92 ± 16.21 **a**	164.40± 16.71 **b**	191.46± 13..79 **c**
Blood urea (mg/dl)	36.71 ± 4.62 **a**	46.31± 12.53**a**	101.68± 38.88**b**
S. creatinine (mg/dl)	1.09 ± 0.10 **a**	1.32± 0.27 **a**	2.64 ± 1.17 **b**
HbA$_1$c (%)	6.69 ± 0.66 **a**	7.90± 0.86 **b**	10.18 ± 1. 48 **c**
Microalbuminuria(mg/l)	15.13 ± 7.92 **a**	58.08± 20.22 **b**	155.50 ± 93**c**
Albumin-creatinine ratio (µg/mg)	18.82 ± 8.04 **a**	63.51± 21.35 **b**	230.17 ± 77.75**c**
CHOL(mg/dl)	169.12 ± 15.93 **a**	187.83± 16.58 **b**	218.50 ± 19.41 **c**
TG(mg/dl)	111.99 ± 25.56 **a**	154.76± 40.85 **b**	213.37 ± 27.68 **c**
HDL(mg/dl)	46.70 ± 8.76 **a**	35.99± 8.99 **b**	30.16 ± 4.30 **c**
LDL(mg/dl)	102.58 ± 15.04 **a**	121.45± 15.96 **b**	145.61 ± 16.16 **c**
VLDL(mg/dl)	22.88±4.93 **a**	30.96±8.15 **b**	42.67±5.53 **c**
Chymase(ng/ml)	2.23 ± 0.13 **a**	2.51± 0.27 **a**	4.01± 1.81 **b**
AngiotensinII(ng/l)	25.78 ± 2.79 **a**	31.20± 6.05 **a**	52.22 ± 16.17 **b**

- **One-Way ANOVA Test (normal distribution) and Kruskal-Wallis Test (abnormal distribution), S.D: Standard Deviation.**
- **Different letters in the same raw refer to significant differences ($p \leq 0.05$)**

3.7. Effect of Sex Groups on Biochemical Parameters of patients groups

The study of the effect of sex on biochemical parameters showed strong a significant difference (P≤0.01) in HDL levels only between males and females, Also there is a statistically significant ≤ 0.05 in blood urea, serum creatinine, age, and LDL, as shown in (Table 3-7).

Table (3-7): The effect of Sex on biochemical parameters of patients groups

Sex Group / Parameters	Mean ± S.D		
	Male N= 35	**Female N= 27**	**p-value**
Age (year)	60.14± 10.34	55.14± 9.61	0.049
BMI(Kg/m^2)	28.53± 4.12	27.31± 4.75	0.284
Duration of Disease(year)	12.42± 6.09	9.77± 6.06	0.094
FBS(mg/dl)	179.08± 20.76	170.03 ± 26.77	0.138
Blood urea (mg/dl)	82.57± 48.75	62.37± 27.40	0.044
S. creatinine (mg/dl)	2.22± 1.03	1.66 ± 0.74	0.040
HbA_1c (%)	9.10± 1.68	8.68± 2.02	0.380
Microalbuminuria(mg/l)	110.24± 50.35	88.85± 52.93	0.310
Albumin-creatinine ratio (µg/mg)	148.85± 92.34	135.06± 99.90	0.672
CHOL(mg/dl)	205.00± 26.04	194.05 ± 28.02	0.118
TG(mg/dl)	185.54± 50.06	165.89 ± 52.91	0.140
HDL(mg/dl)	31. 75± 6.41	39.52 ± 10.69	0.001
LDL(mg/dl)	136.88± 20.53	121.87± 25.09	0.012
VLDL(mg/dl)	37.31± 9.70	33.16 ± 10.57	0.113
Chymase(ng/ml)	3.37 ± 1.58	3.09± 1.54	0.488
AngiotensinII(ng/l)	43.07 ± 16.97	38.86± 17.15	0.343

t-test (normal distribution) and Mann-Whitney Test (abnormal distribution), P≤0.01 highly significant difference, P≤0.05 significant difference, S.D: Standard Deviation.

3.8. Comparison between type 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters

The study of the Comparison between type 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters showed In (HDL, LDL, FBS, CHOL, HbA1c, Macroalbuminuria, TG, VLDL, blood urea, serum creatinine, and

AngiotensinII), there were statistically significant differences between all groups, as well as in the Chymase it was statistically significant differences between the first and third and second and third groups, and either In age, there were statistically significant differences in the first and third groups, as shown in Table (3-8)

Table (3-8): The Comparison between types 2 Diabetes Mellitus with Diabetic Nephropathy groups for Biochemical Parameters

ACR Group / Parameters	Mean ± S.D		
	<30 N= 22	**30-299 N= 20**	**≥ 300 N= 20**
Age (year)	54.09 ± 11.70 **a**	56.75 ± 9.47 **ab**	63.45± 6.79 **b**
BMI(Kg/m^2)	25.18 ± 4.88 **a**	29.36 ± 3.96 **b**	29.74± 2.47 **b**
Duration of Disease	5.63 ± 3.69 **a**	11.40± 4.50 **b**	17.35 ± 3.39 **c**
FBS(mg/dl)	148.88 ± 14.48 **a**	178.30± 5.16 **b**	200.78 ± 6.89 **c**
Blood urea (mg/dl)	36.80 ± 3.43 **a**	62.70± 13.44 **b**	125.52 ± 29. 72 **c**
S. creatinine (mg/dl)	1.11 ± 0.09 **a**	1.55± 0.29 **b**	3.35 ± 0.97 **c**
HbA$_1$c (%)	7.07 ± 0.62 **a**	8.68 ± 0.35 **b**	11.19± 0.93 **c**
Microalbuminuria(mg/l)	14.94 ± 4.23 **a**	99.54±36.03 **b**	196.91 ± 41.78 **c**
CHOL(mg/dl)	176.64± 19.80 **a**	195.19± 11.24 **b**	231.24 ± 11.89 **c**
TG(mg/dl)	117.43 ± 28.19 **a**	187.71± 8.81 **b**	231.77 ± 16.84 **c**
HDL(mg/dl)	43.44 ± 10.14 **a**	30.59± 4.17 **b**	30.54 ± 4.85 **b**
LDL(mg/dl)	111.39 ± 19.38 **a**	127.21± 13.40 **b**	154.33 ± 12.51 **c**
VLDL(mg/dl)	23.18±5.55 **a**	37.51±1.79 **b**	46.53±3.36 **c**
Chymase(ng/ml)	2.28 ± 0.18 **a**	2.76± 0.19 **a**	4.80 ± 1.96 **b**
AngiotensinII(ng/l)	26.21 ± 2.54 **a**	36.72± 5.05 **b**	62.33± 12.18 **c**

- **One-Way ANOVA Test (normal distribution) and Kruskal-Wallis Test (abnormal distribution), S.D: Standard Deviation.**
- **Different letters in the same raw refer to significant differences ($p \leq 0.05$)**

3.9. The relationship between Chymase and AngiotensinII with some parameters

3.9.1 The Correlation of HbA1c, FBS, blood urea, serum creatinine, Chymase, and ANGII levels in patients

To assess the relationship between the blood levels of HbA1c, FBS, blood urea, serum creatinine, Chymase, and ANGII levels in patients, linear regression analysis was used to evaluate the data. See Table (3-9).

Table (3-9): Results of linear regression analysis of HbA1c, FBS, blood urea, serum creatinine, Chymase, and ANGII levels in patients

parameter	FBS		HbA1c		Blood urea		Serum creatinine		Chymase		ANGII	
	r	p	r	p	r	p	r	p	r	p	r	p
FBS			0.94	<0.001	0.84	<0.001	0.80	<0.001	0.71	<0.001	0.88	<0.001
HbA1c	0.94	<0.001			0.88	<0.001	0.87	<0.001	0.79	<0.001	0.93	<0.001
Blood urea	0.84	<0.001	0.88	<0.001			0.96	<0.001	0.75	<0.001	0.90	<0.001
Serum creatinine	0.80	<0.001	0.87	<0.001	0.96	<0.001			0.84	<0.001	0.93	<0.001
Chymase	0.71	<0.001	0.79	<0.001	0.75	<0.001	0.84	<0.001			0.90	<0.001
ANGII	0.88	<0.001	0.93	<0.001	0.90	<0.001	0.93	<0.001	0.90	<0.001		

3.9.1.1. The relationship between Chymase and ANGII Opposite FBS

The study revealed a positive linear regression between Chymase and ANGII with fasting blood glucose. In Chymase the positive linear regression (Figure 3-6) was according to linear equation (Y=0.05X-4.9) at level P<0.001 and correlation coefficient r=0.71.while ANGII the positive linear regression (Figure 3-7) was according to linear equation (Y=0.63X-69.9) at level P<0.001 and correlation coefficient r=0.88.

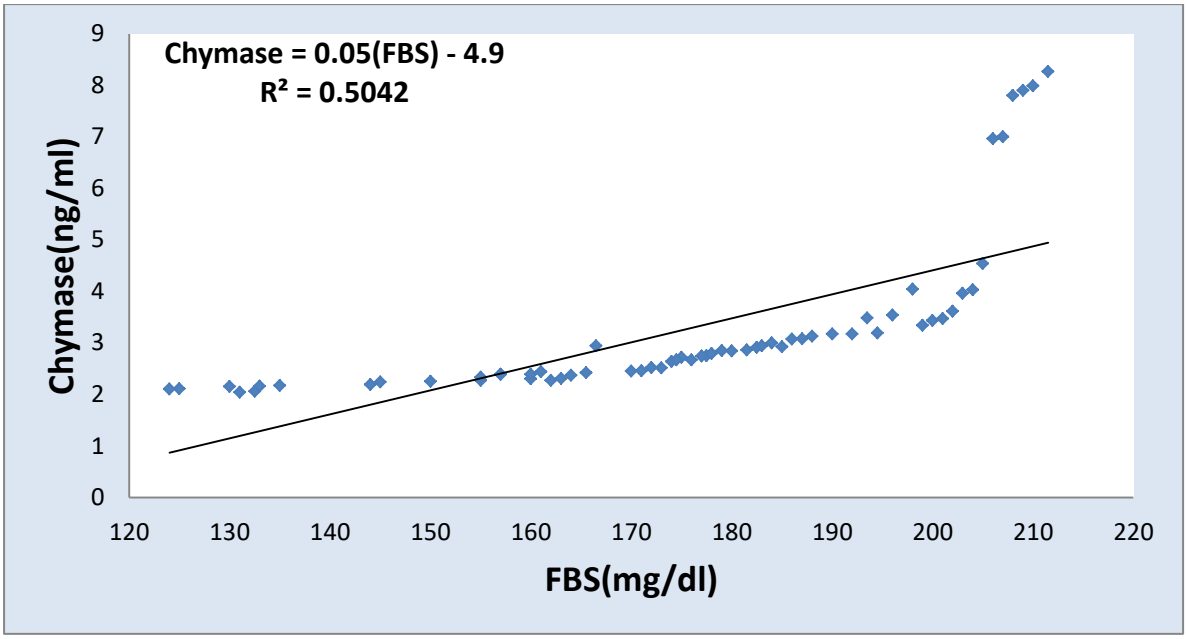

Figure (3-6): The relationship between fasting blood glucose (mg/dl) and Chymase (ng/ml)

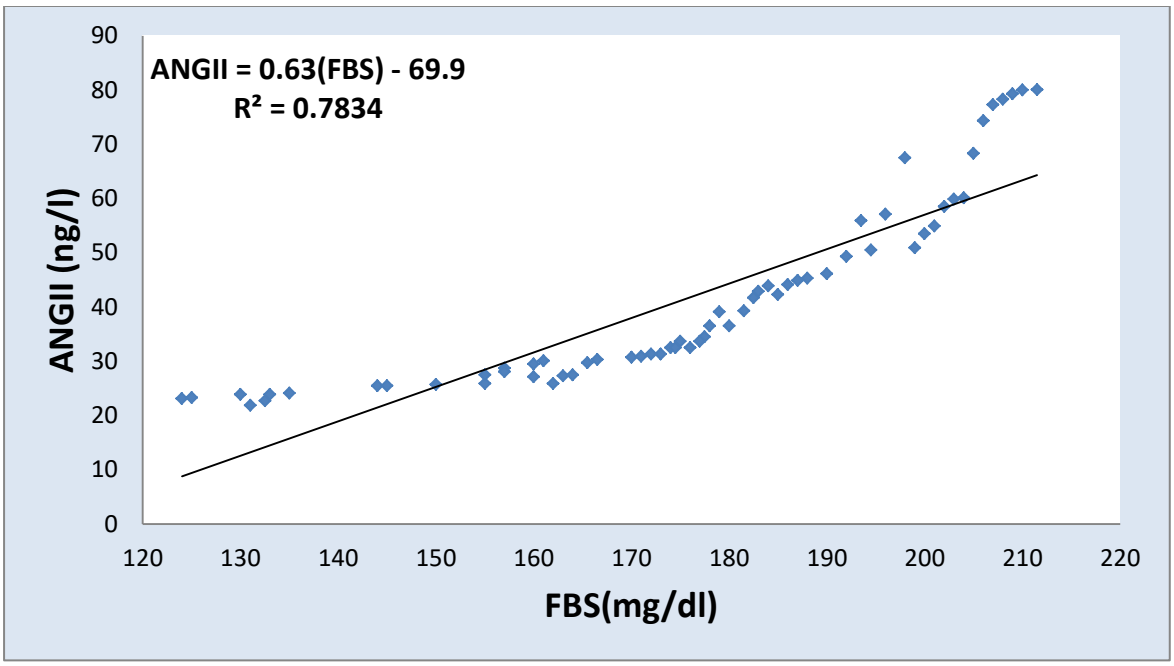

Figure (3-7): The relationship between fasting blood glucose (mg/dl) and ANGII (ng/l)

3.9.1.2. The relationship between Chymase and ANGII Opposite HbA_1c

Figure (3-8) showed a positive correlation between the Chymase level and HbA1c percentage in patients was according to linear equation (Y=0.67X-4.76) at level P<0.001 and correlation coefficient r=0.79, as for Figure (3-9) showed ANGII the positive linear regression was according to linear equation (Y=8.69X-36.3) at level P<0.001 and correlation coefficient r=0.93.

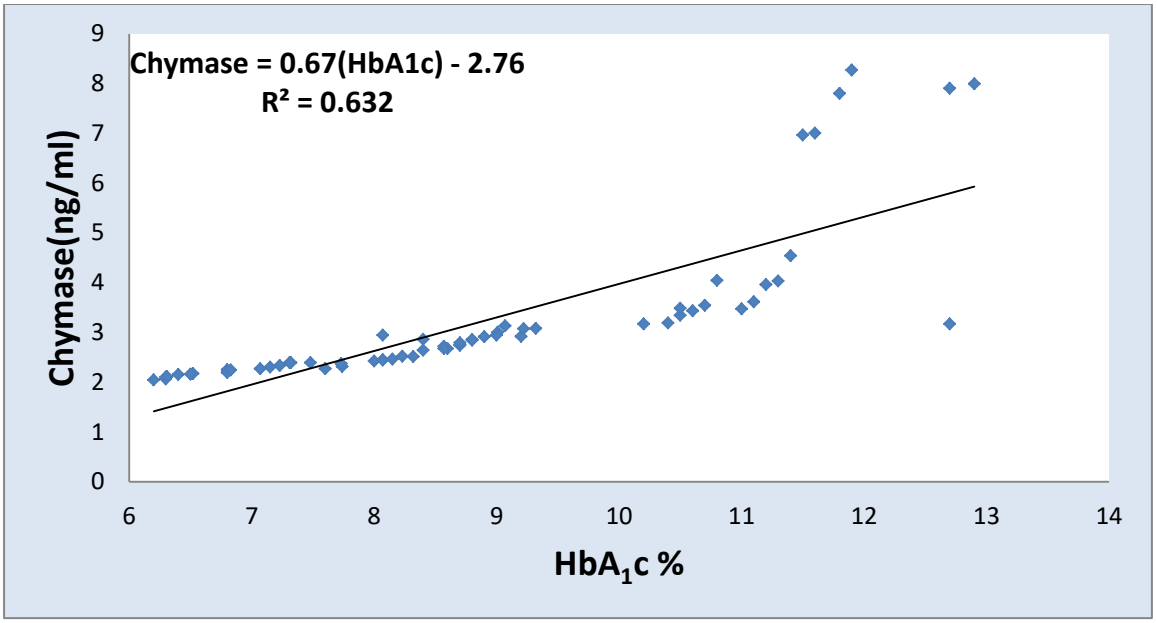

Figure (3-8): The relationship HbA_1c % and Chymase (ng/ml)

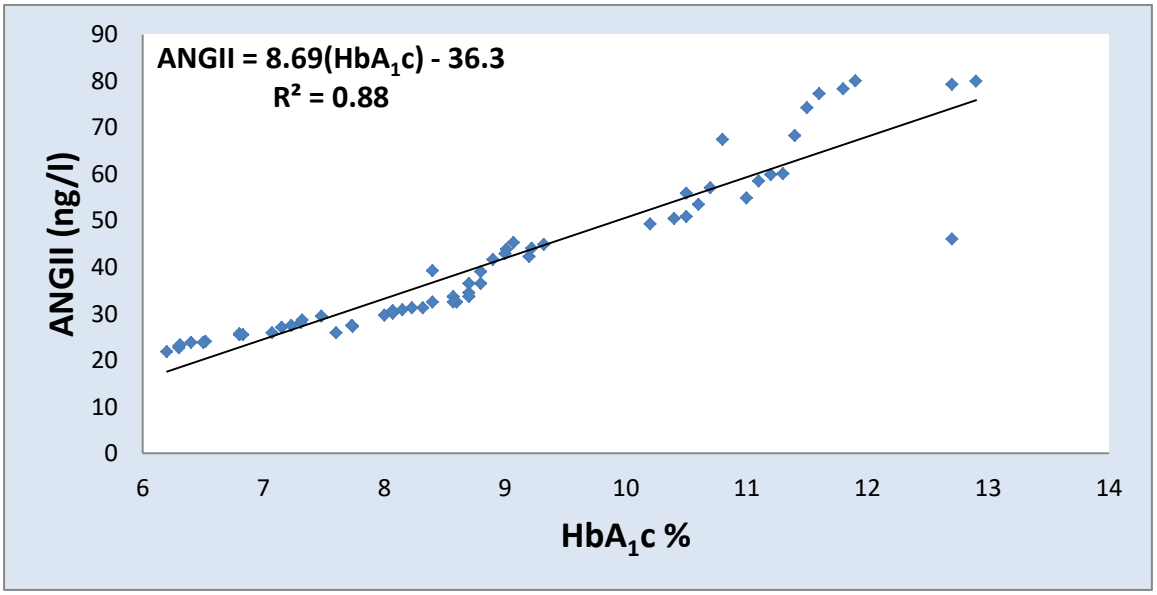

Figure (3-9): The relationship between HbA_1c % and ANGII (ng/l)

3.9.1.3. The relationship between Chymase and ANGII Opposite blood urea

The study showed a positive linear regression between Chymase and ANGII with blood urea, In Chymase the positive linear regression (Figure 3-10) was according to linear equation (Y=0.02X+ 1.13) at level P<0.001 and correlation coefficient r=0.75, while ANGII the positive linear regression (Figure 3-11) was according to linear equation (Y=0.37X-14.07) at level P<0.001 and correlation coefficient r=0.90

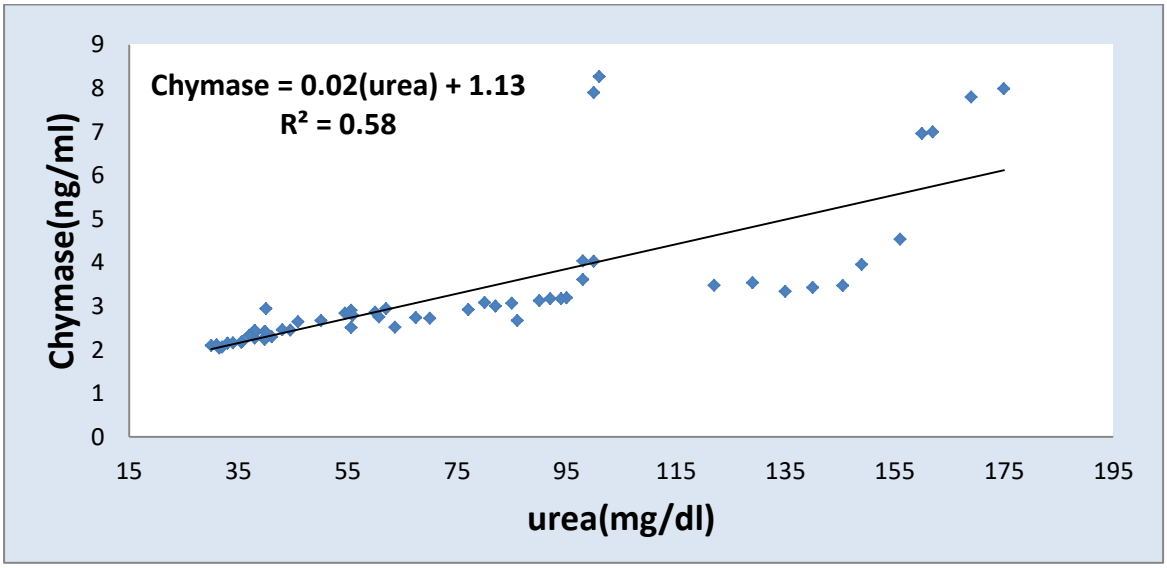

Figure (3-10): The relationship between blood urea (mg/dl) and Chymase (ng/ml)

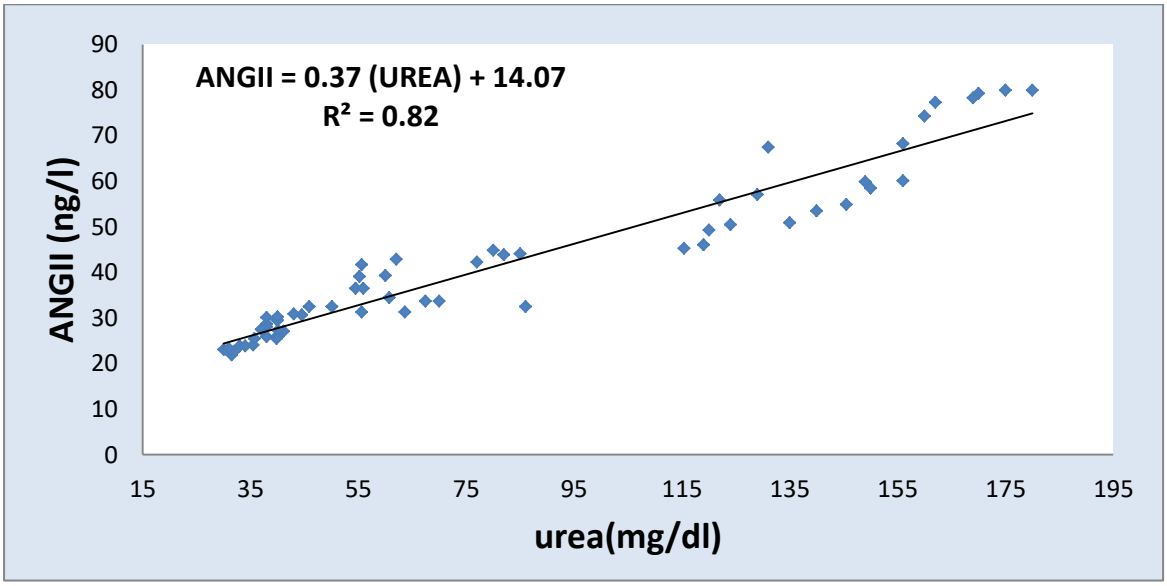

Figure (3-11): The relationship between blood urea (mg/dl) and ANGII (ng/l)

3.9.1.4. The relationship between Chymase and ANGII Opposite serum creatinine

The study found a positive linear regression between Chymase and ANGII with serum creatinine, In Chymase the positive linear regression (Figure 3-12) was according to linear equation (Y=1.16X+0.93) at level P<0.001 and correlation coefficient r=0.84. while ANGII the positive linear regression (Figure 3-13) was according to linear equation (Y=14.08X+13.9) at level P<0.001 and correlation coefficient r=0.93

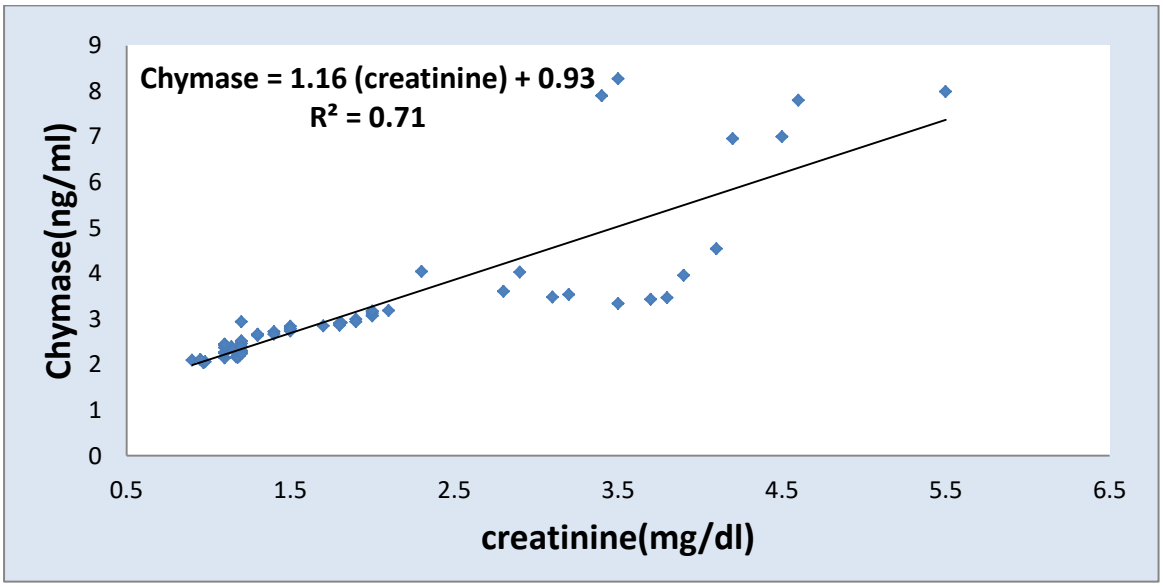

Figure (3-12): The relationship between serum creatinine (mg/dl) and Chymase (ng/ml)

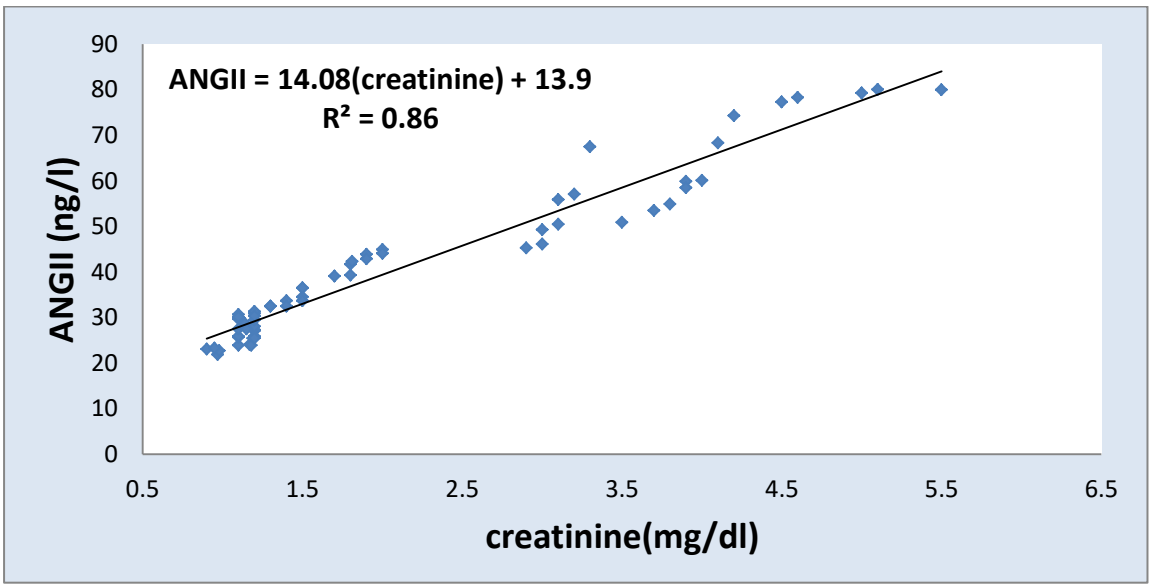

Figure (3-13): The relationship between serum creatinine (mg/dl) and ANGII (ng/l)

3.9.2 The Correlation of lipid profile with Chymase and ANGII levels in patients

To assess the relationship between the blood levels of lipid profile with Chymase and ANGII levels in patients, linear regression analysis was used to evaluate the data. See Table (3-10).

Table (3-10): Results of linear regression analysis of lipid profile with Chymase and ANGII levels in patients

parameter	CHOL		TG		HDL		LDL		VLDL		Chymase		ANGII	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
CHOL			0.88	<0.001	-0.58	<0.001	0.97	<0.001	0.88	<0.001	0.74	<0.001	0.88	<0.001
TG	0.88	<0.001			-0.77	<0.001	0.84	<0.001	0.99	<0.001	0.70	<0.001	0.86	<0.001
HDL	-0.58	<0.001	-0.77	<0.001			-0.68	<0.001	-0.77	<0.001	-0.44	<0.001	-0.56	<0.001
LDL	0.97	<0.001	0.84	<0.001	-0.68	<0.001			0.85	<0.001	0.70	<0.001	0.83	<0.001
VLDL	0.88	<0.001	0.99	<0.001	-0.77	<0.001	0.85	<0.001			0.70	<0.001	0.90	<0.001
Chymase	0.74	<0.001	0.70	<0.001	-0.44	<0.001	0.70	<0.001	0.70	<0.001			0.90	<0.001
ANGII	0.88	<0.001	0.86	<0.001	-0.56	<0.001	0.83	<0.001	0.86	<0.001	0.90	<0.001		

3.9.2.1. The relationship between Chymase and ANGII Opposite CHOL

The study revealed a positive linear regression between Chymase and ANGII with cholesterol. In Chymase the positive linear regression (Figure 3-14) was according to linear equation (Y=0.04X-5.23) at level P<0.001 and correlation coefficient r=0.74.while ANGII the positive linear regression (Figure 3-15) was according to linear equation (Y=0.55X-69.1) at level P<0.001 and correlation coefficient r=0.88.

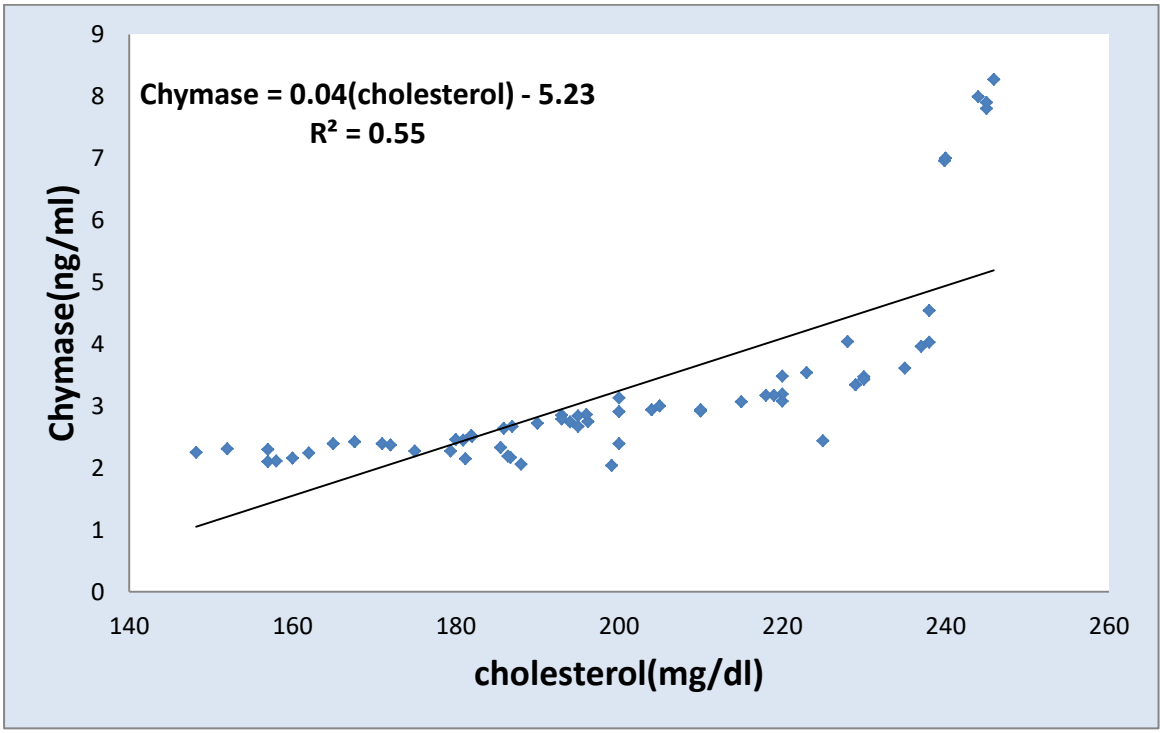

Figure (3-14): The relationship between cholesterol (mg/dl) and Chymase (ng/ml)

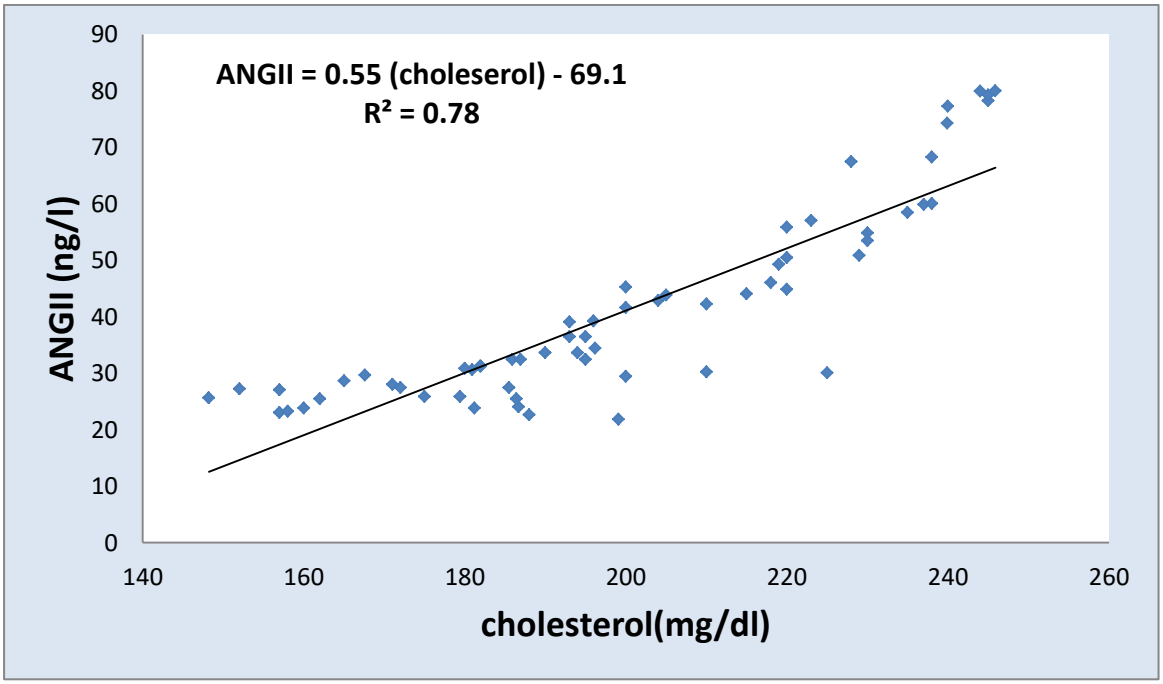

Figure (3-15): The relationship between cholesterol (mg/dl) and ANGII (ng/l)

3.9.2.2. The relationship between Chymase and ANGII Opposite TG

The study showed a positive linear regression between Chymase and ANGII with TG, In Chymase the positive linear regression (Figure 3-16) was according to linear equation (Y=0.02X-0.49) at level P<0.001 and correlation coefficient r=0.70. while ANGII the positive linear regression (Figure 3-17) was according to linear equation (Y=0.28X-8.91) at level P<0.001 and correlation coefficient r=0.86.

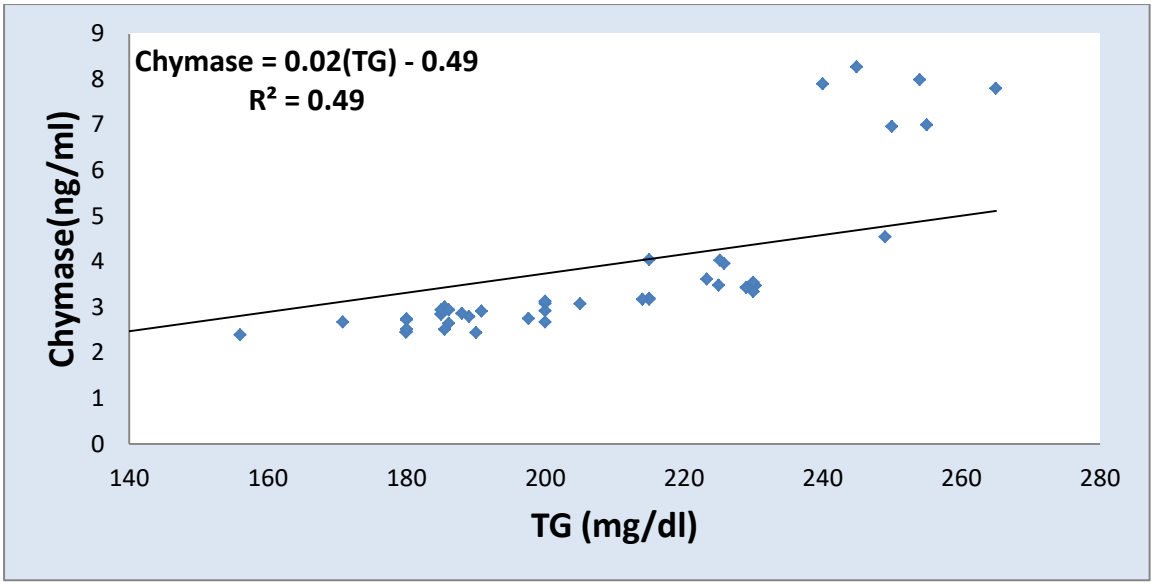

Figure (3-16): The relationship between TG (mg/dl) and Chymase (ng/ml)

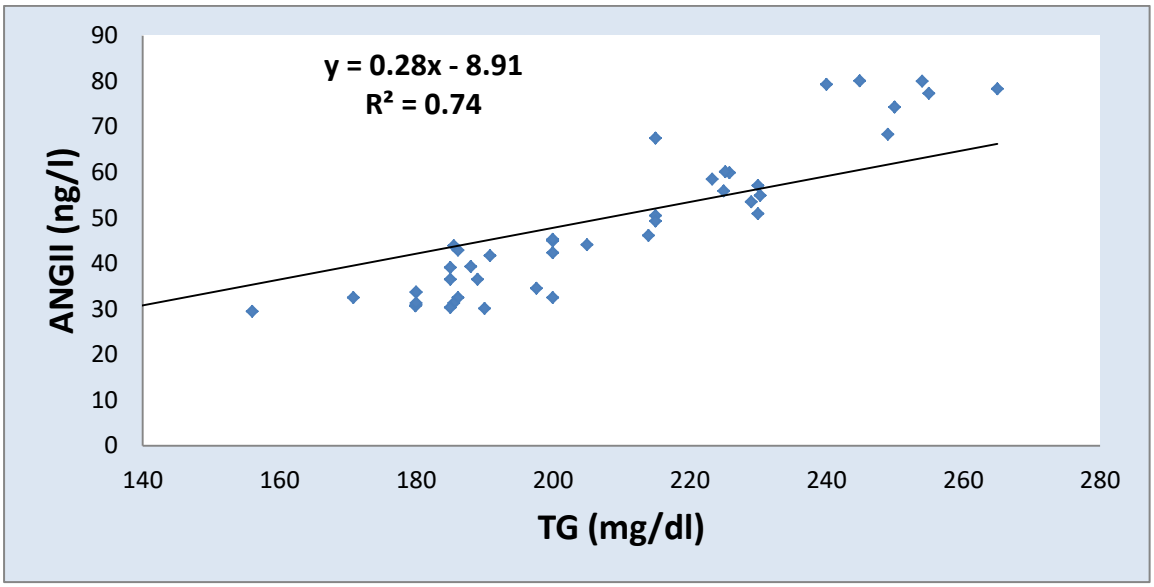

Figure (3-17): The relationship between TG (mg/dl) and ANGII (ng/l)

3.9.2.3. The relationship between Chymase and ANGII Opposite HDL

The study showed a negative linear regression between Chymase and ANGII with HDL, In Chymase the negative linear regression (Figure 3-18) was according to linear equation (Y=0.07X-5.87) at level P<0.001 and correlation coefficient r= -0.44. while ANGII the negative linear regression (Figure 3-19) was according to linear equation (Y=1.04X-77.7) at level P<0.001 and correlation coefficient r= -0.56.

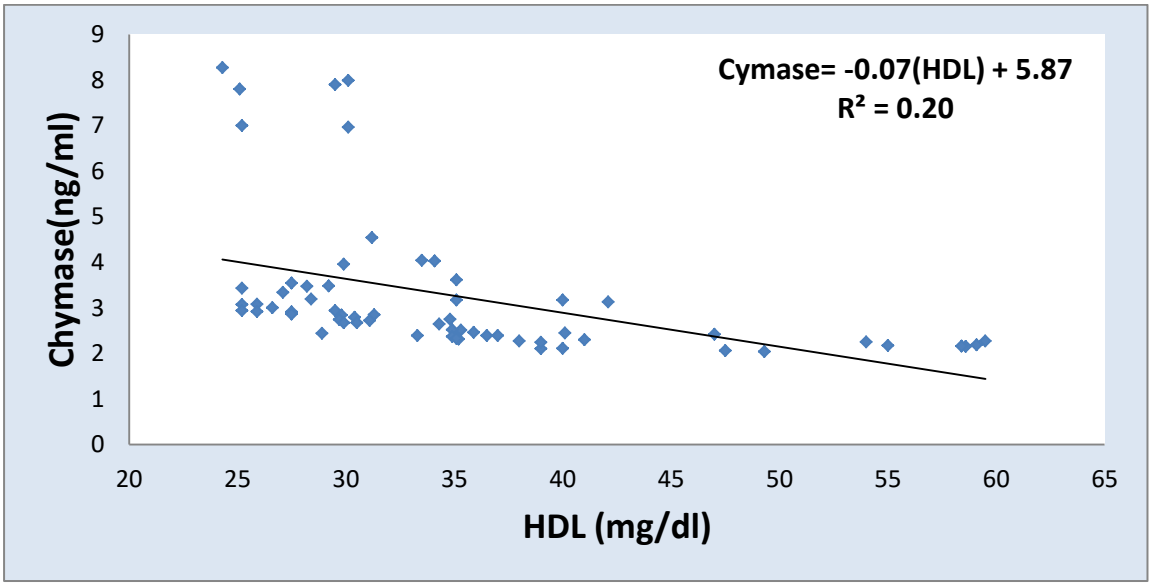

Figure (3-18): The relationship between HDL (mg/dl) and Chymase (ng/ml)

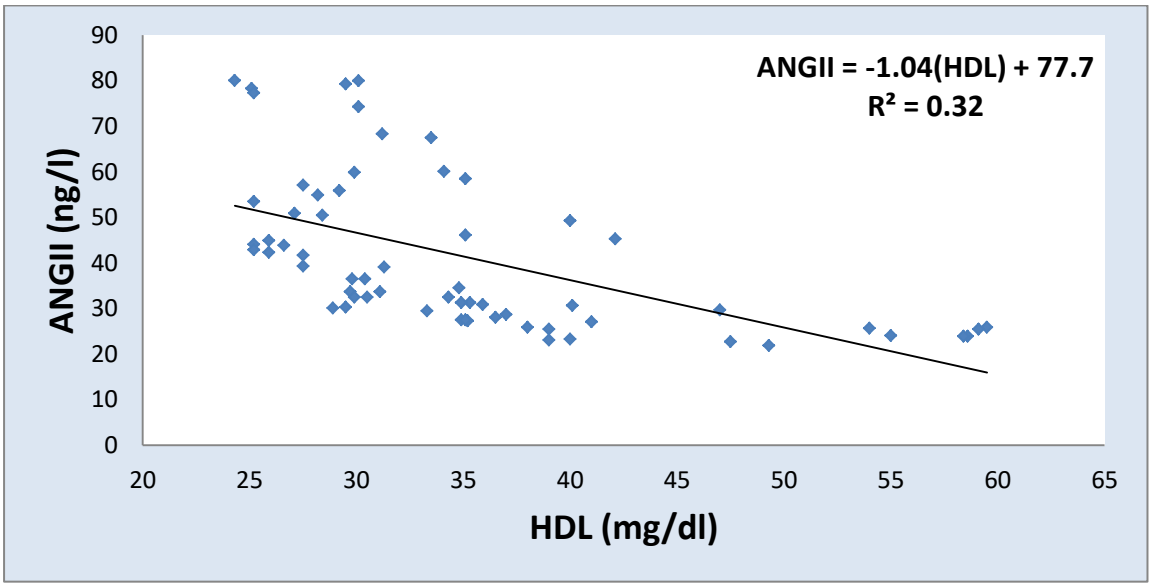

Figure (3-19): The relationship between HDL (mg/dl) and ANGII (ng/l)

3.9.2.4. The relationship between Chymase and ANGII Opposite LDL

The study revealed a positive linear regression between Chymase and ANGII with LDL, In Chymase the positive linear regression (Figure 3-20) was according to linear equation (Y=0.05X-2.82) at level P<0.001 and correlation coefficient r=0.70. while ANGII the positive linear regression (Figure 3-21) was according to linear equation (Y=0.61X-37.8) at level P<0.001 and correlation coefficient r=0.83.

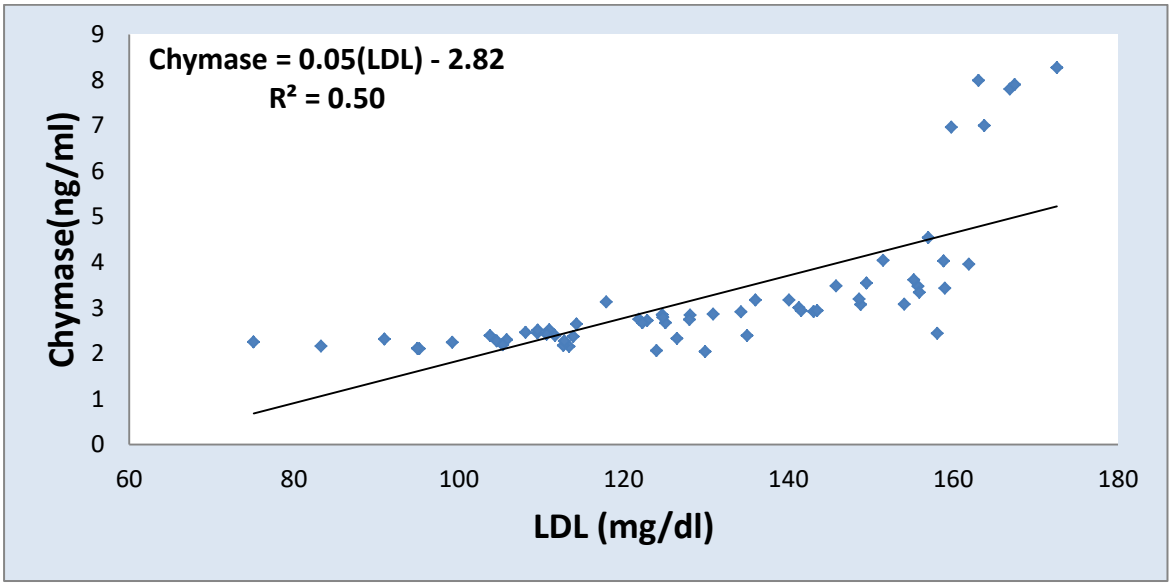

Figure (3-20): The relationship between LDL (mg/dl) and Chymase (ng/ml)

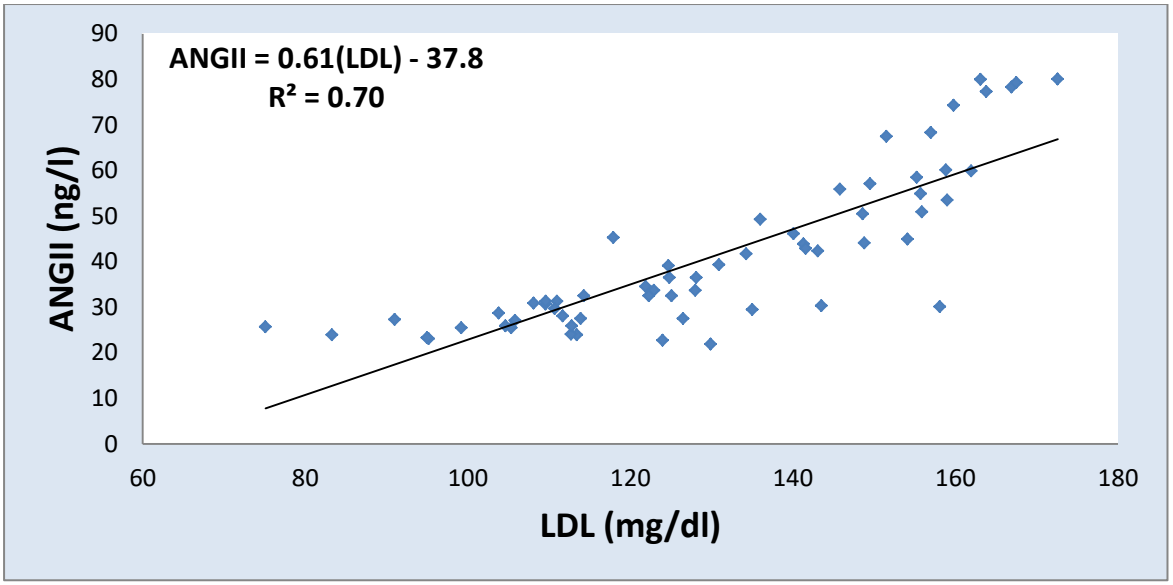

Figure (3-21): The relationship between LDL (mg/dl) and ANGII (ng/l)

3.9.2.5. The relationship between Chymase and ANGII Opposite VLDL

The study found a positive linear regression between Chymase and ANGII with VLDL, In Chymase the positive linear regression (Figure 3-22) was according to linear equation (Y=0.11X-0.57) at level P<0.001 and correlation coefficient r=0.70. while ANGII the positive linear regression (Figure 3-23) was according to linear equation (Y=1.44X-9.8) at level P<0.001 and correlation coefficient r=0.86.

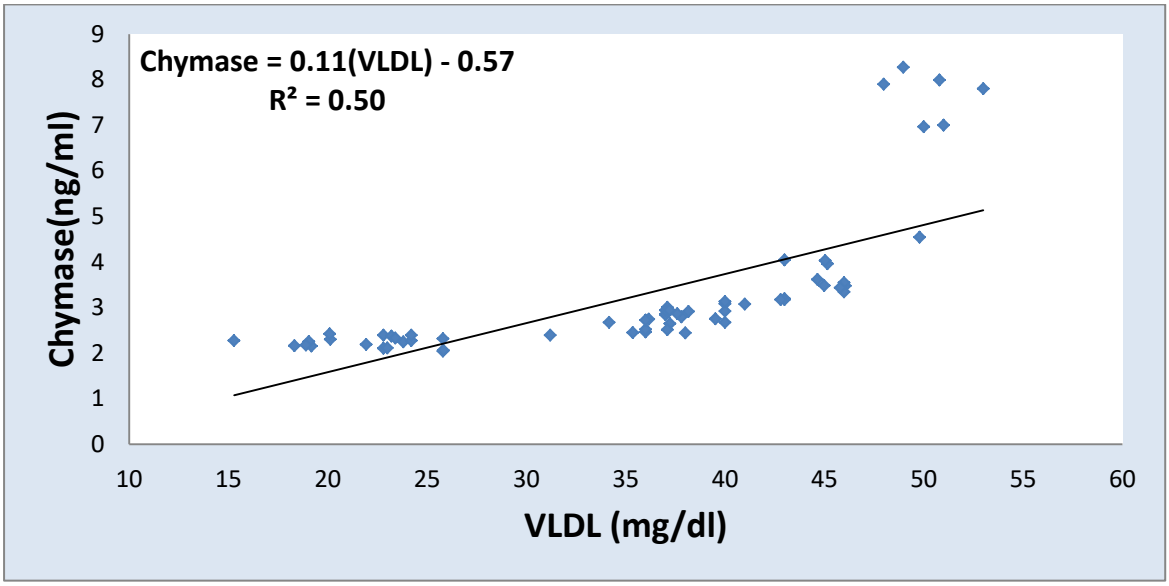

Figure (3-22): The relationship between VLDL (mg/dl) and Chymase (ng/ml)

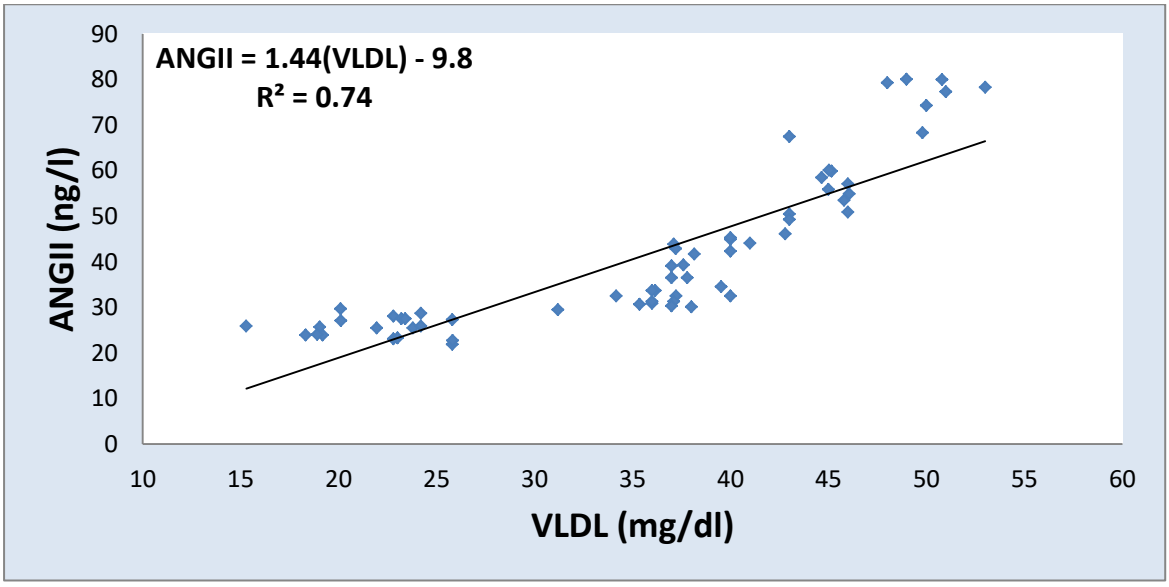

Figure (3-23): The relationship between VLDL (mg/dl) and ANGII (ng/l)

3.9.3. The Correlation of age, BMI, duration of disease, Chymase, and ANGII levels in patients

To assess the relationship between the blood levels of age, BMI, duration of disease, Chymase, and ANGII levels in patients, linear regression analysis was used to evaluate the data. See Table (3-11).

Table (3-11): Results of linear regression analysis of age, BMI, duration of disease, Chymase, and ANGII levels in patients

parameter	AGE		BMI		DURATION DISEASE		Chymase		ANGII	
	r	p	r	p	r	p	r	p	r	p
AGE			0.27	0.082	0.73	<0.001	0.44	<0.001	0.46	<0.001
BMI	0.27	0.028			0.46	<0.001	0.26	0.038	0.34	0.006
DURATION DISEASE	0.73	<0.001	0.46	<0.001			0.67	<0.001	0.80	<0.001
Chymase	0.44	<0.001	0.26	0.083	0.67	<0.001			0.90	<0.001
ANGII	0.46	<0.001	0.34	0.006	0.80	<0.001	0.90	<0.001		

3.9.3.1. The relationship between Chymase and ANGII Opposite age

The study revealed a positive linear regression between Chymase and ANGII with age, In Chymase the positive linear regression (Figure 3-24) was according to linear equation (Y=0.06X-0.51) at level P<0.001 and correlation coefficient r=0.44.while ANGII the positive linear regression (Figure 3-25) was according to linear equation (Y=0.75X-2.42) at level P<0.001 and correlation coefficient r=0.46.

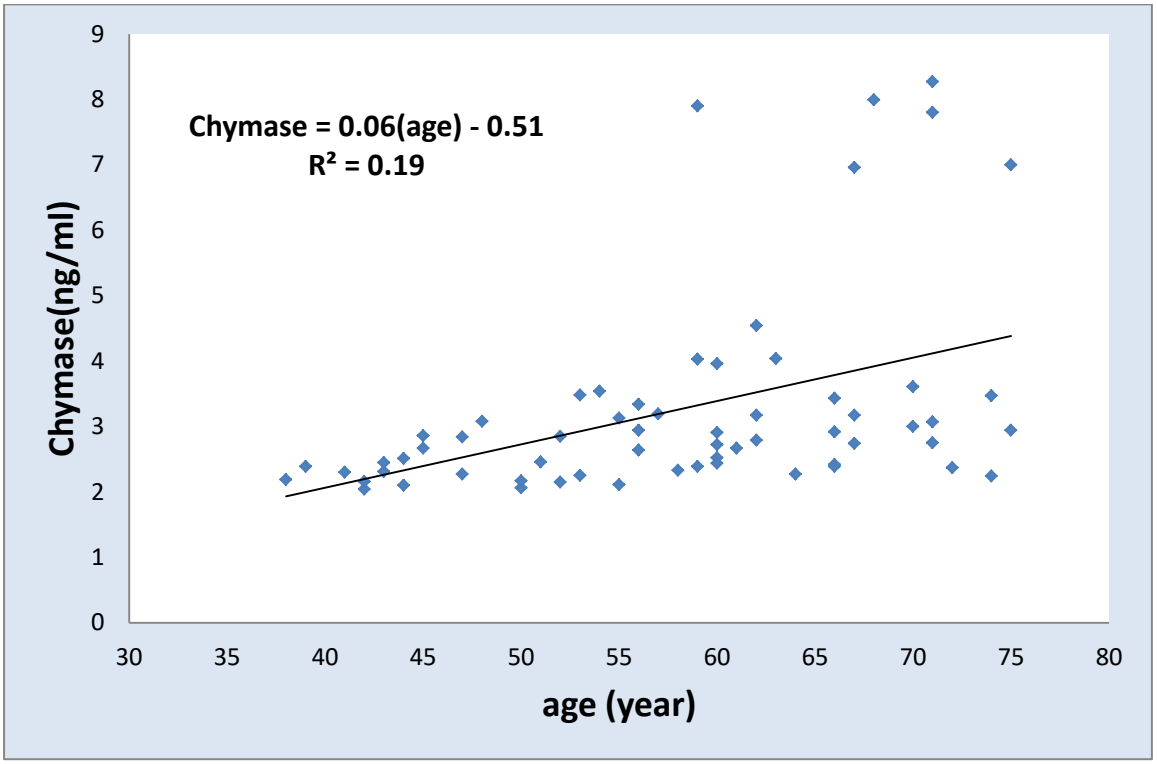

Figure (3-24): The relationship between age (year) and Chymase (ng/ml)

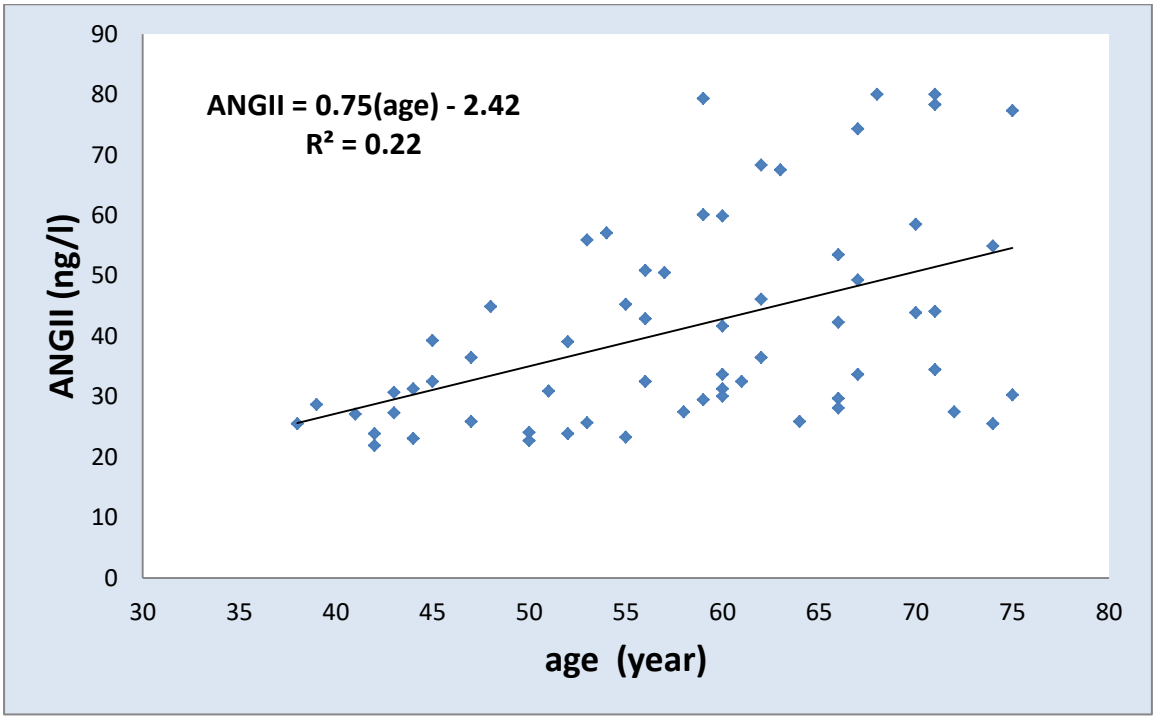

Figure (3-25): The relationship between age (year) and ANGII (ng/l)

3.9.3.2. The relationship between Chymase and ANGII Opposite BMI

The study showed a positive linear regression between Chymase and ANGII with BMI, In Chymase the positive linear regression (Figure 3-26) was according to linear equation (Y=0.09X+0.63) at level P=0.086 and correlation coefficient r=0.26. while ANGII the positive linear regression (Figure 3-27) was according to linear equation (Y=1.34X+3.72) at level P=0.006 and correlation coefficient r=0.34.

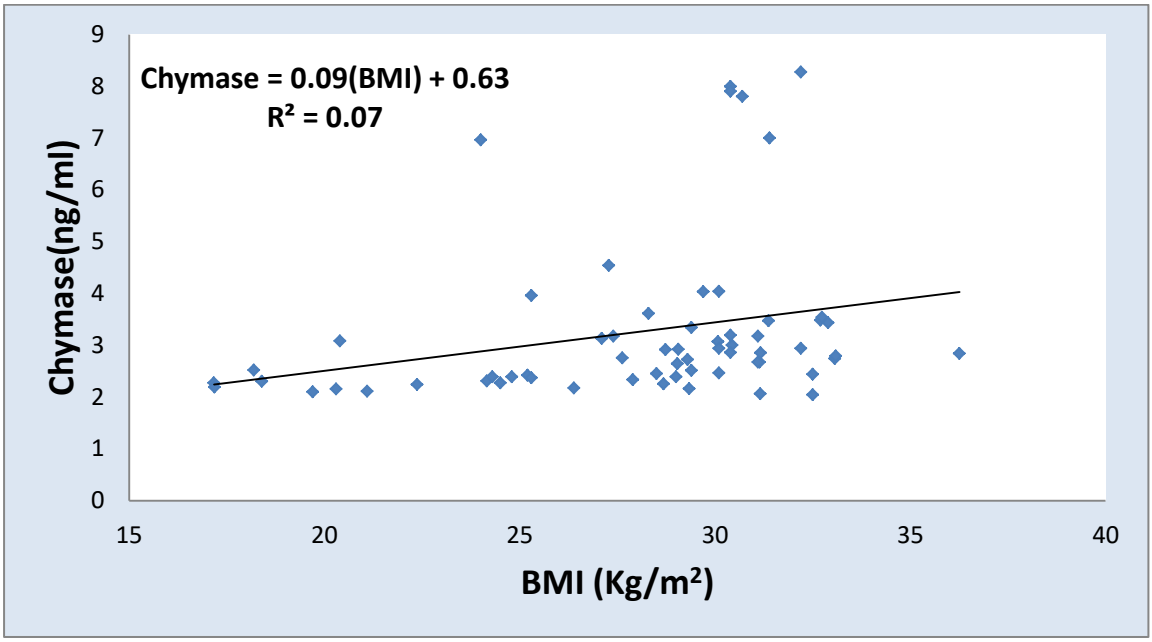

Figure (3-26): The relationship between BMI (Kg/m^2) and Chymase (ng/ml)

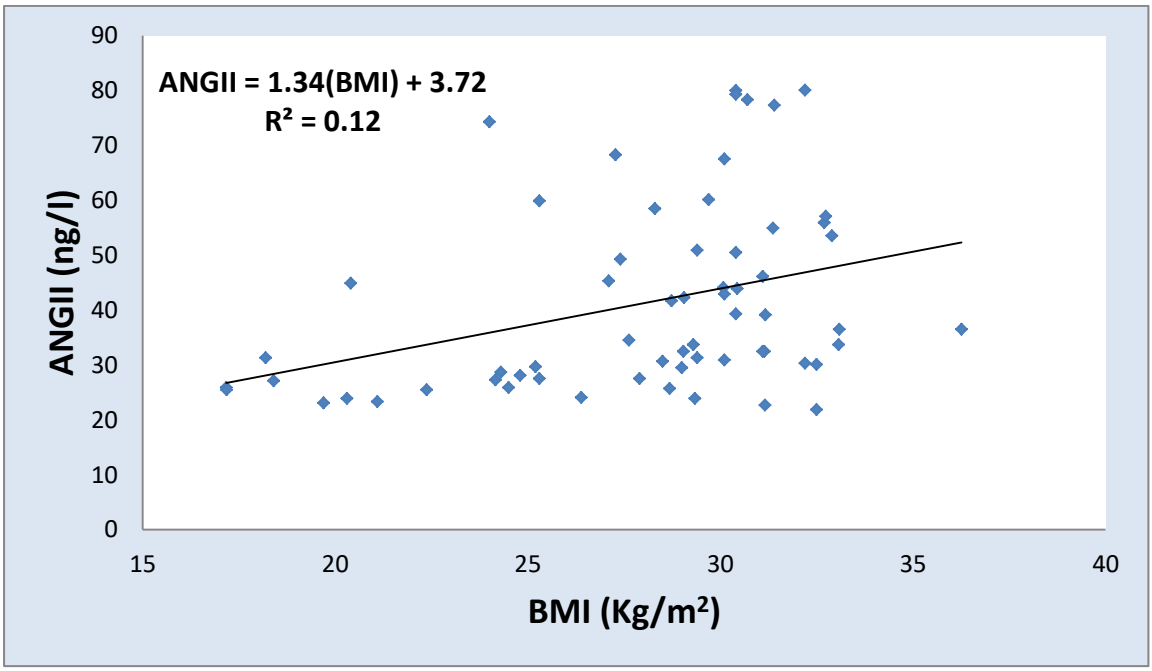

Figure (3-27): The relationship between BMI (Kg/m^2) and ANGII (ng/l)

3.9.3.3. The relationship between Chymase and ANGII Opposite duration disease

The study revealed a positive linear regression between Chymase and ANGII with duration disease, In Chymase the positive linear regression (Figure 3-28) was according to linear equation (Y=0.17X+1.32) at level P<0.001 and correlation coefficient r=0.67. while ANGII the positive linear regression (Figure 3-29) was according to linear equation (Y=2.22X+16.16) at level P<0.001 and correlation coefficient r=0.80.

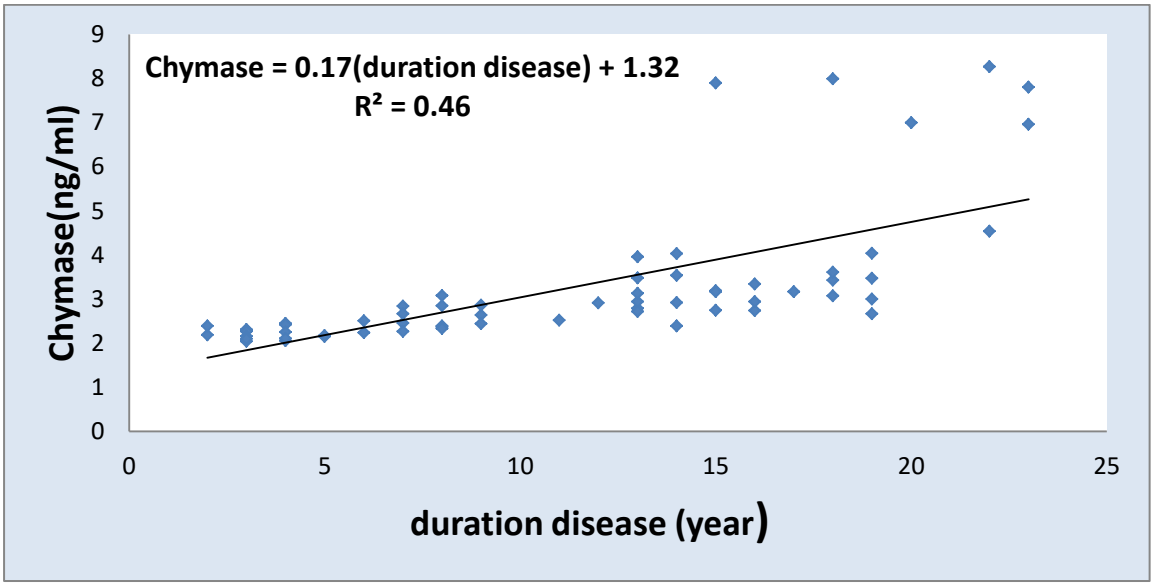

Figure (3-28): The relationship between duration of disease (year) and Chymase (ng/ml)

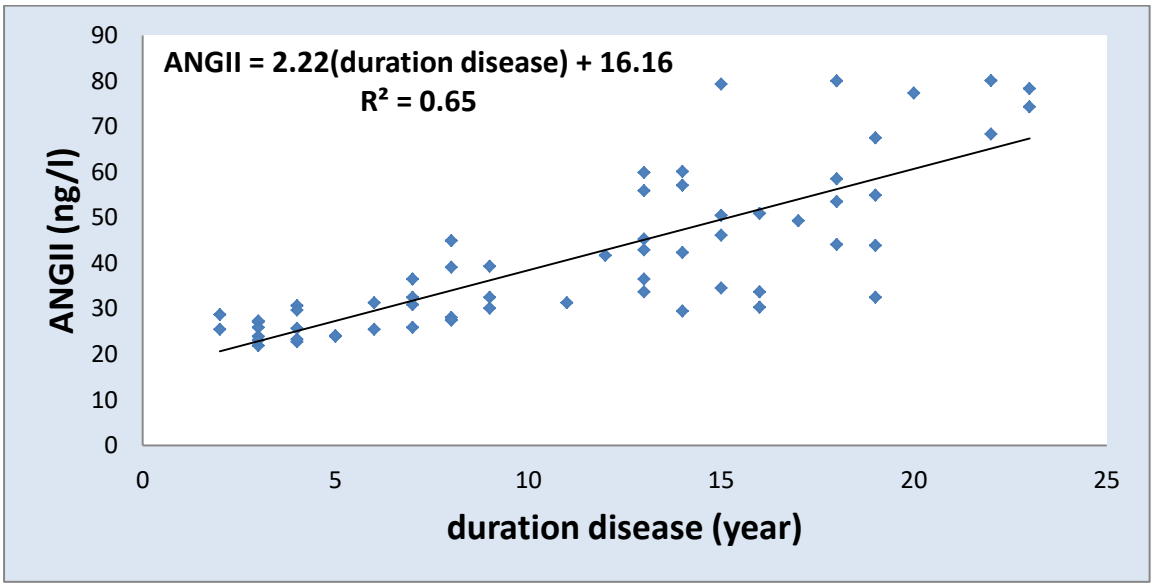

Figure (3-29): The relationship between duration of disease (year) and ANGII (ng/l)

3.9.4. The Correlation of Microalbuminuria, albumin-creatinine ratio (ACR), Chymase, and ANGII levels in patients

To assess the relationship between the levels of Microalbuminuria, albumin-creatinine ratio (ACR), Chymase, and ANGII levels in patients, the linear regression analysis was used to evaluate the data. See Table (3-12).

Table (3-12): Results of linear regression analysis Microalbuminuria, albumin-creatinine ratio (ACR), Chymase, and ANGII levels in patients

parameter	Microalbuminuria		ACR		Chymase		ANGII	
	r	p	r	p	r	p	r	p
Microalbuminuria			0.93	<0.001	0.57	<0.001	0.82	<0.001
ACR	0.93	<0.001			0.70	<0.001	0.91	<0.001
Chymase	0.57	<0.001	0.70	<0.001			0.90	<0.001
ANGII	0.82	<0.001	0.91	<0.001	0.90	<0.001		

3.9.4.1. The relationship between Chymase and ANGII Opposite Microalbuminuria

The study revealed a positive linear regression between Chymase and ANGII with Microalbuminuria, In Chymase the positive linear regression (Figure 3-30) was according to linear equation (Y=0.01X+2.15) at level P<0.001 and correlation coefficient r=0.57 while ANGII the positive linear regression (Figure 3-31) was according to linear equation (Y=0.16X+28.6) at level P<0.001 and correlation coefficient r=0.82.

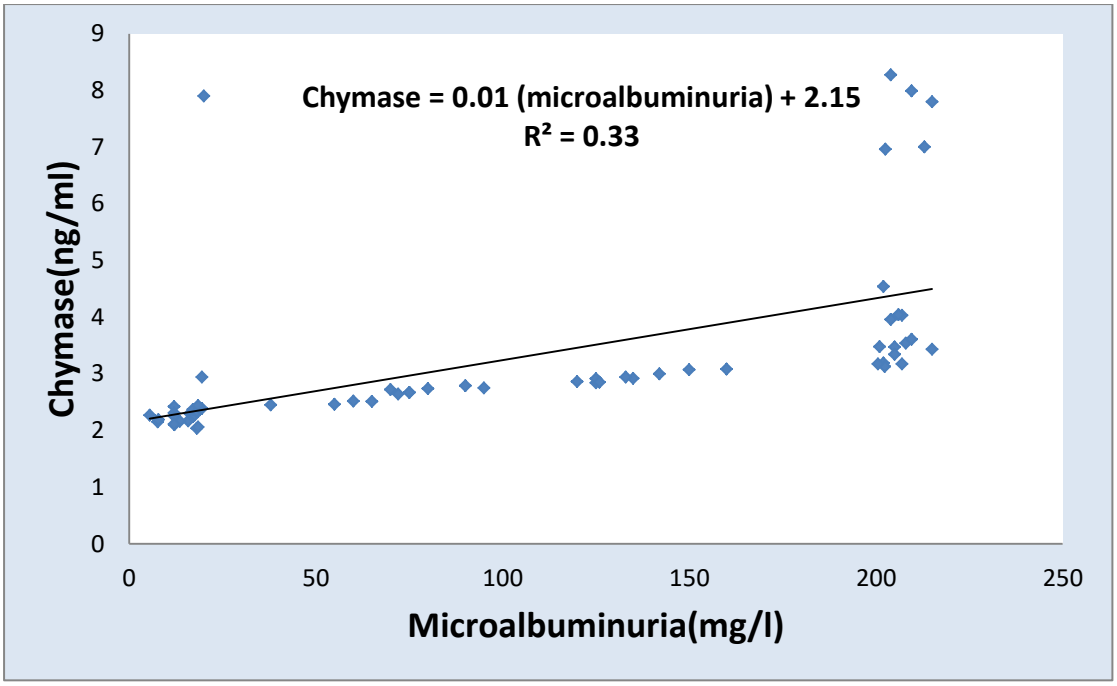

Figure (3-30): The relationship between Microalbuminuria (mg/l) and Chymase (ng/ml)

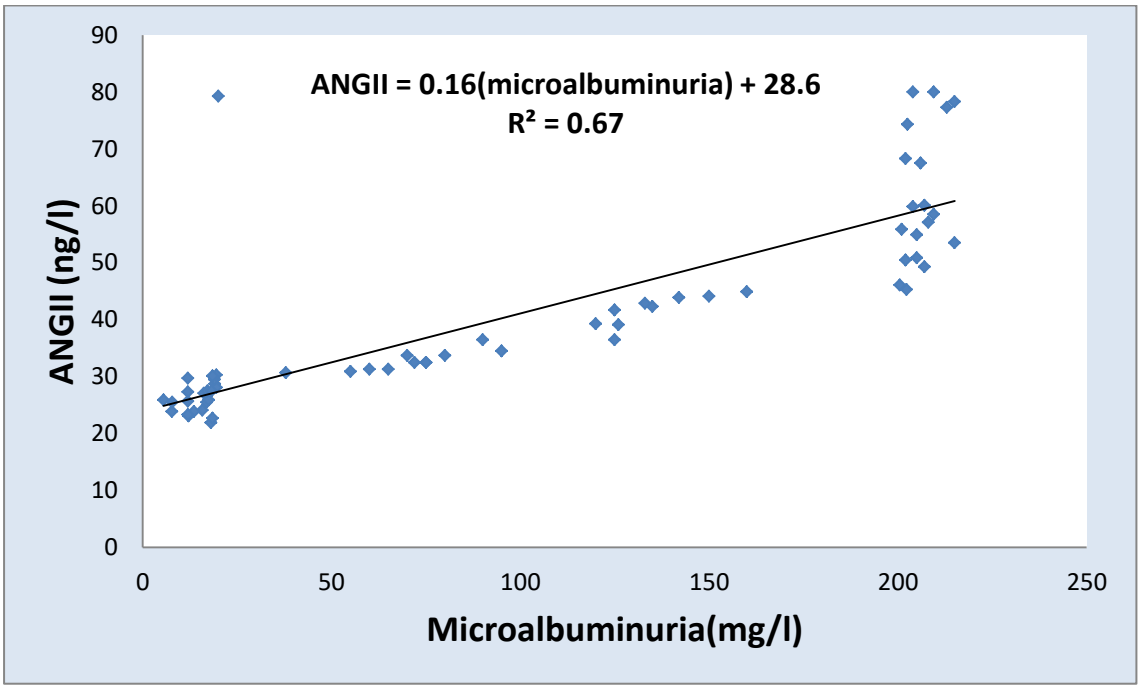

Figure (3-31): The relationship between Microalbuminuria (mg/l) and ANGII (ng/l)

3.9.4.2. The relationship between Chymase and ANGII Opposite ACR

The study revealed a positive linear regression between Chymase and ANGII with ACR, In Chymase the positive linear regression (Figure 3-32) was according to linear equation (Y=0.009X+1.99) at level P<0.001 and correlation coefficient r=0.70, while ANGII the positive linear regression (Figure 3-33) was according to linear equation (Y=0.12X+23.50) at level P<0.001 and correlation coefficient r=0.91.

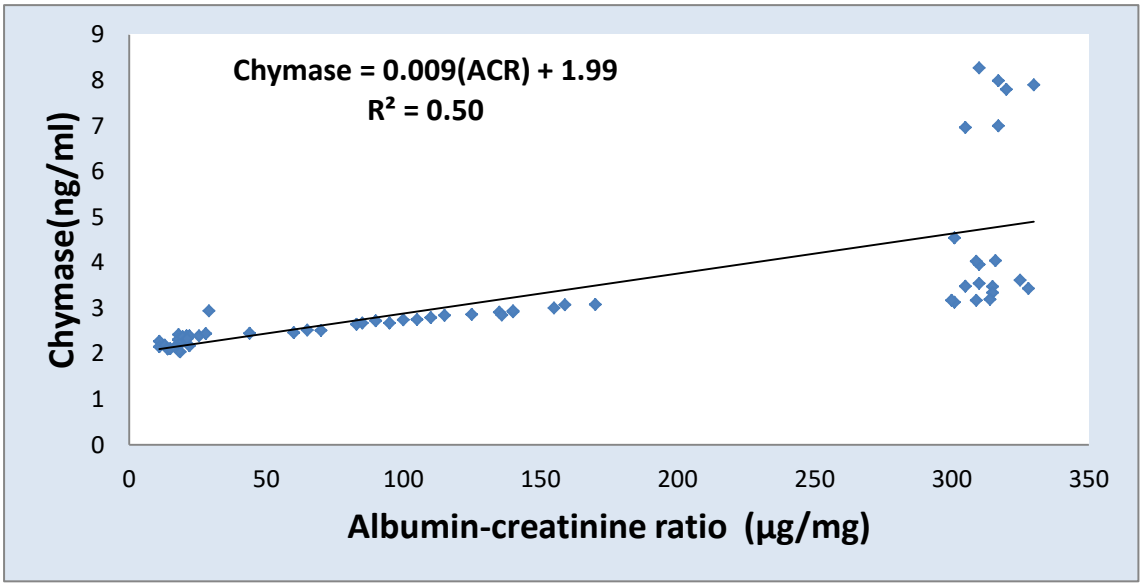

Figure (3-32): The relationship between Albumin-creatinine ratio (µg/mg) and Chymase (ng/ml)

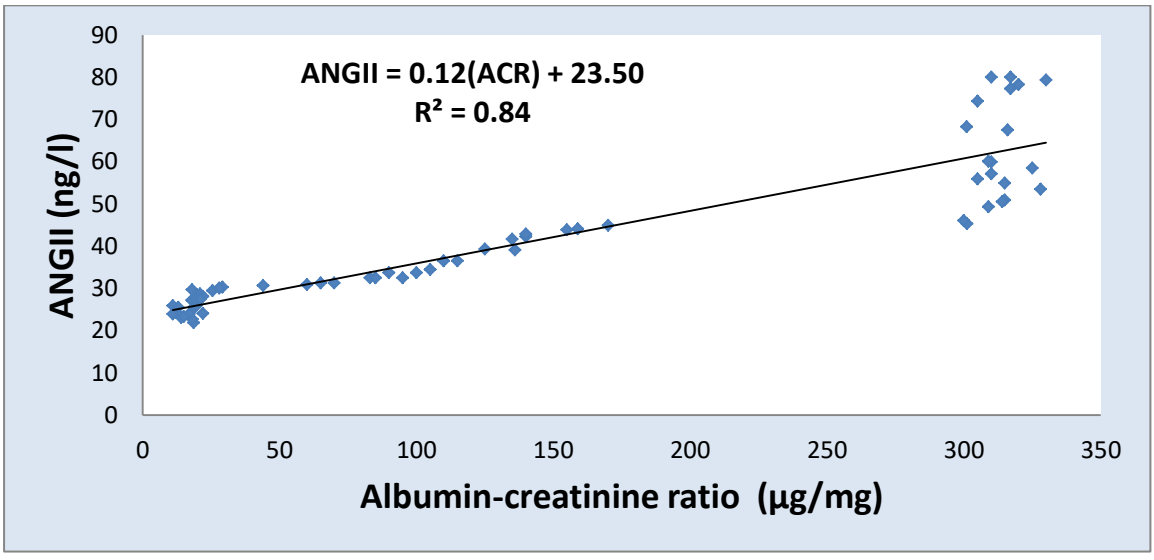

Figure (3-33): The relationship between Albumin-creatinine ratio (µg/mg) and ANGII (ng/l)

Chapter FOUR

Discussion of Result

4. Discussion

Diabetes mellitus has long been recognized as a major health problem with far-reaching consequences, not only for its adverse health impact on individuals but also for its economic burden on the health care system and society at large**.**

It is the sixth leading cause of death by disease in the United States, accounting for almost 18% of all deaths in people over 25 years of age, and it is the leading cause of the end-stage renal disease (ESRD), new cases of blindness, and non – traumatic lower-limb amputations. Cardiovascular disease is the major cause of diabetes-related death and is two to four times more common in patients with diabetes than in the general population, life expectancy in middle-aged patients is reduced by 5 to 10 years due to cardiovascular disease (Barnett and Braunstein, 2001)

The International Diabetes Federation (IDF) in 2003 ranked the UAE's prevalence rate for type 2 diabetes and impaired glucose tolerance (IGT) as the second-highest in the world (20%for DM and 26% for IGT), also it is estimated that the prevalence of type 2 diabetes and IGT in Saudi Arabia in 2003 was 4.4% and its prevalence will increase to 9.6% in 2025 and Iraq in 2003 was 7.7% and in 2025 will become 9.1 (Al-Maskari *et al*., 2007).

4.1. Association of diabetic type2 with diabetic nephropathy groups and control groups with Study Variables

The result of the demographic study showed that there were more patients at age ≥ (45-60) years old (41.9 %) compared to the control group (28.6%) Table (3-1and 3- 2), this study is in agreement with the results of a study conducted in Saudi Arabia in 2018 (Aldossari *et al*., 2018).

The incidence of males is 56.5% higher than that of females 43.5%, and the result of this study was in agreement with the results of a study conducted in Palestine (Shahwan *et al*., 2019), The reason for this study was attributed to the daily effort that men are exposed to compared to women.

Show table (3-1)and (3-2) Body mass index, the results show no significant differences in body mass index in patient and control between patients and control.

These results are supported by another study that found no significant differences in body mass index despite more results falling in the obesity group ($\geq$30kg/m^2) (AL-Saadi, 2005) because insulin resistance, which is commonly associated with this clustering of metabolic factors, frequently precedes the onset of T2DM (Lorenzo *et al*., 2003).

The percent of patients who have hypertension was 62.9% (Table 3-1) and this percent related to the WHO criteria that were found at 40% (Barnett, 2001) The results show a significant elevation in blood pressure in patients compared with the control group, hypertension in type 2 diabetes occurs from insulin resistance and the cause of hypertension in insulin resistance contribute to volume expansion because insulin enhances proximal tubular sodium reabsorption and endothelial dysfunction (Ferrannini, 2005).

4.2. Comparison of biochemical parameters

4.2.1 Fasting blood glucose (FBS)

The current study found As shown in table (3-3) that there were significant differences in fasting glucose levels between the people patients and the healthy, where the results were(175.14 ± 23.75) and (94.13 ± 8.02) mg/dl, respectively, and the differences were significant at a level ($p<0.001$), and the superiority of the group of patients over the control was consistent with What everyone gets(Biadgo et al., 2016), (Lu et al., 2012), (Rusdiana and Amelia, 2018) When studying type 2 diabetes, The cause is insulin resistance and glucose intolerance.

It is also noted from our current study as shown in Table (3-4) the effect of age on fasting glucose, Patients aged 35-45 showed a blood sugar level of 155.86 ± 19.80, and those aged 46-60 had a sugar level of 172.84 ± 24.23, their age is over 60, a sugar level was 186.10 ± 18.84, where we note statistically significant differences between the first group on the one hand and the second and third group on the other hand, and this study is in agreement with (Reaven et al., 1989) This study confirms that glucose intolerance deteriorates as individuals get older That this is due to the development of insulin-stimulated glucose resistance and therefore, plasma glucose concentrations will increase (Reaven *et al*., 1989).

Through table 3-5, it appears that fasting sugar increases with an increase in BMI, where the group ≥30 showed the highest percentage and reached 43.5 % and the sugar level was (184.42 ± 20.60), and this shows that obesity has an effect on increasing the level of sugar and these results are in agreement with a study(Friedman et al., 1994) This is because obesity increases the resistance of cells to insulin(Balkhiyarova *et al*., 2022).

As for the effect of the duration of the disease, table 3-6 shows that those who were less than five years old reached 20.9%, the group 5-10 was 25.8%, and the group greater than 10 reached 53.2%, where the sugar level of them reached 191.46 ± 13.79, were This study found that the longer the illness, the greater the risk of infection, and this is consistent with what was found(Verma et al., 2006) The body becomes more resistant to insulin with increasing duration of diabetes(Verma *et al*., 2006).

Tables 3-7 showed that there was no statistically significant difference between male and female patients.

When comparing fasting sugar with the ACR groups in Table 3-8, the current study found that the Macroalbuminuria group had the highest sugar level, which was 200.78 ± 6.89, while the Microalbuminuria group reached 178.30 ± 5.16, and the Normoalbuminuria group reached 148.88 ± 14.48, where the study showed that the sugar rate increases with ACR increase and this is in agreement with a study(Chowta *et al*., 2009) In the stage of primary renal hyperperfusion, glomerular filtration rises with the absence of albuminuria, the next stage is primary nephropathy, in which glomerular filtration is normal with Microalbuminuria usually appears 5-10 years after diagnosis of diabetes mellitus and in the later stage, it decreases Glomerular filtration with significant proteinuria and clinical manifestations of nephropathy(Chowta *et al*., 2009)

4.2.2. Glycated hemoglobin (HbA_1c)

This study revealed, as shown in Table 3-3, a significant and statistically significant difference between the patient group and the control group with a significant level of p-value = 0.001, This is because glucose uptake by human erythrocytes is not dependent upon insulin and the erythrocyte cell membrane is freely

permeable to glucose (Higgins *et al*., 1982) and the concentration of glucose within the erythrocyte approximates the glucose concentration in the corresponding plasma. Thus, the elevations of HbA_1c provide an integrated measure of plasma glucose concentration during the previous two to three months (Goldstein *et al*., 1980).

The levels of HbA1c in good glycemic control is less than 7% (American Diabetes Association, 2009) and in the present study the percentage of patients who have levels of HbA1c more than 7% (Bad control) was 64.5% (Table 3-8) and this ratio Similar to the percentage obtained from the UAE study (62.4%) (Al-Maskari *et al*., 2008).

4.2.3. Urea and Creatinine

The urea and creatinine results recorded that the patients had higher levels of urea and creatinine compared to the control group (Table 3-3). Urea and creatinine levels are important biomarkers because they play a pivotal role in the diagnosis and follow-up of renal failure. Urea, a byproduct of protein metabolism, accumulates in the blood of patients with renal failure and causes urination in the blood (Merzah *et al.*, 2015), Urea nitrogen is a normal waste nitrogen product found in the blood that comes from the breakdown of protein from foods. Healthy kidneys remove urea nitrogen from the blood, but the level of urea in the blood rises with kidney failure occurs (Rusul Arif *et al*., 2014).

When studying the effect of sex in biochemical parameters there was a significant increase ($P \leq 0.05$) in creatinine between males and females (Table 3-7), Serum creatinine concentration is the most commonly used biomarker to predict the level of kidney function, but it can be affected by various factors such as age, gender, ethnicity, muscle mass, dietary habit and specific drug use total parenteral nutrition and infection in addition to vigorous prolonged exercise may result in increased serum creatinine due to an increase in muscle creatinine generation. Also, ingestion of creatinine supplements may increase serum creatinine as well ingestion of cooked meat may increase serum creatinine (Heymsfield *et al.*, 1983, Levey *et al*., 2007, Star, 1998), Females have less muscle mass as compared to males and the muscle mass is a major determinant of serum creatinine level (Heymsfield *et al*., 1983) The difference

between females and males in glomerular structure, glomerular hemodynamics, and hormone metabolism might play an important role in the gender disparity (Silbiger and Neugarten, 2003), Previous studies demonstrated that the serum creatinine and blood urea nitrogen (BUN) have typically been used to diagnose CKD. Creatinine is a small molecule of 113 Daltons. It is distributed in the body throughout the body water and it is generated in muscle from the non-enzymatic conversion of creatine and phosphocreatine.

Furthermore, serum creatinine may change due to renal factors that are independent of kidney function, for example, several medications, alter the tubular secretion of creatinine leading to changes in serum creatinine independent of GFR, on the other hand, BUN is dependent on non-renal factors independent of kidney function, e.g. protein intake, catabolic state, upper gastrointestinal bleeding, volume status and therapy with high-dose steroids. Thus, alterations in serum creatinine and BUN are not particularly sensitive or specific for small changes in GFR (Herget-Rosenthal *et al*., 2005)

4.2.4. Cholesterol

The results of the current study, as shown in Table (3-3), showed a significant increase in cholesterol level in diabetic patients compared to healthy people, as its level reached (200.23 ± 27.25) and (160.03 ± 11.28) mg/dl respectively, these results It agrees with the findings of many researchers (Bopanna *et al*., 1997, Maghrani *et al*., 2004, Saravanan *et al*., 2003) Which indicated that there is a significant increase in the level of cholesterol in diabetic patients compared to healthy people, and the reason may be due to the increased absorption of cholesterol by the intestine due to the activity of the enzyme Cholesterol Acyl Transferase, and this has been clarified by(Maechler *et al*., 1993) ,the reason may be due to the nutrition pattern, which is one of the factors that cause a rise in the level of fats in the blood plasma, and thus lead to a high level of cholesterol (Kirkpatrick *et al*., 2019), While these results contradicted what was obtained by both (Haffner, 1999, Howard *et al*., 2003) And who indicated that there was no increase in cholesterol in the blood serum when they were studied on people with diabetes.

While did not notice, and as in Table (3-7), we did not find any significant differences in the level of cholesterol between male and female patients.

As for the age groups, there were significant differences in age in the level of blood cholesterol for patients, as shown in Table (3-4), and the older the patient, the higher his cholesterol level, and these results were identical to what was obtained (Maghrani *et al*., 2004, Naoumova *et al*., 1996) While it conflicted with what he got (El-Feki *et al*., 1997) The cause of high cholesterol in diabetic patients may be due to the slow metabolism of elderly people, who have been proven to have no equivalence between building and demolition processes, in which the cuff tends to increase catabolism, which results in an increase in body fat and an increase in the level of blood cholesterol (Bronk, 1999).

4.2.5. Triglyceride

The study included the effect of diabetes on the level of triglycerides (TG) as shown in Table 3-4, where its level reached (176.99 ± 51.83) in the affected people compared to its level in the healthy (109.68 ± 11.82) and the percentage of increase was (+67.3%). Results to previous studies (Bopanna *et al*., 1997, Maghrani et al., 2004, Rubins *et al*., 2003) The reason can be attributed to the fact that the absence of insulin led to the activation of the lipase enzyme in the fat cells, causing an increase in the decomposition of stored glycerides and the release of large amounts of fatty acids and glycerol into the blood, and when they moved to the liver, where they were re-manufactured, and that the increase in fatty acids in the liver led to the conversion of some of them to Phosphorylated fats and glycerol have moved with the triglycerides formed in the liver into the blood, thus increasing the level of fats in the blood. Also, the lack of insulin leads to inhibition of the activity of Lipoprotein Lipase (LPL), which causes a reduction in the process of removing triglycerides in chylomicrons and VLDL, causing an increase in the level of triglycerides (Morigny *et al*., 2021).

When made a comparison between males and females for the level of triglycerides and the extent to which their level was affected by sex as in Table 3-7,

did not notice significant differences in the level between males and females What got matches what he found (Naoumova *et al*., 1996), At the same time, this study differs from another study (Maghrani *et al*., 2004) They indicated that there are significant differences in the concentration of triglycerides between the sexes and that males have a higher concentration compared to females. The reason may be due to the level of some sex hormones such as estrogen, as when the level of this hormone in women decreases, the level of triglycerides decreases by increasing the metabolic rate This leads to lower levels in women compared to men, Our study also showed a significant effect of age on the level of triglycerides, but it was noticed through the results that we obtained an increase in the level of these compounds in the blood with age, which is shown in Table (3-4). Its level increases with age. These results agreed with what was obtained by (Naoumova *et al*., 1996), I also think that the reason is also that excessive intake of foods rich in fat leads to an increase in the production of chylomicron in the intestine, and when it is decomposed, fatty acids are released, which leads to an increase in their quantities in the liver, causing an increase in the release of triglycerides and that This, in the absence of insulin, results in an increase in the release of triglycerides in VLDL. In addition, the lack of insulin leads to an increase in the level of liver glucose, which is also converted into fatty acids and triglycerides and identified in VLDL (Bronk, 1999).

4.2.6. High-density lipoproteins (HDL)

current study, as shown in Table (3-3), showed a decrease in the HDL level, which amounted to (35.13 ± 9.31) mg/dL in patients compared to its level in healthy patients (38.84 ± 5.11) mg/dL, the value of the decrease was (- 3.17). These results are in agreement with what was obtained by (Maghrani *et al*., 2004, Naoumova *et al*., 1996) And who found a significant decrease in the level of HDL in diabetic patients compared to healthy ones, and the reason may be due to the lack of glycolysis of VLDL molecules, which works to deplete HDL by impeding the transfer of both Apo proteins and phosphorylated lipoproteins from triglyceride-rich lipoproteins to HDL. It leads to an increase in the activity of the hepatic lipase enzyme, the latter of which facilitates the removal of HDL.

notice from Table (3-7) that the level of HDL was significant for female patients, compared with male patients, and this agreed with what was found (Rubins

et al., 2003) The reason may be attributed to the high level of estrogen in women and that this hormone leads to an increase in HDL levels (Ji *et al*., 2022).

4.2.7. Very Low-density lipoproteins, Low-density lipoproteins (VLDL, LDL)

Current study, as shown in Table (3-3), showed significant differences in the concentration of VLDL and LDL in diabetic patients when compared with the control group. We notice an increase in the concentration of VLDL, and LDL in patients (130.35 ± 23.75), (35.50 ± 10.21) mg/dl, respectively, while their concentration reached in The control group was (99.23 ± 10.55), (21.91 ± 2.35) mg/dl, and the percentage of this increase amounted to (31.1%), (20.5%), respectively, and these results match what he got (Parmantier et al., 1999, Rubins et al., 2003) They explained that the lack of insulin, which leads to the decomposition of fat stored in adipose tissue, results in a rise in the levels of these compounds, Also, a decrease in the level of this hormone activates the enzyme lipoprotein lipase in the adipose tissue, which results in the release of fat into the blood circulation (Hadcock *et al*., 1991).

In diabetic patients, as shown in Table (3-7), our study showed no significant differences between females and males in VLDL levels, despite the superiority of females over males, while we obtained significant differences between males and females in the level of LDL, as males outperformed over Female patients with a level ($P < 0.05$), and this indicates that the pathological condition of diabetic patients led to a metabolic inversion in both males and females(Rubins *et al*., 2003).

4.2.8. Microalbuminuria

The result shows significantly higher Microalbuminuria in patients compared to the control group as shown in Table 3-3 because Microalbuminuria is a sign or predictor of diabetic nephropathy and cardiovascular disease in patients with type 1 and type 2 diabetes mellitus (Lane, 2004), Hyperglycemia is a critical factor in the development of diabetic nephropathy due to its effects on glomerular and mesangial cells, but alone it is not a causative agent. Melanocytes are essential for maintaining the glomerular capillary structure and for modulating glomerular filtration via smooth

muscle activity. Hyperglycemia is associated with increased proliferation and hyperplasia of mesangial cells, as well as increased matrix production and basement membrane thickness, and consequently increased Microalbuminuria (Marshall, 2016).

In this study, the prevalence of Microalbuminuria was 65.5% (Table 3-8) and this is related to other percentages obtained from other studies in Saudi Arabia where it was 45.6% (Sciences, 2007), and in the United Arab Emirates, it was 61.2% (Sciences, 2007). Other studies revealed that the prevalence of Microalbuminuria is less than this percentage in our study A study in Tanzania revealed that the incidence of Microalbuminuria in type 2 diabetes is 9.8% (Lutale *et al*., 2007) This variation in prevalence can be attributed to factors such as differences in population, method of urine collection or difference in racial susceptibility, differences in race, poor health and education settings.

4.2.9. Chymase

The current study found As shown in table (3-3) that there were significant differences in Chymase levels between the blood of healthy people and the blood of patients, where the results were(1.64 ± 0.39) and (3.25± 1.55) ng/ml, respectively, and the differences were significant at a level ($p<0.001$), This is in agreement with what was obtained by both studies(Cristovam *et al*., 2012, Wang et al., 2011) The reason is that diabetes significantly increases the effect of inflammatory cytokines associated with mast cells, and thus increases Chymase, Chymase is an alternative pathway for the angiotensin-converting enzyme in angiotensin II (Ang II) formation, and its expression is increased in patients with diabetic nephropathy and human mesangial cells (MCs) stimulated with high glucose(Cristovam *et al*., 2012), These authors demonstrated that chymase promotes cell proliferation and collagen synthesis and increases fibroblasts by mechanisms dependent on TGF-B1 and independent of Ang II(Zhao *et al*., 2008).

It is also noted from our current study as shown in Table (3-4) the effect of age on chymase, Patients aged 35-45 showed a blood Chymase level of 2.36 ± 0.24, and those aged 46-60 had a Chymase level of 2.98± 1.14, Their age is over 60, a chymase level was 3.92 ± 1.96, where we note statistically significant differences between the first

group on the one hand and third group on the other hand, and this study is in agreement with(Krüger *et al*., 1981) Where it was found that the content of different granule components increases with age, such as the content of heparin, histamine, and glucuronidase, and thus the Chymase increases(Krüger *et al*., 1981), It may also be because with age, the risk factors for diabetes, high blood pressure, and heart disease increase, and therefore we expect increased levels of Chymase.

From watching Table 3-5, it appears that Chymase increases with an increase in BMI, where the group ≥30 showed the highest Chymase level was (3.83 ± 1.97), and this shows that obesity has an effect on increasing the level of Chymase and these results are in agreement with a study (Fenger *et al*., 2012, Liu **et** *al*., 2009) As they noticed that with obesity, mast cells increased and thus the production of inflammatory cytokines (Altintas *et al*., 2011), Mast cells may contribute to obesity Promote angiogenesis. Mast cells are often localized next to microvessels Microvascular numbers are positively correlated with an increase in mast cell numbers During the development of obesity (Fenger *et al*., 2012).

As for the effect of the duration of the disease, Table 3-6 shows that those who were less than five years reached 2.23 ± 0.13, the group 5-10 was 2.51± 0.27, and the group greater than 10 reached 4.01± 1.81, where This study found that the longer the illness, the greater levels chymase, and this is consistent with what was found (Zheng *et al*., 2012) Mast cells are more abundant as the stages of diabetic nephropathy progress Mast cells secrete a range of factors that may contribute to renal injury, including Endothelin, growth factors, and the proteolytic enzymes implicated in the pathogenesis of the renal disease. For instance, the major mast cell enzyme, tryptase, promotes renal fibroblast proliferation and collagen synthesis, particularly in combination with heparin, another mast cell secretory product (Kondo *et al*., 2001).

Tables 3-7 showed that there was no statistically significant difference between male and female patients.

When comparing Chymase with the ACR groups in Table 3-8, the current study found that the Macroalbuminuria group had the highest Chymase level, which was 4.80 ± 1.96, while the Microalbuminuria group reached 2.76± 0.19, and the Normoalbuminuria group reached 2.28 ± 0.18, where the study showed that the Chymase rate increases with ACR increase and this is in agreement with a

study(Sharma *et al*., 2007) This is due to the association of mast cell infiltration into the kidneys with proteinuria and interstitial fibrosis in various kidney diseases (Sharma *et al*., 2007).

4.2.10. Angiotensin II

The current study found As shown in table (3-3) that there were significant differences in Angiotensin II levels between the healthy people and the patients, where the results were (16.98± 3.55) and (41.25 ± 17.03) ng/l, respectively, and the differences were significant at a level ($p<0.001$), This is in agreement with what was obtained by studies(Chu et al., 2009, Cristovam et al., 2008) Where this study showed Local adipose RAS participates in obesity-associated metabolic alterations. Emerging evidence indicates that the development of insulin resistance and type2 diabetes was associated with the augmented expression of ANG II generating enzymes and RAS activation in adipose tissue(Gorzelniak *et al*., 2002), In addition, this result suggests that an HG-induced increase in the intracellular ANG II generation involves the ACE pathway. Moreover, MC exposed to HG has increased intracellular renin activity paralleled by an increase in the angiotensinogen gene transcription(Cristovam *et al*., 2008).

It is also observed from our current study as shown in Table (3-4) the effect of age on Angiotensin II, the patients aged 35-45 years showed a serum Angiotensin II level of 28.28 ± 5.01, and those aged 46-60 had Angiotensin II level of 39.41± 14.55, their age is over 60, Angiotensin II level of 41.25 ± 17.03, where we notice statistically significant differences between the first group on the one hand and the second and third group on the other hand, and this study is compatible with (Corbatón-Anchuelo *et al*., 2018, Shukri *et al*., 2018) Where it was shown that with age increases the risks of diseases such as high blood pressure, heart disease, and kidney disease, and therefore we expect an increase in the levels of angiotensin II.

Through Table 3-5, it appears that Angiotensin II increases with an increase in body mass index, where the group ≥30 showed the highest Angiotensin II level was (48.02± 18.42), and this shows that obesity has an effect on increasing the level of Angiotensin II and these results are in agreement with a study (Hall *et al*., 2021, South

et al., 2019), Although excessive weight gain and increased adiposity initially cause modest increases in BP, obesity-induced metabolic and inflammatory disorders interact with hypertension to promote gradual injury to blood vessels and various organs, including the heart and kidneys (Hall *et al*., 2021).

Table 3-6 shows the effect of the duration of the disease, that those who were less than five years old reached 25.78 ± 2.79, the group 5-10 was 31.20± 6.05, and the group greater than 10 reached 52.22 ± 16.17, where This study found that the longer the illness, the greater levels Angiotensin II, and this is consistent with what was found (Sabuncu *et al.*, 2021) The body becomes more resistant to insulin with increasing duration of diabetes (Verma *et al*., 2006).

While we did not notice, and as in Table (3-7), we did not find any significant differences in the level of Angiotensin II between male and female patients.

When comparing Angiotensin II with the ACR groups in Table 3-8, the current study found that the Macroalbuminuria group had the highest Angiotensin II level, which was 62.33± 12.18, while the Microalbuminuria group reached 36.72± 5.05, and the Normoalbuminuria group reached 26.21 ± 2.54, where the study showed that the Angiotensin II rate increases with ACR increase and this is in agreement with a study(Kim et al., 2005) In this experiment, glucose stimuli and ANG II rapidly increased the VEGF synthesis in proximal tubular epithelial cells. This finding may provide a clue to understanding why the proximal tubule participates actively in the regulation of intrarenal VEGF synthesis(Kim et al., 2005) The clinical impact of this increased expression of VEGF, is caused by high glucose concentrations and ANG II in proximal tubule cells, is not known. Although it has been suggested that VEGF acts as a potential mediator of glomerular hyperfiltration and albuminuria in the glomerulus, there is also strong evidence for VEGF acting as an important endothelial cell angiogenic, survival, and trophic factor(Takahashi *et al*., 1998).

4.3. The relationship between Chymase and ANGII Opposite some parameters

Correlation between Chymase and ANG II vs. Fasting Blood Glucose The results show a significant association between fasting blood glucose and chymase in

patients (Fig. 3-6) and fasting blood glucose and ANGII in patients (Fig. 3-7) and this is because elevated levels of glucose contribute to the blood dramatically increases the effects of these mast cell-associated molecules, leading to increased levels of chymase, and consequently an increase in ANGII, the development of insulin resistance and type 2 diabetes increased expression of Ang II-generating enzymes and RAS activation in adipose tissue(Chu *et al*., 2009).

While there was a Correlation between Chymase and ANG II Opposite HbA1c showed a positive correlation between HbA1c and chymase in patients (Fig. 3-8) and HbA1c and ANGII in patients (Fig. 3-9) because of poor glycemic control may play a crucial role in the development of DNP and other complications associated with the disease by altered cytokine production MCs infiltrate the kidney and are degranulated in renal diseases, releasing pathologically active substances such as chymase and tryptase (Popoola *et al*., 2016).

The study showed a positive linear regression between chymase and ANGII with serum urea, as in Figure (3-10) and Figure (3-11), as well as a positive regression between chymase and ANGII with creatinine, as in Figure (3-12) and Figure (3-13), the reason is due to the rich mast cells with chymase and such large numbers that when mast cells are recruited during inflammation, this chymase may be released into the inflamed tissue. Several reports have considered mast cells to be a factor associated with diabetic nephropathy. These proteins are involved in damage to the interstitial tube, causing further deterioration in kidney disease (Kaczmarczyk *et al*., 2014, Konishi *et al*., 2008, Okon and Stachura, 2007).

While there was a Correlation between Chymase and ANG II Opposite cholesterol showed a positive correlation between cholesterol and chymase in patients (Fig. 3-14) and cholesterol and ANGII in patients (Fig. 3-15) because chymase in mononuclear cells from peripheral blood is activated by high blood pressure or hypercholesterolemia (Murakami *et al*., 2007).

The study showed a positive linear regression between Chymase and ANGII with TG, as in Figures 3-16 and Figure 3-17 The reason can be attributed to the fact that the lack of insulin led to the activation of the enzyme lipase in the fat cells, causing an increase in the decomposition of stored glycerides and the release of large

amounts of fatty acids and glycerol into the blood leading to an increase in mast cells thus raising both chymase and ANG II (He and Shi, 2013).

The study showed a negative linear regression between Chymase and ANGII with HDL, in the negative linear Chymase regression (Fig. 3-18) while the negative linear regression of ANGII (Fig. 3-19) may be due to the non-degradation of VLDL in diabetic patients leading to HDL depletion by transfusion ApoA1 and phospholipids from triglyceride-rich lipoproteins to HDL as well as proteolysis by chymase, thus reducing the anti-inflammatory properties of ApoAI and impairing the ability of ApoAI to stimulate cholesterol influx from macrophage cells (Maaninka, 2018).

The study showed a positive linear regression between chymase and ANG II with serum LDL, as in Figure (3-20) and Figure (3-21), as well as a positive regression between chymase and ANG II with VLDL, as in Figure (3-22) and Figure (3-23) The reason may be that the lack of insulin that leads to the decomposition of fat stored in the adipose tissue results in an increase in the levels of these compounds, and a decrease in the level of this hormone activates the enzyme lipoprotein lipase in the adipose tissue. , which leads to the release of fats in the blood circulation, which leads to an increase in the number of mast cells, and consequently, an increase in the level of chymase and ANG II (Maaninka, 2018).

While there was a Correlation between Chymase and ANG II Opposite Microalbuminuria showed a positive correlation between Microalbuminuria and chymase in patients (Fig. 3-30) and Microalbuminuria and ANG II in patients (Fig. 3-31) Because poor glycemic control and high blood pressure are risk factors for Microalbuminuria (Zhou et al., 2021) Hyperglycemia is a crucial factor in the development of diabetic nephropathy because of its effects on glomerular and mesangial cells, but alone it is not causative. Mesangial cells are crucial for the maintenance of the glomerular capillary structure and the modulation of glomerular filtration via smooth-muscle activity. Hyperglycemia is associated with an increase in mesangial cell proliferation and hypertrophy, as well as increased matrix production and basement membrane thickening (Sethi *et al.*, 2021) ANG II also has potent proliferative inflammatory and fibrotic activities through its action through the AT1 receptor, leading to disease progression and progression of renal fibrosis (Vibhushan *et al.*, 2020).

Chapter five

Conclusions, Recommendations and Reference

Conclusion

From the result of our study, the following conclusion can be obtained:

1. Increased levels of both chymase and ANG II play a role in the pathogenesis of diabetic nephropathy.
2. Increasing knowledge and a better understanding of the role of chymase and ANG II in the context of diabetic nephropathy will present an important therapeutic opportunity to develop new strategies that can be successfully translated into clinical applications for the treatment of this complication.
3. The poor glycemic control associated with increase of micro and macro albuminuria leading to increase stimulation of chymase that lead to elevation of angiotensin II and increase the risk of hypertension in patient with T2DM and increase of diabetic nephropathy.
4. Age, body mass index and disease duration have a direct effect on the levels of chymase and ANG II concentrations.
5. Through this study, noticed that there is no effect of sex on the levels of chymase and ANG II concentrations.
6. In this study, found a positive relationship between chymase and ANG II on the one hand and between (fasting sugar (FBS), glycated hemoglobin (HbA1c), blood urea, Serum Creatinine, albumin-creatinine ratio (ACR), Microalbuminuria, cholesterol (CHOL), triglyceride (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL)) on the other hand and negative correlation with high-density lipoprotein (HDL).

Recommendations

The following recommendations for the future works are:

1. More studies are needed to evaluate the effect of chymase and ANG II inhibitors on the progressive stages of diabetic nephropathy.
2. Evaluation of other inflammatory markers (IL-6, IL-18, TGF-β, and MMPs) in type 2 diabetic nephropathy patients.
3. Further clinical studies are required to evaluate the effects of chymase and ANG II on other complications of diabetes mellitus like diabetic neuropathy.
4. A study of chymase and ANG II levels can be used to assess the development of heart disease

References

Lancent,1998. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet,* 352**,** 837-853.

Abusaib, M., Ahmed, M., Nwayyir, H. A., Alidrisi, H. A., Al-Abbood, M., Al-Bayati, A., Al-Ibrahimi, S., Al-Kharasani, A., Al-Rubaye, H., Mahwi, T., Ashor, A., Howlett, H., Shakir, M., Al-Naqshbandi, M. & Mansour, A. 2020. Iraqi Experts Consensus On The Management Of Type 2 Diabetes/Prediabetes In Adults. *Clin Med Insights Endocrinol Diabetes,* 13**,** 1179551420942232.

AHMAD, S. & FERRARIO, C. M. 2018. Chymase inhibitors for the treatment of cardiac diseases: a patent review (2010-2018). *Expert Opin Ther Pat,* 28**,** 755-764.

AL-MASKARI, F., EL-SADIG, M. & NORMAN, J. N. J. C. D. 2007. The prevalence of macrovascular complications among diabetic patients in the United Arab Emirates. 6**,** 1-7.

AL-MASKARI, F., EL-SADIG, M. & OBINECHE, E. J. B. N. 2008. Prevalence and determinants of microalbuminuria among diabetic patients in the United Arab Emirates. 9**,** 1-8.

AL-SAADI, H. 2005. *Physiological and biochemical changes of some adrenochortico and pituitary hormones in patients with non-insulin dependent diabetes mellitus.* Ph. D. Thesis, College of Science, University of AL-Mustansiriyah.

ALDOSSARI, K. K., ALDIAB, A., AL-ZAHRANI, J. M., AL-GHAMDI, S. H., ABDELRAZIK, M., BATAIS, M. A., JAVAD, S., NOORUDDIN, S., RAZZAK, H. A. & EL-METWALLY, A. J. J. O. D. R. 2018. Prevalence of prediabetes, diabetes, and its associated risk factors among males in Saudi Arabia: a population-based survey. 2018.

ALEXANDRU, N., TITORENCU, I., FRUNZã, S., WEISS, E., BãDILã, E. & GEORGESCU, A. 2017. Endothelial progenitor cell dysfunction in the pathogenesis of vascular complications of diabetes. *Mechanisms of vascular defects in diabetes mellitus.* Springer.

ALLDRED, S. K., TAKWOINGI, Y., GUO, B., PENNANT, M., DEEKS, J. J., NEILSON, J. P. & ALFIREVIC, Z. J. C. D. O. S. R. 2017. First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening.

ALTINTAS, M. M., ROSSETTI, M. A., NAYER, B., PUIG, A., ZAGALLO, P., ORTEGA, L. M., JOHNSON, K. B., MCNAMARA, G., REISER, J., MENDEZ, A. J. J. L. I. H. & DISEASE 2011. Apoptosis, mastocytosis, and diminished adipocytokine gene expression accompany reduced epididymal fat mass in long-standing diet-induced obese mice. 10**,** 1-12.

ALZAHRANI, B., ALTURKISTANI, A. M., ALZIDANI, T., ALTURKISTANI, A. & ABOZAID, H. J. I. J. O. M. I. D. C. 2019. Prevalence and risk factors for diabetic nephropathy in type 2 diabetic patients, Taif City, Saudi Arabia. 3**,** 167-172.

AMERICAN DIABETES, A. 2011. Standards of medical care in diabetes--2011. *Diabetes Care,* 34 Suppl 1**,** S11-61.

ANDERSSON, M. K., KARLSON, U. & HELLMAN, L. 2008. The extended cleavage specificity of the rodent beta-chymases rMCP-1 and mMCP-4 reveal major functional similarities to the human mast cell chymase. *Mol Immunol,* 45**,** 766-75.

ANDREN, K., WIKKELSö, C., SUNDSTRöM, N., AGERSKOV, S., ISRAELSSON, H., LAURELL, K., HELLSTRöM, P. & TULLBERG, M. J. J. O. N. 2018. Long-term

effects of complications and vascular comorbidity in idiopathic normal pressure hydrocephalus: a quality registry study. 265**,** 178-186.

ASSOCIATION, A. D. 2009. Standards of Medical Care in Diabetes—2009. 32**,** S13-S61.

ATIAKSHIN, D., BUCHWALOW, I., TIEMANN, M. J. H. & BIOLOGY, C. 2020. Mast cells and collagen fibrillogenesis. 154**,** 21-40.

BACCI, S., TINTI, M. G., RAUSEO, A., MASSA, V., VENDEMIALE, G. & DE COSMO, S. J. N. D. T. 2021. Albuminuria in parents with type 2 diabetes is associated with age-related increase in low-density lipoprotein cholesterol and increased albuminuria in non-diabetic offspring. 36**,** 378-379.

BAHREINI, E., REZAEI-CHIANEH, Y. & NABI-AFJADI, M. J. R. O. D. S. 2021. Molecular Mechanisms Involved in Intrarenal Renin-Angiotensin and Alternative Pathways in Diabetic Nephropathy-A Review. 17**,** 1-10.

BALKHIYAROVA, Z., LUCIANO, R., KAAKINEN, M., ULRICH, A., SHMELIOV, A., BIANCHI, M., CHIOMA, L., DALLAPICCOLA, B., PROKOPENKO, I. & MANCO, M. J. H. M. G. 2022. Relationship between glucose homeostasis and obesity in early life—a study of Italian children and adolescents. 31**,** 816-826.

BALTATU, O. C., CAMPOS, L. A. & BADER, M. J. P. 2011. Local renin–angiotensin system and the brain—a continuous quest for knowledge. 32**,** 1083-1086.

BARNETT, A. H. 2001. *Clinical Management of Hypertension in Diabetes: Pocketbook*, CRC Press.

BARNETT, P. & BRAUNSTEIN, G. 2001. Diabetes mellitus. Cecil essentials of medicine. WB Saunders Company.

BIADGO, B., MELKU, M., ABEBE, S. M., ABEBE, M. J. D., METABOLIC SYNDROME, TARGETS, O. & THERAPY 2016. Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. 9**,** 91.

BOPANNA, K., KANNAN, J., SUSHMA, G., BALARAMAN, R. & RATHOD, S. J. I. J. O. P. 1997. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. 29**,** 162.

BOT, I., SHI, G.-P., KOVANEN, P. T. J. A., THROMBOSIS, & BIOLOGY, V. 2015. Mast cells as effectors in atherosclerosis. 35**,** 265-271.

BRONK, J. R. 1999. *Human metabolism: functional diversity and integration*, Addison-Wesley Longman.

CAMPAGNA, D., ALAMO, A., DI PINO, A., RUSSO, C., CALOGERO, A., PURRELLO, F., POLOSA, R. J. D. & SYNDROME, M. 2019. Smoking and diabetes: dangerous liaisons and confusing relationships. 11**,** 1-12.

CAMPION, C. G., SANCHEZ-FERRAS, O., BATCHU, S. N. J. C. J. O. K. H. & DISEASE 2017. Potential role of serum and urinary biomarkers in diagnosis and prognosis of diabetic nephropathy. 4**,** 2054358117705371.

CAñADAS-GARRE, M., ANDERSON, K., MCGOLDRICK, J., MAXWELL, A. & MCKNIGHT, A. J. J. O. P. 2019. Proteomic and metabolomic approaches in the search for biomarkers in chronic kidney disease. 193**,** 93-122.

CARAMORI, M. L., FIORETTO, P. & MAUER, M. 2000. The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? *Diabetes,* 49**,** 1399-408.

CARE, A. D. A. J. D. 2020. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2020. 43**,** S14-S31.

CAREY, R. M. & SIRAGY, H. M. J. E. R. 2003. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. 24**,** 261-271.

CAT, A. N. D. & TOUYZ, R. M. J. C. H. R. 2011a. Cell signaling of angiotensin II on vascular tone: novel mechanisms. 13**,** 122-128.

CAT, A. N. D. & TOUYZ, R. M. J. P. 2011b. A new look at the renin–angiotensin system—focusing on the vascular system. 32**,** 2141-2150.

CATARINO, D., SILVA, D., GUIOMAR, J., RIBEIRO, C., RUAS, L., CARDOSO, L. & PAIVA, I. 2020. Non-immune-mediated versus immune-mediated type 1 diabetes: diagnosis and long-term differences-retrospective analysis. *Diabetol Metab Syndr,* 12**,** 56.

CAUGHEY, G. H., ZERWECK, E. H. & VANDERSLICE, P. 1991. Structure, chromosomal assignment, and deduced amino acid sequence of a human gene for mast cell chymase. *Journal of Biological Chemistry,* 266**,** 12956-12963.

CERVANTES, A., WAYMOUTH, E. K., PETROV, M. S. J. D. D. & SCIENCES 2019. African-Americans and indigenous peoples have increased burden of diseases of the exocrine pancreas: a systematic review and meta-analysis. 64**,** 249-261.

CHO, N. H., SHAW, J. E., KARURANGA, S., HUANG, Y., DA ROCHA FERNANDES, J. D., OHLROGGE, A. W. & MALANDA, B. 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract,* 138**,** 271-281.

CHOWTA, N., PANT, P. & CHOWTA, M. J. I. J. O. N. 2009. Microalbuminuria in diabetes mellitus: Association with age, sex, weight, and creatinine clearance. 19**,** 53.

CHU, K. Y., LEUNG, P. S. J. C. P. & SCIENCE, P. 2009. Angiotensin II in type 2 diabetes mellitus. 10**,** 75-84.

CLIMIE, R. E., VAN SLOTEN, T. T., BRUNO, R.-M., TADDEI, S., EMPANA, J.-P., STEHOUWER, C. D., SHARMAN, J. E., BOUTOUYRIE, P. & LAURENT, S. J. H. 2019. Macrovasculature and microvasculature at the crossroads between type 2 diabetes mellitus and hypertension. 73**,** 1138-1149.

COELHO, S. C., BERILLO, O., CAILLON, A., OUERD, S., FRAULOB-AQUINO, J. C., BARHOUMI, T., OFFERMANNS, S., PARADIS, P. & SCHIFFRIN, E. L. J. H. 2018. Three-month endothelial human endothelin-1 overexpression causes blood pressure elevation and vascular and kidney injury. 71**,** 208-216.

CORBATóN-ANCHUELO, A., MARTíNEZ-LARRAD, M. T., PRADO-GONZáLEZ, N. D., FERNáNDEZ-PEREZ, C., GABRIEL, R. & SERRANO-RíOS, M. J. I. J. O. H. 2018. Prevalence, treatment, and associated factors of hypertension in Spain: a comparative study between populations. 2018.

CRISTOVAM, P. C., ARNONI, C. P., DE ANDRADE, M. C. C., CASARINI, D. E., PEREIRA, L. G., SCHOR, N., BOIM, M. A. J. E. B. & MEDICINE 2008. ACE-dependent and chymase-dependent angiotensin II generation in normal and glucose-stimulated human mesangial cells. 233**,** 1035-1043.

CRISTOVAM, P. C., CARMONA, A. K., ARNONI, C. P., MAQUIGUSSA, E., PEREIRA, L. G., BOIM, M. A. J. E. B. & MEDICINE 2012. Role of chymase in diabetic nephropathy. 237**,** 985-992.

DE MORAES, A. G. & SURANI, S. J. W. J. O. D. 2019. Effects of diabetic ketoacidosis in the respiratory system. 10**,** 16.

DEEBEL, N. A., BRADSHAW, A. W., SADRI-ARDEKANI, H. J. B. P., ENDOCRINOLOGY, R. C. & METABOLISM 2020. Infertility considerations in klinefelter syndrome: From origin to management. 101480.

DIMEGLIO, L. A., EVANS-MOLINA, C. & ORAM, R. A. 2018. Type 1 diabetes. *The Lancet,* 391**,** 2449-2462.

DODIA, R. & SAHOO, S. J. L. S. L. 2021. AA REVIEW OF DRUG-INDUCED DIABETES. 135**,** 1-8.

DONOGHUE, M., HSIEH, F., BARONAS, E., GODBOUT, K., GOSSELIN, M., STAGLIANO, N., DONOVAN, M., WOOLF, B., ROBISON, K., JEYASEELAN, R., BREITBART, R. E. & ACTON, S. 2000. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*, 87**,** E1-9.

DZAU, V. J. J. H. 2001. Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. 37**,** 1047-1052.

EDELMAN, D., OLSEN, M. K., DUDLEY, T. K., HARRIS, A. C. & ODDONE, E. Z. 2004. Utility of hemoglobin A1c in predicting diabetes risk. *J Gen Intern Med*, 19**,** 1175-80.

EL-FEKI, M., RADWAN, Z., ABDEL-MONAIEM, A. & SALAH, E. J. J.-E. G. S. O. Z. 1997. Effect of Nigella sativa and fish oil on alloxan diabetic rats 2-Immunological studies. 23**,** 161-186.

ELIEH ALI KOMI, D., WöHRL, S., BIELORY, L. J. C. R. I. A. & IMMUNOLOGY 2020. Mast cell biology at molecular level: a comprehensive review. 58**,** 342-365.

FAN, Y., LONG, E., CAI, L., CAO, Q., WU, X. & TONG, R. J. F. I. P. 2021. Machine learning approaches to predict risks of diabetic complications and poor glycemic control in nonadherent type 2 diabetes. 12**,** 1485.

FENGER, R., LINNEBERG, A., VIDAL, C., VIZCAINO, L., HUSEMOEN, L., AADAHL, M., GONZALEZ-QUINTELA, A. J. I. A. O. A. & IMMUNOLOGY 2012. Determinants of serum tryptase in a general population: the relationship of serum tryptase to obesity and asthma. 157**,** 151-158.

FERRANNINI, E. J. H. 2005. Insulin and blood pressure: connected on a circumference? : Am Heart Assoc.

FERRARIO, C. M. & VARAGIC, J. J. A. J. O. P.-R. P. 2010. The ANG-(1–7)/ACE2/mas axis in the regulation of nephron function. 298**,** F1297-F1305.

FRANKLIN, R. G., MUTHUKUMAR, B. J. J. O. C. & NANOSCIENCE, T. 2020. A prediction system for diagnosis of Diabetes Mellitus. 17**,** 6-9.

FRIEDEWALD, W. T., LEVY, R. I. & FREDRICKSON, D. S. J. C. C. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. 18**,** 499-502.

FRIEDMAN, J. E., CARO, J., PORIES, W. J., AZEVEDO JR, J. L. & DOHM, G. L. J. M. 1994. Glucose metabolism in incubated human muscle: effect of obesity and non-insulin-dependent diabetes mellitus. 43**,** 1047-1054.

FUCHS, M. M., ATTENHOFER JOST, C., BABOVIC-VUKSANOVIC, D., CONNOLLY, H. M. & EGBE, A. J. J. O. T. A. H. A. 2019. Long-term outcomes in patients with Turner syndrome: A 68-year follow-up. 8**,** e011501.

GHEITH, O., FAROUK, N., NAMPOORY, N., HALIM, M. A. & AL-OTAIBI, T. J. J. O. N. 2016. Diabetic kidney disease: world wide difference of prevalence and risk factors. 5**,** 49.

GOLDSTEIN, D. E., PETH, S. B., ENGLAND, J. D., HESS, R. L. & COSTA, J. D. J. D. 1980. Effects of acute changes in blood glucose on HbA1c. 29**,** 623-628.

GOMEZ, R. A. J. P. N. 1990. Molecular biology of components of the renin-angiotensin system during development. 4**,** 421-423.

GORZELNIAK, K., ENGELI, S., JANKE, J., LUFT, F. C. & SHARMA, A. M. J. J. O. H. 2002. Hormonal regulation of the human adipose-tissue renin–angiotensin system: relationship to obesity and hypertension. 20**,** 965-973.

GRADY, E. F., SECHI, L. A., GRIFFIN, C. A., SCHAMBELAN, M. & KALINYAK, J. E. J. T. J. O. C. I. 1991. Expression of AT2 receptors in the developing rat fetus. 88**,** 921-933.

GRIENDLING, K. K., MURPHY, T. & ALEXANDER, R. W. J. C. 1993. Molecular biology of the renin-angiotensin system. 87**,** 1816-1828.

HADCOCK, S., RICHARDSON, M., WINOCOUR, P. D., HATTON, M. J. A. & BIOLOGY, T. A. J. O. V. 1991. Intimal alterations in rabbit aortas during the first 6 months of alloxan-induced diabetes. 11**,** 517-529.

HAFFNER, S. M. J. T. A. J. O. C. 1999. Diabetes, hyperlipidemia, and coronary artery disease. 83**,** 17-21.

HALL, J. E., MOUTON, A. J., DA SILVA, A. A., OMOTO, A., WANG, Z., LI, X. & DO CARMO, J. M. J. C. R. 2021. Obesity, kidney dysfunction, and inflammation: interactions in hypertension. 117**,** 1859-1876.

HE, A. & SHI, G.-P. J. C. P. D. 2013. Mast cell chymase and tryptase as targets for cardiovascular and metabolic diseases. 19**,** 1114-1125.

HEIKKILÄ, H. M., LÄTTI, S., LESKINEN, M. J., HAKALA, J. K., KOVANEN, P. T., LINDSTEDT, K. A. J. A., THROMBOSIS, & BIOLOGY, V. 2008. Activated mast cells induce endothelial cell apoptosis by a combined action of chymase and tumor necrosis factor-α. 28**,** 309-314.

HERGET-ROSENTHAL, S., PIETRUCK, F., VOLBRACHT, L., PHILIPP, T. & KRIBBEN, A. J. C. N. 2005. Serum cystatin C--a superior marker of rapidly reduced glomerular filtration after uninephrectomy in kidney donors compared to creatinine. 64**,** 41-46.

HEYMSFIELD, S. B., ARTEAGA, C., MCMANUS, C., SMITH, J. & MOFFITT, S. J. T. A. J. O. C. N. 1983. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. 37**,** 478-494.

HIGGINS, P. J., GARLICK, R. L. & BUNN, H. F. J. D. 1982. Glycosylated hemoglobin in human and animal red cells: role of glucose permeability. 31**,** 743-748.

HIGUCHI, S., OHTSU, H., SUZUKI, H., SHIRAI, H., FRANK, G. D. & EGUCHI, S. J. C. S. 2007. Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. 112**,** 417-428.

HOMOUD, B., ALHAKAMI, A., ALMALKI, M., SHAHEEN, M., ALTHUBAITI, A., ALKHATHAAMI, A. & KHATRI, I. A. J. A. O. S. M. 2020. The association of diabetes with ischemic stroke and transient ischemic attacks in a tertiary center in Saudi Arabia. 40**,** 449-455.

HOSTETTER, T. H., RENNKE, H. G. & BRENNER, B. M. J. T. A. J. O. M. 1982. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. 72**,** 375-380.

HOWARD, B. V., CRIQUI, M. H., CURB, J. D., RODABOUGH, R., SAFFORD, M. M., SANTORO, N., WILSON, A. C. & WYLIE-ROSETT, J. J. M. 2003. Risk factor clustering in the insulin resistance syndrome and its relationship to cardiovascular disease in postmenopausal white, black, hispanic, and Asian/Pacific Islander women. 52**,** 362-371.

HUANG, R. Y., BLOM, T. & HELLMAN, L. 1991. Cloning and structural analysis of MMCP-1, MMCP-4 and MMCP-5, three mouse mast cell-specific serine proteases. *Eur J Immunol*, 21**,** 1611-21.

HULKOTI, V. S., GUPTE, Y. & ACHARYA, S. J. A. C. C. H. O. D. M. 2021. Treatment of Acute Complications of Diabetes Mellitus. 64-71.

HUMMA, L. M. & TERRA, S. G. J. A. J. O. H.-S. P. 2002. Pharmacogenetics and cardiovascular disease: impact on drug response and applications to disease management. 59**,** 1241-1252.

IBA, T., LEVY, J. J. J. O. T. & HAEMOSTASIS 2019. Derangement of the endothelial glycocalyx in sepsis. 17**,** 283-294.

JANDELEIT-DAHM, K., COOPER, M. E. J. E. & CLINICS, M. 2006. Hypertension and diabetes: role of the renin-angiotensin system. 35**,** 469-490.

JI, H., KWAN, A. C., CHEN, M. T., OUYANG, D., EBINGER, J. E., BELL, S. P., NIIRANEN, T. J., BELLO, N. A. & CHENG, S. J. C. R. 2022. Sex differences in myocardial and vascular aging. 130**,** 566-577.

JUNG, B., MARTINEZ, M., CLAESSENS, Y.-E., DARMON, M., KLOUCHE, K., LAUTRETTE, A., LEVRAUT, J., MAURY, E., OBERLIN, M. & TERZI, N. J. A. O. I. C. 2019. Diagnosis and management of metabolic acidosis: guidelines from a French expert panel. 9**,** 1-17.

JUNG, S.-H., JUNG, C.-H., REAVEN, G. M. & KIM, S. H. J. D. 2018. Adapting to insulin resistance in obesity: role of insulin secretion and clearance. 61**,** 681-687.

KACZMARCZYK, K., KOSALKA, J., SOJA, J., KUZNIEWSKI, M., MUSIAL, J. & OKON, K. J. F. H. E. C. 2014. Renal interstitial mast cell counts differ across classes of proliferative lupus nephritis. 52**,** 218-224.

KANETO, H., TAKAMI, A., SPRANGER, R., AMANO, A., WATANABE, D., NIEMOELLER, E. J. D., OBESITY & METABOLISM 2020. Efficacy and safety of insulin glargine/lixisenatide fixed-ratio combination (iGlarLixi) in Japanese patients with type 2 diabetes mellitus inadequately controlled on basal insulin and oral antidiabetic drugs: the LixiLan JP-L randomized clinical trial. 22**,** 3-13.

KATSAROU, A., GUDBJORNSDOTTIR, S., RAWSHANI, A., DABELEA, D., BONIFACIO, E., ANDERSON, B. J., JACOBSEN, L. M., SCHATZ, D. A. & LERNMARK, A. 2017. Type 1 diabetes mellitus. *Nat Rev Dis Primers,* 3**,** 17016.

KAWAKAMI, T. & GALLI, S. J. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat Rev Immunol,* 2**,** 773-86.

KIM, N. H., OH, J. H., SEO, J. A., LEE, K. W., KIM, S. G., CHOI, K. M., BAIK, S. H., CHOI, D. S., KANG, Y. S. & HAN, S. Y. J. K. I. 2005. Vascular endothelial growth factor (VEGF) and soluble VEGF receptor FLT-1 in diabetic nephropathy. 67**,** 167-177.

KIRCHHOFF, S. M. J. C. R. S. 2018. Medicare coverage of end-stage renal disease (ESRD).

KIRKPATRICK, C. F., BOLICK, J. P., KRIS-ETHERTON, P. M., SIKAND, G., ASPRY, K. E., SOFFER, D. E., WILLARD, K.-E. & MAKI, K. C. J. J. O. C. L. 2019. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: a scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. 13**,** 689-711. e1.

KONDO, S., KAGAMI, S., KIDO, H., STRUTZ, F., MULLER, G. A. & KURODA, Y. J. J. O. T. A. S. O. N. 2001. Role of mast cell tryptase in renal interstitial fibrosis. 12**,** 1668-1676.

KONISHI, Y., MORIKAWA, T., OKADA, N., MAEDA, I., KITABAYASHI, C., YOSHIOKA, K., OKUMURA, M., NISHIYAMA, A., UEDA, M. & TAKAI, S. J. H. R. 2008. Evidence for abundant presence of chymase-positive mast cells in the kidneys of patients with immunoglobulin A nephropathy: effect of combination

therapy with prednisolone and angiotensin II receptor blocker valsartan. 31**,** 1517-1524.

KOVANEN, P. T., LEE, M., LINDSTEDT, L. & ÖöRNI, K. 2000. Mast Cells in Atherogenesis: A Model for Studying Proteolytic Modification of Low and High Density Lipoproteins. *Lipoprotein Metabolism and Atherogenesis.* Springer.

KOWLURU, R. A., ZHONG, Q. & SANTOS, J. M. J. E. O. O. I. D. 2012. Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9. 21**,** 797-805.

KRUGER, P. G., LAGUNOFF, D. J. I. A. O. A. & IMMUNOLOGY 1981. Effect of age on mast cell granules. 65**,** 291-299.

KUMAR, R., SAHA, P., KUMAR, Y., SAHANA, S., DUBEY, A. & PRAKASH, O. 2020. A REVIEW ON DIABETES MELLITUS: TYPE1 & TYPE2.

LANE, J. T. J. A. J. O. P.-R. P. 2004. Microalbuminuria as a marker of cardiovascular and renal risk in type 2 diabetes mellitus: a temporal perspective. 286**,** F442-F450.

LEE, Y.-Y., LIN, C.-W. & CHEN, I.-W. J. F. I. E. 2020. Exogenous Insulin Injection-Induced Stiff-Person Syndrome in a Patient With Latent Autoimmune Diabetes: A Case Report and Literature Review. 11.

LEMARIE, C. A. & SCHIFFRIN, E. L. J. J. O. T. R.-A.-A. S. 2010. The angiotensin II type 2 receptor in cardiovascular disease. 11**,** 19-31.

LESKINEN, M., WANG, Y., LESZCZYNSKI, D., LINDSTEDT, K. A., KOVANEN, P. T. J. A., THROMBOSIS, & BIOLOGY, V. 2001. Mast cell chymase induces apoptosis of vascular smooth muscle cells. 21**,** 516-522.

LEVEY, A. S., CORESH, J., GREENE, T., MARSH, J., STEVENS, L. A., KUSEK, J. W., VAN LENTE, F. & CHEMISTRY, C. K. D. E. C. J. C. 2007. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. 53**,** 766-772.

LI, X. C., COOK, J. L., RUBERA, I., TAUC, M., ZHANG, F. & ZHUO, J. L. J. A. J. O. P.-R. P. 2011. Intrarenal transfer of an intracellular fluorescent fusion of angiotensin II selectively in proximal tubules increases blood pressure in rats and mice. 300**,** F1076-F1088.

LINDSTEDT, L., LEE, M., CASTRO, G. R., FRUCHART, J.-C. & KOVANEN, P. T. J. T. J. O. C. I. 1996. Chymase in exocytosed rat mast cell granules effectively proteolyzes apolipoprotein AI-containing lipoproteins, so reducing the cholesterol efflux-inducing ability of serum and aortic intimal fluid. 97**,** 2174-2182.

LINDSTEDT, L., LEE, M. & KOVANEN, P. T. 2001. Chymase bound to heparin is resistant to its natural inhibitors and capable of proteolyzing high density lipoproteins in aortic intimal fluid. *Atherosclerosis,* 155**,** 87-97.

LIU, J., DIVOUX, A., SUN, J., ZHANG, J., CLEMENT, K., GLICKMAN, J. N., SUKHOVA, G. K., WOLTERS, P. J., DU, J. & GORGUN, C. Z. J. N. M. 2009. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. 15**,** 940-945.

LIU, Y. 2006. Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int,* 69**,** 213-7.

LOON, N. R. J. C. D. 2003. Diabetic kidney disease: preventing dialysis and transplantation. 21**,** 55-62.

LORENZO, C., OKOLOISE, M., WILLIAMS, K., STERN, M. P. & HAFFNER, S. M. J. D. C. 2003. The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. 26**,** 3153-3159.

LU, H., WU, L., LIU, L., RUAN, Q., ZHANG, X., HONG, W., WU, S., JIN, G. & BAI, Y. J. B. P. 2018. Quercetin ameliorates kidney injury and fibrosis by modulating M1/M2 macrophage polarization. 154**,** 203-212.

LU, T., SHENG, H., WU, J., CHENG, Y., ZHU, J. & CHEN, Y. J. N. R. 2012. Cinnamon extract improves fasting blood glucose and glycosylated hemoglobin level in Chinese patients with type 2 diabetes. 32**,** 408-412.

LUTALE, J. J. K., THORDARSON, H., ABBAS, Z. G. & VETVIK, K. J. B. N. 2007. Microalbuminuria among type 1 and type 2 diabetic patients of African origin in Dar Es Salaam, Tanzania. 8**,** 1-8.

MA, Y., CAI, J., WANG, Y., LIU, J., FU, S. J. D., METABOLIC SYNDROME, TARGETS, O. & THERAPY 2021. Non-Enzymatic Glycation of Transferrin and Diabetes Mellitus. 14**,** 2539.

MAANINKA, K. 2018. Atheroinflammatory Properties of LDL and HDL Particles Modified by Human Mast Cell Neutral Proteases.

MAECHLER, P., WOLLHEIM, C. B., BENTZEN, C., NIESOR, E. J. A. O. N. & METABOLISM 1993. Importance of exogenous cholesterol in diabetic rats: effects of treatment with insulin or with an acyl-CoA: cholesterol acyltransferase inhibitor. 37**,** 199-209.

MAGHRANI, M., LEMHADRI, A., ZEGGWAGH, N.-A., EL AMRAOUI, M., HALOUI, M., JOUAD, H. & EDDOUKS, M. J. J. O. E. 2004. Effects of an aqueous extract of Triticum repens on lipid metabolism in normal and recent-onset diabetic rats. 90**,** 331-337.

MANCUSO, T. J. 2021. Endocrinopathies. *Pediatric Anesthesiology Review*. Springer.

MANSOUR, A. A., AL-MALIKY, A. A., KASEM, B., JABAR, A. & MOSBEH, K. A. 2014. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. *Diabetes Metab Syndr Obes,* 7**,** 139-44.

MARSHALL, C. B. J. A. J. O. P.-R. P. 2016. Rethinking glomerular basement membrane thickening in diabetic nephropathy: adaptive or pathogenic? 311**,** F831-F843.

MASOUDI, A. J. A. O. T. R. S. F. C. B. 2021. Evaluation of Methods for Diagnosing Diabetes Mellitus Due to Hyperglycemia or Insulin Secretion. 25**,** 18410-18423.

MAYEDA, L., KATZ, R., AHMAD, I., BANSAL, N., BATACCHI, Z., HIRSCH, I. B., ROBINSON, N., TRENCE, D. L., ZELNICK, L., DE BOER, I. H. J. B. O. D. R. & CARE 2020. Glucose time in range and peripheral neuropathy in type 2 diabetes mellitus and chronic kidney disease. 8**,** e000991.

MCEUEN, A. R., ASHWORTH, D. M. & WALLS, A. F. 1998. The conversion of recombinant human mast cell prochymase to enzymatically active chymase by dipeptidyl peptidase I is inhibited by heparin and histamine. *Eur J Biochem,* 253**,** 300-8.

MEHTA, P. K. & GRIENDLING, K. K. J. A. J. O. P.-C. P. 2007. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. 292**,** C82-C97.

MERZAH, K. S., HASSON, S. F. J. I. J. O. P. M. & SCIENCES, B. 2015. The Biochemical changes in patients with chronic renal failure. 4**,** 75-79.

MIKHAEL, E., HASSALI, M. & HUSSAIN, S. 2021. Validation of newly developed culturally specific diabetes self-management education and support program for Iraqi type 2 diabetes mellitus patients. 10**,** 357-357.

MISHTO, M., MANSURKHODZHAEV, A., RODRIGUEZ-CALVO, T. & LIEPE, J. J. F. I. I. 2021. Potential mimicry of viral and pancreatic β cell antigens through non-spliced and cis-spliced zwitter epitope candidates in type 1 diabetes. 12**,** 1229.

MIYAZAKI, M., TAKAI, S., JIN, D., MURAMATSU, M. J. P. & THERAPEUTICS 2006. Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models. 112**,** 668-676.

MOGHETTI, P. & TOSI, F. J. J. O. E. I. 2021. Insulin resistance and PCOS: chicken or egg? 44**,** 233-244.

MOHSEN, M., ELBERRY, A. A., MOHAMED RABEA, A., ABDELRAHIM, M. E. & HUSSEIN, R. R. J. I. J. O. C. P. 2021. Saxagliptin and vildagliptin lowered albuminuria in patients with diabetes and hypertension independent on glycaemic control. 75**,** e13769.

MOLINA-VEGA, M., GUTIERREZ-REPISO, C., LIMA-RUBIO, F., SUáREZ-ARANA, M., LINARES-PINEDA, T. M., COBOS DíAZ, A., TINAHONES, F. J., MORCILLO, S. & PICóN-CESAR, M. J. J. J. O. C. M. 2021. Impact of the Gestational Diabetes Diagnostic Criteria during the Pandemic: An Observational Study. 10**,** 4904.

MOLITCH, M. E., DEFRONZO, R. A., FRANZ, M. J., KEANE, W. F., MOGENSEN, C. E., PARVING, H. H. & STEFFES, M. W. 2004. Nephropathy in diabetes. *Diabetes Care,* 27 Suppl 1**,** S79-83.

MORIGNY, P., BOUCHER, J., ARNER, P. & LANGIN, D. J. N. R. E. 2021. Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and therapeutics. 17**,** 276-295.

MORIYA, T., YAMAGISHI, T., MATSUBARA, M., OUCHI, M. J. J. O. D. & COMPLICATIONS, I. 2019. Serial renal biopsies in normo-and microalbuminuric patients with type 2 diabetes demonstrate that loss of renal function is associated with a reduction in glomerular filtration surface secondary to mesangial expansion. 33**,** 368-373.

MURAKAMI, K., UEHARA, Y., ABE, S., INOUE, Y. & IDEISHI, M. J. J. C. 2007. Positive Correlation Between Chymase-Like AngiotensinII-For-ming Activity in Mononuclear Cells and Serum Cholesterol Level. 50**,** 291-298.

MURRAY, I. & PAOLINI, M. A. 2020. Histology, Kidney and Glomerulus.

NAOUMOVA, R., CUMMINGS, M., WATTS, G., RENDELL, N., TAYLOR, G., SöNKSEN, P. & THOMPSON, G. J. E. J. O. C. I. 1996. Acute hyperinsulinaemia decreases cholesterol synthesis less in subjects with non-insulin-dependent diabetes mellitus than in non-diabetic subjects. 26**,** 332-340.

NISHIYAMA, A., KOBORI, H. J. C. & NEPHROLOGY, E. 2018. Independent regulation of renin–angiotensin–aldosterone system in the kidney. 22**,** 1231-1239.

NORRBY, K., JAKOBSSON, A. & SöRBO, J. 1986. Mast-cell-mediated angiogenesis: a novel experimental model using the rat mesentery. *Virchows Arch B Cell Pathol Incl Mol Pathol,* 52**,** 195-206.

OKON, K. & STACHURA, J. J. P. J. P. 2007. Increased mast cell density in renal interstitium is correlated with relative interstitial volume, serum creatinine and urea especially in diabetic nephropathy but also in primary glomerulonephritis. 58**,** 193-197.

OLOKOBA, A. B., OBATERU, O. A. & OLOKOBA, L. B. 2012. Type 2 diabetes mellitus: a review of current trends. *Oman Med J,* 27**,** 269-73.

OWENS, E. P., VESEY, D. A., KASSIANOS, A. J., HEALY, H., HOY, W. E. & GOBE, G. C. 2019. Biomarkers and the role of mast cells as facilitators of inflammation and fibrosis in chronic kidney disease. *Transl Androl Urol,* 8**,** S175-s183.

PADMANABHAN, N., JARDINE, A. G., MCGRATH, J. C. & CONNELL, J. M. J. C. 1999. Angiotensin-converting enzyme–independent contraction to angiotensin I in human resistance arteries. 99**,** 2914-2920.

PAL, S., NATH, S., MEININGER, C. J. & GASHEV, A. A. J. F. I. I. 2020. Emerging roles of mast cells in the regulation of lymphatic immuno-physiology. 11**,** 1234.

PARK, S., KANG, H.-J., JEON, J.-H., KIM, M.-J. & LEE, I.-K. J. A. O. P. R. 2019. Recent advances in the pathogenesis of microvascular complications in diabetes. 42**,** 252-262.

PARMANTIER, E., LYNN, B., LAWSON, D., TURMAINE, M., NAMINI, S. S., CHAKRABARTI, L., MCMAHON, A. P., JESSEN, K. R. & MIRSKY, R. J. N. 1999. Schwann cell–derived Desert hedgehog controls the development of peripheral nerve sheaths. 23**,** 713-724.

PATEL, D. M., BOSE, M. & COOPER, M. E. J. I. J. O. M. S. 2020. Glucose and Blood Pressure-Dependent Pathways–The Progression of Diabetic Kidney Disease. 21**,** 2218.

PAUL, M., POYAN MEHR, A. & KREUTZ, R. J. P. R. 2006. Physiology of local renin-angiotensin systems. 86**,** 747-803.

PEJLER, G., RöNNBERG, E., WAERN, I. & WERNERSSON, S. 2010. Mast cell proteases: multifaceted regulators of inflammatory disease. *Blood,* 115**,** 4981-90.

PETERSMANN, A., MULLER-WIELAND, D., MULLER, U. A., LANDGRAF, R., NAUCK, M., FRECKMANN, G., HEINEMANN, L. & SCHLEICHER, E. 2019. Definition, Classification and Diagnosis of Diabetes Mellitus. *Exp Clin Endocrinol Diabetes,* 127**,** S1-S7.

PIH, G. Y., NA, H. K., AHN, J. Y., JUNG, K. W., KIM, D. H., LEE, J. H., CHOI, K. D., SONG, H. J., LEE, G. H. & JUNG, H.-Y. J. B. G. 2018. Risk factors for complications and mortality of percutaneous endoscopic gastrostomy insertion. 18**,** 1-10.

POPOOLA, O., EPENUSI, O. J. A. O. M. & SCIENCES, H. 2016. Immunoglobulin E is associated with markers of mast cell degranulation and microalbuminuria in obese subjects with type 2 diabetes. 4**,** 175.

PRAIDOU, A., HARRIS, M., NIAKAS, D., LABIRIS, G. J. J. O. D. & COMPLICATIONS, I. 2017. Physical activity and its correlation to diabetic retinopathy. 31**,** 456-461.

PROVENZANO, M., COPPOLINO, G., DE NICOLA, L., SERRA, R., GAROFALO, C., ANDREUCCI, M., BOLIGNANO, D. J. F. I. C. & BIOLOGY, D. 2019. Unraveling cardiovascular risk in renal patients: a new take on old tale. 7**,** 314.

RANJAN, A., SCHMIDT, S., DAMM-FRYDENBERG, C., HOLST, J. J., MADSBAD, S. & NøRGAARD, K. 2017. Short-term effects of a low carbohydrate diet on glycaemic variables and cardiovascular risk markers in patients with type 1 diabetes: A randomized open-label crossover trial. 19**,** 1479-1484.

REAVEN, G., CHEN, N., HOLLENBECK, C. & CHEN, Y. D. J. J. O. T. A. G. S. 1989. Effect of age on glucose tolerance and glucose uptake in healthy individuals. 37**,** 735-740.

RITZ, E. 2003. Chymase: a potential culprit in diabetic nephropathy? : Am Soc Nephrol.

ROSIK, J., SZOSTAK, B., MACHAJ, F. & PAWLIK, A. J. A. O. H. G. 2020. The role of genetics and epigenetics in the pathogenesis of gestational diabetes mellitus. 84**,** 114-124.

ROSZKOWSKA-CHOJECKA, M. M., BARANOWSKA, I., GAWRYS, O., SADOWSKI, J., WALKOWSKA, A., KALISZ, M., LITWINIUK, A., KOMPANOWSKA-JEZIERSKA, E. J. C. & HYPERTENSION, E. 2021. Role of chymase in blood pressure control, plasma and tissue angiotensin II, renal Haemodynamics, and excretion in spontaneously hypertensive rats. 1-10.

RUBINS, H., ROBINS, S. & COLLINS, D. J. J. 2003. Treating Diabetic Dyslipidemia with Gemfibrozil for Secondary Prevention of Coronary Heart Disease. 10.

RUSDIANA, M. S. & AMELIA, R. J. O. A. M. J. O. M. S. 2018. The effect of diabetes self-management education on Hba1c level and fasting blood sugar in type 2 diabetes mellitus patients in primary health care in Binjai City of North Sumatera, Indonesia. 6**,** 715.

RUSUL ARIF, A., HAIDER, S. J. I. J. O. C. M. & SCIENCES, A. 2014. A study of some biochemical changes in patients with chronic renal failure undergoing hemodialysis. 3**,** 581-586.

RUTTER, H. J. C. C. 2018. The complex systems challenge of obesity. 64**,** 44-46.

SABUNCU, T., SONMEZ, A., EREN, M. A., SAHIN, I., ÇORAPCIOĞLU, D., ÜCLER, R., AKIN, Ş., HAYMANA, C., DEMIRCI, I. & ATMACA, A. J. P. C. D. 2021. Characteristics of patients with hypertension in a population with type 2 diabetes mellitus. Results from the Turkish Nationwide SurvEy of Glycemic and Other Metabolic Parameters of Patients with Diabetes Mellitus (TEMD Hypertension Study). 15**,** 332-339.

SALI, A., MATSUMOTO, R., MCNEIL, H. P., KARPLUS, M. & STEVENS, R. L. 1993. Three-dimensional models of four mouse mast cell chymases. Identification of proteoglycan binding regions and protease-specific antigenic epitopes. *J Biol Chem*, 268**,** 9023-34.

SAMADI-NOSHAHR, Z., EBRAHIMZADEH-BIDESKAN, A., SHAFEI, M. N., SALMANI, H., HOSSEINIAN, S. & KHAJAVI-RAD, A. J. B. 2021. trans-Anethole attenuated renal injury and reduced expressions of angiotensin II receptor (AT1R) and TGF-β in streptozotocin-induced diabetic rats. 185**,** 117-127.

SAMSU, N. J. B. R. I. 2021. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. 2021.

SANTOS, R. A., E SILVA, A. C. S., MARIC, C., SILVA, D. M., MACHADO, R. P., DE BUHR, I., HERINGER-WALTHER, S., PINHEIRO, S. V. B., LOPES, M. T. & BADER, M. J. P. O. T. N. A. O. S. 2003. Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. 100**,** 8258-8263.

SARAVANAN, G., PARI, L. J. D., OBESITY & METABOLISM 2003. Effect of Cogent db, a herbal drug, on serum and tissue lipid metabolism in experimental hyperglycaemic rats. 5**,** 156-162.

SAVOIA, C., BURGER, D., NISHIGAKI, N., MONTEZANO, A. & TOUYZ, R. M. J. E. R. I. M. M. 2011. Angiotensin II and the vascular phenotype in hypertension. 13.

SCHILLING, J. A. 1976. Wound healing. *Surg Clin North Am*, 56**,** 859-74.

SCHLATTER, D., BRACK, S., BANNER, D. W., BATEY, S., BENZ, J., BERTSCHINGER, J., HUBER, W., JOSEPH, C., RUFER, A. C., VAN DER KLOOSTER, A., WEBER, M., GRABULOVSKI, D. & HENNIG, M. 2012. Generation, characterization and structural data of chymase binding proteins based on the human Fyn kinase SH3 domain. *mAbs*, 4**,** 497-508.

SCHNAPER, H. W., HAYASHIDA, T., HUBCHAK, S. C. & PONCELET, A. C. 2003. TGF-beta signal transduction and mesangial cell fibrogenesis. *Am J Physiol Renal Physiol*, 284**,** F243-52.

SCIENCES, ا.ع. J. P. J. O. M. 2007. Prevalence of microalbuminuria in type 2 diabetes mellitus at a diabetic clinic in King Abdulaziz university hospital. 23.

SELVARAJAH, D., KAR, D., KHUNTI, K., DAVIES, M. J., SCOTT, A. R., WALKER, J., TESFAYE, S. J. T. L. D. & ENDOCRINOLOGY 2019. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. 7**,** 938-948.

SERAFIN, W. E., SULLIVAN, T. P., CONDER, G. A., EBRAHIMI, A., MARCHAM, P., JOHNSON, S. S., AUSTEN, K. F. & REYNOLDS, D. S. 1991. Cloning of the

cDNA and gene for mouse mast cell protease 4. Demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral proteases of the mouse and rat. *J Biol Chem,* 266**,** 1934-41.

SERDAR, M. A., SERTESER, M., UCAL, Y., KARPUZOGLU, H. F., AKSUNGAR, F. B., COSKUN, A., KILERCIK, M., ÜNSAL, İ., ÖZPıNAR, A. J. A. B. & BIOTECHNOLOGY 2020. An assessment of HbA1c in diabetes mellitus and pre-diabetes diagnosis: a multi-centered data mining study. 190**,** 44-56.

SETHI, S. K., KUMAR, R. J. J. O. A. M. & RESEARCH, D. S. 2021. Prevalence of nephropathy and its parameters in patients with diabetes mellitus-A prospective study in a Central Indian Population. 9**,** 35-39.

SHAHWAN, M. J., GACEM, S. A., ZAIDI, S. K. J. D., RESEARCH, M. S. C. & REVIEWS 2019. Prevalence of diabetic nephropathy and associated risk factors among type 2 diabetes mellitus patients in Ramallah, Palestine. 13**,** 1491-1496.

SHARMA, R., PRASAD, V., MCCARTHY, E. T., SAVIN, V. J., DILEEPAN, K. N., STECHSCHULTE, D. J., LIANOS, E., WIEGMANN, T., SHARMA, M. J. M. & BIOCHEMISTRY, C. 2007. Chymase increases glomerular albumin permeability via protease-activated receptor-2. 297**,** 161-169.

SHI, D., MOTAMED, M., MEJíA-BENíTEZ, A., LI, L., LIN, E., BUDHRAM, D., KAUR, Y. & MEYRE, D. J. O. R. 2021. Genetic syndromes with diabetes: A systematic review. 22**,** e13303.

SHIOTA, N., KAKIZOE, E., SHIMOURA, K., TANAKA, T. & OKUNISHI, H. 2005. Effect of mast cell chymase inhibitor on the development of scleroderma in tight-skin mice. *British journal of pharmacology,* 145**,** 424-431.

SHUKRI, M. Z., TAN, J. W., MANOSROI, W., POJOGA, L. H., RIVERA, A., WILLIAMS, J. S., SEELY, E. W., ADLER, G. K., JAFFE, I. Z. & KARAS, R. H. J. H. 2018. Biological sex modulates the adrenal and blood pressure responses to angiotensin II. 71**,** 1083-1090.

SILBIGER, S. R. & NEUGARTEN, J. J. A. I. R. R. T. 2003. The role of gender in the progression of renal disease. 10**,** 3-14.

SINGH, R., SINGH, A. K., ALAVI, N. & LEEHEY, D. J. J. J. O. T. A. S. O. N. 2003. Mechanism of increased angiotensin II levels in glomerular mesangial cells cultured in high glucose. 14**,** 873-880.

SMITH, M. T. & MURALIDHARAN, A. J. E. O. O. T. T. 2015. Targeting angiotensin II type 2 receptor pathways to treat neuropathic pain and inflammatory pain. 19**,** 25-35.

SOUTH, A. M., NIXON, P. A., CHAPPELL, M. C., DIZ, D. I., RUSSELL, G. B., SHALTOUT, H. A., O'SHEA, T. M. & WASHBURN, L. K. J. T. J. O. P. 2019. Obesity is associated with higher blood pressure and higher levels of angiotensin II but lower angiotensin-(1-7) in adolescents born preterm. 205**,** 55-60. e1.

STAR, R. A. J. K. I. 1998. Treatment of acute renal failure. 54**,** 1817-1831.

STEGBAUER, J., COFFMAN, T. M. J. C. O. I. N. & HYPERTENSION 2011. New insights into angiotensin receptor actions: from blood pressure to aging. 20**,** 84.

SUN, J., ZHANG, J., LINDHOLT, J. S., SUKHOVA, G. K., LIU, J., HE, A., ABRINK, M., PEJLER, G., STEVENS, R. L., THOMPSON, R. W., ENNIS, T. L., GURISH, M. F., LIBBY, P. & SHI, G. P. 2009. Critical role of mast cell chymase in mouse abdominal aortic aneurysm formation. *Circulation,* 120**,** 973-82.

TAKAHASHI, T., HUYNH-DO, U. & DANIEL, T. O. J. K. I. 1998. Renal microvascular assembly and repair: Power and promise of molecular definition. 53**,** 826-835.

TANAKA, A., NOMURA, Y., MATSUDA, A., OHMORI, K. & MATSUDA, H. J. A. J. O. P.-C. P. 2011. Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-delta-12, 14-prostaglandin J2. 301**,** C1360-C1367.

TANKEU, A. T., KAZE, F. F., NOUBIAP, J. J., CHELO, D., DEHAYEM, M. Y. & SOBNGWI, E. J. W. J. O. N. 2017. Exercise-induced albuminuria and circadian blood pressure abnormalities in type 2 diabetes. 6**,** 209.

TATSUMI, Y. & OHKUBO, T. 2017. Hypertension with diabetes mellitus: significance from an epidemiological perspective for Japanese. *Hypertension Research,* 40**,** 795-806.

TCHOUGOUNOVA, E., PEJLER, G. & ABRINK, M. 2003. The chymase, mouse mast cell protease 4, constitutes the major chymotrypsin-like activity in peritoneum and ear tissue. A role for mouse mast cell protease 4 in thrombin regulation and fibronectin turnover. *The Journal of experimental medicine,* 198**,** 423-431.

THORPE, M., FU, Z., CHAHAL, G., AKULA, S., KERVINEN, J., DE GARAVILLA, L. & HELLMAN, L. 2018. Extended cleavage specificity of human neutrophil cathepsin G: A low activity protease with dual chymase and tryptase-type specificities. *PLoS One,* 13**,** e0195077.

TIAN, S., WU, J., LIU, J.-S., ZOU, B.-S. & KONG, L.-Q. J. A. O. I. M. 2020. Type 2 Diabetes. 172**,** 704-705.

TIGERSTEDT, R. & BERGMAN, P. J. S. A. F. P. 1898. Niere und Kreislauf 1. 8**,** 223-271.

URATA, H., KINOSHITA, A., MISONO, K. S., BUMPUS, F. M. & HUSAIN, A. J. J. O. B. C. 1990. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. 265**,** 22348-22357.

VAN NETTEN, J. J., BUS, S. A., APELQVIST, J., LIPSKY, B. A., HINCHLIFFE, R. J., GAME, F., RAYMAN, G., LAZZARINI, P. A., FORSYTHE, R. O., PETERS, E. J. G., SENNEVILLE, E., VAS, P., MONTEIRO-SOARES, M., SCHAPER, N. C. & INTERNATIONAL WORKING GROUP ON THE DIABETIC, F. 2020. Definitions and criteria for diabetic foot disease. *Diabetes Metab Res Rev,* 36 Suppl 1**,** e3268.

VERDONK, K., DANSER, A. J. & VAN ESCH, J. H. J. E. O. O. I. D. 2012. Angiotensin II type 2 receptor agonists: where should they be applied? 21**,** 501-513.

VERMA, M., PANERI, S., BADI, P. & RAMAN, P. J. I. J. O. C. B. 2006. Effect of increasing duration of diabetes mellitus type 2 on glycated hemoglobin and insulin sensitivity. 21**,** 142-146.

VIBHUSHAN, S., BRATTI, M., MONTERO-HERNáNDEZ, J. E., EL GHONEIMI, A., BENHAMOU, M., CHARLES, N., DAUGAS, E. & BLANK, U. J. I. J. O. M. S. 2020. Mast cell chymase and kidney disease. 22**,** 302.

VISSE, R. & NAGASE, H. 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res,* 92**,** 827-39.

WåGSäTER, D., ZHU, C., BJöRKEGREN, J., SKOGSBERG, J. & ERIKSSON, P. J. I. J. O. M. M. 2011. MMP-2 and MMP-9 are prominent matrix metalloproteinases during atherosclerosis development in the Ldlr-/-Apob100/100 mouse. 28**,** 247-253.

WANG, I.-K., LIN, C.-L., CHEN, H.-C., LIN, S.-Y., CHANG, C.-T., YEN, T.-H. & SUNG, F.-C. J. N. D. T. 2018a. Risk of new-onset diabetes in end-stage renal disease patients undergoing dialysis: analysis from registry data of Taiwan. 33**,** 670-675.

WANG, Y., QU, H., XIONG, X., QIU, Y., LIAO, Y., CHEN, Y., ZHENG, Y. & ZHENG, H. J. M. O. I. 2018b. Plasma asprosin concentrations are increased in individuals

with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion. 2018.

WANG, Z., ZHANG, H., SHEN, X.-H., JIN, K.-L., YE, G.-F., QIAN, L., LI, B., ZHANG, Y.-H. & SHI, G.-P. J. P. O. 2011. Immunoglobulin E and mast cell proteases are potential risk factors of human pre-diabetes and diabetes mellitus. 6**,** e28962.

WARREN, A. M., KNUDSEN, S. T. & COOPER, M. E. J. E. O. O. T. T. 2019. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. 23**,** 579-591.

WOLF, G. J. E. J. O. C. I. 2004. New insights into the pathophysiology of diabetic nephropathy: from haemodynamics to molecular pathology. 34**,** 785-796.

XIANG, M., SUN, J., LIN, Y., ZHANG, J., CHEN, H., YANG, D., WANG, J. & SHI, G.-P. J. A. 2011. Usefulness of serum tryptase level as an independent biomarker for coronary plaque instability in a Chinese population. 215**,** 494-499.

XUE, J., MIN, F. & MA, F. Research on Diabetes Prediction Method Based on Machine Learning. Journal of Physics: Conference Series, 2020. IOP Publishing, 012062.

XUE, K., ZHANG, J., LI, C., LI, J., WANG, C., ZHANG, Q., CHEN, X., YU, X., SUN, L., YU, X. J. J. O. C. & MEDICINE, M. 2019. The role and mechanism of transforming growth factor beta 3 in human myocardial infarction-induced myocardial fibrosis. 23**,** 4229-4243.

YAHAYA, T. O. & UFUOMA, S. B. J. O. M. J. 2020. Genetics and Pathophysiology of Maturity-onset Diabetes of the Young (MODY): A review of current trends. 35**,** e126.

YALE, J.-F., PATY, B. & SENIOR, P. A. J. C. J. O. D. 2018. Hypoglycemia. 42**,** S104-S108.

YAN, P., TANG, Q., WU, Y., WAN, Q., ZHANG, Z., XU, Y., ZHU, J., MIAO, Y. J. D. & SYNDROME, M. 2021. Serum albumin was negatively associated with diabetic peripheral neuropathy in Chinese population: A cross-sectional study. 13**,** 1-9.

YOUNAN, G., SUBER, F., XING, W., SHI, T., KUNORI, Y., ABRINK, M., PEJLER, G., SCHLENNER, S. M., RODEWALD, H.-R., MOORE, F. D., JR., STEVENS, R. L., ADACHI, R., AUSTEN, K. F. & GURISH, M. F. 2010. The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5. *Journal of immunology (Baltimore, Md. : 1950),* 185**,** 7681-7690.

ZAMOLODCHIKOVA, T. S., SMIRNOVA, E. V., ANDRIANOV, A. N., KASHPAROV, I. V., KOTSAREVA, O. D., SOKOLOVA, E. A., IGNATOV, K. B. & PEMBERTON, A. D. 2005. Cloning and molecular modeling of duodenase with respect to evolution of substrate specificity within mammalian serine proteases that have lost a conserved active-site disulfide bond. *Biochemistry (Mosc),* 70**,** 672-84.

ZELMANOVITZ, T., GERCHMAN, F., BALTHAZAR, A. P., THOMAZELLI, F. C., MATOS, J. D. & CANANI, L. H. 2009. Diabetic nephropathy. *Diabetol Metab Syndr,* 1**,** 10.

ZHANG, X.-X., KONG, J. & YUN, K. J. J. O. D. R. 2020. Prevalence of diabetic nephropathy among patients with type 2 diabetes mellitus in China: a meta-analysis of observational studies. 2020.

ZHAO, L., GAO, Y., CAO, X., GAO, D., ZHOU, S., ZHANG, S., CAI, X., HAN, F., WILCOX, C. S. & LI, L. J. A. P. 2017. High-salt diet induces outward remodelling of efferent arterioles in mice with reduced renal mass. 219**,** 654-661.

ZHAO, X.-Y., ZHAO, L.-Y., ZHENG, Q.-S., SU, J.-L., GUAN, H., SHANG, F.-J., NIU, X.-L., HE, Y.-P., LU, X.-L. J. M. & BIOCHEMISTRY, C. 2008. Chymase induces

profibrotic response via transforming growth factor-β1/Smad activation in rat cardiac fibroblasts. 310**,** 159-166.

ZHENG, J., YAO, G., CHENG, Z., WANG, R. & LIU, Z. J. D. 2012. Pathogenic role of mast cells in the development of diabetic nephropathy: a study of patients at different stages of the disease. 55**,** 801-811.

ZHOU, Y., CHEN, K., DU, X., TANG, J. & SHI, B. J. D. T. 2021. Risk Factors for Albuminuria in Normotensive Older Adults with Type 2 Diabetes Mellitus and Normal Renal Function: A Cross-Sectional Study. 12**,** 697-705.

الخلاصة

داء السكري (DM) هو مجموعة من الأمراض الأيضية التي تتميز بارتفاع السكر في الدم الناتج عن عيوب في إفراز الأنسولين أو عمل الأنسولين أو كليهما. يرتبط ارتفاع السكر في الدم المزمن في مرض السكري بضرر طويل الأمد واختلال وظيفي وفشل أعضاء مختلفة ، وخاصة العين والكلى والأعصاب والقلب والأوعية الدموية.

تهدف هذه الدراسة إلى التحقق من الارتباط بين مستويات الكيماز والأنجيوتنسين في مرضى السكري من النوع 2 المصابين باعتلال الكلية السكري المبكر والمزمن ، وكذلك دراسة تأثير الجنس ومؤشر كتلة الجسم ومدة المرض والفئات العمرية على قيم المتغيرات المدروسة مع الآخرين. المتغيرات في المرضى والأشخاص الأصحاء وكذلك هل توجد فروق ذات دلالة إحصائية بين مستويات الكيماز و الأنجيوتنسين 2 ومرضى السكري من النوع 2 المصابين باعتلال الكلية السكري المبكر والمزمن

أُجريت هذه الدراسة للاصحاء كضوابط والأفراد المصابين بداء السكري من النوع 2 في الفترة من تشرين الثاني (نوفمبر) 2021 إلى ايار (مايو) 2022. وقد أجريت هذه الدراسة على 62 مريضًا يعانون من مرض السكري من النوع 2 الذين حضروا مدينة الإمام الحسين الطبية في كربلاء. و 28 حالة في مجموعة الضبط . المرضى تم تقسيمهم إلى ثلاث مجموعات وفقًا لنسبة الألبومين البولي لديهم إلى نسبة الكرياتينين (ACR) ، والمجموعة الأولى (بيلة نورمالبومينية) حيث كان معدل ACR أقل من 30 وعددهم 22 مريضًا ، والمجموعة الثانية (البيلة الألبومينية الزهيدة) كانت ACR من 30-299 وعددهم 20 مريض والمجموعة الثالثة (Macroalbuminuria) حيث كان ACR أكبر أو يساوي 300 ، وكان عددهم 20 مريضاً بالإضافة إلى تم تقسيمهم إلى أربع مجموعات حسب مؤشر كتلة جسمهم ، المجموعة الأولى (مؤشر كتلة الجسم أقل من 18) عددهم 4 ، والمجموعة الثانية (مؤشر كتلة الجسم 18-24.9) عددهم 10 والمجموعة الثالثة (مؤشر كتلة الجسم 25-29.9) عددهم 21 ، المجموعة الرابعة (مؤشر كتلة الجسم أكبر أو يساوي 30) وعددهم 27 مريض ، كما تم تقسيمهم إلى ثلاث مجموعات حسب العمر ، المجموعة الأولى كانت عمر (35-45) وعددهم 11 ، والمجموعة الثانية 46 -60 وعددهم 26 والمجموعة الثالثة (فوق 60) وعددهم 25. وكانوا قسمة ايضا قسمت إلى ثلاث مجموعات حسب مدة المرض ، المجموعة الأولى (مدة المرض أقل من 5 سنوات) وعددها 13 ، والمجموعة الثانية (مدة المرض 5-10 سنوات) والمجموعة الثانية عددهم 16 والمجموعة الثالثة (مدة المرض أكبر من 10 سنوات) وعددهم 33 ، وتم تقسيمهم حسب الجنس إلى 35 ذكر و 27 أنثى.

شملت الدراسة البيوكيميائية مجموعة المريض والمجموعة الضابطة. قياس مستويات السكر الصائم (FBS) ، الهيموجلوبين السكري (HbA1c) ، اليوريا في الدم ، مصل الكرياتينين ، نسبة الألبومين إلى الكرياتينين (ACR) ، بيلة الألبومين الزهيدة ، الكوليسترول (CHOL) ، الدهون الثلاثية (TG) ، مستويات البروتين الدهني عالي الكثافة (HDL) تم أيضًا قياس البروتين الدهني منخفض الكثافة (LDL) والبروتين الدهني منخفض الكثافة جدًا (VLDL) و Chymase و AngiotensinII.

أظهرت هذه الدراسة فرقا كبيرا معنويا ($P\leq0.01$) في سكر الصيام (FBS) ، الهيموجلوبين السكري (HbA1c) ، اليوريا في الدم ، مصل الكرياتينين ، البول الزلالي الدقيق ، نسبة الألبومين إلى الكرياتينين (ACR) ، الكوليسترول (CHOL) ، الدهون الثلاثية (TG) والبروتين الدهني عالي الكثافة (HDL) والبروتين الدهني منخفض الكثافة (LDL) والبروتين الدهني منخفض الكثافة جدًا (VLDL) والبروتين الدهني عالي الكثافة (HDL) والكيماز والأنجيوتنسين II ، بين مرضى السكري ومجموعات التحكم.

أظهرت هذه الدراسة أيضا أنه وفقا لتأثير العمر في (FBS ، يوريا الدم ، HbA1c، CHOL، LDL، VLDL، AngiotensinII Microalbuminuria ، نسبة الألبومين - الكرياتينين (ACR)) كانت هناك فروق ذات دلالة إحصائية بين المجموعة الأولى والثانية ، وكذلك بين المجموعتين الأولى والثالثة. أما بالنسبة لمركبات (TG ، كرياتينين المصل ، HDL ، و Chymase) ، فقد كانت هناك فروق ذات دلالة إحصائية بين المجموعتين الأولى والثالثة.

كما أظهرت هذه الدراسة تأثير مؤشر كتلة الجسم ، في (TG، VLDL ، ومدة المرض) كانت هناك فروق ذات دلالة إحصائية بين كل من المجموعتين الأولى والرابعة ، الأولى والثالثة ، والثانية والرابعة كذلك. كما لاحظنا في (يوريا الدم ، بيلة الألبومين الدقيقة ، نسبة الألبومين - الكرياتينين (ACR) ، مصل الكرياتينين والأنجيوتنسين 2) كانت هناك فروق ذات دلالة إحصائية بين المجموعة الأولى والرابعة ، وكذلك في (HDL، LDL، FBS، CHOL، HbA1c). توجد فروق ذات دلالة إحصائية بين المجموعة الأولى والرابعة والمجموعة الثانية والرابعة ، وفي المجموعة (Chymase) كانت هناك فروق ذات دلالة إحصائية في المجموعة الأولى والرابعة والمجموعة الثانية والرابعة والمجموعة الثالثة والرابعة.

أظهرت هذه الدراسة أيضًا وفقًا لتأثير مدة المرض ، على (HDL، LDL، FBS، CHOL، HbA1c ، بيلة الألبومين الزهيدة ، نسبة الألبومين - الكرياتينين (ACR، TG ، وVLDL) ، كانت هناك فروق ذات دلالة إحصائية بين جميع المجموعات ، كما لاحظنا في (يوريا الدم ، كرياتينين

المصل ، أنجيوتنسين 2 ، وكيماز) فروق ذات دلالة إحصائية بين المجموعتين الأولى والثالثة والثانية والثالثة.

أظهرت دراسة المقارنة بين داء السكري من النوع 2 ومجموعات اعتلال الكلية السكري للمعلمات البيوكيميائية في (HDL و LDL و FBS و CHOL و HbA1c و Macroalbuminuria و TG و VLDL واليوريا في الدم وكرياتينين المصل والأنجيوتنسين 2) ، كان هناك إحصائيًا. فروق ذات دلالة إحصائية بين جميع المجموعات ، وكذلك في Chymase كان هناك فروق ذات دلالة إحصائية بين المجموعة الأولى والثالثة والثانية والثالثة.

أظهرت النتائج وجود علاقة ارتباط موجبة معنوية بين مستويات HbA1c و FBS واليوريا في الدم وكرياتينين المصل والبيلة الألبومينية الزهيدة و CHOL و TG و LDL و VLDL مقابل Chymase ومستويات ANGII لدى المرضى ، كما أظهرت الدراسة سلبية الانحدار الخطي بين Chymase و ANGII مع HDL .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب

قسم الكيمياء الحيوية السريرية

تقييم الكايميز والأنجيوتنسين II في تطور النوع الثاني لمرضى السكري المصابين بالاعتلال الكلوي

رسالة

مقدمة الى مجلس كلية الطب / جامعة كربلاء كجزء من متطلبات نيل شهادة الماجستير في الكيمياء الحياتية السريرية

من قبل

ستار جابر حسن

بكالوريوس كيمياء كلية العلوم جامعة بابل

2001

باشراف

الاستاذ المساعد الدكتورة / شيماء زهراو ندى

الاستاذ الدكتور فراس شوقي الجبوري

2022 ميلادي 1443 هجري