

**Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Al-Qadisiyah / College of Science
Department of Biological**



**Evaluation the levels and functions of some biochemical parameters in
patients with diabetic foot ulcer**

A Thesis

**Submitted to The Council of The Collage of The Science University of Al-
Qadisiyah it is partial of the requirement for obtaining a Master's Degree in
biology /zoology**

By

Aseel Abdul Amir Kamash Al-Khalidi

B.S.C Biology(2008)

underSupervision of

Assistant. professor. Dr. Rasha Muzahim Hatem

July /2025

1447/ محرم

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا

الْعِلْمَ دَرَجَاتٍ ۖ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

(صدق الله العلي العظيم)

سورة المجادلة، الآية ﴿ 11 ﴾

Supervision Certification

I hereby declare that this thesis (**Evaluation the levels and functions of some biochemical parameters in patients with diabetic foot ulcer**)

was prepared by **Aseel Abdul Amir Kamash**, under my supervision at the Department of Biology, College of Science / the University of Al-Qadisiyah, as partial fulfilment of the requirements for the degree the master in Biology

Supervisor

Assistant. professor.. Dr. Rasha Muzahim Hatem

Supervisor

The recommendation of the Department

In the view of the available recommendation,for wared this thesis for debate
By the examining committee.

Prof. Dr. Laith Saree Matar

Head of Department of Biology College of Science, Al-Qadisiyah University

The Rectified Scientific Declaration

I certify that the thesis prepared by **Aseel Abdul Amir Kamash (Evaluation the levels and functions of some biochemical parameters in patients with diabetic foot ulcer)** has been revised from the scientific point of view, and the scientific errors in it have been corrected; thus, the thesis became suitable for discussion.

Signature:

Name:

Scientific degree:

Address:

Date: / / 2025

The Rectified Scientific Declaration

I certify that the thesis prepared by **Aseel Abdul Amir Kamash (Evaluation the levels and functions of some biochemical parameters in patients with diabetic foot ulcer)** has been revised from the scientific point of view, and the scientific errors in it have been corrected; thus, the thesis became suitable for discussion.

Signature:

Name:

Scientific degree:

Address:

Date: / / 2025

The Rectified Linguistic Declaration

I certify that the thesis prepared by **Aseel Abdul Amir Kamash (Evaluation the levels and functions of some biochemical parameters in patients with diabetic foot ulcer)** has been revised from the linguistic point of view, and the linguistic and expressive errors in it have been corrected; thus, the thesis became suitable for discussion as far as the safety of style and the validity of expression.

Signature:

Name:

Scientific degree:

Address:

Date: / / 2025

Committee Certificate

We are examining committee, certify that we have read this thesis **(Evaluation the levels and functions of some biochemical parameters in patients with diabetic foot ulcer)** by **Aseel Abdul Amir Kamash** in its contents, we find it adequate as a thesis for the award of the Degree of Master in Biology

Approved for the collage of science was met at which held in
/ /2025 and decided to grant her a Master's degree of science in biology.

Signature:

Assist. Prof. Dr.

Address:

Date: / / 2025

Dedication

To the Creator of souls and the Originator of life, to the One who brought forth existence from nothingness, to the All-Knowing, the All-Merciful...

To the one who conveyed the divine message, fulfilled the trust, and guided the nation – to the Prophet of Mercy and the Light of the Worlds, Muhammad (peace be upon him and his purified progeny), the noble household, and the steadfast rope of divine guidance.

To the light of my heart, the vision of the unseen, the Hidden Imam, ever-present in the hearts of the faithful – the awaited savior, Imam Al-Mahdi (may Allah hasten his reappearance).

To....

the one who taught me that life is a struggle, and that its weapons are knowledge and perseverance...

To the man who never failed me, who supported me with all he had – my dearest father.

To....

the purest soul, the one whose prayers guided my steps, who endured for my sake, and whose love is eternal – my beloved mother.

To....

My companion... to the one who held my hand in every endeavor I pursue, with his endless support... with him I share every dream and success, My dear husband.

To....

those who stood by me through every moment – my brothers and sisters, and children, and my dear friends – I dedicate this humble effort with all my love.

And to anyone fighting a silent battle – know that you, too, can succeed. Never give up.

To all those chasing their dreams – never let go.

ASEEL

Acknowledgments

I would want to express my gratitude to Almighty God for assisting me in meeting the requirements of this thesis. Assisst. Prof. Dr. my Rasha Muzahem Hatem supervisors, deserve my greatest gratitude and sincere thanks for this keen interest, kind assistance, and important advice. With a heart full of gratitude, I extend my deepest thanks and appreciation to my esteemed professors at the University of Al-Qadisiyah – College of Science – Department of Biology. They have been guiding lights on my academic journey, instilling in me the values of integrity and dedication to science. I also wish to express my sincere gratitude to the honorable members of the Discusion committee for their valuable time, insightful comments, and constructive feedback. Their guidance has significantly enriched this thesis and will remain a beacon that lights my way in future academic endeavors. I extend my sincere thanks and appreciation to the medical and nursing staff at Al-Hashimiya General Hospital and Al-Qasim General Hospital in Babylon Province for their valuable cooperation in collecting samples and accomplishing the practical aspect of this study. I would also like to express my deepest gratitude to the patients who participated in this study. Despite their suffering, their kind consent and valuable contribution are greatly appreciated. I pray to Almighty God to grant them a speedy recovery and reward them abundantly. Finally, to my beloved family my true support in every moment thanks you from the bottom of my heart To all, please accept my truthful thanks.

ASEEL

Summary

Summary

Diabetic foot ulcers (DFUs) are among the most serious complications of diabetes, significantly impairing patients' quality of life and often leading to persistent infections and potential limb loss. DFUs occurs due to poor circulation caused by atherosclerosis, Venous insufficiency, Problems with the valves in leg veins – wich affecting the safety and speed of blood flow, Blood clotting, coagulation. This study focused on four key immune markers: interleukin IL-10, IL-18, Tumor Necrosis Factor-alpha (TNF- α), Basic Fibroblast Growth Factor (BFGF), C-Reactive Protein (CRP), and lipid profile parameters including Triglycerides (Trig), Total Cholesterol (Tc), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), along with Hemoglobin A1c (HbA1c), among individuals with diabetic foot ulcers to gain a deeper understanding of the causes behind delayed wound healing. The study involved 90 participants: 30 patients with diabetic foot ulcers, 30 diabetic patients without foot ulcers, and 30 healthy controls. Participants were recruited from diabetes centers at Al-Hashimiyah General Hospital and Al-Qasim General Hospital in Babil Governorate between September 2024 and February 2025. Statistical analyses compared results across the groups. The results showed no statistically significant difference in the mean age among the three groups (DFU patients, diabetic patients without ulcers, and healthy controls) ($P = 0.158$). Similarly, age group distribution showed no significant difference ($P = 0.132$). A significant difference was observed in sex distribution ($P = 0.001$), with males predominating among DFU patients, suggesting sex plays an important role in the development of diabetic foot ulcers. No significant differences were found concerning smoking status and hypertension ($P > 0.05$). However, insulin usage showed a significant difference ($P = 0.015$), indicating that patients with ulcers were more likely to be on insulin therapy. No significant differences were observed in ulcer location, duration,

or swelling ($P > 0.05$). Regarding ulcer types, a significant difference was found in distribution ($P = 0.025$), indicating varied ulcer severity among patients. Mean glucose and HbA1c levels showed significant differences ($P < 0.001$), although no significant difference was found between DFU and diabetic non-ulcer groups. Serum triglycerides and LDL levels were higher in both DFU and diabetic groups compared to healthy controls, indicating lipid profile disturbances, with statistically significant differences ($P < 0.05$). DFU patients exhibited lower HDL cholesterol levels compared to diabetic and healthy groups, which may reflect an increased risk of cardiovascular diseases ($P > 0.05$). Mean CRP levels also showed significant differences among DFU, diabetic, and healthy groups ($P < 0.05$), with significant differences between the two patient groups themselves ($P < 0.05$). IL-18 levels were markedly elevated in DFU patients compared to diabetic patients without ulcers and healthy individuals ($P < 0.001$). Similarly, TNF- α levels were significantly higher in the DFU group ($P < 0.001$). Conversely, IL-10 and BFGF levels were significantly increased in both DFU and diabetic patients compared to healthy controls ($P < 0.001$).

It can be conclude that from our current study that impaired healing of diabetic foot ulcers results from an interplay of inflammatory factors such as IL-18 and TNF- α along with biochemical and environmental factors. Chronic inflammation caused by elevated inflammatory markers, lipid profile disturbances, and poor glycemic control leads to delayed tissue repair. Although the body attempts to mitigate inflammation by increasing IL-10 and BFGF concentrations, effective therapeutic strategies should focus on improving glycemic control and correcting lipid abnormalities to enhance wound healing and reduce complications.

List of contents

No.	Subject	Page No.
	Summary	A
	List of contents	C
	List of tables	F
	List of figures	H
	List of abbreviation	I
1	Chapter One Introduction	
	Introduction	1
	Aim of study	2
2	Chapter Two Literatures Review	
2.1	Diabetes Mellitus	4
2.2	Three main Types of Diabetes Mellitus	4
2.2.1	Type 1diabetes mellitus (T1DM)	4
2.2.2	Type 2 Diabetes Mellitus (T 2 DM)	6
2.2.3	Gestational diabetes	7
2.3	Other specific types of diabetes	9
2.4	Glycated hemoglobin	
2.4.1	Types of Hemoglobin	
2.5	Diabetic Foot Ulcers (DFUs)	
2.5.1	Epidemiology of DFUs	9
2.5.2	Etiology of Diabetic Foot Ulcers	10
2.5.3	Diagnosis of Diabetic Foot Ulcers	11
2.5.4	Pathophysiology of Diabetic Foot Ulcers	11

2.5.5	Classification of Diabetic Foot Ulcers (DFUs)	12
2.5.6	Diabetic Ulcer Healing Process	14
2.5.6.1	Hemostasis	
2.4.6.2	Inflammation	
2.5.6.3	Proliferation	
2.5.6.4	Maturation	
2.5.7	Factors Affecting Diabetic foot ulcers Healing	17
2.5.8	Healing of Diabetic Foot Ulcers (DFU)	18
2.5.9	Management of Diabetic Foot Ulcers (DFUs)	19
2.5.9.1	Clinical Evaluation	
2.5.9.2	Pressure offloading	
2.5.9.3	Infection control	
2.5.9.4	Local woundcare	
2.5.9.5	Systemic optimization	
2.5.9.6	Surgical and vascular interventions	
2.5.10	Immune Dysregulation in Diabetes Mellitus	21
2.6	Immunological Markers in Diabetic	23
2.6.1	Interleukin-10 (IL-10)	23
2.6.2	Interleukin-18 (IL-18)	24
2.6.3	Tumor Necrosis Factor-Alpha (TNF- α)	26
2.6.4	Basic fibroblast growth factor (BFGF)	28
3	Chapter Three Materials and Methods	
3.1	Materials	30

3.1.1	Instruments and Equipment Used in the Study	30
3.1.2	ELISA Kits	32
3.2	Methods	32
3.2.1	Study of Design and sample Collecting	32
3.2.2	Design of Study	33
3.3	Blood Samples Collection	35
3.3.1	Biochemical Tests	36
3.3.2	Biomarkers Profile Assay by ELISA Technique	39
3.4	Statistical analysis	51
	Chapter Four/Results & discussion	
4.1	Demographic characteristics	52
4.2	Sex	54
4.3	Some risk factors	55
4.4	Some clinical features associated diseases with Ulcer	57
4.5	Results of metabolic factors (Glucose and HbA1c) in patients and healthy controls	59
4.5.1	Correlation between metabolic factors (Glucose and HbA1c) in both group patients	63
4.6	Results of lipid profile (Triglycerides, Cholesterol, LDL and HDL) in patients and healthy controls	72
4.6.1	Correlation between lipid profile parameters and other parameters in both group patients	77
4.7	Results of C-reactive protein (CRP) in patients and healthy controls	81
4.7.1	Correlation between C-reactive protein (CRP) and other parameter in both group patients	83
4.8	Subjects immunological parameters Analysis Results	85

4.8.1	Interleukin-10 (IL-10) level in study groups	85
4.8.2	Interleukin-18 (IL-18) level in study groups	91
4.8.3	Interleukin-18 (IL-18) level in study groups	97
4.8.4	Interleukin-18 (IL-18) level in study groups	103
4.9	Correlation between immunological parameters in both group patients	110
	Chapter Five Conclusions and Recommendations	
5.1	Conclusions	113
5.2	Recommendations	114
	References	116
	الخلاصة العربية	

List of Tables

Table No.	Title	Page No.
2.1	Wagner Classification System for Diabetic Foot Ulcers	12
2.2	University of Texas Wound Classification System for Diabetic Foot Ulcers	13
3.1	Instruments and Equipment Used in the Study	30
3.2	chemical and reagents used in the study	31
3.3	Devices &Analytical methods used for measuring HbA1c, CRP and Lipid profile parameters	31
3.4	ELISA Devices and Kits Used in the Study	32
4.1	Comparison between patients and control groups in Age group	53
4.2	Comparison between patients and control groups inSex	54
4.3	The frequency distribution Some risk factors	56

4.4	The frequency distribution Ulcer patients according to some clinical features	58
4.5	Comparison of metabolic factors (Glucose and HbA1c) in patients and healthy controls	60
4.6	Correlation between metabolic factors (Glucose and HbA1c) in Ulcer patients	63
4.7	Correlation between metabolic factors (Glucose and HbA1c) in DM patients	63
4.8	Results of lipid profile (Triglycerides, Cholesterol, LDL and HDL) in patients and healthy controls	73
4.9	Correlation between lipid profile and other parameters in Ulcer patients	78
4.10	Correlation between lipid profile and other parameters in DM patients	79
4.11	Comparison of C-reactive protein (CRP) in patients and healthy controls	81
4.12	The correlation between C-reactive protein (CRP) and other parameter in both groups of patients	84
4.13	Interleukin-10 (IL-10) level in study groups	86
4.14	Roc curve of Interleukin-10 (IL-10) level	88
4.15	The correlation between IL-10 levels and other parameter in both groups of patients	90
4.16	Interleukin-18 (IL-18) level in study groups	92
4.17	Roc curve of Interleukin-18 (IL-18) level	94
4.18	The correlation between IL-18 levels and other parameter in both groups of patients	95
4.19	Tumor Necrosis Factor Alpha (TNF- α) level in study groups	97
4.20	Roc curve of Tumor Necrosis Factor Alpha (TNF- α) level	100

4.21	The correlation between TNF- α level and other parameter in both groups of patients	102
4.22	Basic fibroblast growth factor (bFGF) level in study groups	104
4.23	Roc curve of basic fibroblast growth factor (bFGF) level	106
4.24	The correlation between bFGF level and other parameter in both groups of patients	108
4.25	Correlation between immunological parameters in Ulcer patients	110
4.26	Correlation between immunological parameters in DM patients	111

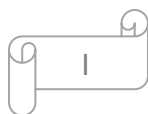
List of Figures

Figure No.	Title	Page No.
3-1	Design of Study	34
3-2	Dilution of standard solutions	41
3-3	standard curve for the IL-18 levels in test samples	41
3-4	Dilution of standard solutions (IL-10)	43
3-5	This curve is used to interpolate the IL-10 levels in test samples	44
3-6	Dilution of standard solutions(bFGF)	45
3-7	Standard Curve for Human TNF- α measured by ELISA	46
3-8	Dilution of standard solutions TNF- α	49
3-9	Standard Curve for Human bFGF measured by ELISA	50
4-10	(A) Receiver operating characteristic curve for IL-10 levels to distinguish Ulcer patients from healthy control subjects. (B)	89

	Receiver operating characteristic curve for IL-10 levels to distinguish DM patients from healthy control subjects.	
4-11	(A) Receiver operating characteristic curve for IL-18 levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for IL-18 levels to distinguish DM patients from healthy control subjects.	
4-12	(A) Receiver operating characteristic curve for TNF- α levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for TNF- α levels to distinguish DM patients from healthy control subjects.	
4-13	(A) Receiver operating characteristic curve for (bFGF) levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for (bFGF) levels to distinguish DM patients from healthy control subjects.	

List of Abbreviations

Abbreviations	Description
4-PL	Four-Parameter Logistic Model
ABI	Ankle-Brachial Index
ADA	American Diabetes Association
AGEs	Advanced Glycation End Products
ANOVA	Analysis of Variance
AUC	Area Under the Curve
CI	Confidence Interval
CRP	C-Reactive Protein
CT / MRI	Computed Tomography / Magnetic Resonance Imaging
CV	Coefficient of Variation
DFU	Diabetic Foot Ulcer
DM	Diabetes Mellitus
DMRT	Duncan's Multiple Range Test
ELISA	Enzyme-Linked Immunosorbent Assay
EPCs	Endothelial Progenitor Cells
GDM	Gestational Diabetes Mellitus
HDL-C	High-Density Lipoprotein Cholesterol
HRP	Horseradish Peroxidase



HbA1c	Hemoglobin A1c
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-1 β	Interleukin-1 Beta
IL-6	Interleukin-6
IRS-1	Insulin Receptor Substrate-1
LDL-C	Low-Density Lipoprotein Cholesterol
NF-K β	Nuclear Factor Kappa B
NLRP3	NOD-, LRR- and Pyrin Domain-Containing Protein 3 Inflammasome
NPV	Negative Predictive Value
NS	Non-Significant
Nm	Nanometer
OD	Optical Density
PAD	Peripheral Arterial Disease
PBS	Phosphate Buffered Saline
PLT	Platelets
PPV	Positive Predictive Value
RBC	Red Blood Cells
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
S	Significant
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TCHO	Total Cholesterol
TMB	Tetramethylbenzidine
TNF- α	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor
VLDL	Very Low-Density Lipoprotein
WBC	White Blood Cells
WHO	World Health Organization
bFGF (FGF-2)	Basic Fibroblast Growth Factor
μ L	Microliter

Chapter One

Introduction

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, action, or both (American Diabetes Association, 2020). According to the World Health Organization, approximately 422 million individuals globally are affected by diabetes, and projections estimate an additional 271 million people will be diagnosed by 2045 (World Health Organization, 2018; Cho *et al.*, 2018). Poor glycemic control leads to chronic complications, some of which may be present even at the time of diagnosis. Among these, diabetic foot ulcers (DFUs) stand out as one of the most common and debilitating outcomes, affecting 15–25% of diabetic individuals and contributing significantly to morbidity and mortality (Zhang *et al.*, 2017). Long-term complications associated with diabetes include cardiovascular diseases, nephropathy, retinopathy, peripheral neuropathy, and chronic lower-limb ulcers (Al-Saliha and Ali, 2021). Diabetes mellitus is generally classified into three main types: type 1 diabetes, characterized by autoimmune destruction of pancreatic beta cells; type 2 diabetes, resulting from peripheral insulin resistance combined with a relative insulin deficiency and gestational diabetes, which occurs during pregnancy due to hormonal changes affecting insulin sensitivity (American Diabetes Association, 2007).

DFUs represent one of the most serious complications of diabetes mellitus and are a leading cause of plantar ulceration (Ali, 2022). These ulcers typically affect the sole of the foot and involve all layers of the skin. They are often associated with peripheral neuropathy or peripheral arterial disease, poor glycemic control, and structural foot deformities (Al-Rubeaan *et al.*, 2015). Chronic hyperglycemia induces oxidative stress and vascular endothelial dysfunction by generating reactive oxygen species, which impairs wound healing in diabetic patients (Al-Salih *et al.*,

2021). If untreated, DFUs frequently progress to amputation, Research indicated that healthcare costs are substantially higher for diabetic patients with DFUs compared to those without ulcers (Anyim *et al.*, 2019). DFUs are characterized by a prolonged and dysregulated inflammatory phase, with an overproduction of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α . This imbalance between pro- and anti-inflammatory macrophages impairs proper wound resolution and contributes to chronicity (Kadhim, 2021a).

Aim of the study

The present study aims to evaluate the role of cytokines, hematological parameters, and inflammatory markers in patients with diabetic foot ulcers. To achieve this objective, the following parameters were assessed:

1. The effect of age, gender, smoking, insulin therapy, blood pressure, and several ulcer-related characteristics such as ulcer location, type, presence of swelling, and duration—among diabetic patients with and without foot ulcers. These variables were compared with those of healthy control.
2. Determination (HbA1c) levels and Glucose.
3. Determination parameters included lipid profile components including, TcTG, LDL), and (HDL), in order to assess metabolic control among diabetic patients with and without foot ulcers, as well as in healthy controls.
4. Determination the level of (CRP).
5. Additionally, the study evaluated selected immunological markers in diabetic patients compared to healthy individuals. These included (IL-18), (IL-10), (TNF- α), and (bFGF).

Chapter Two

Literatures

review

2. Literatures Review

2.1. Diabetes Mellitus

Diabetes mellitus is a group of chronic metabolic disorders characterized by elevated blood glucose levels due to either impaired insulin secretion, insulin resistance, or both (Seidu *et al.*, 2022). This condition affects vital organs such as the eyes, kidneys, nerves, heart, and blood vessels due to disruptions in carbohydrate, protein, and fat metabolism (Liu *et al.*, 2022a). One of the most prominent complications is peripheral neuropathy, which results from reduced blood flow to nerves, causing sensory loss, especially in areas like the metatarsophalangeal joints and heels, leading to ulcer formation (Pallela *et al.*, 2017). The disease originates from dysfunction in pancreatic islets, where beta cells produce insulin and alpha cells secrete glucagon. Any imbalance between these hormones leads to hyperglycemia (Lenzen, 2021).

2.2. Three main Types of Diabetes Mellitus

Diabetes mellitus is categorized into three major types: T1DM, T2DM and G DM (American Diabetes Association, 2018).

2.2.1. Type 1 diabetes mellitus (T1DM)

T1DM (due to autoimmune β -cell destruction) was identified as an autoimmune disease due to the complete absence of insulin or receptor damage since birth. Researchers identified this T1DM as a T-cell mediated acquired autoimmunity disorder. T1DM patients were found with a low life span, and they were highly prone to cardio-vascular diseases and severe metabolic conditions in their medical history (Lind *et al.*, 2019). The condition is usually diagnosed in children and young people so it used to be called juvenile diabetes. A condition called secondary diabetes is like

type 1 but beta cells are wiped out by something else, like a disease or an injury to pancreas, rather than by immune system (Manchanda *et al.*, 2021). In individuals with type 1 diabetes, elevated blood glucose levels adversely affect the wound healing process. One major complication is poor blood circulation due to blood vessel damage, which reduces the delivery of oxygen and essential nutrients to the wound site, thereby delaying healing. Additionally, hyperglycemia impairs immune cell function, weakening the immune response and increasing the risk of infection, which further hinders wound recovery (Tamarai *et al.*, 2021; Manchanda *et al.*, 2021; Verhulst *et al.*, 2019).

2.2.2. Type 2 Diabetes Mellitus (T 2 DM)

Was first described as a component of metabolic syndrome in 1988. (Patlak, 2002). T2DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia insulin Type 2 DM results from interaction between genetic, environmental and behavioral risk factors (ChL *et al.*, 2011). People living with type 2 DM are more vulnerable to various forms of both short- and long-term complications which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM because of the commonness of this type of DM its insidious onset and late recognition especially in resource-poor developing countries like Africa (Azevedo and Alla, 2008). Insulin peripheral resistance is the cause of type 2 (American Diabetes Association. 2007). Type 2 diabetes is the most prevalent form and results from insulin resistance combined with relative insulin deficiency, It often develops in adulthood and is strongly associated with obesity and sedentary lifestyle, Obesity accounts for approximately 55% of cases, and other contributing factors include smoking, physical inactivity, and diets high in calories and fats. It is predicted that

the prevalence of DM in adults of which type 2 DM is becoming prominent will increase in the next two decades where the majority of patients are aged between 45 and 64 years (Wild *et al.*, 2004). Genetics and lifestyle factors are the main causes of type 2 diabetes. (Ripsin *et al.*, 2009). Type 2 diabetes is known to be influenced by a variety of lifestyle variables. These include a sedentary lifestyle excessive alcohol use cigarette smoking and physical inactivity. (Hu *et al.*, 2001). Overweight, it is increasingly seen in children, adolescents, and younger adults due to rising levels of obesity, physical inactivity, and energy dense diets (Goyal and Jalal, 2018).

2.2.3. Gestational diabetes

Is a temporary condition that typically appears during the second or third trimester of pregnancy and is characterized by elevated blood glucose levels in women without previous diabetes diagnosis (American Diabetes Association, 2018). It affects about 14% of pregnancies globally, translating to 18 million cases annually (International Diabetes Federation, 2017). Risk factors include obesity, advanced maternal age, family history of diabetes, and polycystic ovary syndrome (Haroush *et al.*, 2004; Feig *et al.*, 2011; Castillo *et al.*, 2015). Diets high in fiber and unsaturated fats are protective, while those rich in red and processed meats and simple carbohydrates increase the risk (Yeung *et al.*, 2006; Bowers *et al.*, 2012; Bao *et al.*, 2014; Charron *et al.*, 2017). Although gestational diabetes usually resolves after delivery, it increases the risk of developing type 2 diabetes and cardiovascular diseases later in life (Cianni *et al.*, 2003). One common pregnancy issue is diabetes which is characterized by spontaneous hyperglycemia. Type 1 or type 2 diabetes that is not evidently preexisting is diagnosed in the second or third trimester of pregnancy (American Diabetes Association, 2018). GDM often goes away after birth but it can have long-term health effects such as raising the mother's risk of type 2 diabetes

(T2DM) and future obesity cardiovascular disease type 2 diabetes and/or gestational diabetes mellitus in the mother. (Feig *et al.*, 2011; Castillo *et al.*, 2015). One of the most frequent pregnancy complications is sudden hyperglycemia. (American Diabetes Association, 2018). Unfortunately, there is currently no generally recognized method of treating or preventing GDM other than lifestyle changes (diet and exercise) and occasionally insulin medication, which is only partially effective because of the frequently existing insulin resistance. Insulin sensitivity is a significant metabolic adaption. Depending on the demands of pregnancy insulin sensitivity changes during gestation. In order to prepare for the energy demands of later pregnancy early gestation promotes the absorption of glucose into adipose reserves via increasing insulin sensitivity. Insulin sensitivity is a significant metabolic adaption. Depending on the demands of pregnancy, insulin sensitivity changes during gestation. In order to prepare for the energy demands of later pregnancy early gestation promotes the absorption of glucose into adipose reserves via increasing insulin sensitivity (Cianni *et al.*, 2003). and other diseases of insulin resistance, such as polycystic ovarian syndrome (Haroush *et al.*, 2004).

2.3. Other specific types of diabetes (reviewed by Li et al., 2013).

- Genetic defects of the β -cell
- Genetic defects in insulin action
- Diseases of the exocrine pancreas
- Endocrinopathies).
- Drug- or chemical-induced diabetes:
- Other genetic syndromes associated with diabetes

2.4. Glycated hemoglobin

an important biological marker used to measure the average blood glucose level over a period of 8 to 12 weeks, expressed as a percentage of hemoglobin bound to glucose in red blood cells. According to a master's thesis titled "Assessment of glycemic control among type 2 diabetes mellitus patients using HbA1c test in tertiary care hospitals" (Khan *et al.*, 2018). HbA1c is one of the most important biomarkers reflecting long-term glucose control, providing a more accurate assessment than daily blood glucose measurements. This test is based on the principle of non-enzymatic glucose binding to hemoglobin molecules, reflecting the average glucose concentration over the lifespan of red blood cells (Ahmed, 2020). Diabetic foot ulcers are common and serious complications of diabetes, mainly caused by nerve damage (diabetic neuropathy) and poor blood circulation to the extremities (peripheral vascular disease). The HbA1c level is a key indicator reflecting long-term blood sugar control and plays a direct role in the occurrence and severity of foot ulcers. High HbA1c levels indicate elevated average blood glucose, which increases the risk of nerve and blood vessel damage, leading to loss of sensation and poor wound healing. Studies have confirmed that patients with elevated HbA1c are at higher risk of developing chronic foot ulcers and experience difficulties in healing, while good HbA1c control helps reduce ulcer occurrence, improves healing chances, and lowers the risk of infections and gangrene that may lead to amputation (Singh *et al.*, 2005 and Alavi *et al.*, 2016).

2.4.1 Types of Hemoglobin Mayo Clinic Staff. (2023).

1. Hemoglobin A (HbA):

Main type in adult blood
Composed of 2 alpha and 2 beta chains

2.Hemoglobin A2 (HbA2):

Makes up 2-3.5% of hemoglobin Composed of 2 alpha and 2 delta chains

Levels increase in disorders like thalassemia.

3.Hemoglobin F (HbF) – Fetal Hemoglobin:

Main hemoglobin in fetuses and infants (up to 6 months) Composed of 2 alpha and 2 gamma chains Higher oxygen affinity than other types

4. Abnormal Hemoglobins:

Includes Hemoglobin S (HbS) causing sickle cell anemia Includes Hemoglobin C (HbC) linked to some blood disorders

2.5. Diabetic Foot Ulcers (DFUs)

Diabetic Foot Ulcers (DFUs) are among the most serious and debilitating complications of diabetes mellitus, posing a significant risk for infection, gangrene, and limb amputation (Petersen *et al.*, 2022; Rawaan, 2022). These ulcers frequently develop due to underlying foot deformities, peripheral neuropathy, and peripheral vascular disease, which are exacerbated by chronic hyperglycemia and poor glycemic control (Al-Rubeaan *et al.*, 2015). Hyperglycemia leads to the excessive production of reactive oxygen species (ROS), causing endothelial dysfunction and vascular injury (Al-Salih and Ali, 2021,b). One of the critical pathological hallmarks of DFUs is the prolonged and unresolved inflammatory phase marked by excessive production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , resulting in an imbalance between M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages (Bjarnsholt *et al.*, 2008; Kadhim, 2021,b).

The study that 4–10% of diabetic patients will develop foot ulcers annually, with a lifetime risk reaching up to 25% (Fanxing *et al.*, 2013). Furthermore, nearly half

of the individuals with DFUs also suffer from lower extremity peripheral artery disease, which significantly compromises wound healing (Prompers *et al.*, 2007). If untreated, DFUs can lead to soft tissue infections, osteomyelitis, gangrene, and ultimately limb loss (Ndosi *et al.*, 2018).

The burden of DFUs extends beyond physical symptoms to include decreased mobility, reduced quality of life, and higher healthcare utilization (Armstrong *et al.*, 2017; Petersen *et al.*, 2022).

2.5.1. Epidemiology of DFUs

The global rise in diabetes prevalence has intensified the public health and economic burden of chronic wounds, especially DFUs (Sen *et al.*, 2009; Petersen *et al.*, 2022). It is estimated that the number of individuals with diabetes will increase from 171 million in 2000 to 366 million by 2030, a trend largely attributed to population aging and the obesity epidemic (Wild *et al.*, 2004). Patients with diabetes face a lifetime risk of up to 25% for developing lower extremity ulcers, with over 7% of neuropathic DFU cases progressing to amputation (Margolis *et al.*, 2005). The wound healing process consists of four sequential phases: hemostasis, inflammation, proliferation, and remodeling. This process is tightly regulated by interactions among hematopoietic, immune, and resident skin cells (Sun *et al.*, 2014). Diabetic foot disease is a leading cause of non-traumatic lower-limb amputation and contributes to substantial morbidity, mortality, and healthcare costs (Martins-Mendes *et al.*, 2014; Jupiter *et al.*, 2016; Zhang *et al.*, 2017).

2.5.2. Etiology of Diabetic Foot Ulcers

The development of diabetic foot ulcers (DFUs) is multifactorial and stems from several overlapping pathophysiological conditions, with peripheral neuropathy

being the most prominent contributor (Singh *et al.*, 2005; Soewondo *et al.*, 2013; Stacy, 2021). Sensory neuropathy leads to the loss of protective sensation, making patients vulnerable to unnoticed trauma due to impaired thermal, pain, and proprioceptive perception, Motor neuropathy contributes to foot deformities, such as hammer toes and hallux rigidus, which alter biomechanics and increase focal pressure points, Autonomic neuropathy results in reduced sweat and oil gland function, leading to xerosis (dry skin), fissures, and an increased risk of infection due to impaired skin integrity (Oliver and Mutluoglu, 2019).

Elevated plantar pressure is another significant factor, commonly linked to limited joint mobility and structural abnormalities in the foot, particularly in the ankle, subtalar, and metatarsophalangeal joints, Study suggest that approximately 28% of patients with peripheral neuropathy and elevated plantar pressure develop DFUs within 2.5 years (Soewondo *et al.*, 2013). Trauma, particularly repetitive friction from ill-fitting footwear, accounts for 21% of ulcerations, while acute injury contributes to 11%, fungal infections such as tinea pedis to 4%, and nail-trimming accidents to another 4% (Singh *et al.*, 2005). Peripheral arterial disease due to atherosclerosis, especially affecting the femoropopliteal and infrapopliteal arteries, is also a key factor that impairs blood flow and tissue oxygenation, thereby delaying wound healing (Tellechea *et al.*, 2010). The overall risk of foot ulcers is nearly twice as high in diabetic individuals compared to those without diabetes (Singh *et al.*, 2005).

2.5.3. Diagnosis of Diabetic Foot Ulcers

A comprehensive diagnostic approach is essential in assessing diabetic foot ulcers, The workup typically includes blood tests, plain radiographs, ankle-brachial index (ABI), toe pressure, pulse-volume recordings, and duplex ultrasonography

(Stacy, 2021). Advanced imaging such as computed tomography scans, magnetic resonance imaging, bone scans, and angiography may be indicated in complex or non-healing ulcers to assess for osteomyelitis or vascular compromise, Physical examination involves evaluating peripheral pulses, inspecting for anatomical deformities, ulcer location, callus formation, and signs of vascular insufficiency such as hair loss or muscle wasting (Boulton *et al.*, 2008). Monofilament is commonly used to assess for peripheral neuropathy, along with inspection for purulence, scabs, or odor indicating infection (Oliver and Mutluoglu, 2019).

2.5.4. Pathophysiology of Diabetic Foot Ulcers

Diabetic foot ulcers (DFUs) develop due to a mix of nerve damage, poor blood flow, and weakened immune function, all of which slow down the healing process (Wild *et al.*, 2010; Sharp & Clark, 2011). Damage to the autonomic nerves affects sweat and oil gland activity, causing the skin to become dry and fragile, making it more prone to injury. When sensory nerves are damaged, people may not feel pain properly, allowing wounds to go unnoticed and worsen over time without treatment (Clayton and Elasy, 2009). Diabetes also weakens the body's natural defenses and reduces the production of collagen and new blood vessels, both of which are essential for healing. In advanced stages of diabetes, prolonged high blood sugar causes permanent harm to the skin, blood vessels, and nerves, and even good blood sugar control can't undo this damage. If left untreated, DFUs can lead to serious issues like skin infections, bone infections, abscesses, gangrene, and even blood poisoning, increasing the risk of severe illness or death (Sharp and Clark, 2011).

2.5.5. Classification of Diabetic Foot Ulcers (DFUs)

The ulcer should be staged after diagnosed. Diabetic foot ulcers have been categorized using various methods. the Wagner-Ulcer Classification System and the

University of Texas Wound Classification System are the most widely utilized (Syafil, 2018). Wagner Classification for Diabetic Foot Ulcer the Wagner system classifies ulcers into six stages based on depth: a superficial ulcer, a deep ulcer involving tendon, bone, or joint, osteomyelitis or a deep ulcer with an abscess, gangrene localized to the forefoot, and gangrene affecting the entire foot (Monteiro-Soares *et al.*, 2020). This classification evaluates ulcer depth and tissue involvement. It includes six grades:

Table (2-1) Wagner Classification System for Diabetic Foot Ulcers (Monteiro-Soares *et al.*, 2020).

Grade	Clinical Description
0	No ulceration present, but foot deformities or high-risk signs (e.g., callus, neuropathy).
1	Superficial ulcer involving only the skin layer.
2	Ulcer extends to tendon or muscle without signs of infection.
3	Deep ulcer with associated abscess formation or osteomyelitis.
4	Localized gangrene affecting the forefoot or heel.
5	Extensive gangrene involving the entire foot.

The University of Texas classification system is a comprehensive tool developed to assess and grade diabetic foot ulcers based on three critical parameters: the depth of the ulcer, the presence of infection, and the degree of ischemia. Unlike simpler classification models that focus solely on wound depth, such as the Wagner system, the Texas classification provides a more nuanced approach by incorporating clinical factors that significantly affect wound healing and limb prognosis. (Bravo-Molina *et al.*, 2018).

Table 2-2 University of Texas Wound Classification System for Diabetic Foot Ulcers (Bravo-Molina *et al.*, 2018).

Grade	Wound Description	Clinical Condition
0	Pre- or post-ulcerative lesion that is completely epithelialized.	Non-infected, non-ischemic
1	Superficial ulcer not involving tendon, capsule, or bone.	Infected only
2	Ulcer penetrates to tendon or joint capsule.	Ischemic only
3	Ulcer with associated abscess, osteomyelitis, or joint sepsis.	Both infected and ischemic

2.5.6. Diabetic Ulcer Healing Process

Wound healing is a complex and ever-changing biological process aimed at restoring both the structure and function of damaged tissue. In individuals with diabetes, this healing process is notably disrupted due to the intricate interaction of high blood sugar levels, nerve damage, and poor blood circulation (Romo *et al.*, 2008; Polat *et al.*, 2010). The process of healing generally unfolds in four interrelated yet separate stages: hemostasis, inflammation, proliferation, and maturation (Oberszyn, 2007).

2.5.6.1. Hemostasis

Hemostasis is the body's immediate reaction to blood vessel injury, aiming to prevent blood loss through blood vessel constriction and the creation of a clot. This process begins with a series of coagulation factors that ultimately transform fibrinogen, a soluble protein in plasma, into an insoluble fibrin mesh that reinforces the clot (Lundblad *et al.*, 2004). Thrombin (Factor IIa) plays a crucial role by not only converting fibrinogen into fibrin but also intensifying the coagulation cascade through the activation of factors V, VIII, XI, XIII, and protein

C (Hoffman and Monroe, 2001). Moreover, thrombin increases vascular permeability, promoting the entry of immune cells and initiating the subsequent stage of healing (Esmon, 2005).

2.5.6.2. Inflammation

The inflammatory phase lasts from 2 to 6 days after an injury and plays a key role in eliminating pathogens and clearing damaged tissue, Neutrophils are the first immune cells to arrive, reaching their peak between 24 and 48 hours, followed by macrophages that clean up cellular debris and release cytokines and growth factors. This stage is characterized by the classic symptoms of inflammation: redness, warmth, swelling, Fluid drainage from the foot, Bad smell and pain, In individuals with diabetes, ongoing low-level inflammation and dysfunctional neutrophils extend this phase, hindering the progression to tissue repair. (Polat *et al.*, 2010).

2.5.6.3. Proliferation

At this phase, fibroblasts move into the wound area, multiply, and start producing type III collagen, which serves as a temporary framework for tissue healing, At the same time, blood vessel formation, known as angiogenesis, begins with the help of vascular endothelial growth factor (VEGF), encouraging new capillary growth. Skin cells called keratinocytes from the wound edges also multiply and spread over the wound in a process called re-epithelialization, However, in diabetic ulcers, this phase often doesn't work properly due to low oxygen levels, the negative effects of high blood sugar on fibroblast function, and a decrease in essential growth factors (Romo *et al.*, 2008).

2.5.6.4. Maturation

The last stage of wound healing, known as maturation or remodeling, can extend from a few weeks to over a year, depending on how severe the wound is. During this period, type III collagen is gradually replaced by the stronger type I collagen, and the collagen fibers are reorganized to improve the wound's tensile strength. Thrombin continues to play a role in this phase by affecting fibroblast activity and the remodeling of the extracellular matrix (Lundblad *et al.*, 2004). In individuals with diabetes, persistent high blood sugar and the formation of advanced glycation end products (AGEs) disrupt the matrix remodeling process, often resulting in delayed or incomplete healing. Other factors such as age, poor nutrition, reduced blood flow (ischemia), infection, and excessive moisture (maceration) can also negatively impact the healing outcome (Polat *et al.*, 2010).

2.5.7. Factors Affecting Diabetic foot ulcers Healing

Diabetic foot ulcers (DFUs) are a serious and prevalent complication that arise from a multifaceted interaction of physiological, metabolic, and behavioral elements. A leading factor is diabetic neuropathy, which causes a gradual loss of sensation in the feet. Consequently, patients often overlook minor injuries, enabling the wounds to deteriorate over time (Pop-Busui *et al.*, 2023). Peripheral arterial disease (PAD) is another key contributor, as it diminishes blood circulation to the limbs, hinders oxygen delivery to tissues, and slows the healing process. Research indicates that more than half of DFU instances is associated with some level of ischemia (Armstrong *et al.*, 2023). Inadequate glycemic control is closely linked with slower healing and higher susceptibility to infections, given that elevated blood sugar impairs immune response and the regulation of inflammation (Zhao *et al.*, 2022). Additionally, persistent bacterial infections—especially those involving

antibiotic-resistant strains like *Staphylococcus aureus*—lead to extended inflammation and suboptimal wound healing Beyond, physiological concerns, behavioral and socioeconomic aspects such as smoking, poor nutrition, and lack of education on foot care greatly heighten the risk of developing ulcers(Lipsky, *et al.*, 2022).

2.5.8. Healing of Diabetic Foot Ulcers (DFU)

Wound healing is a complex biological process that involves a well-orchestrated sequence of events, typically divided into four overlapping phases: hemostasis, inflammation, proliferation (including extracellular matrix formation and angiogenesis), and remodeling, In non-diabetic individuals, these stages proceed in a tightly regulated and timely manner, ensuring efficient tissue repair, However, in diabetic patients, multiple disruptions impair this cascade and delay healing (Jones *et al.*, 2019).highlights the critical role of macrophages in modulating inflammation and initiating tissue repair, These cells exhibit functional plasticity, transitioning from a pro-inflammatory to an anti-inflammatory phenotype during wound healing. Diabetes interferes with this transition by impairing monocyte recruitment, reducing phagocytic activity, and sustaining a chronic inflammatory state, ultimately disrupting the progression to the proliferative phase (Dekker *et al.*, 2019). Moreover, the regenerative functions of keratinocytes and fibroblasts are compromised in diabetes, especially during the later stages of healing, These disruptions hinder epithelial cell migration and collagen deposition, key components of re-epithelialization and dermal matrix reconstruction (Alavi *et al.*, 2014). At the molecular level, healing depends on the activation of multiple cell types-keratinocytes, fibroblasts, platelets, endothelial cells, and macrophages, Hypoxia within the wound microenvironment typically stimulates the release of vascular

endothelial growth factor (VEGF), which in turn promotes endothelial nitric oxide synthase (eNOS) activation, an essential step in angiogenesis, In diabetic wounds, however, this hypoxic response is blunted, leading to poor neovascularization (Rupert, 2016). Additionally, diabetes impairs the mobilization and signaling of endothelial progenitor cells (EPCs), which are crucial for targeting wound sites and facilitating vascular repair, The reduction in EPC activity results in diminished angiogenic response and impaired tissue regeneration (Omanakuttan *et al.*, 2012).

Several systemic factors also exacerbate impaired healing in diabetic patients, including vascular complications, chronic inflammation, oxidative stress, and dysregulated cytokine and growth factor expression, Clinical evidence suggests that adequate glycemic control at the time of surgical intervention enhances the likelihood of complete wound closure, whereas persistent hyperglycemia compromises leukocyte function, immune defense, and nutrient delivery to injured tissues, resulting in prolonged or incomplete healing (Pande *et al.*, 2013).

2.5.9. Management of Diabetic Foot Ulcers (DFUs)

Diabetic foot ulcers (DFUs) are a serious and complex complication of diabetes that call for a comprehensive, multidisciplinary approach. This includes prevention strategies, early diagnosis, and intensive care. The main objectives are to lower infection risks, support tissue repair, and protect limb function (Armstrong *et al.*, 2023).

2.5.9.1. Clinical Evaluation

The first step in management involves a detailed evaluation of the ulcer, assessing its depth, size, presence of dead tissue, and any discharge, Vascular health is checked

using tools like the Ankle-Brachial Index (ABI), and peripheral neuropathy is screened through sensory testing (Lipsky *et al.*, 2022).

2.5.9.2. Pressure Offloading

Relieving pressure from the affected area is crucial for healing, Devices such as total contact casts and specially designed offloading footwear are often used and have been shown in studies to improve healing outcomes (Bus *et al.*, 2020).

2.5.9.3. Infection Control

Managing infection is a top priority in DFU care, Antibiotic treatment is tailored based on clinical symptoms and wound culture results. Early, targeted intervention helps prevent complications like bone infection or the need for amputation (Lipsky *et al.*, 2022).

2.5.9.4. Local Wound Care

Wound care involves routine cleaning, removal of dead tissue, and the use of dressings that retain moisture to promote healing. More advanced dressings may include antimicrobial agents or compounds that stimulate tissue growth (Game *et al.*, 2020).

2.5.9.5. Systemic Optimization

Healing is also influenced by overall health, requiring strict blood sugar control, proper nutrition, and management of other health conditions, Improved glucose control has been linked to stronger immune responses and better healing (Zhao *et al.*, 2022).

2.5.9.6. Surgical and Vascular Interventions For patients with severe poor circulation or structural foot issues, surgical procedures might be necessary, These can include restoring blood flow, removing infected tissue, or performing corrective surgeries to reduce pressure and support healing (Prompers *et al.*, 2019)

2.5.10. Immune Dysregulation in Diabetes Mellitus

Diabetes mellitus causes a wide range of changes in the immune system that weaken the body's defenses, making individuals more prone to infections and slowing down wound healing, These changes impact both the innate and adaptive immune systems, resulting in persistent inflammation and poor tissue recovery,

High blood sugar levels negatively affect the function of white blood cells, especially neutrophils and macrophages, These cells become less capable of sticking to surfaces, moving toward infection sites, and engulfing harmful pathogens, Their ability to produce substances that kill microbes is also reduced, leading to a weakened first line of immune defense (Wolcott *et al.*, 2021). In people with diabetes, the balance of cytokines is disturbed, There is an increase in inflammatory cytokines like IL-6 and TNF- α , while anti-inflammatory ones like IL-10 are decreased. This imbalance extends the period of inflammation and interferes with the body's ability to repair tissue (Zhao *et al.*, 2022). T-cell function is also weakened in diabetes, These cells don't multiply or produce signaling molecules as effectively, which disrupts the adaptive immune system and limits the body's capacity to remember and fight off pathogens (Sadeghi *et al.*, 2020). Diabetes hampers the growth of fibroblasts and the movement of keratinocytes—both of which are crucial for skin healing and wound closure, It also reduces collagen production, which results in poor tissue regeneration (Guo & DiPietro, 2019). Altogether, these immune system issues increase the risk of infections and chronic wounds in diabetic

patients, Common complications include skin and soft tissue infections, urinary tract infections, respiratory infections, and diabetic foot ulcers, If untreated, these ulcers can worsen and lead to bone infections (Lipsky *et al.*, 2022). The role and therapeutic possibilities of IL-10 in diabetes and diabetic foot ulcers are areas of ongoing research.

2.6. Immunological Markers in Diabetic

Interleukins (ILs) represent a varied group of cytokines mainly released by blood monocytes and tissue macrophages, These signaling substances play a critical role in orchestrating both innate and adaptive immune responses, When an infection or injury occurs, ILs attach to designated receptors on target cells, triggering signaling cascades that influence cell behavior, Among diabetic individuals, especially those with diabetic foot ulcers (DFUs), increased IL levels are commonly detected, contributing to chronic inflammation and impaired wound healing (Berlanga-Acosta *et al.*, 2013).

2.6.1. Interleukin-10 (IL-10)

Interleukin-10 (IL-10) is a cytokine with anti-inflammatory properties that plays a crucial role in moderating immune responses and protecting tissues from damage. It is predominantly produced by monocytes, regulatory T cells, B cells, and dendritic cells. IL-10 functions by suppressing the production of inflammatory cytokines like $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6, and by hindering antigen presentation by dendritic cells and macrophages. This modulatory role is vital for preserving immune balance, especially during infections and inflammatory conditions. Nonetheless, high levels of IL-10 have also been linked to persistent infections and the ability of tumors to evade immune detection (O'Gara & Vieira, 2007).

Responses in individuals with diabetes. It acts by dampening the heightened immune activity associated with chronic inflammation, a hallmark of diabetes pathophysiology (Couper *et al.*, 2008). In type 2 diabetes patients, diminished IL-10 levels in the blood have been linked to greater insulin resistance and increased levels of pro-inflammatory markers like CRP and TNF- α , suggesting that low IL-10 may worsen the body's inflammatory state in diabetes (van Exel *et al.*, 2002). Additionally, higher IL-10 levels are tied to a reduced risk of both small and large blood vessel complications (Esposito *et al.*, 2006). When it comes to diabetic foot ulcers (DFUs), IL-10 has a protective role by controlling inflammation and supporting wound repair. It curbs the activation of immune cells like neutrophils and macrophages and reduces levels of pro-inflammatory cytokines such as IL-1 β and IL-6, helping to minimize tissue damage and support healing (Mills *et al.*, 2017). Research in both clinical and experimental settings show that increasing IL-10 levels, either through topical application or systemic delivery, speeds up healing by promoting blood vessel formation and regulating fibroblast activity (Parenteau *et al.*, 2008). Furthermore, a lack of IL-10 gene expression has been associated with slower wound healing in diabetic patients, highlighting its potential as a treatment target (Berlanga-Acosta *et al.*, 2013). Because of IL-10's promising healing properties, new therapeutic strategies are exploring its use in topical or gene-based treatments for chronic diabetic wounds. In preclinical models, applying IL-10 directly to wounds significantly improved healing by reducing inflammation and boosting growth factors like VEGF (Parenteau *et al.*, 2008). Gene therapy using viral vectors to deliver IL-10 has also shown long-lasting anti-inflammatory benefits and improved tissue regeneration in diabetic wounds (Sarkar *et al.*, 2010). These discoveries highlight IL-10's diverse functions in shifting chronic inflammation toward healing. However, despite encouraging results from animal studies, large-

scale clinical trials in humans are still needed to confirm the safety and effectiveness of IL-10 treatments, especially in diabetic patients with compromised vascular and immune systems (Kwak and Kim, 2022)

2.4.2. Interleukin-18 (IL-18)

Is a cytokine that promotes inflammation and is part of the IL-1 family, It plays an essential role in managing the immune system by stimulating the production of interferon-gamma (IFN- γ) and supporting Th1-type immune responses, This molecule is mainly released by activated macrophages, monocytes, and epithelial cells when they encounter pathogens or inflammatory signals (Dinarello *et al.*, 2013). In individuals with type 2 diabetes mellitus (T2DM), IL-18 levels are notably higher and closely linked to poor blood sugar control, as indicated by elevated HbA1c levels and fasting blood glucose measurements (Hung *et al.*, 2005). IL-18 helps maintain a state of chronic, low-level inflammation by activating nuclear factor kappa B (NF- κ B) and the NLRP3 inflammasome, These processes interfere with insulin signaling and harm the function of insulin-producing β -cells in the pancreas (Stienstra *et al.*, 2012). Besides its effects on metabolism, IL-18 also contributes to blood vessel issues in diabetes, High levels of IL-18 are related to impaired blood vessel function and the development of atherosclerosis, which can lead to the small and large blood vessel damage often found in people with diabetes (Mallat *et al.*, 2001). In diabetic foot ulcers (DFUs), IL-18 levels are notably elevated in the wound area, especially within inflammatory cells and keratinocytes located at the edges of the ulcer, This prolonged inflammatory response disrupts the normal healing process by hindering the shift from inflammation to tissue repair (Wetzler *et al.*, 2000). Experimental study suggest that blocking IL-18 signaling can promote faster skin

regeneration and the formation of new blood vessels, pointing to a potential new treatment strategy for chronic diabetic wounds (Dinarello *et al.*, 2013).

This bar chart illustrates the average serum IL-18 levels among four clinical categories: healthy individuals, individuals with type 2 diabetes without foot ulcers, diabetic individuals with active foot ulcers, and diabetic individuals with healed foot ulcers. The chart reveals a marked increase in IL-18 levels in those with active foot ulcers compared to all other groups, highlighting its involvement in sustaining chronic inflammation and hindering wound healing. Following the healing of ulcers, a significant decline in IL-18 levels is noted, indicating a transition toward immune balance and tissue repair (Wetzler *et al.*, 2000 and Hung *et al.*, 2005 and Dinarello *et al.* 2013),

2.6.3. Tumor Necrosis Factor-Alpha (TNF- α)

TNF- α is a key pro-inflammatory cytokine that plays a vital role in regulating the immune system and driving systemic inflammation. It is mainly produced by activated macrophages, but T lymphocytes and monocytes also contribute to its secretion. High levels of TNF- α are consistently found in people with type 2 diabetes mellitus (T2DM), indicating its significant role in ongoing metabolic inflammation (Lampropoulou *et al.*, 2014).

One of the well-known ways TNF- α contributes to insulin resistance is by promoting serine phosphorylation of insulin receptor substrate-1 (IRS-1). This action interferes with normal insulin signaling in muscles, the liver, and fat tissues, leading to reduced glucose uptake. This mechanism is a central link between inflammation and metabolic problems (Kang, 2021). In diabetic foot ulcers (DFUs), TNF- α has a complex role. While it is important for triggering the initial inflammatory response necessary for wound healing, prolonged or excessive

levels—common in chronic wounds—can block the shift to tissue regeneration. Research has shown that TNF- α levels are elevated in endothelial and skin cells around the ulcer edges, where they disrupt new blood vessel formation and impair fibroblast function, ultimately slowing wound healing (Salajegheh *et al.*, 2016).

TNF- α is also involved in diabetic neuropathy, a serious complication of diabetes. Chronic high blood sugar causes excessive TNF- α production, which harms both small and large nerve fibers, leads to loss of nerve insulation (demyelination), and increases nerve sensitivity. These effects make injuries more likely and delay tissue healing, highlighting TNF- α 's role in nerve inflammation and the development of ulcers (Stomnaroska, 2019). Because of its broad impact, TNF- α is seen as a promising target for treatment. Blocking its pathways could help reduce chronic inflammation, speed up wound healing, and prevent diabetes-related complication

2.6.4. Basic fibroblast growth factor (BFGF)

Also known as FGF-2, is a multifunctional glycoprotein with the capacity to stimulate the proliferation and activity of various cell types, including dermal fibroblasts, keratinocytes, endothelial cells, and melanocytes. Its mitogenic and angiogenic characteristics make it an essential mediator in processes such as tissue regeneration, neovascularization, and wound healing (Akita, 2010). The involvement of BFGF in both angiogenesis and neurogenesis has been well documented, and studies have highlighted its significant impact on tissue remodeling (Cao *et al.*, 2012). Clinical evidence further supports its efficacy, as patients with diabetic ulcers treated with BFGF-based interventions demonstrated accelerated healing rates and improved epithelial regeneration (Uchi *et al.*, 2009). BFGF mitogenic properties have made it a valuable target in therapeutic wound care. Research indicates that controlled-release systems delivering bFGF can notably

enhance wound closure and promote faster epithelial coverage in diabetic animal models (Akita *et al.*, 2013). These benefits are largely attributed to its role in stimulating the proliferative phase of wound repair, while also facilitating apoptosis of fibroblasts and myofibroblasts in later stages—an effect that helps limit excessive scar formation (Huang *et al.*, 2016). However, in diabetic wounds, the natural expression of FGF-2 is frequently reduced. This downregulation is associated with impaired angiogenesis, decreased fibroblast activity, and insufficient collagen deposition, all of which contribute to the delayed healing commonly observed in diabetic foot ulcers (Brizeno *et al.*, 2016). In the advanced stages of wound healing, pathological disruptions become increasingly apparent, Persistent inflammation evidenced by sustained TNF- α expression further hinders angiogenesis and extracellular matrix remodeling, Basic fibroblast growth factor (bFGF) is a pivotal regulator of cellular and molecular mechanisms involved in tissue repair, Its diminished expression in diabetic environments underscores its therapeutic relevance, particularly in overcoming the chronic, non-healing nature of diabetic foot ulcers (Heng *et al.*, 2023).

Chapter Three

Materials and Methods

3. Materials and method

3.1. Materials

3.1.1. Instruments and Equipment Used in the Study

Table (3-1): Instruments and Equipment Used in the Study

Instrument/Device Name	Usage	Manufacturer	Country of Origin
Centrifuge	Blood component separation (serum/plasma)	Kokusan	Japan
Cool Box	Sample transportation at low temperature	VB	China
Cotton	Cleaning	BDH	England
Deep Freezer	Long-term sample freezing	Hettich	South Korea
EDTA Tubes (Purple Cap)	Whole blood collection for CBC test	VACUETTE	Austria or Germany
Eppendorf Tubes 0.5 ml	Storage and transfer of micro-samples	ALS	China
Gel Tubes 6 ml	Serum collection for biochemical analysis	Arrow	Egypt
Gloves	Personal protection during procedures	ALS	China
Incubator	Sample incubation	Memmert	Germany
Micropipette Set	Precise pipetting of small volumes	SLAMED	Germany
Refrigerator	Sample refrigeration	Panasonic	South Korea
Syringe 5 ml	Biological sample collection	ALS	China

Table (3:2)chemical and reagents used in the study

No.	Chemical Name	Manufacturer (if available)	Country of Origin
1	70% Ethanol	BDH Chemicals	United Kingdom
3	Albumin Bovine Serum (BSA)	Thermo Scientific / Sigma-Aldrich	USA / Germany
2	Biotinylated Detection Antibody	Elabscience®	China
4	Deionized/Distilled Water	Local lab / Distillation system	Local
5	HRP-Conjugate (Avidin-HRP)	Elabscience®	China
6	Phosphate Buffered Saline (PBS)	Thermo Fisher / Sigma-Aldrich	USA / Germany
7	Standard & Sample Diluent	Elabscience®	China
8	Stop Solution (Diluted H ₂ SO ₄)	Elabscience® / Merck	China / Germany
9	Substrate Reagent (TMB)	Sigma-Aldrich / Elabscience®	Germany / China
10	Tween-20	Sigma-Aldrich	Germany
11	Wash Buffer (25×)	Elabscience®	China

Table (3-3) Devices &Analytical methods used for measuring HbA1c, CRP and Lipid profile parameters

Device Name	Type of Analysis	Measured Parameters	Manufacturer	Country of Origin
Fuji Dri-Chem Nx600i	Dry chemistry analysis (Glucose, Total Lipids, Cholesterol, Triglycerides)	Glucose, Total Lipids, Cholesterol, Triglycerides	Fujifilm Corporation	Japan
Ichroma II	Fluorescence immunoassay (CRP)	CRP	Boditech Med Inc.	Boditech Med Inc.
Ichroma II	Fluorescence immunoassay (HbA1c)	HbA1c	Boditech Med Inc.	South Korea

3.1.2. ELISA Kits

Table (3-4): ELISA Devices and Kits Used in the Study

Device/Kit Name	Application	Manufacturer	Country of Origin
Human IL-10 ELISA Kit	Measurement of IL-10 in serum	Elabscience	China
Human IL-18 ELISA Kit	Measurement of IL-18 in serum	Elabscience	China
Human TNF- α ELISA Kit	Measurement of TNF- α in serum	Elabscience	China
Human bFGF ELISA Kit	Measurement of bFGF in serum	Elabscience	China
Microplate Reader (800 TS)	Absorbance reading for ELISA	BioTek Instruments (Agilent)	USA
ELISA Plate (96-well)	Microplate for ELISA testing	Elabscience	China
Micropipette Set	Precise dispensing of reagents and samples	SLAMED	Germany
Incubator	Incubation of ELISA microplates	Memmert	Germany

3.2. Methods

3.2.1. Study Design and sample collecting

Individuals with type 2 diabetes and diabetic foot ulcers (DFU) who attended the Diabetes Centers at Al-Hashimiyah General Hospital and Al-Qassim General Hospital in Babylon Province between September 2024 and February 2025 were included in the study. Each patient was diagnosed with DFU by a specialist, with confirmation provided through X-ray imaging. It included a total of 90 participants 60 patients and 30 healthy controls and confirmed DFU consistently attended

follow-up appointments and received standard wound care (SWO) throughout the study period.

3.2.2. Study of Design

The study was conducted at the Diabetes Center in Hashimiyah General Hospital and Al-Qassam General Hospital in Babylon province during the period from September 2024 to February 2025. It included a total of 90 participants: 60 patients and 30 healthy controls. The participants were divided into three groups as follows:

Group 1: This group consisted of 30 diabetic patients with Diabetic Foot Ulcer (DFU), selected from the Diabetes Center in Babylon province. Among them, 22 were male and 8 were female.

Group 2: This group included 30 diabetic patients without Diabetic Foot Ulcer (DFU), also selected from the Diabetes Center in Babylon province. It comprised 5 males and 25 females.

Group 3: The control group consisted of 30 healthy individuals without diabetes or DFU. They were randomly selected from the general population while visiting Al-Aamil Laboratory for routine checkups. This group included 16 males and 14 females.

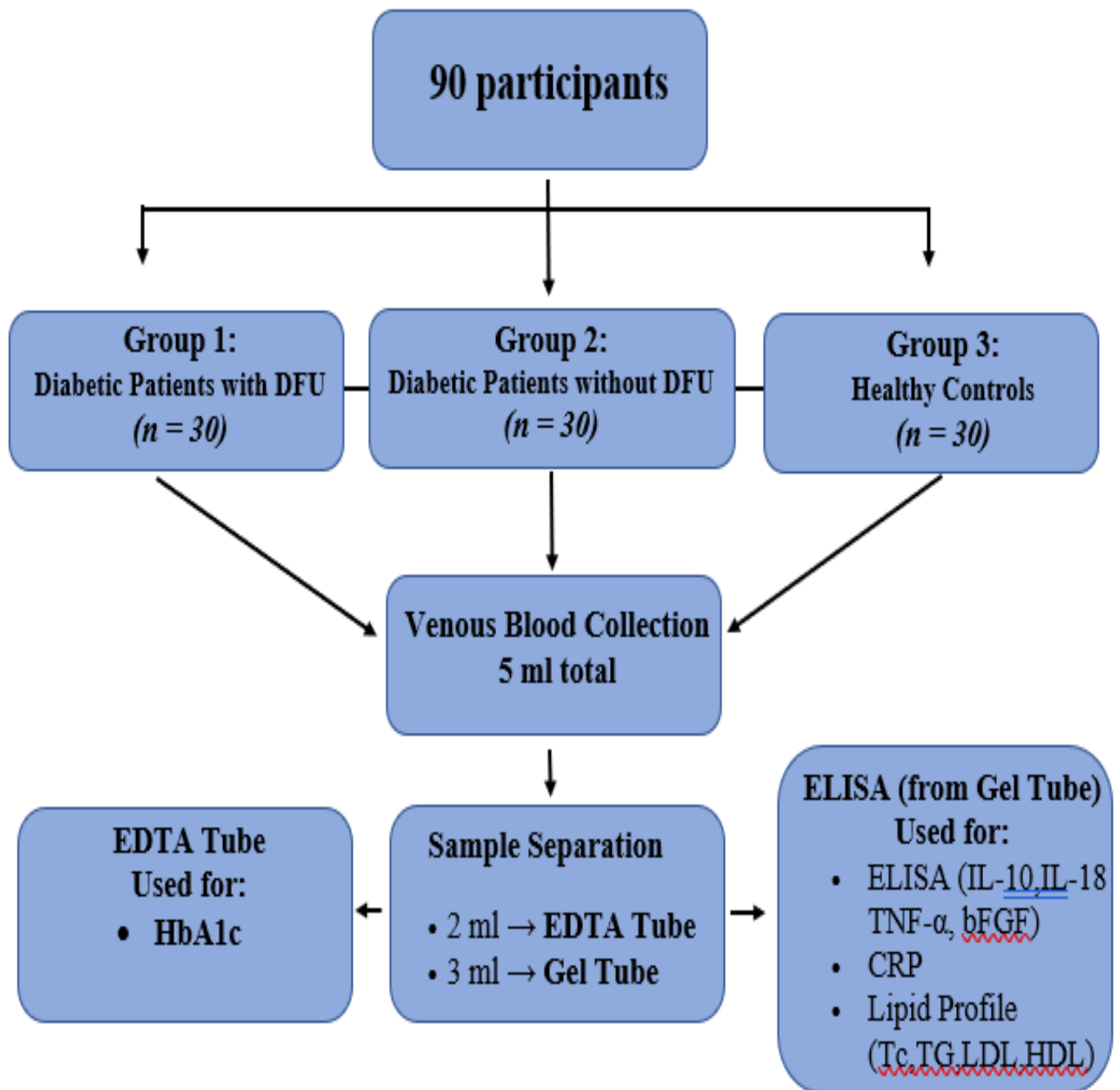


Figure (3:1): Study of Design

3.3. Blood Samples Collection

Approximately 5 ml of venous blood was collected from the participant after the cubital fossa was disinfected with 70% ethanol, A tourniquet was applied, and venipuncture was performed using disposable syringes, For hematological analysis, 2 ml of the blood was transferred into an EDTA tube, The remaining 3 ml was placed in a gel tube, allowed to clot, and then centrifuged at 4000 RPM for 10 minutes to separate the serum, The serum was subsequently transferred to an Eppendorf tube and stored at -20°C for use in immunological testing.

3.3.1. Biochemical Tests

Blood samples 3ml were collected in gel tube and left to clot for 15 minutes in room temperature (20-25) °C, Then, after collecting, it was centrifuged at (4000 rpm) for approximately (10) min period to obtain serum, the isolated serum of samples was distributed into 3 aliquots (0.5ml) in tightly closed Eppendorf tubes, and then the tubes were stored at -20 C until assayed for immunological markers testing by ELIZA.

3.3.1.1. Conduct the C-reactive protein (CRP) test

We conduct the C-reactive protein (CRP) test using the ichroma™ II system by following these steps:

1. Use the empty sample collector to pierce the top of the detection solution container.
2. Draw a 10 µL aliquot of the sample (whole blood, serum, plasma, or control) using the sample collector.
3. Dispense the collected sample into the detection solution container. Mix thoroughly by stirring at least ten times to ensure complete release of the sample from the collector.
4. The prepared mixture must be used within 30 seconds to maintain test accuracy.

Remove the cap from the assembled tube. Discard the first two drops of the mixture.

5. Dispense exactly two drops of the mixture into the sample well of the test cartridge.

6. Let the cartridge stand at room temperature for 3 minutes before inserting it into the ichroma™ instrument.

7. Insert the cartridge into the holder. Press the "Select" or "Start" button to automatically begin the scanning process.

8. The test results will be displayed on the screen after the analysis is completed.

(Boditech Med Inc., 2022).

3.3.1.2. HbA1c Test Procedure

The Hemoglobin A1c (HbA1c) test was conducted to estimate the average blood glucose level over the past two to three months using a point of care testing system according to the following steps.

1. A blood sample was collected either via fingerstick or venipuncture using a sterile collection tool. Approximately 4 µL of blood was obtained for the test.

2. The collected blood was transferred into a reagent tube containing a specific hemoglobin detection buffer. The tube was tightly sealed and gently inverted 6 to 8 times to ensure thorough mixing.

3. The reaction mixture was incubated at room temperature for 3 to 5 minutes, as recommended by the manufacturer's protocol.

4. A portion of the prepared sample was dispensed into the sample well of a single-use test cartridge.

5. The cartridge was allowed to rest undisturbed for approximately 3 minutes to stabilize the reaction components.

6. The cartridge was inserted into the iChroma II device slot, and the test was initiated by pressing the “Start” button.

7. The analyzer automatically processed the sample and displayed the HbA1c result within 3 to 15 minutes. Results were reported as a percentage of total hemoglobin concentration. Interpretation followed the criteria of the American Diabetes Association (ADA) HbA1c < 5.7%: Normal glycemic control HbA1c between 5.7% and 6.4%: Prediabetes HbA1c $\geq$ 6.5%: Indicative of diabetes mellitus (Boditech Med Inc. 2022).

3.3.1.3. The lipid profile test on the FUJIFILM DRI-CHEM NX600 is a total Cholesterol (TCHO), Triglycerides (TG), HDL-Cholesterol (HDL-C) and LDL-Cholesterol (LDL-C) tests in blood. It is performed using a FUJI DRI-CHEM SLIDE designed for this test. The detailed steps are as per the manufacturer's instructions: Sample Preparation:

1. Serum or plasma samples were utilized for the analysis.
2. Plasma was collected using anticoagulants such as **EDTA**, both of which were considered acceptable for use.
3. All samples were carefully inspected to ensure they were free from clots and air bubbles, as these could compromise analytical accuracy.
4. Test slides were inserted into the designated compartment of the machine according to manufacturer instructions.
5. Approximately 10 μ L of the prepared sample was applied to the sample pad using the designated applicator.
6. The analysis was started by pressing the Start button on the instrument.

7. The FUJIFILM DRI-CHEM NX600 analyzer (FUJIFILM Corporation. (2020). was powered on and allowed to initialize until it was ready for operation

3.3.2. Biomarkers Profile Assay by ELISA Technique:

The serum level of interleukin 18, interleukin 10, TNF- α & BFGF were determined by classic sandwich-ELISA using ELISA research kits.

3.3.2.1. Measuring the Concentration of Serum Human interleukin 18 in Patients' Blood.

The serum level of IL-18 was measured using Ela science® ELISA kit (Intra-Assay: CV<5.39 %, Inter-Assay: CV<7.43%, and sensitivity: 9.38 pg/mL). The quantitative sandwich enzyme immunoassay method was used in this test.

***Test Principle**

This assay employs a sandwich ELISA technique. The provided microplate is pre-coated with an antibody specific to human IL-18. Standards or samples are added to the wells and bind to the specific antibody. A biotinylated detection antibody and avidin-HRP conjugate are added successively. After washing away unbound substances, a substrate solution is added that produces a blue color, turning yellow after adding the stop solution. The optical density is measured at 450 ± 2 nm and is proportional to the concentration of IL-18 in the sample (Elabscience,2024a).

***Reagent Preparation**

Use high-purity deionized or distilled water at room temperature (18–25°C). Wash Buffer: Dilute 30 mL of 25× Wash Buffer with 720 mL distilled water to prepare 750 mL working solution. Warm if crystals form.

Standard: Reconstitute the standard with 1 mL of Standard & Sample Diluent after centrifuging at 10,000×g. Prepare serial dilutions: 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL. Biotinylated Detection Ab: Dilute 1:99 with its diluent (prepare fresh). HRP Conjugate: Also dilute 1:99 with its specific diluent before use.

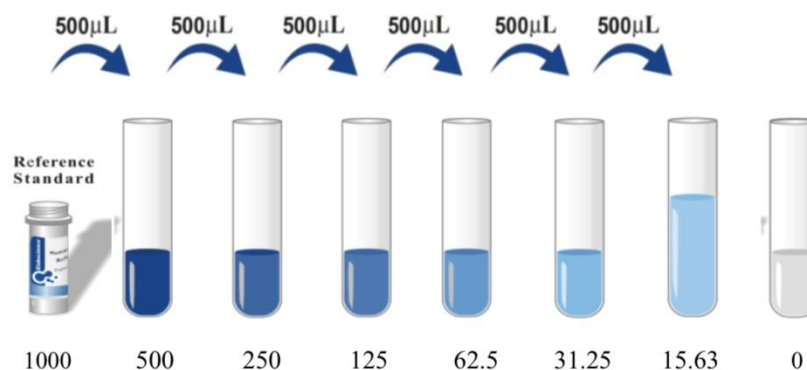


Figure (3-2) Dilution of standard solutions

The concentration of IL-18 in unknown samples is calculated using the following proportional formula: Total IL-18 concentration

$$\text{Total IL-18 concentration} = \frac{A_{\text{Test}}}{A_{\text{STD}}} \times \text{Concentration of STD}$$

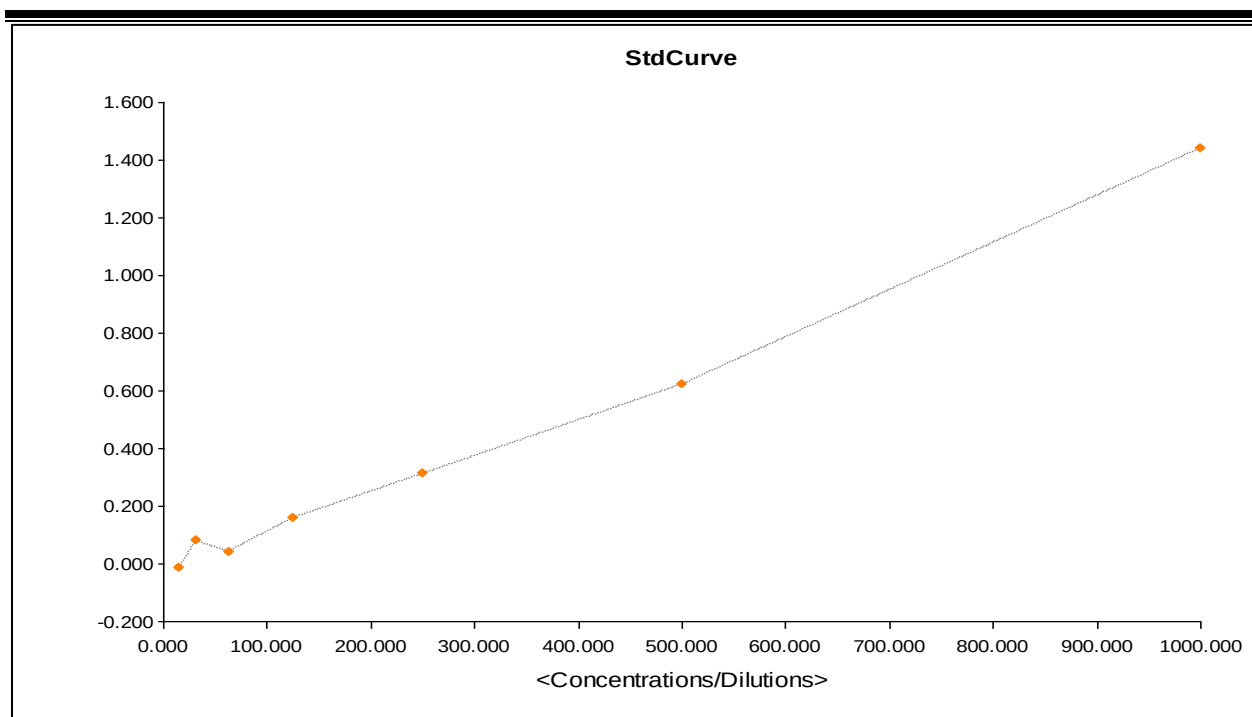


Figure (3-3).standard curvefor the IL-18 levels in test samples.

3.3.2.2. Measuring the Concentration of Serum Human interleukin 10in Patients' Blood.

The serum level of IL-10 was measured using using Elabscience® Catalog No: E-EL-H6154) This ELISA kit offers a high sensitivity of 0.94 pg/mL, with a detection range from 1.56 to 100 pg/mL. No significant cross-reactivity or interference with IL-10 analogues was observed. The coefficient of variation (CV) is less than 10%.

***Test Principle**

This assay employs the Sandwich-ELISA technique. The microplate is pre-coated with an antibody specific to human IL-10. Samples or standards are added to the wells and bind to the immobilized antibody. A biotinylated detection antibody specific to IL-10 is added, followed by Avidin-HRP conjugate. After washing,

substrate solution is added, which turns blue in the presence of IL-10, and then yellow after adding stop solution. The optical density (OD) is read at 450 ± 2 nm and correlates with the IL-10 concentration in the sample. concentration in the sample. (Elabscience,2024b).

*** Preparation of Serial Dilutions for Human Interleukin-10 (IL-10) Standard in ELISA Assay**

To construct the standard curve for the quantitative determination of Human Interleukin-10 (IL-10) using an ELISA assay, a series of two-fold serial dilutions was prepared starting from a 100 pg/mL reference standard. In each step, 500 μ L of the previous concentration was transferred into a new tube containing 500 μ L of diluent, resulting in the following final concentrations: 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 pg/mL. An additional tube containing only diluent was used as the zero standard (0 pg/mL).

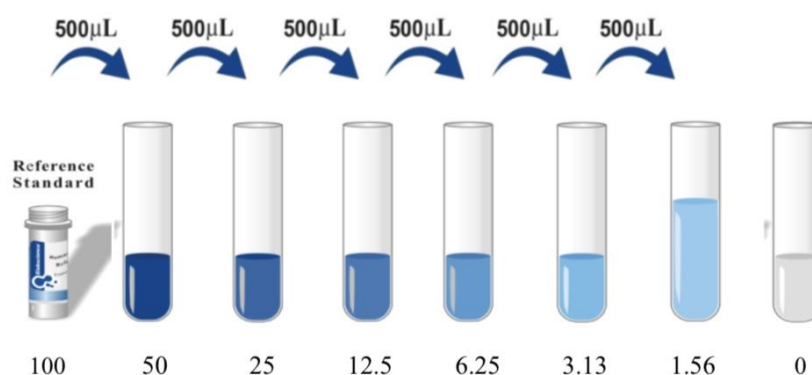


Figure (3-4) Dilution of standard solutions

***Assay Procedure**

Add 100 μ L of standards, blank, and samples to each well. Incubate for 90 minutes at 37°C. Decant the solution. Add 100 μ L of biotinylated antibody. Incubate for 1 hour at 37°C. Wash 3 times with Wash Buffer. Add 100 μ L of HRP conjugate.

Incubate for 30 minutes at 37°C. Wash 5 times with Wash Buffer. Add 90 µL of Substrate Reagent. Incubate for 15 minutes at 37°C in the dark. Add 50 µL of Stop Solution. Measure absorbance at 450 nm immediately.

***Results Analysis**

This graph illustrates the standard curve showing the relationship between known concentrations of Interleukin-10 (IL-10) and the corresponding optical density (OD) values measured using the ELISA method. As observed, there is a direct linear correlation, indicating that OD increases proportionally with the concentration of IL-10. This confirms the reliability of the standard preparation and the validity of the curve for quantifying unknown sample concentrations. The standard curve serves as a foundational tool to translate OD readings into actual IL-10 concentrations using either a linear or logarithmic regression model.

Mathematical Equation for IL-10 Concentration Calculation The concentration of IL-10 in unknown samples can be estimated using the following formula:

Total IL-10 Concentration = $A_{\text{Test}} / A_{\text{STD}} \times \text{Concentration of ST.}$

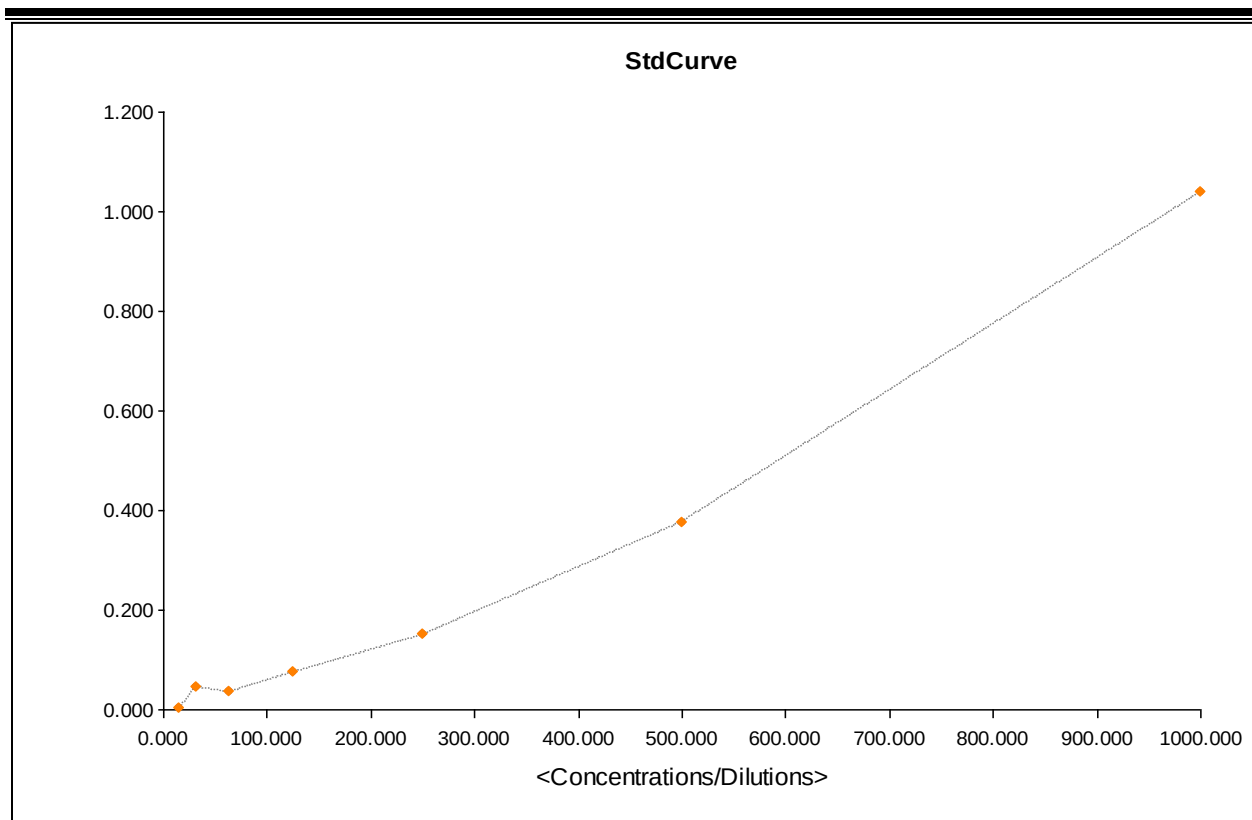


Figure (3-5) This curve is used to interpolate the IL-10 levels in test samples.

3.3.2.3. Measuring the Concentration of Serum Human Tumor Necrosis Factor-alpha (TNF- α in Patients' Blood).

The serum level of Human Tumor Necrosis Factor-alpha was measured using Elabscience®.ELISA-Based Quantification of Human TNF- α Using the Elabscience® Kit (E-EL-H0109) The kit exhibits a sensitivity of 4.69 pg/mL and a detection range from 7.81 to 500 pg/mL. It demonstrates high specificity for human TNF- α with no significant cross-reactivity with related cytokines or proteins.

*Assay Principle

The assay employs the Sandwich-ELISA technique. A microplate pre-coated with a monoclonal antibody specific for human TNF- α captures the target analyte. After sample or standard addition, a biotinylated detection antibody is introduced,

followed by HRP-conjugated avidin. The reaction is visualized using a TMB substrate, which yields a blue color that turns yellow upon the addition of stop solution. The optical density (OD) is measured at 450 ± 2 nm, and the concentration is determined using a standard curve. (Elabscience,2024c).

*Reagent Preparation

Distilled or Deionized Water: Used to dilute the concentrated wash buffer.
Wash Buffer: Prepare by diluting 30 mL of 25× Wash Buffer with 720 mL distilled water to make 750 mL of 1× Wash Buffer.
Standard Solution: Reconstitute the lyophilized standard with 1 mL of Reference Standard & Sample Diluent, allow it to dissolve completely, then perform serial dilutions to prepare a standard curve ranging from 500 pg/mL to 0 pg/mL.
Detection Antibody: Dilute the 100× Biotinylated Detection Antibody at a 1:99 ratio with its specific diluent.
HRP Conjugate: Similarly, dilute the 100× HRP Conjugate at a 1:99 ratio with its diluent just before use.

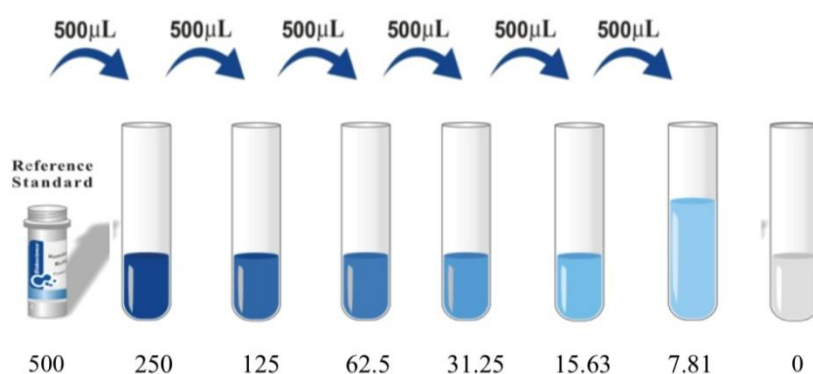


Figure (3-6) Dilution of standard solutions

*Assay Procedure

Add 100 μ L of standards, blank, or samples to each well. Incubate at 37°C for 90 minutes. Add 100 μ L of Biotinylated Detection Antibody and incubate for 60

minutes at 37°C. Wash wells three times with 350 µL of wash buffer. Add 100 µL of HRP Conjugate and incubate for 30 minutes at 37°C. Wash wells five times. Add 90 µL of Substrate Reagent and incubate 15 minutes at 37°C in the dark. Add 50 µL of Stop Solution to each well in the same order a substrate addition. Read absorbance at 450 nm immediately.

***Result Interpretation**

The OD values are averaged for duplicate wells and the blank OD is subtracted. A standard curve is constructed using a four-parameter logistic (4-PL) model. Sample concentrations are calculated from this curve and adjusted by the dilution factor, if applicable.

Mathematical Equation for IL-10 Concentration Calculation
The concentration of IL-10 in unknown samples can be estimated using the following formula:

Total TNF-α concentration = $A_{\text{Test}} / A_{\text{STD}} \times \text{Concentration of STD}$.

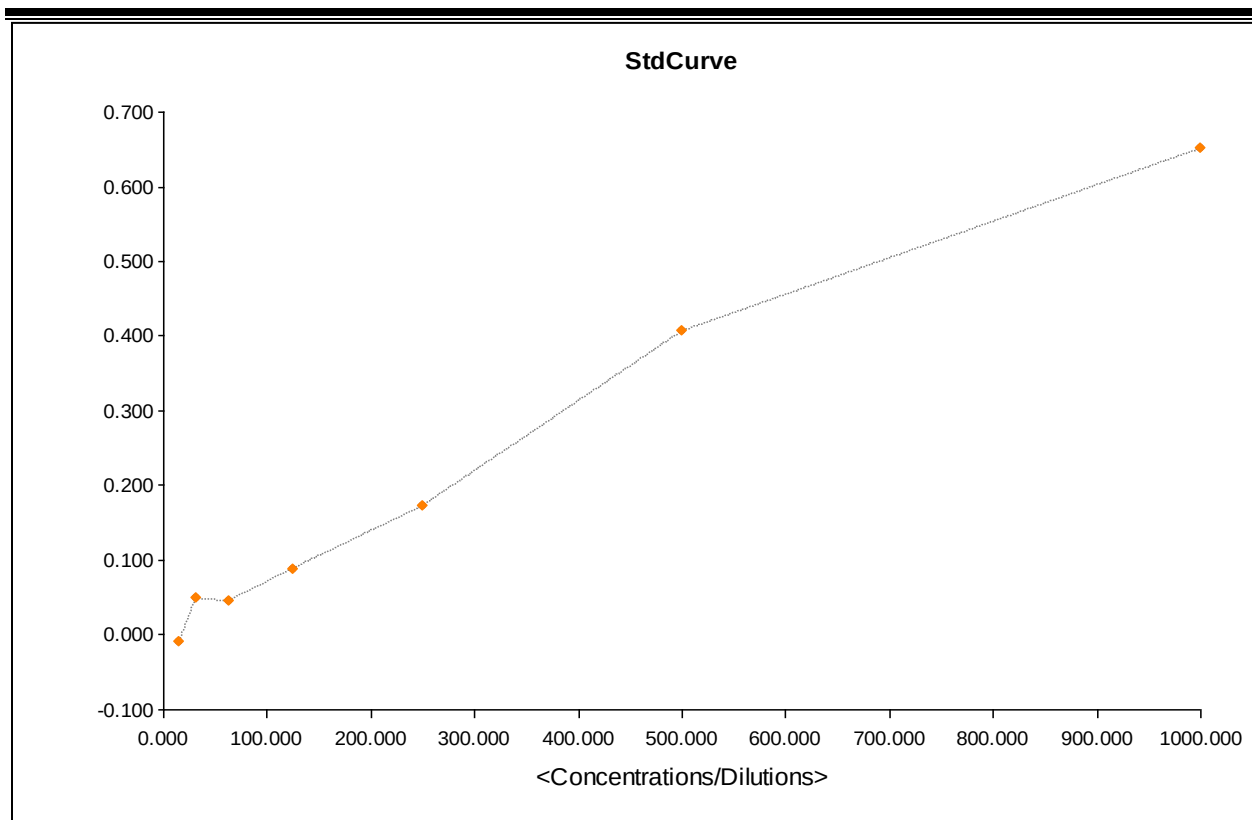


Figure (3-7) Standard Curve for Human TNF- α measured by ELISA.

3.3.2.4. Measuring the Concentration of Serum Human bFGF/FGF2 (Basic Fibroblast Growth Factor) in Patients' Blood

The serum level of bFGF/FGF2 was measured using Elabscience® ELISA kit (Intra-Assay: CV<5.99 %, Inter-Assay: CV< 8.69%, and sensitivity: 18.75pg/mL). The quantitative sandwich enzyme immunoassay method was used in this test.

***Principle of the Assay**

This kit utilizes the Sandwich ELISA technique. The 96-well microplate is pre-coated with an antibody specific to human BFGF/FGF2. Samples or standards are added to the wells, allowing the BFGF/FGF2 to bind to the immobilized antibody. A biotinylated detection antibody specific for BFGF/FGF2 is then added, followed by the addition of Avidin-Horseradish Peroxidase (HRP) conjugate. After incubation and washing, a substrate solution (TMB) is added, producing a blue color

that turns yellow upon the addition of stop solution. The absorbance is measured at 450 ± 2 nm, and the concentration is determined by comparison to a standard curve. (Elabscience,2024d).

*Reagent Preparation

Wash Buffer: Dilute 30 mL of the 25 $\times$ concentrated wash buffer with 720 mL of distilled or deionized water. Standard Solution: Centrifuge and reconstitute with 1 mL of diluent. Perform serial dilutions to generate standards. Biotinylated Detection Antibody: Dilute 1:99 with the provided diluent (e.g., 10 μ L stock + 990 μ L diluent). HRP Conjugate: Dilute 1:99 with the HRP diluent (e.g., 10 μ L stock + 990 μ L diluent).

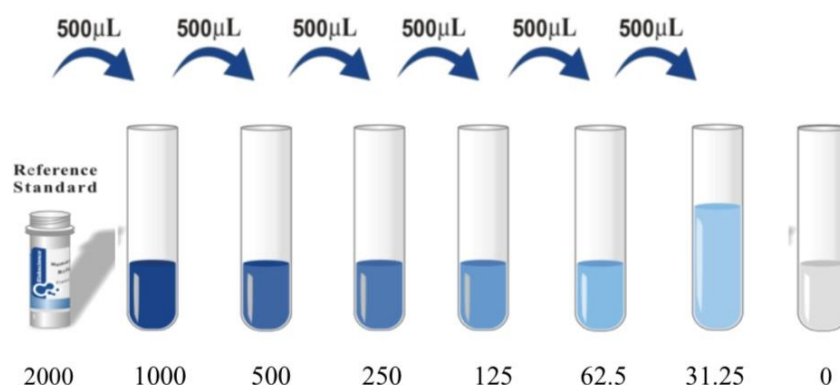


Figure (3-8) Dilution of standard solutions

*Assay Procedure

Add 100 μ L of standards, samples, and blank to wells. Incubate for 90 min at 37°C. Discard and add 100 μ L of detection antibody. Incubate for 1 hour at 37°C.. Wash 3 times with 350 μ L wash buffer. Add 100 μ L of HRP conjugate. Incubate 30 min at 37°C. Wash 5 times. Add 90 μ L of substrate. Incubate ~15 min at 37°C in the dark. Add 50 μ L of stop solution. Read OD at 450 nm.

***Results Interpretation**

Calculate the average OD for duplicates. Subtract blank OD. Construct a standard curve using a 4-PL fit. Determine sample concentration by comparing with the curve. Dilute and reanalyze sample Standard Curve of Human bFGF ELISA Assay. As the bFGF concentration increases, the absorbance rises correspondingly, allowing the quantification of unknown sample concentrations through interpolation.

Total bFGF concentration = $A_{\text{Test}} / A_{\text{STD}} \times \text{Concentration of STD}$

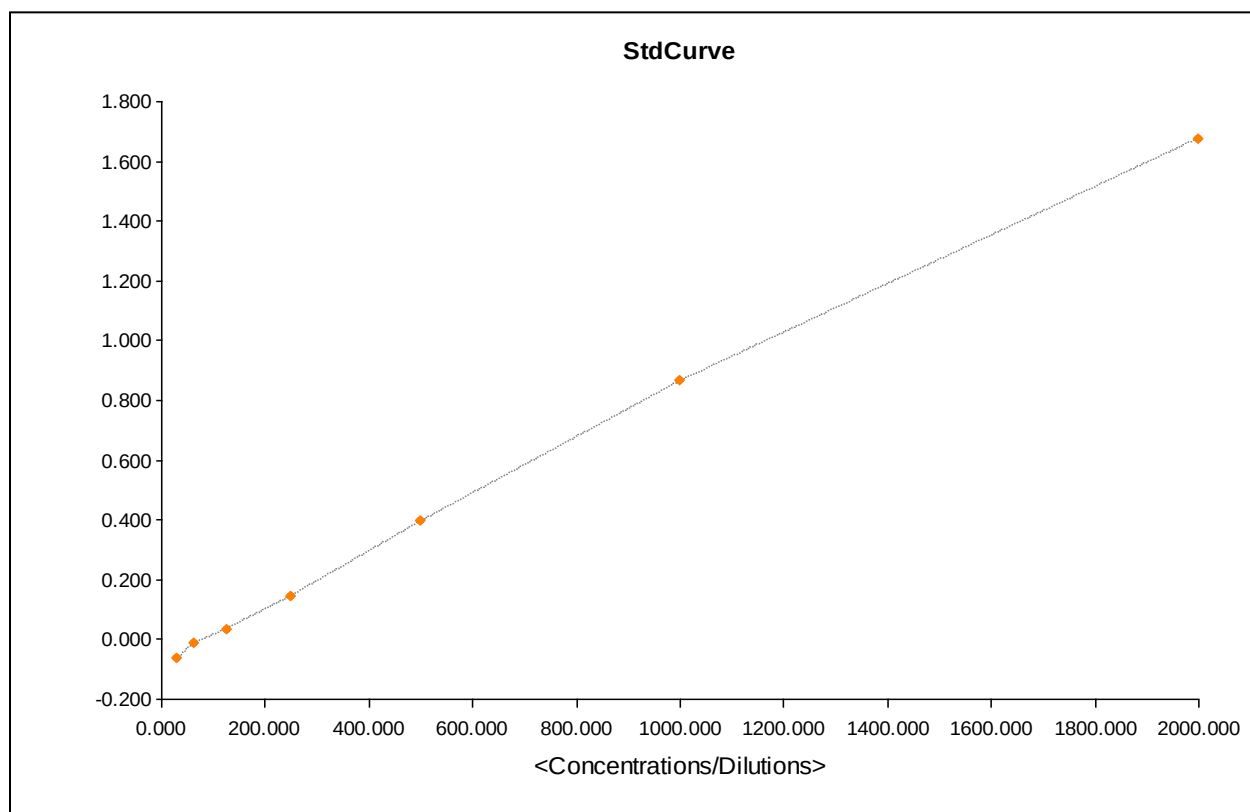


Figure (3-9) Standard Curve for Human bFGF measured by ELISA.

3.4. Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, SE: standard Error after performance of Kolmogorov-Smirnov normality test and making decision about normally and non-normally distributed variables. **Independent sample t-test** was used to study difference in mean between any two groups provided that the variable is normally distributed. **Mann Whitney test** was used to study difference in median between two non-parametric groups. **One-way a nova test** was used to study difference in mean among more than two groups provided that the variable is normally distributed. **Chi-square test** was used to study association between any two categorical variables. In order to detect the cutoff value that predict a positive finding, **receiver operator characteristic (ROC)** curve analysis was used with its corresponding area under the curve (AUC), accuracy level, sensitivity, specificity and level of significance (P). **Pearson correlation** was used to evaluate the correlation between any 2 numeric variables and the results were expressed as correlation co-efficient (r) and the level of significance (P). The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less (Daniel, 2018).

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Demographic characteristics

According to age the mean age of Ulcer patients was 59.93 ± 10.8 years old, 58.76 ± 9.3 years old for DM patients, and that of control subjects was 54.40 ± 8.60 years old and there was non-significant difference between patients and control subjects in mean age ($P = 0.158$). Regarding age group, Ulcer patients included 4 (13.3%) less than 50 years, 8 (26.7%) between 50-59 years, and 18 (60.0%) more than 60 years, DM patients included 7 (23.3%) less than 50 years, 3 (10.0%) between 50-59 years, and 20 (66.7%) more than 60 years, while control group included 9 (30.0%) less than 50 years, 9 (30.0%) between 50-59 years, and 12 (40.0%) more than 60 years and there was non-significant difference in the frequency distribution of patients and control subjects according to age groups ($P = 0.132$).

Table (4-1) Comparison between patients and control groups in Age group.

Characteristic	Ulcer <i>n</i> = 30	DM patients <i>n</i> = 30	Control <i>n</i> = 30	<i>P</i>
Age (years)				
Mean $\pm$ SD	59.93 $\pm$ 10.8	58.76 $\pm$ 9.3	54.40 $\pm$ 8.60	0.158 †
Range	32–85 years	28– 83 years	33– 71 years	
41-50, <i>n</i> (%)	4 (13.3%)	7 (23.3%)	9 (30.0%)	0.132 ¥ NS
51-60, <i>n</i> (%)	8 (26.7%)	3 (10.0%)	9 (30.0%)	
61-70years	18 (60.0%)	20 (66.7%)	12 (40.0%)	

n: number of cases; SD: standard deviation; †: one way ANOVA; ¥: Chi-square test; NS: non-significant at $P > 0.05$

the results showed that patients with DM age(51-60) years had a higher risk of (DFU) than those in other age groups. There was no significant difference in mean age between the patient and control groups ($P = 0.158$). As in the table (4.1) displays the age group distribution of participants. Analysis of this distribution revealed a clear trend: most patients with diabetic foot ulcers (DFU) and diabetic patients without ulcers were over 60 years old, comprising 60.0% and 66.7% of those groups. In comparison, only 40.0% of the control group was in this age range. The difference in age group distribution between patients and controls was not statistically significant ($P = 0.132$). This indicates that although older age was more common among patients, it did not independently differentiate the groups. This finding was somewhat similar to that of (Al-Rubeaan *et al.*, 2015) who reported that older patients tend to experience significantly more complications than younger ones. This may be risk factors like age related decline in healing, reduced mobility, co-existing health conditions severity of narrowed blood vessels and the generally weaker immune systems of elderly individuals. These findings also in agreement with the study by (McHugh, 2013). The higher percentage of individuals over 60 in the diabetic groups supports existing research indicating that the risk of developing type 2 diabetes and its complications increases steadily after the age of 50, with a notable rise after 60. This pattern is thought to result from age-related declines in insulin sensitivity, beta-cell function, and levels of physical activity (Kirkman *et al.*, 2012).

4.2. Sex

Regarding to sex in overall, 43 (47.8%) male and 47 (52.2%) female were included, Ulcer patients included 22 (73.3%) cases were male gender and 8 (26.7%) cases were female, DM patients included 5 (16.7%) cases were male gender and 25

(83.3%) cases were female, while control group included 16 (53.3%) cases were male gender and 14 (46.7%) cases were female and there was significant difference in the frequency distribution of patients and control subjects according to gender ($P=0.001$).As in table (4:2).

Table (4-2) :Comparison between patients and control groups in gender.

Study groups		sex		Total	p-value
		Male	Female		
Groups	Ulcer patients	22 (73.3%)*	8 (26.7%)	30	0.001 ¥ S
	DM patients	5 (16.7%)	25 (83.3%)	30	
	Control	16 (53.3%)	14 (46.7%)	30	
Total		43 (47.8%)	47 (52.2%)	90	

¥: Chi-square test; S: significant at ($P < 0.05$)

Regarding gender, a higher prevalence of DFU was observed among males. The study identified a statistically significant difference in gender distribution among the three groups ($P < 0.05$) as shown in table (4-2), with males appearing more susceptible. This result is consistent with earlier studies indicating that men have a higher incidence of foot ulcers, potentially due to behavioral and physiological factors such as increased physical activity, lower adherence to self-care practices, and higher exposure to foot injuries. Moreover, sex hormones like estrogen, testosterone, may influence immune function and wound healing, which could explain the poorer outcomes in males with poorly managed diabetes (Armstrong *et al.*, 2017; Zhang *et al.*, 2022; American Diabetes Association, 2023).

4.3. Some risk factors

The frequency distribution participant groups according to some features are shown in table (4-3), The frequency distribution of patients according to smoking the difference was non-significant ($P = 0.638$), Furthermore, the frequency distribution of patients according to hypertension was as the difference was non-significant ($P = 0.551$), Also, the frequency distribution of patients according to insulin intake, and the difference was significant ($P = 0.015$), where half patients with ulcer have insulin intake.

Table (4-3): Frequency distribution of participant groups according to some risk factors features.

Characteristic	Ulcer foot <i>n</i> = 30	DM patients <i>n</i> = 30	Control <i>n</i> = 30	P value
Smoking				
Positive, <i>n</i> (%)	5 (16.7%)	7 (23.3%)	8 (26.7%)	0.638 ¥ NS
Negative, <i>n</i> (%)	25 (83.3%)	23 (76.7%)	22 (73.3%)	
Insulin intake				
Positive, <i>n</i> (%)	15 (50.0%)*	6 (20.0%)		0.015 ¥ S
Negative, <i>n</i> (%)	15 (50.0%)	24 (80.0%)		
Hypertension				
Positive, <i>n</i> (%)	14 (46.7%)	13 (43.3%)	10 (33.3%)	0.551 ¥ NS
Negative, <i>n</i> (%)	16 (53.3%)	17 (56.7%)	20 (66.7%)	

n: number of cases; ¥: Chi-square test; NS: not significant at ($P > 0.05$) S: significant at ($P < 0.05$). * significant

In table (4-3) revealed that smoking prevalence among the three groups did not exhibit a statistically significant difference ($P = 0.638$). Specifically, 16.7% of patients with diabetic foot ulcers (DFUs) were smokers, in comparison to 23.3% of diabetic individuals without ulcers and 26.7% of the healthy control group. This association might be confounded by variables such as the duration of diabetes and the level of glycemic control. These findings Conflicts with those of (Abeer Elnour ,2021); however, they diverge from earlier studies, which have demonstrated that smoking elevates the risk of diabetic neuropathy and atherosclerosis—both critical contributors to the development of diabetic foot ulcers (Armstrong *et al.*, 2017).

Regarding hypertension, findings indicated that 46.7% of DFU patients had hypertension, versus 43.3% of non-ulcer diabetic individuals and 33.3% of the healthy control group, with no statistically significant difference ($P = 0.551$) as in table (4-3) This finding is supported by certain studies suggesting that although hypertension is a recognized risk factor for cardiovascular disease, it does not serve as a specific predictor for the occurrence of diabetic foot ulcers (Boulton *et al.*, 2005). Hypertension which damages small blood vessels in the skin ,leading to reduced blood flow tissue damage

In contrast, insulin therapy demonstrated a statistically significant difference among diabetic patients ($P = 0.015$). 50.0% of patients with foot ulcers were on insulin treatment compared to only 20.0% of diabetic patients without ulcers. The results further indicated that a greater proportion of DFU patients had poor glycemic control (93.1%) relative to diabetic individuals without ulcers, who generally maintained better glycemic control. The higher rate of insulin use among DFU patients may suggest that these individuals are in a more advanced stage of diabetes or have more severe disease progression, where oral medications are no longer sufficient, and insulin becomes necessary, Poor glycemic control is a well-known

risk factor for the development of diabetic complications, including foot ulcers, These findings are consistent with those reported by (Purwanti *et al.*, 2016).

4.4. Some clinical features associated with Ulcer

The frequency distribution of Ulcer patients according to some clinical features are shown in table (4-4). The frequency distribution of ulcer patients according to ulcer site was as of ulcer patients with sole site and of ulcer patients with back site, there was non-significant difference ($P= 0.446$) The frequency distribution of ulcer patients according to ulcer type was as with superficial type, with deep type, and of ulcer patients with bone type, and the differences was significant ($p= 0.025$). The frequency distribution of ulcer patients according to Ulcer swelling was as) there was non-significant difference ($P= 0.144$) Regarding to Duration of Ulcer was non-significant difference ($P= 0.273$).

Table (4-4): The frequency distribution Ulcer patients according to some clinical features

Characteristic	Ulcer Patients <i>n</i> (%)	P value
Ulcer site		
Sole, <i>n</i> (%)	13 (43.3%)	0.465 ¥ NS
hind, <i>n</i> (%)	17 (56.7%)	
Ulcer type		
Superficial, <i>n</i> (%)	13 (43.3%)	0.025* ¥ S
Deep, <i>n</i> (%)	14 (46.7%)	
With bone, <i>n</i> (%)	3 (10.0 %)	
Ulcer swelling		

Swelling, <i>n</i> (%)	19 (43.3%)	0.144 ¥ NS
Without swelling, <i>n</i> (%)	11 (46.7%)	
Duration of Ulcer		
<1 year, <i>n</i> (%)	18 (60.0%)	0.273 ¥ NS
≥ 1 year, <i>n</i> (%)	12 (40.0%)	

n: number of cases; ¥: Chi-square test; S: significant at($P < 0.05$). * significant/
nS non sig($P > 0.05$)

Regarding ulcer site, most cases had ulcers located on the hind of the foot (17 patients, 56.7%), compared to 13 patients (43.3%) with ulcers on the sole. Although this difference was not statistically significant ($p=0.465$), As in table (4:4) mechanical factors such as increased pressure or friction in that area especially in diabetic patients with peripheral neuropathy and loss of protective sensation, these results are comparable to the results reported by by (Driver *et al.*, 2015).

Ulcer type is classified into superficial ulcer, deep ulcer, ulcer with bone involvement, and ulcer with gangrene, the majority of cases were of deep ulcers (46.7%), followed by superficial ulcers (43.3%), while bone-exposing ulcers were the least common (10%), and the difference was statistically significant ($p=0.025$). due to delayed diagnosis or poor glycemic control. This result identical with study of (Lavery *et al.*, 2006).

As for swelling, with no significant difference ($p=0.144$). This suggests that swelling may not be a defining feature or an independent marker of ulcer severity in all cases, but could reflect stages of localized inflammation or infection These results are comparable to the results reported by (Jeffcoate & Harding, 2003).

In terms of ulcer duration, most patients (60%) had ulcers persisting for more than a year, while 40% had ulcers of less than one year duration, with no statistical

significance ($p=0.273$). due to impaired tissue regeneration and associated vascular or immune problems These findings are consistent with those reported by (Prompers *et al.*, 2008).

4.5. Results of metabolic factors (Glucose and HbA1c) in patients and healthy controls.

The comparison of metabolic factors (Glucose and HbA1c) between patients and control group has been carried out and the results were demonstrated in table (4-5). Mean levels of Glucose were, in Ulcer patients, DM patients and healthy control group respectively; the mean levels were higher in both groups of patients (Ulcer patients and DM patients) in compared to healthy control and the difference was highly significant ($P < 0.001$). But there was non-significant difference between Ulcer patients and DM patients themselves. The mean of HbA1c were in Ulcer patients, DM patients and healthy control group respectively; the mean was higher in both groups of patients (Ulcer patients and DM patients) in compared to healthy control and the difference was highly significant ($P < 0.001$). But there was non-significant difference between Ulcer patients and DM patients themselves.

Table (4-5) :Comparison of metabolic factors (Glucose and HbA1c) in patients and healthy controls

Groups		Glucose (mg/dl)	HbA1c (%)
Ulcer patients	Mean $\pm$ SD	243.45 $\pm$ 27.85 ^B	9.51 $\pm$ 2.1 ^B
	Range	165.00-353.00	6.80-13.50
DM patients	Mean $\pm$ SD	227.03 $\pm$ 28.61 ^B	8.61 $\pm$ 1.8 ^B
	Range	134.00-393.00	6.20-12.90
Control	Mean $\pm$ SD	85.20 $\pm$ 6.93A	5.27 $\pm$ 0.49A

	Range	72.00-109.00	4.30-6.00
p-value		0.001**	0.001**
Different letters denote to the significant differences at $p < 0.05$			

SD: standard deviation; †: one way ANOVA; **: significant at $P < 0.05$ Different letters represent significantly higher than control

The results shown in table (4-5) indicate a clear elevation in fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) levels among both diabetic foot ulcer (DFU) patients and diabetic patients without ulcers, compared to the healthy, the mean glucose (DFU), diabetic patients, and healthy controls. Similarly, the mean (HbA1c), These results show a statistically significant difference ($P < 0.001$) between patient groups and healthy individuals, the difference between DFU and diabetic patients was not statistically significant, The HbA1c levels are significantly elevated in both diabetic groups compared to controls, indicating chronic poor glycemic control. The value is even higher in patients with foot ulcers, suggesting that inadequate long-term glucose management may contribute to the development of diabetic foot complications. this result can be compared with the findings of a previous by (Dekker *et al.*, 2016) suggesting poor glycemic control in both groups regardless of ulcer status. the occurrence of diabetic foot ulcers has been linked to high blood sugar levels an investigation conducted in Iran found a connection between inadequate blood glucose regulation and the presence of diabetic foot ulcers (Kateel *et al.*, 2018). Research by (Kumar *et al.*, 2016) indicated that ulcer recovery progressed more rapidly among individuals with reduced levels of glycosylated hemoglobin. Additionally, (Hemmingsen *et al.*, 2013) identified a 35% decrease in the likelihood of lower-limb amputations among those with strict blood sugar control. The data on fasting blood glucose and HbA1C is displayed in Table (4.5). It revealed a higher average HbA1C (mean = 9.47) in ulcer patients compared to

healthy controls, although the variation was not statistically significant ($p = 0.098$). Elevated HbA1c levels serve as a robust indicator for diabetes-related complications (Al-Shaheeb *et al.*, 2022). Elevated blood glucose has been recognized as a contributing factor in the emergence of diabetic foot ulcers due to its role in triggering peripheral neuropathy and microvascular issues (Al Kafrawy *et al.*, 2014). Insufficient glycemic management stands as a primary contributor to the onset of diabetes-related health issues.

Effective regulation of blood sugar levels can prevent all associated complications, including diabetic foot ulcers (Marzoq *et al.*, 2019). The study confirmed that diabetes-related complications are closely tied to average blood sugar levels, as determined by HbA1c measurements (White *et al.*, 2010)

4.5.1. Correlation between metabolic factors (Glucose and HbA1c) in both group patients.

The correlations between metabolic factors (Glucose and HbA1c) in both group patients were shown in tables (4-6) and (4-7). The present results show there was significant correlation between Glucose level and HbA1c ($r = 0.531$, $p = 0.004$) in Ulcer patients and Glucose level and HbA1c ($r = 0.685$, $p = 0.001$) in DM patients.

Table (4-6): Correlation between metabolic factors (Glucose and HbA1c) in Ulcer patients

Parameters	Metabolic factors			
	Glucose		HbA1c	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	1			
HbA1c	0.531	0.004**	1	

r: correlation coefficient.

Table (4-7): Correlation between metabolic factors (Glucose and HbA1c) in DM patients

Parameters	Metabolic factors			
	Glucose		HbA1c	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	1			
HbA1c	0.685	0.001**	1	

r: correlation coefficient.

The results in Tables (4-6) and (4-7) indicate a statistically significant positive correlation between blood glucose levels and HbA1c concentrations in both diabetic foot ulcer patients and diabetic patients without ulcers. The correlation coefficient was ($r = 0.531$, $p = 0.004$) in the ulcer group, and higher ($r = 0.685$, $p = 0.001$) in the diabetic group, suggesting a to Moderate positive correlation relationship in both groups, For patients with ulcers, the correlation coefficient was $r = 0.531$ and the p-value was 0.004, signifying a moderate positive association. Among diabetic individuals without ulcers, the correlation was stronger, with a coefficient of $r = 0.685$ ($p = 0.001$), indicating a strong positive relationship, higher glucose levels mean more glucose hemoglobin this process is the basis for using HbA1c as a marker for long-term blood sugar control in DM patients,. Elevated blood levels typically lead to increased hemoglobin glycation (Sherwani *et al.*, 2016; American Diabetes Association, 2024). The more pronounced correlation in the non-ulcer diabetic group may point to more consistent blood sugar in these individuals, in contrast to those with ulcers who may experience variable glucose levels due to infections, inflammation, or stress responses linked to wound healing (Zubair *et al.*, 2021).

4.6. Results of lipid profile (Triglycerides, Cholesterol, LDL and HDL) in patients and healthy controls.

The comparison of lipid profile (Triglycerides, Cholesterol, LDL and HDL) between patients and control group has been carried out and the results were in table (4-11) Mean levels of serum triglycerides were, in Ulcer patients, DM patients and healthy control group respectively; the mean levels was higher in both group of patients in compared to control group and the difference was significant ($P < 0.05$). But the mean levels were non-significant difference between Ulcer patients and DM patients themselves ($P > 0.05$). The mean levels of serum cholesterol were, in Ulcer patients, DM patients and healthy control group respectively; the mean levels were lower in Ulcer patients in comparison to other groups and the difference was significant ($P < 0.05$). Regarding the mean levels of serum low-density lipoprotein (LDL) were, in Ulcer patients, DM patients and healthy control group respectively; the mean levels was lower in both group of patients in compared to control group and the difference was significant ($P < 0.05$). But the mean levels were non-significant difference between Ulcer patients and DM patients themselves ($P > 0.05$). The mean levels of serum High-density lipoprotein (HDL) were, in Ulcer patients, DM patients and healthy control group respectively; the mean levels were lower in Ulcer patients in comparison to other groups and the difference was significant ($P < 0.05$).

Table (4-8): Results of lipid profile (Triglycerides, Cholesterol, LDL and HDL) in patients and healthy controls.

Characteristic	Ulcer patients n=30	DM patients n=30	Control n=30	P
Triglycerides (mg/dl)				
Mean $\pm$ SE	158.07 $\pm$ 23.6 ^A	174.6 $\pm$ 27.9 ^A	116.9 $\pm$ 20.4 ^B	0.014

Range	73.0- 321.0	77.0 – 351.0	77.0 – 368.0	† S
Cholesterol (mg/dl)				
Mean± SE	126.2 ± 21.5 ^A	162.4 ± 19.9 ^B	165.8 ± 16.9 ^B	0.001
Range	62.0- 196.0	90.0 – 235.00	124.0 – 234.0	† S
low-density lipoprotein (LDL) (mg/dl)				
Mean± SE	108.5 ± 10.8 ^A	101.7 ± 11.3 ^A	75.0±8.11 ^B	0.001
Range	71.0- 138.00	65.0–150.0	55.0 – 144.0	† S
High-density lipoprotein (HDL) (mg/dl)				
Mean± SE	26.7 ± 6.4 ^A	36.50 ± 8.5 ^B	65.61 ± 8.3 ^B	0.011
Range	10.0- 49.0	16.00 – 66.0	51.00 – 75.00	† S
Different latter denote to the significant differences at p< 0.05				

n: number of cases; SE: standard Error; †: one way ANOVA; HS: Highly significant at P <0.001

Different latter denote to the significant differences at(p< 0.05)

n: number of cases; SE: standard Error; †: one way ANOVA; HS: Highly significant at(P <0.001)

This study examined lipid components triglycerides, total cholesterol, LDL (low-density lipoproteins), and HDL (high-density lipoproteins) across three groups: diabetic patients with foot ulcers, diabetic patients without ulcers, and healthy individuals, as shown in table (4-8), The findings revealed significant alterations in these lipid markers, indicating disrupted lipid metabolism in diabetic patients, particularly those with foot ulcers. Both DFU and DM groups exhibited significantly elevated serum triglyceride levels compared to the control group (P = 0.014). In a healthy state, insulin suppresses the activity of hormone-sensitive lipase in fat cells, thereby limiting fat breakdown. But when insulin resistance occurs, this suppression becomes less effective, causing an increased breakdown of fat and a surge of free

fatty acids (FFAs) into the bloodstream (Samuel and Shulman, 2016). These FFAs are taken up by the liver and converted back into triglycerides, which are then packed into VLDL particles, leading to their excessive production and elevated blood levels (Adiels *et al.*, 2008). These findings are in agreement with the results reported by (Al-Maskari and El-Sadig, (2007) Patients with diabetic foot ulcers tend to have elevated triglyceride (TG) levels, which are linked to reduced blood flow and slower wound healing. Patients with foot ulcers had notably lower total cholesterol levels (126.2 mg/dL) than those in other groups, Several physiological and medical factors help explain why people with diabetic foot ulcers (DFUs) tend to have lower total cholesterol levels compared to healthy individuals, A major reason is that many diabetic patients are prescribed statins and similar cholesterol-lowering drugs to help reduce their risk of heart disease, These medications work by blocking the liver's ability to produce cholesterol, which leads to lower levels in the blood. healing chronic wounds like DFUs requires the body to use more phospholipids and cholesterol to rebuild cell membranes and support the controlled inflammation needed for recovery, Poor nutrition, which is often seen in people with long-term diabetes complications, may further reduce the raw materials needed to make cholesterol diabetes and certain medications can affect how well the liver works, potentially disrupting cholesterol production and regulation, The results of our study can be compared with those of previous studies. (Goldberg, 2020; Ali *et al.*, 2020; Zhao *et al.*, 2021; Ahmed *et al.*, 2021; Jones *et al.*, 2022). LDL levels were reduced in patients with DFU and DM, possibly as a result of medication or enhanced lipid usage. This result agrees with by (Idogun & Ajala ,2010). The lowest HDL level was observed in patients with foot ulcers (26.7 mg/dL), serving as a negative indicator for delayed wound healing, This association is supported by the study of (Magbanua *et al.* 2020), which demonstrated that reduced HDL is linked to complications of diabetic foot and a heightened risk of amputation. HDL plays an important protective

role against atherosclerosis by helping to transport cholesterol from peripheral tissues back to the liver, A recent study found that diabetic patients with foot ulcers have significantly lower HDL levels compared to those without ulcers, indicating that this drop may be associated with heightened inflammation, more severe infections, and poor blood sugar control (Zhao *et al.*, 2021).

4.6.1. Correlation between lipid profile parameters and other parameters in both group patients.

The correlations between lipid profile parameters and other parameters in both group patients were shown in tables (4-9) and (4-10). There are positive correlation between Triglyceride levels and cholesterol ($r=0.401$ and $p=0.021$), cholesterol levels and LDL ($r=0.638$ and $p=0.001$), cholesterol levels and HDL ($r=0.598$ and $p=0.001$) and LDL levels and HDL ($r=0.531$ and $p=0.001$) in Ulcer patients. Also, there are positive correlation between Triglyceride levels and cholesterol ($r=0.362$ and $p=0.028$), Triglyceride and HBA1c ($r=0.457$ and $p=0.008$), cholesterol levels and LDL ($r=0.533$ and $p=0.001$), cholesterol levels and HBA1c ($r=0.384$ and $p=0.024$) in DM patients. But there are negative correlation between Triglyceride levels and HDL ($r=0.542$ and $p=0.001$) in DM patients.

Table (4-9): Correlation between lipid profile and other parameters in Ulcer patients.

Characteristic	lipid profile parameters							
	TG		Chole		LDL		HDL	
	R	P	R	P	R	P	R	P
TG	1							
Chole	0.401	0.021*	1					

LDL	-0.023	0.909	0.638	0.001*	1			
HDL	0.171	0.385	0.598	0.001*	0.531	0.001*	1	
Glucose	0.135	0.493	0.004	0.983	-0.004	0.984	0.210	0.282
HBA1c	0.029	0.882	0.258	0.185	0.057	0.772	0.112	0.571

r: correlation coefficient.

Table (4-10): Correlation between lipid profile and other parameters in DM patients

Characteristic	lipid profile parameters							
	TG		Chole		LDL		HDL	
	R	P	R	P	R	P	R	P
TG	1							
Chole	0.362	0.028*	1					
LDL	0.029	0.879	0.533	0.001*	1			
HDL	-0.542	0.001*	0.309	0.077	0.097	0.611	1	
Glucose	0.292	0.117	0.477	0.001*	0.217	0.250	0.059	0.759
HBA1c	0.457	0.008*	0.384	0.024*	0.219	0.244	0.003	0.986

r: correlation coefficient.

The results presented in tables (4-9) and (4-10) reveal positive correlation between lipid profile parameters and other clinical indicators among diabetic patients and those with diabetic foot ulcers, reflecting the intricate relationship between metabolic dysregulation and diabetic complications. In Ulcer Patients a positive correlation between triglycerides and total cholesterol ($r = 0.401$, $p = 0.021$) indicates that elevated triglycerides often accompany high cholesterol levels. This is

common in metabolic syndrome, particularly in chronic inflammatory states like foot ulcers (Mooradian, 2009; Almdal *et al*, 2020). Positive correlations between total cholesterol and LDL ($r = 0.638$, $p = 0.001$) suggest an overall lipid imbalance, likely reflecting increased hepatic lipid production due to chronic inflammation and insulin resistance (Goldberg, 2021). strong correlation is expected as LDL and total cholesterol, in DFU, elevated LDL may exacerbate vascular inflammation and complications (Jeffcoat & Harding, 2003) There are negative correlation between LDL and HDL ($r = 0.531$, $p = 0.001$) may reflect disturbed reverse cholesterol transport, often impaired in chronic tissue damage Total Cholesterol and HDL ($r = 0.598$, $p = 0.001$): Despite typically low HDL in dyslipidemia, the positive correlation may indicate simultaneous lipid elevation due to impaired reverse cholesterol transport or oxidative stress. The negative correlation between LDL and HDL ($r = 0.531$, $p = 0.001$) may reflect disturbed reverse cholesterol transport, often impaired in chronic tissue damage. Although a negative association between HDL and LDL is not commonly observed, it has been reported in patients with metabolic syndrome or elevated triglycerides (Shamai *et al.*, 2011). (Feingold & Grunfeld, 2000). In Diabetic Patients Triglycerides correlate positively with cholesterol ($r = 0.362$, $p = 0.028$) and HbA1c ($r = 0.457$, $p = 0.008$), indicating that poor glycemic control is associated with elevated triglycerides, due to increased lipolysis and hepatic lipid synthesis (Wong *et al.*, 2022). Cholesterol levels correlate with LDL ($r = 0.533$, $p = 0.001$) and HbA1c ($r = 0.384$, $p = 0.024$), showing how dyslipidemia is tightly linked to glucose dysregulation (Goldberg, 2021). A significant negative correlation between triglycerides and HDL ($r = -0.542$, $p = 0.001$) supports the known pattern of abnormal blood lipid levels, which increases cardiovascular risk (Mooradian, 2009).

4.7. Results of C-reactive protein (CRP) in patients and healthy controls.

The comparison of C-reactive protein (CRP) between patient and control groups has been carried out and the results were demonstrated in table (4-11) and Figure (3-11): Mean levels of C-reactive protein (CRP) were 128.4 ± 13.3 , 47.78 ± 12.7 and 7.03 ± 1.3 , in Ulcer patients, DM patients and healthy control group respectively; the mean levels was higher in both group of patients in compared to healthy control and the difference was significant ($P < 0.05$). Also, there was significant difference between both group of patients (Ulcer patients and DM patients) themselves, ($P < 0.05$).

Table (4.11) :Comparison of C-reactive protein (CRP) in patients and healthy controls

Groups		C-reactive protein (CRP) levels mg/L
Ulcer patients	Mean $\pm$ SE	128.4 ± 13.3^A
	Range	16.0-295.0
DM patients	Mean $\pm$ SE	47.78 ± 12.7^B
	Range	1.20-293.0
Control	Mean $\pm$ SE	7.03 ± 1.3^C
	Range	1.00-41.00
p-value		0.001*
Different latters denote to the significant differences at($p < 0.05$)		

SE: standardError; †: one way ANOVA; **: significant at($p < 0.05$)

C-reactive protein (CRP) is an acute phase reactant protein made by the liver that increases in response to inflammation, which can be triggered by infection, injury, or chronic illness. It is considered one of the most sensitive and early indicators of systemic inflammation. CRP levels start to increase within a few hours after the onset of inflammation and usually peak within 24 to 48 hours. Its production is mainly controlled by the inflammatory molecule interleukin-6 (IL-6). CRP contributes to the body's first line of defense, known as the innate immune response, by helping activate the complement system and aiding in the removal of pathogens and damaged cells (Pepys and Hirschfield, 2003). As explained in table (4-11), the average serum levels of C-reactive protein (CRP) were 128.4 ± 13.3 mg/L in diabetic patients with foot ulcers, 47.78 ± 12.7 mg/L in diabetic patients without ulcers, and 7.03 ± 1.3 mg/L in the healthy control group. The findings demonstrated a statistically significant increase in CRP levels in both groups of diabetic patients when compared to the healthy controls ($P < 0.05$), along with a significant difference between the two diabetic groups themselves ($P < 0.05$). This finding aligns with previous research that documented significantly elevated serum CRP levels in patients with diabetic foot ulcers when compared to healthy individuals. The elevation of CRP is widely recognized as a biomarker of systemic inflammation and is often associated with tissue injury or infection, both of which are common in diabetic foot conditions (Herder *et al.*, 2019; Shrestha *et al.*, 2021). In this research, the DFU group exhibited significantly higher CRP levels compared to both healthy individuals and diabetic patients without ulcers. This observation aligns with the findings of (Kahraman *et al.* 2014), who highlighted that systemic inflammation commonly influenced by unhealthy lifestyle choices and obesity can result in increased CRP levels through cytokine-driven mechanisms, involving IL-6 and TNF- α (Ehiaghe *et al.*, 2013).

4.7.1. Correlation between C-reactive protein (CRP) and other parameter in both group patients.

The correlation between C-reactive protein (CRP) and other parameter in both group patients were shown in tables (4-12). The present results show there was significant positive correlation between CRP levels and glucose level ($r=0.389$ and $p=0.034$), , CRP levels and LDL level ($r=-0.364$ and $p=0.041$) and CRP levels and HDL level ($r=-0.387$ and $p=0.035$) in DM patients. But the present results show non-significant correlation between CRP and all other parameters in both groups of patients.

Table (4-12): The correlation between C-reactive protein (CRP) and other parameter in both groups of patients.

Other parameters	C-reactive protein (CRP)			
	Ulcer patients		DM patients	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	0.389	0.034*	0.315	0.090
HbA1c	0.241	0.216	0.323	0.082
TG	0.250	0.299	0.169	0.372
Cholesterol	0.033	0.866	0.233	0.216
LDL	0.289	0.136	-0.364	0.041*
HDL	0.155	0.432	-0.387	0.035*

ur: Pearson correlation.

The results in Table (4-12) show statistically significant correlations between C-reactive protein (CRP) levels in the diabetic foot ulcer group (Ulcer patients): CRP levels were significantly positively correlated with Glucose level ($r = 0.389$, $p = 0.034$), suggesting that hyperglycemia may be associated with systemic inflammation. In addition, inflammation disrupts iron metabolism and impairs

hemoglobin synthesis (Ganz & Nemeth, 2015). LDL levels ($r = -0.364$, $p = 0.041$), Usually, higher LDL levels are linked with elevated CRP because of atherogenesis, but the observed inverse relationship might stem from changes in lipid metabolism during prolonged inflammation. Inflammatory cytokines could decrease the production of lipoproteins or boost the removal of LDL by activated immune cells (Khovidhunkit *et al.*, 2004). HDL levels ($r = -0.387$, $p = 0.035$) This finding is consistent with earlier research indicating that reduced HDL levels are frequently linked to chronic inflammation. HDL has anti-inflammatory and antioxidant effects and helps remove cholesterol from immune cells (Barter *et al.*, 2004). However, during inflammation, the structure and function of HDL can be impaired, weakening its protective effects and potentially worsening inflammation (Feingold and Grunfeld, 2010).

4.8. Subjects immunological parameters Analysis Results

4.8.1. Interleukin-10 (IL-10) level in study groups.

The comparison of Interleukin-10 (IL-10) level between patients and control groups has been carried out and the results were demonstrated in table (4-13), the mean levels were higher in DM patients in compared to healthy control and the difference was significant ($P < 0.05$). But the mean levels were non-significant difference between other both groups (Ulcer patients and healthy control) themselves ($P > 0.05$). Also, the mean levels were non-significant difference between both patients' groups (Ulcer patients and DM patients) themselves ($P > 0.05$).

Table (4-13) :Interleukin-10 (IL-10) level in study groups.

Groups		Interleukin-10 (IL-10) level pg/mL.
Ulcer patients	Mean $\pm$ SE	41.09 $\pm$ 10.5 ^{AB}

	Range	12.40-211.0
DM patients	Mean $\pm$ SE	54.70 $\pm$ 16.05^A
	Range	11.70-346.00
Healthy control	Mean $\pm$ SE	19.92 $\pm$ 1.59^B
	Range	12.00-41.00
p-value		0.048* †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SE: standard error; †: Kruskal Wallis test; **: significant at group ($P < 0.05$),

This study found a statistically significant elevation in serum Interleukin-10 (IL-10) levels in both diabetic groups—those with and without foot ulcers—when compared to healthy individuals ($P = 0.048$). Diabetic patients without foot ulcers exhibited the highest average IL-10 level, followed in patients with ulcers. The healthy control group showed the lowest levels, averaging. However, the difference in IL-10 concentrations between the two diabetic subgroups was not statistically significant. IL-10 is a powerful anti-inflammatory cytokine mainly secreted by regulatory T cells and monocytes. It plays a critical role in downregulating the production of pro-inflammatory cytokines such as TNF- α and IL-1 β , thereby helping maintain immune system balance (Akdis *et al.*, 2011). The observed increase in IL-10 levels among diabetic individuals may indicate a compensatory anti-inflammatory mechanism in response to the persistent, low-grade inflammation typically seen in diabetes mellitus (Donath and Shoelson, 2011). The lack of significant differences in IL-10 levels between diabetic patients with and without foot ulcers suggests that the cytokine's elevated presence may be attributed more to

the systemic inflammatory milieu of diabetes itself rather than being directly associated with ulcer formation, delayed wound healing, similar results were observed in the study by (Moore *et al.*, 2001). suggesting a compensatory immune mechanism to counteract the chronic inflammation linked to diabetes. In contrast, there was no statistically significant difference in IL-10 levels between ulcer patients and the control group ($P > 0.05$), nor between ulcer and DM patients ($P > 0.05$). This may be attributed to variations in clinical presentation or other factors such as ongoing local infections, which could disrupt the balance between pro- and anti-inflammatory cytokines (Ip *et al.*, 2016). Regarding the impact of hyperglycemia and oxidative stress, elevated glucose levels are known to induce oxidative stress and cellular damage, which suppress IL-10 gene expression while stimulating the production of pro-inflammatory cytokines. This indicates a negative relationship between blood glucose regulation and IL-10 levels (Mirza *et al.*, 2014).

4.8.1.1. Diagnostic accuracy of Interleukin-10 (IL-10) level

Receiver operating characteristic (ROC) analysis was performed to reveal the prognostic accuracy of using Interleukin-10 (IL-10) concentrations to distinguish Ulcer patients from healthy control subjects, An optimal IL-10 cut-off value more than of 15.9 resulted in an AUC value of 0.577 (95% confidence interval [CI], 0.429-0.724, $P = 0.438$), sensitivity of 53.3%, specificity of 56.7%, PPV of 55.2%, and NPV of 54.8%, The present results indicate IL-10 is considered as weak prognostic marker to distinguish Ulcer patients from healthy control, In addition, an optimal IL-10 cut-off value of more than 18.9 could be used to distinguish DM patients from healthy control subjects with a sensitivity of 63.3%, specificity of 63.3%, PPV of 63.3%, and NPV of 63.3% and 0.634 (0.483- 0.785). The present results indicate IL-10 is considered as weak prognostic marker to distinguish DM patients from healthy control.

Table (4-14): Roc curve of Interleukin-10 (IL-10) level

Characteristic	Ulcer patients / control	DM patients /control
Cutoff value	>15.9	>18.9
P value	0.438	0.039
Sensitivity %	53.3 %	63.3 %
Specificity %	56.7 %	63.3 %
PPV %	55.2 %	63.3 %
NPV %	54.8 %	63.3 %
AUC (95% CI)	0.577 (0.429- 0.724)	0.634 (0.483- 0.785)

CI: Confidence interval, AUC: Area under curve.

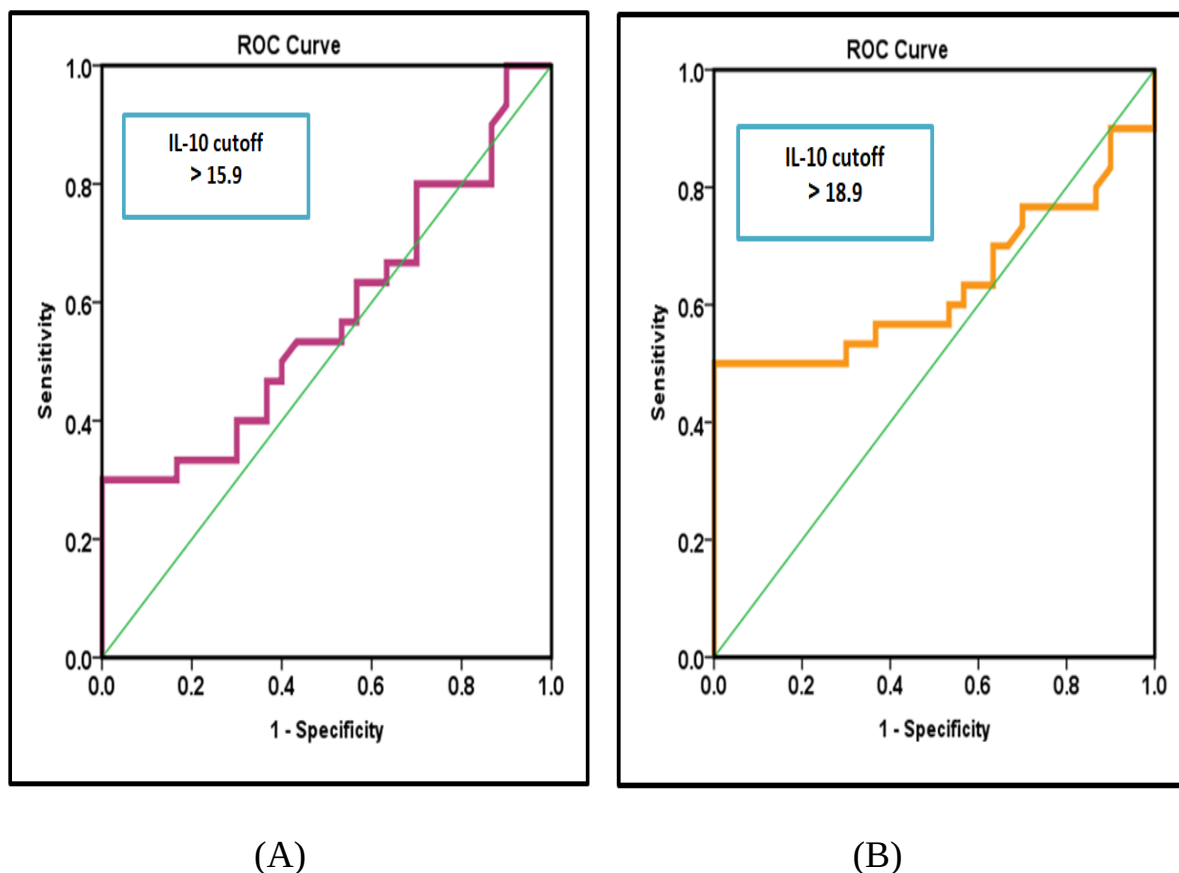


Figure (4:1): (A) Receiver operating characteristic curve for IL-10 levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for IL-10 levels to distinguish DM patients from healthy control subjects.

Figure (4-1) and Table (4-14) demonstrate that Interleukin-10 (IL-10) exhibits limited diagnostic utility in distinguishing between patients with foot ulcers and healthy individuals, as reflected by an AUC of 0.577 and a non-significant p-value ($P = 0.438$). These findings are consistent with those reported by (Liu *et al.* 2022b), who investigated IL-10 levels in diabetic patients with foot ulcers and observed similarly poor performance in ROC analysis, reinforcing the notion that IL-10 lacks sufficient value as an independent diagnostic marker. Furthermore, IL-10 showed weak discriminatory power between diabetic patients and healthy controls. Prior studies support these results, by (Ghosh *et al.* 2024) .reported elevated IL-10 levels in individuals with diabetes, suggesting that IL-10 may not serve as a reliable indicator for differentiating patients from healthy subjects. Similarly, (Zhao *et al.* 2012). concluded that IL-10 alone is ineffective as a diagnostic biomarker for distinguishing diabetic individuals from healthy populations.

4.8.1.2. Correlation between IL-10 level and other parameter in both group patients.

The correlation between IL-10 levels and other parameter in both group patients were shown in tables (4-15). The present results show there was non-significant correlation between IL-10 levels and all other parameters in both groups of patients.

Table (4-15): The correlation between IL-10 levels and other parameter in both groups of patients.

Other parameters	IL-10 level			
	Ulcer patients		DM patients	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	0.184	0.348	0.093	0.625
HbA1c	0.011	0.955	0.044	0.818
TG	0.196	0.317	0.081	0.669

Cholesterol	0.266	0.171	0.038	0.841
LDL	-0.138	0.482	0.096	0.615
HDL	-0.170	0.387	-0.114	0.550
CRP	0.014	0.943	0.136	0.475

r: Pearson correlation

The findings presented in Tables (4–15) demonstrate that there was no statistically significant correlation between interleukin-10 (IL-10) levels and any of the other investigated parameters in both patient groups those with diabetic foot ulcers and those without. In both cohorts, IL-10 did not exhibit significant associations with the measured biochemical or hematological markers ($P > 0.05$). These results suggest that IL-10, a key anti-inflammatory cytokine, may not be directly linked to systemic blood or metabolic indicators in diabetic individuals, regardless of the presence of foot ulcers (Esposito *et al.*, 2003). The absence of significant correlations could reflect the complex regulatory mechanisms governing IL-10 expression, which are influenced by various local and systemic factors, including inflammatory status, oxidative stress, and the dynamic balance between pro- and anti-inflammatory mediators (Dinarello, 2007). Moreover, while study has suggested that reduced IL-10 production may be associated with the development of metabolic syndrome and diabetes, these associations are not consistently observed and may depend on individual variability, such as genetic predisposition and age (van Exel *et al.*, 2002).

4.8.2. Interleukin-18 (IL-18) level in study groups.

The comparison of Interleukin-18 (IL-18) level between patients and control groups has been carried out and the results were demonstrated in table (4-19), the mean levels were higher in both group of patients in compared to healthy control and the difference was significant ($P < 0.05$). But the mean levels were non-

significant difference between both patients' groups (Ulcer patients and DM patients) themselves ($P > 0.05$).

Table (4-16) :Interleukin-18 (IL-18) level in study groups.

Groups		Interleukin-18 (IL-18) level pg/mL.
Ulcer patients	Mean $\pm$ SE	556.1 $\pm$ 36.8 ^A
	Range	265.07-956.19
DM patients	Mean $\pm$ SE	530.62 $\pm$ 43.4 ^A
	Range	155.64-1038.00
Healthy control	Mean $\pm$ SE	315.4 $\pm$ 12.9 ^B
	Range	143.79-553.46
p-value		0.001* †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SE: standard error; †: Kruskal Wallis test; **: significant at ($P < 0.05$)

Interleukin-18 (IL-18) levels were analyzed in both patient groups and healthy individuals, as illustrated in Table (4-16). Results showed that both patient groups exhibited significantly elevated IL-18 levels compared to the healthy, However, there was no meaningful difference in IL-18 concentrations between the ulcer and diabetic patient groups themselves ($P > 0.05$), This lack of significant variation suggests that increased IL-18 may be a common feature in diabetic patients rather than a specific marker for ulcers, Despite this, the ulcer group did display slightly higher IL-18 levels, which could point to more pronounced inflammation or additional factors such as localized infection or delayed wound healing, These findings in agreement with those of (Zhang *et al.* (2020). who reported increased IL-18 expression in ulcerated tissue as a result of targeted immune responses, other

research has also linked elevated IL-18 to insulin resistance, systemic inflammation, and the onset of both microvascular and macrovascular complications (Esposito *et al.*, 2002; Neeta *et al.*, 2010).

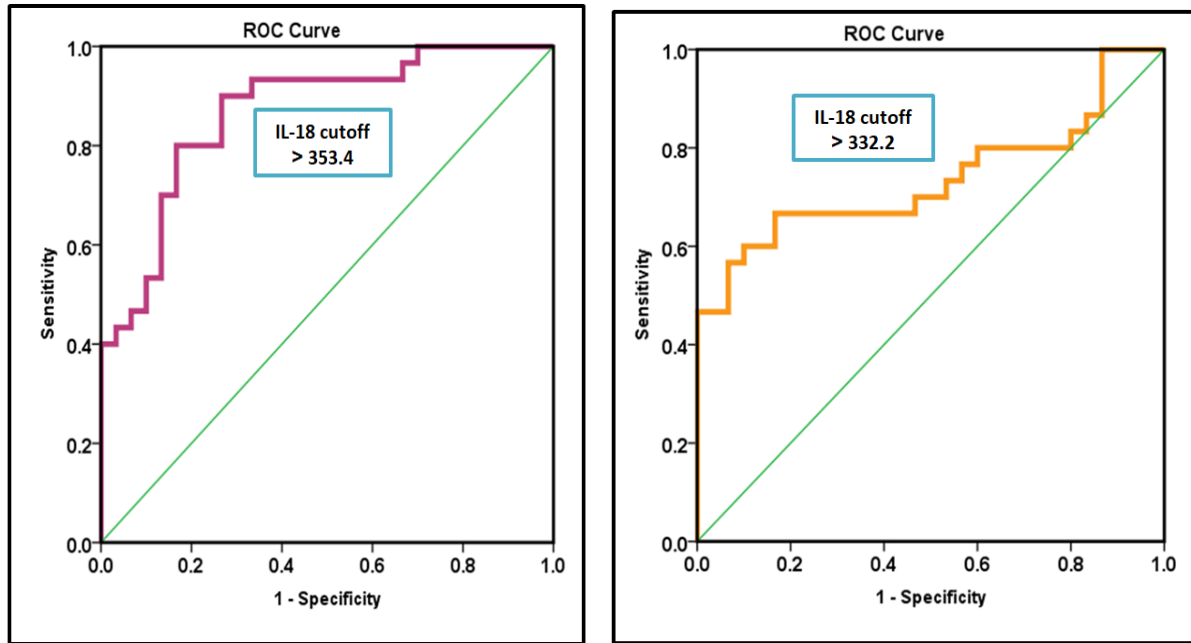
4.8.2.1. Diagnostic accuracy of Interleukin-18 (IL-18) level

Receiver operating characteristic (ROC) analysis was performed to reveal the prognostic accuracy of using Interleukin-18 (IL-18) concentrations to distinguish Ulcer patients from healthy control subjects. An optimal IL-18 cut-off value more than of 353.4 resulted in an AUC value of 0.868 (95% confidence interval [CI], 0.777-0.958, $P= 0.001$), sensitivity of 86.7%, specificity of 90.0%, PPV of 89.7%, and NPV of 87.1%. The present results indicate IL-18 is considered as a good prognostic marker to distinguish Ulcer patients from healthy control. In addition, an optimal IL-18 cut-off value of more than 332.2 could be used to distinguish DM patients from healthy control subjects with a sensitivity of 73.3%, specificity of 76.7%, PPV of 75.9%, and NPV of 74.2% and 0.737 (0.603- 0.870). The present results indicate IL-18 is considered as acceptable prognostic marker to distinguish DM patients from healthy control.

Table (4-17): Roc curve of Interleukin-18 (IL-18) level

Characteristic	Ulcer patients / control	DM patients /control
Cutoff value	>353.4	>332.2
P value	0.001	0.001
Sensitivity %	86.7 %	73.3 %
Specificity %	90.0 %	76.7 %
PPV %	89.7 %	75.9 %
NPV %	87.1 %	74.2 %
AUC (95% CI)	0.868 (0.777- 0.958)	0.737 (0.603- 0.870)

CI: Confidence interval, AUC: Area under curve.



(A)

(B)

Figure (4:2): (A) Receiver operating characteristic curve for IL-18 levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for IL-18 levels to distinguish DM patients from healthy control subjects.

In Figure (4-2), table (4-17): the Receiver Operating Characteristic (ROC) analysis demonstrates that IL-18 serves as a reliable prognostic marker for distinguishing patients with ulcers from healthy individuals. IL-18 is recognized as a significant biomarker in assessing chronic inflammation in diabetes, especially in advanced stages associated with complications such as foot ulcers. These findings are in agreement with those of (Dinarello, 2018). Furthermore, the current study suggests that IL-18 is a reasonably effective prognostic marker for differentiating diabetic patients from healthy controls. It is proposed that interleukin-18 (IL-18) acts as a moderately dependable indicator of inflammation in distinguishing diabetic individuals from those without the condition, supporting the results reported by

(Zaki *et al.*, 2013), who observed markedly elevated IL-18 levels in diabetic patients compared to healthy subjects.

4.8.2.2. Correlation between IL-18 level and other parameter in both group patients.

The correlation between IL-18 levels and other parameter in both group patients were shown in tables (4-18). The present results show there was non-significant correlation between IL-18 levels and all other parameters in both groups of patients.

Table (4-18): The correlation between IL-18 levels and other parameter in both groups of patients.

Other parameters	IL-18 level			
	Ulcer patients		DM patients	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	0.067	0.733	0.034	0.859
HbA1c	0.229	0.242	0.170	0.368
TG	0.011	0.958	0.068	0.780
Cholesterol	0.169	0.390	0.107	0.573
LDL	0.153	0.437	-0.222	0.239
HDL	0.234	0.230	-0.078	0.681
CRP	0.214	0.275	0.310	0.075

r: Pearson correlation.

Table (4-18) presents the relationship between interleukin-18 (IL-18) levels and several clinical and biochemical indicators in diabetic patients, with and without foot ulcers. The findings showed no statistically significant associations between IL-18 and any of the evaluated parameters in either group ($P > 0.05$), indicating that IL-18 is not directly linked to these variables. This suggests that IL-18 levels are not

significantly impacted by blood glucose regulation or long-term glycemic control. These results align with those reported by (Kaplanski *et al.* 2003). Previous research, including (Esposito *et al.* 2002), has also proposed that IL-18 functions as an inflammatory cytokine largely separate from conventional metabolic pathways in diabetes. While a slight positive correlation was observed between IL-18 and CRP in diabetic patients without foot ulcers ($r = 0.310$), it did not reach statistical significance ($P = 0.075$), indicating a potential trend worth further investigation in studies with larger participant groups (Vogelzangs *et al.*, 2007). IL-18 does not show direct correlations with the biochemical markers analyzed in this study, reinforcing the need for deeper exploration of its immunological and inflammatory roles in diabetic and ulcer-affected patients within a broader cytokine context.

4.8.3. Tumor Necrosis Factor Alpha (TNF- α) level in study groups.

The comparison of Tumor Necrosis Factor Alpha (TNF- α) level between patients and control groups has been carried out and the results were demonstrated in table (4-19). the mean levels were higher in both group of patients in compared to healthy control and the difference was significant ($P < 0.05$). But the mean levels was non-significant difference between both patients groups (Ulcer patients and DM patients) themselves ($P > 0.05$).

Table (4-19) :Tumor Necrosis Factor Alpha (TNF- α) level in study groups.

Groups		Tumor Necrosis Factor Alpha (TNF- α) level pg/mL
Ulcer patients	Mean $\pm$ SE	91.67 $\pm$ 15.5 ^A
	Range	11.70-368.26
DM patients	Mean $\pm$ SE	96.42 $\pm$ 14.7 ^A
	Range	5.38-364.78

Healthy control	Mean $\pm$ SE	32.64 $\pm$ 5.7 ^B
	Range	3.51-122.7
p-value		0.006* †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SE: standard error; †: Kruskal Wallis test; **: significant at ($P < 0.05$)

The data presented in Table (4-19) revealed that serum concentrations of Tumor Necrosis Factor-alpha (TNF- α) were markedly elevated in both groups of diabetic patients those with foot ulcers and those without when compared to healthy controls. This elevation was statistically significant ($P < 0.05$). However, there was no notable difference in TNF- α levels between the two diabetic subgroups ($P > 0.05$). TNF- α , a key proinflammatory cytokine predominantly secreted by activated macrophages, plays an essential role in the body's innate immune response. It is strongly implicated in the onset of insulin resistance, as it inhibits insulin receptor phosphorylation, thereby disrupting glucose metabolism and fostering systemic inflammation (Hotamisligil, 2006). Moreover, TNF- α stimulates the production of other inflammatory markers such as IL-6 and CRP, your study and are consistent with previous research indicating that systemic TNF- α levels may not reliably represent the localized inflammatory activity within diabetic foot ulcers (DFUs). For instance, studies such as those by (Herder *et al.*, 2005) and (Brem *et al.*, 2007) have demonstrated that while TNF- α is elevated in diabetic conditions, its serum concentration often fails to correlate with the severity or presence of localized ulceration. This supports the notion that local tissue cytokine expression, rather than circulating levels, may serve as a more accurate indicator of the inflammatory milieu within DFUs. Therefore, while TNF- α remains a key mediator in the pathophysiology of chronic inflammation and impaired healing, The main cause of

elevation TNF- α in diabetic patients is oxidative stress, which promotes inflammation, its diagnostic or prognostic utility in serum may be limited in the context of foot ulceration (Zhang *et al.* 2021), This study demonstrates that TNF- α levels measured directly at the wound site are more indicative of ulcer severity and healing progression than systemic levels in agreement with previous findings. For instance, (Pickup, 2004) described diabetes as a low-grade inflammatory condition in which TNF- α plays a pivotal role in activating the innate immune system and promoting metabolic dysfunction. In the context of diabetic foot ulcers, (Jeffcoate *et al.* 2018) emphasized that chronic systemic inflammation marked by elevated pro-inflammatory cytokines such as TNF- α can impede wound healing and contribute to the persistence of chronic ulcers. Despite elevated TNF- α levels in diabetic patients, no statistically significant difference was observed between the ulcer and non-ulcer groups. This finding suggests that TNF- α expression may predominantly reflect the systemic inflammatory state associated with diabetes itself, rather than being specifically linked to the presence of foot ulcers that TNF- α is elevated in individuals with diabetes due to persistent low-grade inflammation, regardless of wound status. Previous studies have demonstrated (Pickup *et al.*, 2000; Al-Mashat *et al.*, 2006). Furthermore, immune cells from diabetic patients have been shown to release TNF- α in response to hyperglycemic and pro-inflammatory conditions, reinforcing the notion that its upregulation is not exclusive to ulcer formation (Dasu *et al.*, 2008). The absence of significant intergroup differences may also be attributed to confounding factors such as limited sample size or a lack of variability in disease severity across the studied cohorts.

4.8. 3.1. Diagnostic accuracy of Tumor Necrosis Factor Alpha (TNF- α) level

Receiver operating characteristic (ROC) analysis was performed to reveal the prognostic accuracy of using TNF- α concentrations to distinguish Ulcer patients

from healthy control subjects. An optimal TNF- α cut-off value more than of 22.6 resulted in an AUC value of 0.769 (95% confidence interval [CI], 0.658-0.886, P= 0.001), sensitivity of 76.7%, specificity of 76.7%, PPV of 76.7%, and NPV of 76.7%. The present results indicate TNF- α is considered as acceptable prognostic marker to distinguish Ulcer patients from healthy control.

In addition, an optimal TNF- α cut-off value of more than 22.7 could be used to distinguish DM patients from healthy control subjects with a sensitivity of 76.7%, specificity of 80.0%, PPV of 79.3%, and NPV of 77.4% and 0.767 (0.648- 0.886). The present results indicate TNF- α is considered as acceptable prognostic marker to distinguish DM patients from healthy control.

Table (4-20): Roc curve of Tumor Necrosis Factor Alpha (TNF- α) level

Characteristic	Ulcer patients / control	DM patients /control
Cutoff value	>22.6	>22.7
P value	0.001	0.001
Sensitivity %	76.7 %	73.3 %
Specificity %	76.7 %	80.0 %
PPV %	76.7 %	79.3 %
NPV %	76.7 %	77.4 %
AUC (95% CI)	0.769 (0.651- 0.886)	0.767 (0.648- 0.886)

CI: Confidence interval, AUC: Area under curve. PPV % Positive Predictive Value

NPV % Negative Predictive Value

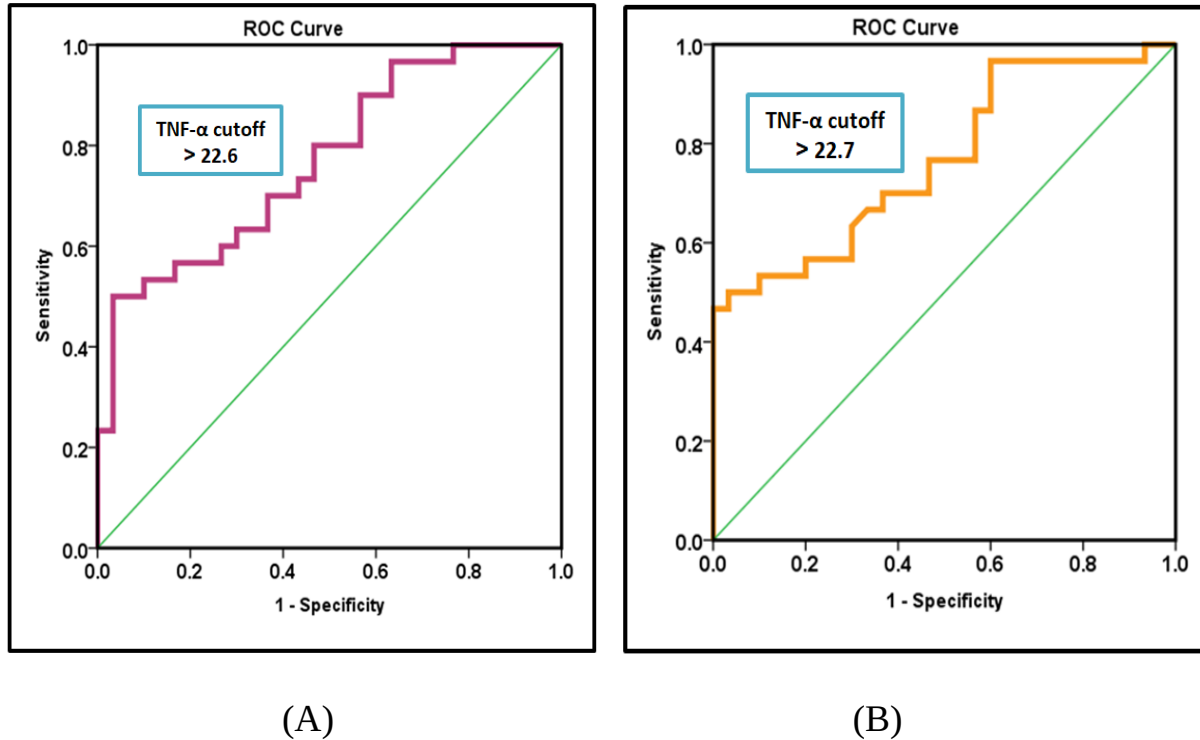


Figure (4:3): (A) Receiver operating characteristic curve for TNF- α levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for TNF- α levels to distinguish DM patients from healthy control subjects.

Receiver operating characteristic (ROC) analysis in table (4-20) Figure (4-3) was conducted to assess the prognostic accuracy of TNF- α concentrations in differentiating ulcer patients from healthy controls. The current findings suggest that TNF- α serves as a reasonably effective prognostic marker for distinguishing ulcer patients from healthy individuals. These findings are in line with previous research demonstrating elevated TNF- α levels in patients with chronic non-healing wounds, including diabetic foot ulcers, where TNF- α contributes to sustained inflammation and impaired healing (Falanga, 2005 and Gupta *et al.*, 2021). The pro-inflammatory role of TNF- α , primarily via activation of NF- κ B pathways, leads to the recruitment of immune cells and the release of further inflammatory mediators, thus exacerbating tissue damage. Furthermore, the results indicate that TNF- α is also a reliable prognostic marker for differentiating diabetic mellitus (DM) patients from healthy

controls. TNF- α demonstrated a significant capability to distinguish between diabetic individuals and healthy controls. These findings further support TNF- α as a promising biomarker for systemic inflammation in diabetes, potentially indicating underlying vascular inflammation and endothelial dysfunction that are commonly seen in people with diabetes (Donath and Shoelson, 2011)

4.8.3.2. Correlation between TNF- α level and other parameter in both group patients.

The correlation between TNF- α level and other parameter in both group patients were shown in tables (4-21). The present results show there was non-significant correlation between TNF- α levels and all other parameters in both groups of patients.

Table (4-21): The correlation between TNF- α level and other parameter in both groups of patients.

Other parameters	TNF- α level			
	Ulcer patients		DM patients	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	0.243	0.213	0.048	0.800
HbA1c	0.162	0.409	0.087	0.647
TG	0.158	0.422	0.004	0.984
Cholesterol	0.058	0.770	0.046	0.808
LDL	-0.142	0.472	0.023	0.903
HDL	0.173	0.379	-0.032	0.866
CRP	0.284	0.142	0.153	0.421

r: Pearson correlation.

The information in table (4-21) indicates that there were no significant statistical links between serum TNF- α levels and the biochemical parameters analyzed in either group of patients (those with diabetic foot ulcers and those with only diabetes

mellitus) at ($P > 0.05$), These findings support earlier research suggesting that TNF- α levels don't necessarily match the extent of metabolic or inflammatory issues in diabetic patients. Instead, other elements like insulin resistance, localized immune responses, or ongoing tissue damage at ulcer sites might also affect TNF- α levels (Sears and Perry, 2010 and Nawaz *et al.*, 2016). These findings are consistent with previous research suggesting that although TNF- α plays a central role in inflammatory and immune responses, it may not always exhibit a strong or direct relationship with certain clinical or biochemical parameters, particularly in chronic or stable disease states (Patel *et al.*, 2019; Chen *et al.*, 2020). These results imply that TNF- α levels don't consistently vary in line with these clinical measures, This could mean that the role of TNF- α in immune and inflammatory responses in diabetes and diabetic foot ulcers is complex and cannot be entirely understood through general inflammation markers or blood sugar levels (Pickup and Crook, 1998).

4.8.4. Basic fibroblast growth factor (bFGF) level in study groups.

The comparison of Basic fibroblast growth factor (bFGF) level between patients and control groups has been carried out and the results were demonstrated in table (4-25). the mean levels were higher in Ulcer patients in compared to other groups and the difference was significant ($P < 0.05$). But the mean levels were non-significant difference between other both groups (DM patients and healthy control) themselves ($P > 0.05$).

Table (4-22) :Basic fibroblast growth factor (bFGF) level in study groups.

Groups		Basic fibroblast growth factor (bFGF) level pg/mL
Ulcer patients	Mean $\pm$ SE	89.06 $\pm$ 10.4 ^A
	Range	31.25-276.86

DM patients	Mean $\pm$ SE	40.81 $\pm$ 1.7 ^B
	Range	31.75-60.56
Healthy control	Mean $\pm$ SE	42.27 $\pm$ 1.04 ^B
	Range	31.88-55.00
p-value		0.001* †
Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different.		

SE: standard error; †: Kruskal Wallis test; **: significant at(P <0.0 5)

Data shown in Table (4-22) indicate that average serum levels of basic fibroblast growth factor (bFGF) were significantly higher in patients with diabetic foot ulcers (89.06 ± 10.4) than in diabetics without ulcers (40.81 ± 1.7) and healthy individuals (42.27 ± 1.04), with the difference being statistically significant (P < 0.05). This elevated bFGF level in ulcer patients may be attributed to the essential role of this factor in promoting wound healing through stimulating fibroblast proliferation and angiogenesis. These results are consistent with earlier study by (Zubair *et al.*, 2012). It is believed to be secreted by the body as a compensatory mechanism in response to chronic tissue injury to accelerate repair.

These results suggest that the elevated bFGF levels in ulcer patients likely represent a natural response aimed at aiding tissue repair at the wound site, particularly considering the ongoing inflammation and poor blood circulation typically seen in diabetic ulcers (Falanga, 2005). Conversely, there was no significant difference in bFGF levels between diabetics without ulcers and healthy subjects (P > 0.05), indicating that chronic high blood sugar alone might not be enough to change systemic bFGF levels unless there is tissue injury. These findings are consistent with earlier study by (Li *et al.* 2015). which found that bFGF levels

tend to increase locally where tissue damage occurs rather than being elevated systemically due to diabetes. In contrast, diabetic patients without ulcers did not show elevated bFGF levels, suggesting that diabetes alone does not significantly alter bFGF concentration in the absence of ulceration. In fact, previous studies have shown that diabetics may suffer from impaired production or response to growth factors like bFGF, which contributes to delayed wound healing (Falanga, 2005).

4.8.4.1. Diagnostic accuracy of basic fibroblast growth factor (bFGF) level

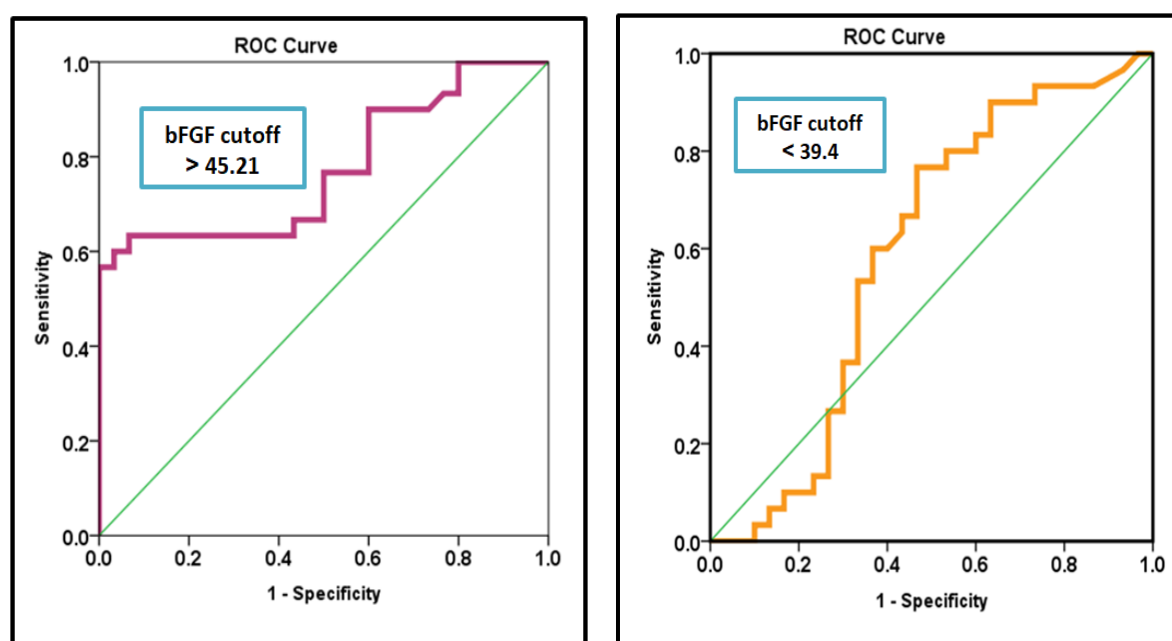
Receiver operating characteristic (ROC) analysis was performed to reveal the prognostic accuracy of using bFGF concentrations to distinguish Ulcer patients from healthy control subjects. An optimal bFGF cut-off value more than of 45.21 resulted in an AUC value of 0.809 (95% confidence interval [CI], 0.679-0.896, $P=0.001$), sensitivity of 80.0%, specificity of 80.0%, PPV of 80.0%, and NPV of 80.0%. The present results although bFGF is not considered a direct prognostic marker, its reduced activity may serve as an indirect indicator of impaired wound healing. considered as a good prognostic marker to distinguish Ulcer patients from healthy control. In addition, an optimal bFGF cut-off value of less than 39.4 could be used to distinguish DM patients from healthy control subjects with a sensitivity of 60.0%, specificity of 56.7%, PPV of 58.1%, and NPV of 58.6% and 0.592 (0.442-0.743). The present results indicate bFGF is considered as a weak prognostic marker to distinguish DM patients from healthy control.

Table (4-23): Roc curve of basic fibroblast growth factor (bFGF) level.

Characteristic	Ulcer patients / control	DM patients /control
Cutoff value	>45.21	<39.4
P value	0.001	0.001

Sensitivity %	80.0 %	60.0 %
Specificity %	80.0 %	56.7 %
PPV %	80.0 %	58.1 %
NPV %	80.0 %	58.6 %
AUC (95% CI)	0.809 (0.679- 0.896)	0.592 (0.442- 0.743)

CI: Confidence interval, AUC: Area under curve.



(A)

(B)

Figure (4:4) (A) Receiver operating characteristic curve for bFGF levels to distinguish Ulcer patients from healthy control subjects. (B) Receiver operating characteristic curve for bFGF levels to distinguish DM patients from healthy control subjects.

In this study in table (4-23), figure (4-4) the Receiver Operating Characteristic (ROC) curve was employed to evaluate the diagnostic potential of bFGF levels in distinguishing among various patient groups namely, individuals with ulcers and diabetic patients without ulcers when compared to healthy controls, The analysis

showed that bFGF effectively differentiates ulcer patients from healthy individuals. This outcome was statistically significant ($P = 0.001$), indicating a strong diagnostic capability. These results are consistent with previous research by (Zhou *et al.*, 2011). This high AUC suggests that bFGF is a reliable biomarker for identifying patients with diabetic foot ulcers, likely due to its key role in tissue regeneration, angiogenesis, and wound repair processes, which are impaired in chronic ulcerative conditions (Li *et al.*, 2015). With both sensitivity and specificity recorded at 80%, the test demonstrates a balanced ability to correctly identify affected individuals and accurately exclude those without the condition. Additionally, the high positive and negative predictive values underscore the reliability of bFGF as a diagnostic marker in clinical environments (Pepe *et al.*, 2001). Conversely, bFGF levels did not show substantial efficacy in distinguishing diabetic patients without ulcers from healthy individuals, reflecting low diagnostic precision (Hajian-Tilaki, 2013). The findings indicate that changes in bFGF levels in this specific group are not significant enough to allow for clear clinical differentiation, thus reducing its usefulness as a diagnostic tool in this study (Pepe *et al.*, 2001). Nonetheless, the results highlight bFGF's strong potential as a biomarker for diagnosing diabetic foot ulcers. Still, its ability to distinguish between diabetics without ulcers and non-diabetics seems to be limited (Zhou *et al.*, 2011).

4.8.4.2. Correlation between bFGF level and other parameter in both group patients.

The correlation between bFGF level and other parameter in both group patients were shown in tables (4-24). The present results show there was significant positive correlation between bFGF levels and TG level ($r=0.381$, $p= 0.036$) and bFGF levels and LDL level ($r=0.429$, $p= 0.013$) in Ulcer patients.

Table (4-24): The correlation between bFGF level and other parameter in both groups of patients.

Other parameters	bFGF level			
	Ulcer patients		DM patients	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Glucose	0.202	0.279	-0.084	0.658
HbA1c	0.197	0.315	-0.168	0.374
TG	0.380	0.036*	-0.283	0.130
Cholesterol	0.258	0.185	-0.331	0.056
LDL	0.429	0.013*	-0.218	0.087
HDL	0.088	0.655	0.179	0.343
CRP	0.123	0.532	-0.241	0.199

r: Pearson correlation.

The results of the study demonstrated varying degrees of correlation between basic fibroblast growth factor (bFGF) levels and a range of clinical and biochemical parameters, as presented in Table (4-24). Notably, significant correlations were observed exclusively in the subgroup of diabetic patients with foot ulcers. Specifically, a statistically significant positive correlation was found between bFGF and triglyceride (TG) levels ($r = 0.380$, $p = 0.036$), suggesting that elevated TG levels may be associated with increased bFGF production. This association may reflect a compensatory response aimed at enhancing angiogenesis at the site of tissue injury (Zhao *et al.*, 2020). Additionally, a significant positive correlation was detected between bFGF and low-density lipoprotein (LDL) levels ($r = 0.429$, $p = 0.013$). This finding indicates that LDL may influence bFGF expression in damaged tissues, potentially through mechanisms related to inflammation or ongoing microvascular injury (Wang *et al.*, 2018). Conversely, no statistically significant associations were found between bFGF and any of the studied parameters in diabetic patients without

foot ulcers. This absence of correlation may be attributed to the lack of active inflammation or tissue injury, as bFGF is typically released in response to such physiological triggers. These observations are consistent with previous findings reported by (Ortega *et al.* 2017).

In summary, the results suggest that bFGF levels in diabetic foot ulcer patients may be modulated by specific lipid abnormalities, highlighting the complex interplay between chronic metabolic disturbances and impaired wound healing in this population.

4.9. Correlation between immunological parameters in both group patients.

The correlations between immunological parameters in both group patients were shown in tables (4-25) and (4-26). There is positive correlation between IL-10 levels and IL-18 ($r=0.380$ and $p=0.035$), IL-18 levels and bFGF ($r=0.434$ and $p=0.017$), in Ulcer patients. But there is negative correlation between IL-10 levels and TNF- α ($r=-0.341$ and $p=0.045$) in DM patients.

Table (4-25): Correlation between immunological parameters in Ulcer patients.

Characteristic	immunological parameters							
	IL-10		IL-18		TNF- α		bFGF	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
IL-10	1							
IL-18	0.380	0.035*	1					
TNF- α	0.069	0.729	0.002	0.993	1			

Bfgf	0.157	0.424	0.434	0.017*	- 0.107	0.588	1
-------------	-------	-------	-------	--------	------------	-------	---

r: correlation coefficient.

Table (4-26): Correlation between immunological parameters in DM patients.

Characteristic	immunological parameters							
	IL-10		IL-18		TNF- α		bFGF	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
IL-10	1							
IL-18	0.292	0.118	1					
TNF-α	- 0.341	0.045*	- 0.076	0.689	1			
bFGF	0.329	0.055	0.170	0.370	- 0.219	0.244	1	

r: correlation coefficient.

Tables (4-25) and (4-26) show how selected immune system markers relate to each other in two groups: individuals with diabetic foot ulcers and those with diabetes but no ulcers. In patients with ulcers, a statistically significant positive correlation was found between IL-10 and IL-18 ($r = 0.380$, $p = 0.035$), suggesting a possible immune regulatory link between the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine IL-18. In patients with ulcers, IL-18 functions as a pro-inflammatory cytokine, while IL-10 serves as an anti-inflammatory cytokine. This suggests that the body may be striving to balance and regulate the inflammatory response (Aggarwal, *et al.*, 2014). Additionally, a notable positive correlation was observed between IL-18 and bFGF ($r = 0.434$, $p = 0.017$). The positive correlation

between IL-18 and the growth factor bFGF indicates that increased inflammation may stimulate growth factors involved in tissue repair and wound healing. This makes sense in the context of ulcers, where the body needs to promote healing despite ongoing inflammation, implying that the inflammatory response may be associated with the activation of tissue repair processes driven by bFGF, a factor known to support blood vessel formation and tissue healing (Tonnesen *et al.*, 2000). In contrast, negative relationship was observed in diabetic patients between IL-10 and TNF- α ($r = -0.341$, $p = 0.045$). The significant negative correlation between IL-10 (an anti-inflammatory cytokine) and TNF- α (a pro-inflammatory cytokine) reflects the usual immune balance, where an increase in one is associated with a decrease in the other. This suggests a normal immune regulation in diabetic patients without ulcers, where IL-10 works to suppress the inflammatory activity of TNF- α , suggesting a mechanism of immune regulation where IL-10 suppresses TNF- α production, which may help mitigate chronic inflammation (Moore *et al.*, 2001)..

Chapter Five

Conclusions and

Recommendations

5.1. Conclusion

1. The prevalence of diabetes and its complications increases with advancing age, and males are more likely to develop (DFUs) compared to females, The dorsal part of the foot was identified as the most frequent site of ulceration , deep ulcers emerged as the most common type.

2.C-reactiveare protein (CRP) necessary for a precise evaluation of inflammatory status.

3.High levels of inflammatory markers like IL-18, TNF- α and bFGF in DFU patients, notably High levels of IL-10 in Dm patients.

4. Individuals with (DFUs) exhibit clear metabolic imbalances, including elevated C-reactive protein (CRP), increased LDL levels, and decreased HDL levels, all of which hinder the heahealin process.

5. ROC analysis revealed that IL-10 poorly differentiated ulcer patients from healthy controls, while IL-18 and bFGF showed good diagnostic performance. TNF- α appears to be a fairly effective prognostic marker for this distinction.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed to improve the diagnosis, management, and clinical outcomes of diabetic foot ulcers (DFUs)

1. Raising awareness among diabetic patients—especially men and older adults about the importance of proper glycemic control to reduce the risk of foot ulcers, and conducting larger-scale studies to examine the influence of treatment methods and ulcer classification on disease progression.

2. Conducting additional cytokine assessments, including interleukins such as IL-12, IL-22, and IL-6, in patients with diabetes and diabetic foot ulcers to better understand the immunological responses and their potential role in disease progression.

3. more research is needed to validate the use of the studied biomarkers as prognostic indicators for DFU progression and healing

4. Regularly monitor lipid levels and inflammation in diabetic patients, especially those with foot ulcers. Focus on controlling blood sugar and improving lifestyle to reduce inflammation.

References

References.....

- Adiels, M., Taskinen, M. Rand Boren, J. (2008).** Fatty liver, insulin resistance, and dyslipidemia. *Current Diab Rep*, 8(1), 60–64.
- Aggarwal, B. B., Gupta, S. C., & Kim, J. H. (2014).** Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood*, 119(3), 651–665. <https://doi.org/10.1182/blood-2011-04-325225>
- Ahmed, M., Khalid, M., and Rasheed, U. (2021).** Lipid abnormalities in type 2 diabetes patients with and without diabetic foot ulcers. *Pakistan Journal of Medical Sciences*, 37(2), 495–500.
- Akita, S. (2010).** Basic fibroblast growth factor in wound healing and its application for diabetic foot ulcer. *Current Pharmaceutical Biotechnology*, 11(6), 643–650.
- Akita, S., Akino, K., Hirano, A., and Imaizumi, T. (2013).** A basic fibroblast growth factor-containing collagen gelatin sponge in the treatment of skin ulcers: An open-label clinical trial. *Wound Repair and Regeneration*, 21(4), 486–493.
- Al Kafrawy, N. A., Abdelaziz, D. A., and El-Fetouh, M. A. (2014).** Diabetic foot: A clinical and microbiological study. *Egyptian Journal of Medical Microbiology* 23(2), 47–54.
- Alavi, A., Sibbald, R. G., Mayer, D., Goodman, L., Botros, M., Armstrong, D. G., and Kirsner, R. S. (2014).** Diabetic foot ulcers: Part II. Management. *Journal of the American Academy of Dermatology*, 70(1), 21.e1–21.e24. <https://doi.org/10.1016/j.jaad.2013.06.055>
- Ali, A. T., Hochfeld, W. E., Myburgh, R., & Pepper, M. S. (2020).** Adipocyte and lipid metabolism in the pathogenesis of insulin resistance and type 2 diabetes. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, 25(3), 25–32.
- Al-Mashat, H., Chen, D., Liu, W., Tseng, H., & Mustoe, T. A. (2006).** Deletion of tumor necrosis factor- α receptor 1 accelerates wound healing in diabetic mice. *The American Journal of Pathology*, 169(2), 673–685. <https://doi.org/10.2353/ajpath.2006.060095>

References.....

- Al-Rubeaan, K., Al Derwish, M., Ouizi, S., Youssef, A. M., Subhani, S. N., Ibrahim, H. M., & Alamri, B. N. (2015).** Diabetic foot complications and their risk factors from a large retrospective cohort study. *PLoS One*, 10(5), e0124446. <https://doi.org/10.1371/journal.pone.0124446>
- Al-Salih, R. M., and Ali, H. M. (2021).** Oxidative stress and diabetic foot ulceration: A review. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 15(2), 645–650.
- Al-Salih, S. A., & Ali, M. M. (2021a).** The role of oxidative stress in diabetic complications. *Journal of Diabetes Research Article ID 6655522*. <https://doi.org/10.1155/2021/6655522>
- Al-Shaheeb, I. S., Al-Otaibi, F., and Alzahrani, H. (2022).** Relationship between glycated hemoglobin and diabetic complications: A cross-sectional study. *Saudi Medical Journal*, 43(1), 28–35. <https://doi.org/10.15537/smj.2022.43.1.20210789>
- American Diabetes Association. (2007).** Standards of medical care in diabetes—2007. *Diabetes Care*, 30(Supplement_1), S4–S41. <https://doi.org/10.2337/dc07-s004>
- American Diabetes Association. (2018).** Classification and diagnosis of diabetes: Standards of medical care in diabetes—2018. *Diabetes Care*, 41(Supplement_1), S13–S27. <https://doi.org/10.2337/dc18-S002>
- American Diabetes Association. (2020).** Standards of medical care in diabetes—2020. *Diabetes Care*, 43(Suppl 1), S1–S212.
- American Diabetes Association. (2023).** Standards of Medical Care in Diabetes—2023. *Diabetes Care*, 46(Supplement_1), S1–S291. <https://doi.org/10.2337/dc23-Sint>
- American Diabetes Association. (2024).** Standards of Medical Care in Diabetes—2024. *Diabetes Care*, 47(Suppl 1), S1–S292. <https://doi.org/10.2337/dc24-S001>
- Anyim, O. B., Okonkwo, E. Cand Chukwuonye, I. I. (2019).** Cost implications of diabetic foot ulcers in a developing country. *Nigerian Journal of Clinical Practice*, 22(5), 670–674.

References.....

Armstrong, D. G., Boulton, A. J. M and Bus, S. A. (2017). Diabetic foot ulcers and their recurrence. *New England Journal of Medicine*, 376(24), 2367–2375.

Armstrong, D. G., Boulton, A. J. M and Bus, S. A. (2017). Diabetic foot ulcers and their recurrence. *New England Journal of Medicine*, 376(24), 2367–2375.
<https://doi.org/10.1056/NEJMra1615439>.

Armstrong, D. G., Boulton, A. J and Bus, S. A. (2023). Diabetic foot ulcers and their recurrence. *New England Journal of Medicine*, 388(1), 2367–2375.

Almdal, R., Nielsen, A. B. S., Nielsen, M. B., and Tarnow, L. (2020). Inflammatory markers and the development of diabetic foot ulcers in patients with type 2 diabetes: A cohort study. *Diabetes Research and Clinical Practice*, 163, 108141.
<https://doi.org/10.1016/j.diabres.2020.108141>

Akdis, M., Burgler, S., Cramer, R., Eiwegger, T., Fujita, H., Gomez, E and Akdis, C. A. (2011). Interleukins, from 1 to 37, and interferon- γ : receptors, functions, and roles in diseases. *Journal of Allergy and Clinical Immunology*, 127(3), 701-721.
<https://doi.org/10.1016/j.jaci.2010.11.050>

Alavi, A., Sibbald, R. G., Mayer, D., Goodman, L., Botros, M., Armstrong, D. Gand Woo, K. Y. (2016). Diabetic foot ulcers: Part I. Pathophysiology and prevention. *Journal of the American Academy of Dermatology*, 74(1), 31-45.

Azevedo, M and Alla, S. (2008). Diabetes in sub-Saharan Africa: Kenya, Mali, Mozambique, Nigeria, South Africa and Zambia. *International Journal of Diabetes in Developing Countries*, 28 (4), 101–108. <https://doi.org/10.4103/0973-3930.45268>.

Abeer Abdelrahman Elnour Eltilib (2021). Association Between Smoking and Foot Ulcer Among Patients with Diabetes Mellitus, Wad Medani, Sudan. *Sudan Journal of Medical Sciences*, Vol. 16, Issue 4, pp. 450–463.
DOI: 10.18502/sjms.v16i4.9943

Bao, W., Bowers, K., Tobias, D. K., Hu, F. B and Zhang, C. (2014). Prepregnancy dietary protein intake, major dietary protein sources, and the risk of gestational diabetes mellitus: A prospective cohort study. *Diabetes Care*, 36 (7), 2001–2008.
<https://doi.org/10.2337/dc13-2513>.

References.....

- Barter, P. J., Nicholls, S., Rye, K. A., Anantharamaiah, G. M., Navab, M and Fogelman, A. M. (2004).** Antiinflammatory properties of HDL. *Circulation Research*, 95(8), 764–772.
- Berlanga-Acosta, J., Schultz, G. S., López-Mola, E., Guillen-Nieto, G and García-Siverio, M. (2013).** Cellular and molecular mechanisms in the healing of diabetic foot ulcers. *Angiogenesis*, 16(3), 523–538.
- Bjarnsholt, T., Kirketerp-Møller, K., Jensen, P. Ø., Madsen, K. G., Phipps, R., Krogh, K., Høiby, N and Givskov, M. (2008).** Why chronic wounds will not heal: A novel hypothesis. *Wound Repair and Regeneration*, 16(1), 2–10. <https://doi.org/10.1111/j.1524-475X.2007.00283.x>.
- Boulton, A. J. M., Vileikyte, L., Ragnarson-Tennvall, G and Apelqvist, J. (2005).** The global burden of diabetic foot disease. *The Lancet*, 366(9498), 1719–1724.
- Bowers, K., Tobias, D. K., Yeung, E., Hu, F. B and Zhang, C. (2012).** A prospective study of prepregnancy dietary fat intake and risk of gestational diabetes. *American Journal of Clinical Nutrition*, 95 (2), 446–453. <https://doi.org/10.3945/ajcn.111.025334>.
- Brizeno, L., Sanchez, M and Rios, M. (2016).** Downregulation of FGF-2 in diabetic foot ulcers: Implications for impaired healing. *Journal of Wound Care*, 25(6), 304–310
- Bravo-Molina, A., Linares-Palomino, J. P., Vera-Arroyo, B., Salmerón-Febres, L. M and Ros-Díe, E. (2018).** Inter-observer agreement of the Wagner, University of Texas and PEDIS classification systems for the diabetic foot syndrome. *Foot and Ankle Surgery*, 24(1), 60–64.
- Bus, S. A., Van Netten, J. J., Lavery, L. A., Monteiro-Soares, M., Rasmussen, A., Jubiz, Y., & Price, P. E. (2020).** IWGDF guidelines on the prevention of foot ulcers in persons with diabetes. IWGDF Guidelines.
- Boulton, A. J. M., Armstrong, D. G., Albert, S. F., Frykberg, R. G., Hellman, R., Kirkman, M. S., ... & Lavery, L. A. (2008).** Comprehensive foot examination and risk assessment: a report of the task force of the foot care interest group of the

References.....

- American Diabetes Association, with endorsement by the American Association of Clinical Endocrinologists. *Diabetes Care*, 31(8), 1679–1685. <https://doi.org/10.2337/dc08-9021>
- Bain, B. J. (2021).** *Blood Cells: A Practical Guide* (6th ed.). Wiley-Blackwell
- Boditech Med Inc. (2022).** *iChroma™ II HbA1c Test Instruction Manual* (Document No. OPM-IR2-EN; Rev.08, June 1, 2018). Boditech Med Inc., Chuncheon, Gangwon-do, Republic of Korea.
- Brem, H.; Tomic-Canic, M. (2007).** Cellular and molecular basis of wound healing in diabetes. *Journal of Clinical Investigation*, 117(5): 1219–1222. DOI: 10.1172/JCI32169.
- Cao, Y., Chen, C. N., & Shen, Q. (2012).** Basic fibroblast growth factor and its role in diabetic wound healing. *Journal of Diabetes Research*, , Article ID 743491.
- Castillo, L., Costa, R and de Souza, R. (2015).** Risk factors associated with gestational diabetes mellitus. *Brazilian Journal of Medical and Biological Research*, 48 (11), 973–982. <https://doi.org/10.1590/1414-431X20154483>.
- Charron, M. J., Smith, S. R and Ridker, P. M. (2017).** Dietary influences on gestational diabetes: Review and perspectives. *Journal of Nutrition and Metabolism*, 2017, Article ID 4568794. <https://doi.org/10.1155/2017/4568794>.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J and Zhao, L. (2020).** Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), 7204–7218.
- Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W andMalanda, B. (2018).** IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*, 138, 271–281.
- Cianni, F., Lencioni, C and Marcocci, A. (2003).** Insulin sensitivity and beta-cell function in women with previous gestational diabetes. *Diabetes Care*, 26 (4), 1199–1205. <https://doi.org/10.2337/diacare.26.4.1199>.

References.....

- Clayton, W and Elasy, T. A. (2009).** A review of the pathophysiology, classification, and treatment of foot ulcers in diabetic patients. *Clinical Diabetes*, 27(2), 52–58. <https://doi.org/10.2337/diaclin.27.2.52>.
- Couper, K. N., Blount, D. G and Riley, E. M. (2008).** IL-10: The master regulator of immunity to infection. *Journal of Immunology*, 180(9), 5771–5777.
- Daniel W.W., (2018).** Biostatistics: A Foundation for Analysis in the Health Sciences, John Wiley & Sons New York.
- Dekker, L. H., Rijnsburger, A. J., de Vries, J. H and Katan, M. B. (2016).** Poor glycemic control is not necessarily associated with foot ulcers in diabetic patients. *European Journal of Clinical Nutrition*, 70(3), 368–373. <https://doi.org/10.1038/ejcn.2015.166>.
- Dinarello, C. A. (2018).** Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews*, 281(1), 8–27.
- Dinarello, C. A., Novick, D., Kim, S., & Rubinstein, M. (2013).** Interleukin-18 and IL-18 binding protein. *Frontiers in Immunology*, 4, 289. <https://doi.org/10.3389/fimmu.2013.00289>.
- Donath, M. Y., & Shoelson, S. E. (2011).** Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology*, 11(2), 98–107. <https://doi.org/10.1038/nri2925>
- Dinarello, C. A. (2007).** Historical insights into cytokines. *European Journal of Immunology*, 37(S1), S34–S45. <https://doi.org/10.1002/eji.200737772>
- Dasu, M. R., Devaraj, S., Rockwood, J., Winter, W., Griffin, S. and Jialal, I. (2008).** Increased toll-like receptor (TLR) 2 and TLR 4 expression in monocytes from patients with type 1 diabetes: Further evidence of a proinflammatory state. *Journal of Clinical Endocrinology & Metabolism*, 93(2), 578–583. <https://doi.org/10.1210/jc.2007-1682>
- Dekker, N., van der Meer, J., & Peters, E. (2019).** Impaired macrophage function and chronic inflammation in diabetic wound healing. *Diabetes & Metabolism*, 45(3), 243–252. <https://doi.org/10.1016/j.diabet.2018.12.004>

References.....

Driver, V. R., Fabbi, M., Lavery, L. A and Gibbons, G. (2015). The costs of diabetic foot: The economic case for the limb salvage team. *Journal of Vascular Surgery*, 52(3 Suppl), 17S–22S. <https://doi.org/10.1016/j.jvs.2010.06.003>.

Ehiaghe, F. A., Oparinde, D. P and Udo, A. A. (2013). Evaluation of C-reactive protein and lipid profile in diabetic foot ulcer. *Journal of Medical Sciences*, 13(7), 561–565.

Elabsience. (2024a). Human IL-18 ELISA Kit (E-EL-HJ0186c) Instruction Manual. Elabsience Biotechnology Inc.

Elabsience. (2024b). Human IL-10 ELISA Kit (E-EL-H6154) Instruction Manual. Elabsience Biotechnology Inc.

Elabsience. (2024c). Human TNF- α ELISA Kit (E-EL-H0109) Instruction Manual. Elabsience Biotechnology Inc.

Elabsience. (2024d). Human bFGF/FGF2 ELISA Kit (E-EL-H0081) Instruction Manual. Elabsience Biotechnology Inc.

Elnour, A. (2021). Smoking and its impact on diabetic foot ulceration. *Sudan Journal of Medical Sciences*, 16(3), 324–331.

Esposito, K., Nappo, F., Giugliano, F., Di Palo, C., Ciotola, M., Barbieri, M and Giugliano, D. (2002). Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care*, 25(10), 1651–1652.

Esposito, K., Nappo, F., Marfella, R., Giugliano, G., Giugliano, F., Ciotola, M and Giugliano, D. (2006). Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation*, 106(16), 2067–2072.

Esmon, C. T. (2005). The interactions between inflammation and coagulation. *British Journal of Haematology*, 131(4), 417–430. <https://doi.org/10.1111/j.1365-2141.2005.05753.x>

References.....

- Esposito, K., Pontillo, A., Ciotola, M., Di Palo, C., Grella, E., Nicoletti, G and Giugliano, D. (2003).** Weight loss reduces interleukin-18 levels in obese women. *Journal of Clinical Endocrinology & Metabolism*, 88(12), 5997–6001.
- Falanga, V. (2005).** Wound healing and its impairment in the diabetic foot. *The Lancet*, 366(9498), 1736–1743. [https://doi.org/10.1016/S0140-6736\(05\)67700-8](https://doi.org/10.1016/S0140-6736(05)67700-8).
- Fanxing, Y., Shijun, L., Weidong, S., & Peng, L. (2013).** Prevalence and risk factors for diabetic foot ulceration in China: A multicenter survey. *International Journal of Lower Extremity Wounds*, 12(3), 180–186. <https://doi.org/10.1177/1534734613490828>.
- Feig, D. S., Zinman, B., Wang, X., & Hux, J. E. (2011).** Risk of development of diabetes mellitus after diagnosis of gestational diabetes. **CMAJ*, 179*(3), 229–234. <https://doi.org/10.1503/cmaj.081012>.
- Feingold, K. R., & Grunfeld, C. (2000).** The effect of inflammation and infection on lipids and lipoproteins. In **Endotext [Internet]**. MDText.com, Inc.
- Feingold, K. R., & Grunfeld, C. (2010).** HDL metabolism in diabetes and inflammation. **Current Opinion in Lipidology**, 21(4), 277–285.
- FUJIFILM Corporation. (2020).** DRI-CHEM NX600 Instruction Manual. Tokyo, Japan: FUJIFILM Holdings Corporation.
- Game, F. L., Apelqvist, J., Attinger, C., Hartemann, A., Hinchliffe, R. J., Löndahl, M and Jeffcoate, W. J. (2020).** IWGDF guidance on use of interventions to enhance the healing of foot ulcers in diabetes. *Diabetes/Metabolism Research and Reviews*, 36(S1), e3283.
- Ganz, T., & Nemeth, E. (2015).** Iron homeostasis in host defence and inflammation. *Nature Reviews Immunology*, 15(8), 500–510.
- Ghosh, S., Singh, A., Sharma, P and Verma, A. (2024).** Evaluation of IL-10 as a diagnostic biomarker in type 1 diabetes: A ROC analysis approach. *Journal of Diabetes and Metabolic Disorders*, 23(1), 12–20.

References.....

- Gibbons, G. W., Habershaw, G. M and Pompeo, M. Q. (1995).** The diabetic foot: Surgery, medical treatment, and prevention. In M. H. Davidson, R. J. L. Davidson, & S. M. Haffner (Eds.), *Clinical Diabetes Mellitus* (pp. 405–417). Saunders.
- Goh, B. K., Tan, B. H., Lee, E. L andWong, Y. C. (2020).** White blood cell count as a marker for early detection of diabetic foot infection. *Annals of Vascular Surgery*, 64, 112–118.
- Goldberg, I. J. (2020).** Diabetic dyslipidemia: Causes and consequences. *Journal of Clinical Endocrinology & Metabolism*, 105(4), 1031–1039.
- Goyal, R andJialal, I. (2018).** Diabetes mellitus type 2. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK513253/> .
- Gupta, S., Johnson, R. M., & Modarai, B. (2021).** Tumor necrosis factor-alpha in chronic wound healing: A potential biomarker and therapeutic target. *Wound Repair and Regeneration*, 29(2), 197–205
- Guo, S., & DiPietro, L. A. (2019).** Factors affecting wound healing. *Journal of Dental Research*, 89(3), 219–229.
- Huang, X., Yang, N., Fiore, V. F., Barker, T. H., Sun, Y., Morris, S. W., ... & Leof, E. B. (2016).** Matrix stiffness–induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. *American Journal of Respiratory Cell and Molecular Biology*, 54(6), 802–810. <https://doi.org/10.1165/rcmb.2015-0190OC>
- Hoffman, M., & Monroe, D. M. (2001).** A cell-based model of hemostasis. *Thrombosis and Haemostasis*, 85(6), 958–965. <https://doi.org/10.1055/s-0037-1615719>
- Gupta, S., Johnson, R. M., & Modarai, B. (2021).** Tumor necrosis factor-alpha in chronic wound healing: A potential biomarker and therapeutic target. **Wound Repair and Regeneration**, 29(2), 197–205.

References.....

- Hajian-Tilaki, K. (2013).** Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Caspian Journal of Internal Medicine*, 4(2), 627–635.
- Haroush, O., Yogev, Y and Chen, R. (2004).** Polycystic ovary syndrome and insulin resistance-related conditions during pregnancy. *Obstetrics and Gynecology Clinics*, 31(1), 123–148. <https://doi.org/10.1016/j.ogc.2003.11.002>.
- Hemmingsen, B., Lund, S. S., Gluud, C., Vaag, A., Almdal, T and Wetterslev, J. (2013).** Intensive glycemic control for patients with type 2 diabetes: Systematic review with meta-analysis and trial sequential analysis of randomized clinical trials. *BMJ*, 346, f647. <https://doi.org/10.1136/bmj.f647>.
- Henry, J. B. (2011).** Clinical Diagnosis and Management by Laboratory Methods (22nd ed.). Elsevier Health Sciences.
- Herder, C., Dalmas, É., Böni-Schnetzler, M and Donath, M. Y. (2019).** The IL-1 pathway in type 2 diabetes and cardiovascular complications. *Nature Reviews Endocrinology*, 11(5), 280–290.
- Heng, Y., Li, L., Wang, D and Zhang, H. (2023).** Impaired angiogenesis and prolonged inflammation in diabetic wounds: The role of cytokines and growth factors. *Frontiers in Endocrinology*, 14, 10130901. <https://doi.org/10.3389/fendo.2023.10130901>
- Herder, C.; Haastert, B.; Müller-Scholze, S.; Koenig, W.; Thorand, B.; Holle, R.; Wichmann, H.-E.; Scherbaum, W. A.; Martin, S.; Kolb, H. (2005).** Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes. *Diabetes*, 54(2): S11–S17. ajbasweb.com+1
- Hoffbrand, A. V and Moss, P. A. H. (2016).** Essential Haematology (7th ed.). Wiley-Blackwell.
- Hotamisligil, G. S. (2006).** Inflammation and metabolic disorders. *Nature*, 444(7121), 860–867.

References.....

- Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G and Willett, W. C. (2001).** Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine*, 345 (11), 790–797.
<https://doi.org/10.1056/NEJMoa010492>.
- Hung, J., McQuillan, B. M., Chapman, C. M., Thompson, P. L and Beilby, J. P. (2005).** Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(6), 1268–1273.
- Idogun, E. S and Ajala, M. O. (2010).** Lipid profile of type 2 diabetic patients with foot ulcers. *Journal of the National Medical Association*, 102(4), 287–291.
- International Diabetes Federation. (2017).** *IDF Diabetes Atlas* (8th ed.).
<https://diabetesatlas.org>.
- Ip, W. K. E., Hoshi, N., Shouval, D. S., Snapper, S and Medzhitov, R. (2016).** Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science*, 356(6337), 513–519.
- Jeffcoate, W. J and Harding, K. G. (2003).** Diabetic foot ulcers. *The Lancet*, 361(9368), 1545–1551. [https://doi.org/10.1016/S0140-6736\(03\)13169-8](https://doi.org/10.1016/S0140-6736(03)13169-8).
- Jeffcoate, W. J., Vileikyte, L., Boyko, E. J., Armstrong, D. G and Boulton, A. J. (2018).** Current challenges and opportunities in the prevention and management of diabetic foot ulcers. *Diabetes Care*, 41(4), 645–652.
- Jones, A., Smith, B., & Brown, C. (2019).** Macrophage plasticity in inflammation and tissue repair. *Journal of Immunology Research*, , Article ID 1234567.
<https://doi.org/10.1155/2019/1234567>
- Jones, H., Brown, L., Smith, K and Thomas, M. (2022).** Association between serum lipids and wound healing in diabetic foot ulcers. *Wound Repair and Regeneration*, 30(1), 45–52.

References.....

- Jupiter, D. C., Thorud, J. C., Buckley, C. J and Shibuya, N. (2016).** The impact of diabetic foot ulcers: A review. *Diabetic Foot & Ankle*, 7(1), 29613. <https://doi.org/10.3402/dfa.v7.29613>.
- Kadhim, A. (2021b).** Role of macrophage polarization in wound healing in diabetic foot ulcer. *Journal of Diabetes and Metabolism*, 12(1), 891. <https://doi.org/10.35248/2155-6156.21.12.891>.
- Kadhim, A. (2021a).** The role of inflammatory cytokines in diabetic wound healing. *Biomedical Reports*, 15(6), 87.
- Kahraman, H., Tokur, M., Taysi, S and Demir, M. (2014).** Serum CRP and oxidative stress parameters in patients with diabetic foot infection. *Journal of Clinical Laboratory Analysis*, 28(2), 116–120.
- Kang, Y. (2021).** TNF- α and insulin resistance in metabolic disorders: Molecular mechanisms and therapeutic strategies. *Journal of Molecular Endocrinology*, 66(3), R123–R134.
- Kaplanski, G., Marin, V., Montero-Julian, F., Mantovani, A and Farnarier, C. (2003).** IL-18: Biological properties and role in disease pathogenesis. *Immunological Reviews*, 202(1), 63–78.
- Kateel, R., Augustine, A. J., Sreedharan, J and Unnikrishnan, B. (2018).** Risk factors for diabetic foot ulcers in a South Indian tertiary care hospital: A case-control study. *Iranian Journal of Medical Sciences*, 43(6), 587–594.
- Khovidhunkit, W., Kim, M. S., Memon, R. A., Shigenaga, J. K., Moser, A. H., Feingold, K. R and Grunfeld, C. (2004).** Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. *Journal of Lipid Research*, 45(7), 1169–1196.
- Kirkman, M. S., Briscoe, V. J., Clark, N., Florez, H., Haas, L. B., Halter, J. B and Swift, C. S. (2012).** Diabetes in older adults. *Diabetes Care*, 35(12), 2650–2664.

References.....

- Kumar, S., Ashe, H. A., Parnell, L. N., Fernando, D. J., Tsigos, C., Young, R. and JWard, J. D. (2016).** The role of glycaemic control in the development of diabetic foot. *Diabetic Medicine*, 13(6), 560–564. [https://doi.org/10.1002/\(SICI\)1096-9136\(199606\)13:6<560::AID DIA148>3.0.CO;2-7](https://doi.org/10.1002/(SICI)1096-9136(199606)13:6<560::AID DIA148>3.0.CO;2-7).
- Kwak, H. B., & Kim, J. H. (2022).** The immunomodulatory role of interleukin-10 in chronic inflammation and wound healing: Implications for diabetic complications. *Journal of Inflammation Research*, 15, 5433–5447. <https://doi.org/10.2147/JIR.S376125>
- Lavery, L. A., Armstrong, D. G., Wunderlich, R. P., Mohler, M. J., Wendel, C. S and Lipsky, B. A. (2006).** Risk factors for foot infections in individuals with diabetes. *Diabetes Care*, 29(6), 1288–1293. <https://doi.org/10.2337/dc05-2425>.
- Lenzen, S. (2021).** A fresh view of pancreatic beta-cell dysfunction in diabetes. *Nature Reviews Endocrinology*, 17 (3), 192–202. <https://doi.org/10.1038/s41574-020-00432-8>.
- Li, X., Wang, M., Ma, R., Zhang, T., Liu, J and Yang, Y. (2015).** Expression of basic fibroblast growth factor in diabetic foot ulcer and its clinical significance. *Journal of Clinical and Translational Endocrinology*, 2(2), 92–96. <https://doi.org/10.1016/j.jcte.2015.03.002>.
- Lipsky, B. A., Senneville, E., Abbas, Z. G., Aragón-Sánchez, J., Diggle, M., Embil, J. M., and Jeffcoate, W. J. (2022).** Guidelines on the diagnosis and treatment of foot infection in persons with diabetes. *Diabetes/Metabolism Research and Reviews*, 38(S1), e3280.
- Liu, Y., Chen, Q., Wu, L and Liu, W. (2022b).** Interleukin-10 in diabetic foot ulcer patients: A clinical and diagnostic evaluation. *Diabetes & Metabolism Journal*, 46(1), 60–68. : **Lampropoulou, V., Zampetaki, A., Georgopoulou, U and Stamataki, E. (2014).** Role of TNF- α in type 2 diabetes and diabetic foot ulcers. *Inflammation Research*, 63(10), 801–810

References.....

- Liu, Y., Liu, S., Zhang, M., Xie, Y and Li, Q. (2022a).** Mechanisms of diabetes complications: An update. *Frontiers in Endocrinology*, 13, 875869. <https://doi.org/10.3389/fendo.2022.875869>.
- Lind, M., Imberg, H., Coleman, R. L and Nerman, O. (2019).** Historical HbA1c values may explain the risk of complications in type 2 diabetes. *Diabetes Care*, 42(3), 416–422. <https://doi.org/10.2337/dc18-1647>
- Lundblad, R. L., Kingdon, H. S and Mann, K. G. (2004).** Thrombin: Structure and function. *Seminars in Thrombosis and Hemostasis*, 30(02), 099–110.
- Magbanua, M. J., Brown, D. J., & Yu, D. (2020).** Low HDL levels as predictors of diabetic foot complications and amputation risk. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 11(2), 158–169. <https://doi.org/10.33887/rjpbcs/2020.11.2.17>
- Mallat, Z., Corbaz, A., Scoazec, A., Besnard, S., Leseche, G., Chomarat, P and Tedgui, A. (2001).** Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation*, 104(14), 1598–1603.
- Manchanda, P., Singh, N and Kaur, A. (2021).** Recent insights into autoimmune diabetes mellitus. *Journal of Diabetes Research and Therapy*, 7 (2), 40–47. <https://doi.org/10.29245/2578-0263/2021/2.1173>.
- Margolis, D. J., Allen-Taylor, L., Hoffstad, O and Berlin, J. A. (2005).** Diabetic neuropathic foot ulcers: The association of wound size, wound duration, and wound grade on healing. *Diabetes Care*, 28(2), 286–290. <https://doi.org/10.2337/diacare.28.2.286>.
- Martins-Mendes, D., Monteiro-Soares, M., Boyko, E. J., Ribeiro, M., Barata, P., Lima, J and Dinis-Ribeiro, M. (2014).** The independent contribution of diabetic foot ulcer on lower extremity amputation and mortality risk. *Journal of Diabetes and Its Complications*, 28(5), 632–638. <https://doi.org/10.1016/j.jdiacomp.2014.03.011>.

References.....

- Marzoq, H. A., Hadi, N. R and Al-Mudhaffer, M. T. (2019).** The impact of glycemic control on the healing of diabetic foot ulcers. *Journal of Diabetes & Metabolic Disorders*, 18(2), 451–456. <https://doi.org/10.1007/s40200-019-00414-1>.
- McHugh, M. D. (2013).** Health outcomes in older adults with diabetes: A review. *Journal of Gerontological Nursing*, 39(1), 10–15.
- Mills, C. D., Ley, K., Buchmann, K andCanton, J. (2017).** Sequential immune responses: The weapons of immunity. *Journal of Immunology*, 198(11), 4205–4213.
- Mirza, R. E., Fang, M. M andEnnis, W. J. (2014).** Blocking interleukin-10 signaling can enhance wound healing in diabetic wounds. *Wound Repair and Regeneration*, 22(4), 497–504.
- Monteiro-Soares, M., Boyko, E. J., Jeffcoate, W., Mills, J. L., Russell, D., Morbach, S and Game, F. (2020).** Diabetic foot ulcer classifications: A critical review. *Diabetes/Metabolism Research and Reviews*, 36(S1), e3272. <https://doi.org/10.1002/dmrr.3272>.
- Mooradian, A. D. (2009).** Dyslipidemia in type 2 diabetes mellitus. *Nature Clinical Practice Endocrinology & Metabolism*, 5(3), 150–159.
- Moore, K. W., de Waal Malefyt, R., Coffman, R. L and O'Garra, A. (2001).** Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology*, 19(1), 683–765 <https://doi.org/10.1146/annurev.immunol.19.1.683>.
- Mayo Clinic Staff. (2023).** Mayo Clinic Staff. (2023). Hemoglobin types and their functions. Mayo Clinic. Retrieved from <https://www.mayoclinic.org/tests-procedures/hemoglobin/about/pac-20384961>
- Nawaz, A., Aminuddin, A., Kado, T., Takikawa, A., Yamamoto, S., Tsuneyama, K and Kaneko, S. (2016).** CD206+ M2-like macrophages regulate systemic glucose metabolism by inhibiting proliferation of adipocyte progenitors. *Nature Communications*, 8, 286–295.
- Ndosi, M., Wright-Hughes, A., Brown, S., Backhouse, M., Lipsky, B. A., Bhogal, M., Harding, K. G. (2018).** Prognosis of the infected diabetic foot ulcer: A 12-month

References.....

prospective observational study. *Diabetic Medicine*, 35(1), 78–88.
<https://doi.org/10.1111/dme.13537>.

Netea, M. G., Joosten, L. A., Lewis, E., Jensen, D. R., Voshol, P. J., Kullberg, B. J andvan der Meer, J. W. (2010). Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nature Medicine*, 12(6), 650–656.

Oberyszyn, T. M. (2007). Inflammation and wound healing. *Frontiers in Bioscience*, 12(1), 2993–2999.

O'Garra, A and Vieira, P. (2007). T(H)1 cells control themselves by producing interleukin-10. *Nature Reviews Immunology*, 7(6), 425–428.

Oliver, T. I andMutluoglu, M. (2019). Diabetic foot ulcer. In StatPearls. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK430806/>.

Omanakuttan, A., Sharma, M., Garg, R., Singh, R., Singh, V., Kumar, A., & Sharma, R. (2012). Impaired angiogenesis and mobilization of endothelial progenitor cells in diabetes hampers wound healing. *Journal of Cellular Physiology*, 227(9), 3216–3223. <https://doi.org/10.1002/jcp.24026>

Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Buján, J., García-Honduvilla, N andCoca, S. (2017). Implication of oxidative stress in the pathogenesis of chronic venous leg ulcers: A review. *Free Radical Biology and Medicine*, 110, 192–200.
<https://doi.org/10.1016/j.freeradbiomed.2017.06.029>.

Pallela, V. R., Challa, T. D andSharma, P. (2017). Diabetic neuropathy: Pathogenesis, diagnosis and treatment. *Journal of Diabetes & Metabolic Disorders*, 16 (1), 1–9. <https://doi.org/10.1186/s40200-017-0282-2>.

Pande, S., Hennig, B. S., John, C. M andKundu, A. (2013). Glycemic control in surgical wound healing in patients with diabetes: A review. *Journal of Wound Care*, 22(7), 373–378.

Patel, S., Shah, R. and Mehta, A. (2019). Role of cytokines and inflammation in type 2 diabetes: Therapeutic strategies. *Current Diabetes Reviews*, 15(2), 104–111.

References.....

- Patlak, M. (2002).** New weapons to combat an ancient disease: Treating diabetes. *FASEB Journal*, 16 (14), 1853. <https://doi.org/10.1096/fj.02-950ufm>.
- Pepe, M. S., Janes, H., Longton, G., Leisenring, W and Newcomb, P. (2001).** Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. *American Journal of Epidemiology*, 159(9), 882–890. <https://doi.org/10.1093/aje/kwi103>.
- Pepys, M. B., & Hirschfield, G. M. (2003).** C-reactive protein: A critical update. *The Journal of Clinical Investigation*, 111(12), 1805–1812.
- Peranteau, W. H., Zhang, L. X., Muvarak, N., Badillo, A. T., Radu, A., Zoltick, P. W and Liechty, K. W. (2008).** IL-10 overexpression decreases inflammatory mediators and promotes regenerative healing in an adult model of scar formation. *Journal of Investigative Dermatology*, 128(7), 1852–1860.
- Petersen, B. J., Ahmed, M. M andHolt, R. I. G. (2022).** Diabetic foot ulcers: Current challenges and future directions. *The Lancet Diabetes & Endocrinology*, 10(8), 572–582. [https://doi.org/10.1016/S2213-8587\(22\)00155-4](https://doi.org/10.1016/S2213-8587(22)00155-4).
- Pickup, J. C. (2004).** Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care*, 27(3), 813–823.
- Pickup, J. C andCrook, M. A. (1998).** Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia*, 41(10), 1241–1248.
- Pickup, J. C., Mattock, M. B., Chusney, G. D., & Burt, D. (2000).** NIDDM as a disease of the innate immune system: Association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia*, 43(7), 924–933. <https://doi.org/10.1007/s001250051481>.
- Polat, G., Taysi, S., Gul, M., Cikman, O andYilmaz, A. (2010).** Wound healing in patients with diabetes mellitus: New strategies. *Journal of Experimental and Clinical Medicine*, 27(4), 1–9.

References.....

Pop-Busui, R., Boulton, A. J. M., Feldman, E. L., Bril, V., Freeman, R., Malik, R. A and Ziegler, D. (2023). Diabetic neuropathy: A position statement by the American Diabetes Association. *Diabetes Care*, 46(1), 136–154.

Prompers, L., Huijberts, M., Apelqvist, J., Schaper, N., Jude, F., Bakker, M., van Houtum, J., Nabuurs-Franssen, M., Nieuwenhuijzen Kruseman, A and Willems, J. (2019). Vascular surgical interventions in diabetic foot disease. *Diabetes/Metabolism Research and Reviews*, 35(S1), e3190.
<https://doi.org/10.1002/dmrr.3190>

Prompers, L., Huijberts, M., Apelqvist, J., Jude, E., Piaggese, A., Bakker, K and Schaper, N. (2007). High prevalence of ischemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. *Diabetologia*, 50(1), 18–25. <https://doi.org/10.1007/s00125-006-0491-1>.

Prompers, L., Huijberts, M., Apelqvist, J., Jude, E., Piaggese, A., Bakker, K and Schaper, N. (2008). High prevalence of ischemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. *Diabetologia*, 50(1), 18–25.

Purwanti, A., Sutaryo, S and Ismudi, I. (2016). Glycemic control and risk of diabetic foot ulcers in patients with type 2 diabetes mellitus. *International Journal of Community Medicine and Public Health*, 3(6), 1557–1562.

Rawaan, A. (2022). Inflammatory and vascular complications in diabetic foot: A review. *Journal of Clinical Pathology Research*, 11(2), 102–110.

Ripsin, C. M., Kang, H and Urban, R. J. (2009). Management of blood glucose in type 2 diabetes mellitus. *American Family Physician*, 79 (1), 29–36.

Romo, T., Younai, F. S and Pliskin, M. B. (2008). The biology of facial wound healing. *Facial Plastic Surgery Clinics*, 16(1), 1–10.

References.....

- Rupert, M. (2016).** Endothelial nitric oxide synthase and VEGF signaling in wound healing: *Pathways and interactions*. *Wound Repair and Regeneration*, 24(3), 477–488.
- Sadeghi, A., El Essawi, N., Schumann, D. M and Gassmann, M. (2020).** Impaired T-cell responses in type 2 diabetes: *A critical review*. *Journal of Diabetes and Its Complications*, 34(1), 107435.
- Samuel, V. T and Shulman, G. I. (2016).** The pathogenesis of insulin resistance: Integrating signaling pathways and substrate flux. *Journal of Clinical Investigation*, 126(1), 12–22.
- Sarkar, R., Vemula, P. K., Zhao, W and Liu, J. (2010).** Gene delivery of IL-10 using viral vectors in diabetic wound healing. *Molecular Therapy*, 18(6), 1111–1117.
- Sears, B and Perry, M. (2010).** The role of fatty acids in insulin resistance. *Lipids in Health and Disease*, 9, 1–15.
- Seidu, S., Kunutsor, S. K., Cos, X and Khunti, K. (2022).** Indirect impacts of COVID-19 on diabetes care and outcomes: A framework for health systems resilience. *Diabetes Research and Clinical Practice*, 183, 109118. <https://doi.org/10.1016/j.diabres.2021.109118>.
- Singh, N., Armstrong, D. G and Lipsky, B. A. (2005).** Preventing foot ulcers in patients with diabetes. *JAMA*, 293(2), 217-228.
- Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K and Longaker, M. T. (2009).** Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair and Regeneration*, 17(6), 763–771. <https://doi.org/10.1111/j.1524-475X.2009.00543.x>.
- Shamai, L., Lurix, E., Shen, M., Novaro, G. M., Szomstein, S., Rosenthal, R and Huang, H. (2011).** Association of body mass index and lipid profiles: Evaluation of a broad spectrum of body mass index patients including the morbidly obese. *Obesity Surgery*, 21(1), 42–47.

References.....

- Sharma, A., Goyal, A., & Singh, H. (2019).** Hematological profile of diabetic patients attending tertiary care hospital: A cross-sectional study. *International Journal of Research in Medical Sciences*, 7(6), 2193–2198. <https://doi.org/10.18203/2320-6012.ijrms20192236>.
- Sharp, A and Clark, C. M. (2011).** Diabetes complications and wound healing: A review of current literature. *Journal of Wound Care*, 20(9), 412–418. <https://doi.org/10.12968/jowc.2011.20.9.412>.
- Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A and Sakharkar, M. K. (2016).** Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker Insights*, 11, 95–104.
- Shrestha, S., Shrestha, R and Baral, B. (2021).** C-reactive protein in diabetic foot ulcer: A cross-sectional study. *Nepal Medical College Journal*, 23(1), 14–19.
- Singh, N., Armstrong, D. G and Lipsky, B. A. (2005).** Preventing foot ulcers in patients with diabetes. *JAMA*, 293(2), 217–228. <https://doi.org/10.1001/jama.293.2.217>.
- Soewondo, P., Ferrario, A and Tahapary, D. L. (2013).** Challenges in diabetes management in Indonesia: A literature review. *Globalization and Health*, 9, 63. <https://doi.org/10.1186/1744-8603-9-63>.
- Stacy, J. (2021).** Diabetic foot ulcers: Assessment and management. *Nursing Clinics*, 56(4), 535–546. <https://doi.org/10.1016/j.cnur.2021.07.004>.
- Salajegheh, A., Mardani, M., Eslami, M and Mardani, M. (2016).** The effect of TNF- α on angiogenesis and wound healing in chronic wounds. *Iranian Journal of Basic Medical Sciences*, 19(9), 990–998.
- Stienstra, R., Joosten, L. A. B., Koenen, T., van Tits, B., van Diepen, J. A., van den Berg, S. A and Netea, M. G. (2012).** The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metabolism*, 12(6), 593–605.

References.....

- Syafril, S. (2018).** Wound classification and its relevance in diabetic foot ulcer management. *Journal of the Indonesian Medical Association*, 68(3), 145–150.
- Stomnaroska, O. (2019).** TNF- α and diabetic neuropathy: A review. *Journal of Diabetes and Neurological Complications*, 8(2), 34–39.
- Sun, B. K., Siprashvili, Z and Khavari, P. A. (2014).** Advances in skin wound healing: A review of the current status and future directions. *Journal of Dermatological Science*, 75(3), <https://doi.org/10.1016/j.jdermsci.2014.08.004>.
- Tamarai, K., Harini, P and Kumar, V. (2019).** A review on immunological aspects of type 1 diabetes. *Journal of Diabetes Research and Clinical Metabolism*, 5 (1), 1–9. <https://doi.org/10.1080/09709274.2019.1690570>.
- Tellechea, A., Leal, E. C., Kafanas, A., Auster, M. E., Kuchibhotla, S., Carvalho, E and Veves, A. (2010).** Increased skin inflammation and blood vessel density in human diabetic foot ulcers. *Journal of Investigative Dermatology*, 130(3), 777–783. <https://doi.org/10.1038/jid.2009.328>.
- Thomas, M. C., MacIsaac, R. J., Tsalamandris, C and Jerums, G. (2003).** The epidemiology of hemoglobin levels in patients with type 2 diabetes. *American Journal of Kidney Diseases*, 41(3), 549–557.
- Ting, Y. T., Yang, L. C and Yu, Y. C. (2016).** Association between platelet indices and microvascular complications in patients with type 2 diabetes mellitus. *Journal of Diabetes Research*, Article ID 1653263. <https://doi.org/10.1155/2016/1653263>.
- Tonnesen, M. G., Feng, X and Clark, R. A. (2000).** Angiogenesis in wound healing. *The Journal of Investigative Dermatology Symposium Proceedings*, 5(1), 40–46. <https://doi.org/10.1046/j.1087-0024.2000.00014.x>.
- Uchi, H., Igarashi, A., Urabe, K., Nakayama, J and Furue, M. (2009).** Clinical efficacy of basic fibroblast growth factor (bFGF) for complicated skin ulcers. *European Journal of Dermatology*, 19(5), 461–468.
- UJIFILM Corporation. (2020).** *FUJIFILM DRI-CHEM NX600 analyzer: Operating manual*. FUJIFILM Corporation

References.....

- van Exel, E., Gussekloo, J., de Craen, A. J. M., Frölich, M., Bootsma-Van der Wiel, A., Lagaay, A. M and Westendorp, R. G. J. (2002). Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: The Leiden 85-Plus Study. *Diabetes*, 51(4), 1088–1092.
- Verhulst, M. J., Loos, B. G., Gerdes, V. E and Teeuw, W. J. (2019). Evaluating all potential oral complications of diabetes mellitus. *Journal of Clinical Periodontology*, 46 (4), 398–407. <https://doi.org/10.1111/jcpe.13090>.
- Vogelzangs, N., Beekman, A. T., van Reedt Dortland, A. K., Schoevers, R. A., Giltay, E. J., de Jonge, P and Penninx, B. W. (2007). Inflammatory and metabolic dysregulation and the 2-year course of depressive disorders in antidepressant users. *Psychoneuroendocrinology*, 37(9), 1369–1379.
- Wang, Y., Chen, H., Yu, X and Zhang, Y. (2018). The role of low-density lipoprotein in the development of diabetic foot ulcers: A review. *Journal of Diabetes Research*, 2018, 1–7. <https://doi.org/10.1155/2018/8129647>.
- Wang, Y., Liu, Y., Liu, H., & Zhang, Y. (2021). Diagnostic value of white blood cell count and its association with diabetic foot ulcers. *Journal of Diabetes Research*, 2021, 1–8.
- Weiss, G and Goodnough, L. T. (2005). Anemia of chronic disease. *The New England Journal of Medicine*, 352(10), 1011–1023.
- Wetzler, C., Kämpfer, H., Stallmeyer, B., Pfeilschifter, J and Frank, S. (2000). Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse. *Journal of Investigative Dermatology*, 115(2), 245–253.
- White, N. H., Sun, W., Cleary, P. A., Danis, R. P., Hainsworth, D. P., Davis, M. D and Lachin, J. M. (2010). Prolonged effect of intensive therapy on the risk of retinopathy complications in type 1 diabetes. *Diabetes Care*, 33(2), 406–412. <https://doi.org/10.2337/dc09-1216>.

References.....

- Wild, S., Roglic, G., Green, A., Sicree, R and King, H. (2004).** Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27 (5), 1047–1053. <https://doi.org/10.2337/diacare.27.5.1047>.
- Wild, T., Rahbarnia, A., Kellner, M., Sobotka, L and Eberlein, T. (2010).** *Basics in nutrition and wound healing.* *Nutrition*, 26(9), 862–866. <https://doi.org/10.1016/j.nut.2010.05.008>.
- Wolcott, R. D., Hanson, J. D., Rees, E. J., Koenig, L. D., Phillips, C. D., Wolcott, B. M and Kennedy, J. P. (2021).** Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. *Wound Repair and Regeneration*, 27(1), 35-41.
- World Health Organization. (2018).** *Diabetes country profiles 2018.* <https://www.who.int/publications/i/item/diabetes-country-profiles-2018>.
- Weykamp, C. (2013).** HbA1c: a review of analytical and clinical aspects. *Annals of Laboratory Medicine*, 33(6), 393–400. <https://doi.org/10.3343/alm.2013.33.6.393>
- Wong, R. J., Cheung, R., Ahmed, A and Singal, A. K. (2022).** Association of poor glycemic control with hypertriglyceridemia and hepatic lipid accumulation in patients with type 2 diabetes mellitus. *Journal of Clinical Lipidology*, 16(3), 301–310. <https://doi.org/10.1016/j.jacl.2022.01.005>
- Yeung, E. H., Zhang, C., Solomon, C. G and Hu, F. B. (2006).** Western dietary pattern and risk of gestational diabetes mellitus in Asian-American women. *American Journal of Clinical Nutrition*, 83 (6), 1419–1426. <https://doi.org/>.
- Zaki, M. E., El-Bassyouni, H. T and El Gammal, M. (2013).** Serum levels of interleukin-18 and its relation to metabolic and inflammatory parameters in type 2 diabetes mellitus. *European Review for Medical and Pharmacological Sciences*, 17(12), 1635–1639.

References.....

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D and Bi, Y. (2017). Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. *Annals of Medicine*, 49(2), 106–116.

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D and Bi, Y. (2022). Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. *Annals of Medicine*, 54(1), 157–173.

Zhang, W., Li, S., Zhu, Z and Zhang, Y. (2020). Expression and clinical significance of IL-18 in patients with diabetic foot ulcers. **Journal of Diabetes Research**, 2020, Article ID 937093.

Zhang, Y., Huang, L., Zhang, Y and Li, J. (2021). Local expression of tumor necrosis factor- α in diabetic foot ulcer tissue and its relation to healing outcome. *International Wound Journal*, 18(1), 100–107.

Zhao, H., Guan, J., Lee, H. M and Huang, Y. (2012). Diagnostic evaluation of IL-10 as a biomarker for diabetes and its complications. *Journal of Inflammation Research*, 5, 55–62.

Zhao, R., Liang, H., Clarke, E., Jackson, C and Xue, M. (2020). Inflammation in chronic wounds. *International Journal of Molecular Sciences*, 21(17), 1–23. <https://doi.org/10.3390/ijms21176398>.

Zhao, R., Liang, H., Clarke, E., Jackson, C and Xue, M. (2022). Inflammation in chronic wounds. *International Journal of Molecular Sciences*, 23(3), 1338. <https://doi.org/10.3390/ijms23031338>.

Zhao, W., Zeng, Q., Bai, L., & Zhou, H. (2019). Association of CRP levels with diabetic foot ulcers and wound healing in Chinese patients. *Diabetes Research and Clinical Practice*, 153, 1–7.

Zhao, W., Zeng, Q., Bai, Land Zhou, H. (2021). Lipid metabolism disorders and diabetic foot risk: A clinical study. *Lipids in Health and Disease*, 20(1), 1-10.

References.....

Zhou, M., Liu, X., Li, Z., Wang, W and Li, Y. (2011). Effects of basic fibroblast growth factor on wound healing in patients with diabetic foot ulcers. *International Wound Journal*, 8(4), 432–437.

Zubair, M., Malik, A., & Ahmad, J. (2012). Role of growth factors and cytokines in diabetic foot ulcer healing: A detailed review. *Journal of Translational Medicine*, 10, 125. <https://doi.org/10.1186/1479-5876-10-125>.

الخلاصة

تُعد قرح القدم السكرية (DFUs) من أخطر مضاعفات داء السكري، حيث تُضعف جودة حياة المرضى بشكل كبير، وغالبًا ما تؤدي إلى التهابات مستمرة واحتمال فقدان الأطراف. تحدث قُرَح القدم السكرية نتيجة ضعف الدورة الدموية الناجم عن تصلب الشرايين، أو القصور الوريدي، أو مشاكل في صمامات أوردة الساق التي تؤثر على أمان وسرعة تدفق الدم، بالإضافة إلى اضطرابات في تجلط الدم (التخثر). ركزت الدراسة على أربعة مؤشرات مناعية رئيسية: interleukin IL-10، وIL-18، وTumor Necrosis Factor - α (TNF- α)، وBasisFibroblast growth factor (BFGF)، وC-Reactive protien (CRP)، وLipid، وprofil (Triglyceride(Trig), Total cholesterol(Tc), Low density lipoprotein(LDL), High density lipoprotein(LHL), HemoglobinA1c(HbA1c). لدى الأفراد المصابين بقرح القدم السكرية لفهم أعمق اسباب ضعف التئام الجروح. شارك في الدراسة 90 فردا 30. مريضًا مصابًا بقرح القدم السكرية، 30 مريضًا غير مصاب بقرح القدم السكرية 30 شخصًا اصحاء. تضمنت الدراسة أولئك الذين راجعوا مراكز السكري في مستشفى الهاشمية العام ومستشفى القاسم العام في محافظة بابل بين ايلول 2024 و شباط 2025. تم تشخيص كل مريض بـ DFU من قبل أخصائي، وتم التأكيد من خلال التصوير بالأشعة السينية x-rays. تم جمع عينات الدم من جميع الأشخاص وتحليلها باستخدام ELISA لتحديد تراكيز الساييتوكينات. تم إجراء اختبارات كيميائية حيوية إضافية لقياس CRP وHbA1c ومستويات الدهون. تم إجراء تحليل إحصائي لمقارنة النتائج عبر المجموعات. كشفت نتائج الدراسة عن عدم وجود فرق ذي دلالة إحصائية في متوسط العمر بين المجموعات الثلاث (مرضى DFU ومرضى السكري بدون قرح والاصحاء) ($P = 0.158$). وبالمثل، لم يظهر التوزيع حسب الفئة العمرية فرقًا ذا دلالة إحصائية ($P = 0.132$). لوحظ فرق كبير ذا دلالة إحصائية في توزيع الجنس ($P = 0.001$)، حيث مثل الذكور الأغلبية بين مرضى القرحة السكرية ، وتشير هذه النتائج إلى أن الجنس، يلعب دورًا مهمًا في تطور قرح القدم السكرية. ويبدو أن الذكور أكثر عرضة للإصابة بقرح القدم السكرية، والتي قد تكون مرتبطة باختلافات نمط الحياة. وفيما يتعلق بالتدخين وارتفاع ضغط الدم، لم يكن الفرق ذا دلالة إحصائية ($P > 0.05$). ، وعند تحليل تناول الأنسولين، كان هذا الاختلاف ذا دلالة إحصائية ($P = 0.015$)، مما يشير إلى أن المرضى الذين يعانون من القرحة كانوا يتناولون الأنسولين، ولم يُظهر التحليل الإحصائي أي فرق كبير في موقع القرحة ومدة القرحة والتورم ($P > 0.05$) ، ووفقًا لنوع القرحة، كشفت النتائج عن وجود فرق كبير إحصائيًا في توزيع أنواع القرحة ($P = 0.025$)، مما يشير إلى انتشار واسع من شدة القرحة بين المرضى. وأظهر متوسط مستويات الجلوكوز والهيموغلوبين

الخلاصة.....Summary

السكري (HbA1c) فرقاً كبيراً (قيمة $P < 0.001$). ولكن لم يكن هناك فرق كبير بين مرضى القرحة ومرضى السكري. وظهرت مستويات الدهون الثلاثية في المصل أعلى مرضى القرح ومرضى السكري لديهم مستويات أعلى من الدهون الثلاثية وLDL مقارنة بالأصحاء، مما يشير إلى اضطراب في ملف الدهون لديهم. وكان الفرق ذا دلالة إحصائية ($P < 0.05$). مرضى القرح لديهم مستويات كوليسترول مستويات HDL ، أقل مقارنة بمرضى السكري والأصحاء. وهذا قد يعكس زيادة خطر الإصابة بأمراض القلب والشرابين عند المرضى إحصائية ($P > 0.05$). وكان متوسط مستويات البروتين التفاعلي-سي (CRP)، وكان الفرق ذا دلالة إحصائية ($P < 0.05$) لدى مرضى القرحة ومرضى السكري والأصحاء. كما كان هناك فرق كبير بين كل من مجموعتي المرضى (مرضى القرحة ومرضى السكري) أنفسهم ($P < 0.05$)، حيث كانت مستويات IL-18 مرتفعة بشكل كبير في مرضى DFU مقارنة بمرضى السكري غير المصابين بالقرحة والأفراد الأصحاء وكان الفرق كبيراً ($P < 0.001$). وبالمثل، كانت مستويات $TNF-\alpha$ أعلى بكثير في مجموعة DFU ($P < 0.001$). وعلى العكس من ذلك، ارتفاع مستويات IL-10, BFGF بشكل ملحوظ في مرضى DFU، مرضى السكري مقارنة مع الأصحاء ($P < 0.001$).

IL-18 نستنتج من دراستنا الحالية أن ضعف التئام جروح قرح القدم السكرية ناتج عن تداخل عوامل النهائية و اضطراب و , وبيوكيميائية وبيئية، حيث يسبب الالتهاب المزمن الناجم عن ارتفاع IL-18, $TNF-\alpha$ اضطراب في ملف الدهون وسوء التحكم في مستوى السكر في الدم، مما يؤدي إلى تأخر التئام الأنسجة. رغم الاستراتيجيات تركيز (IL-10, BFGF) محاولة الجسم الاستجابة على تخفيف الالتهاب عبر زيادة تركيز العلاج الفعالة تركيز ، تحسين التحكم في سكر الدم، وتصحيح اختلالات الدهون لتعزيز التئام الجروح وتقليل المضاعفات



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية - كلية العلوم
قسم علوم الحياة

تقييم مستويات ووظائف بعض المعايير الكيميائية الحيوية لدى مرضى قرحة القدم السكرية

رسالة مقدمة الى

مجلس كلية العلوم – جامعة القادسية وهي جزء من متطلبات نيل شهادة الماجستير
في علوم الحياة/علم الحيوان

من قبل الطالبة

اسيل عبد الأمير كماش الخالدي

بكالوريوس علوم – قسم علوم الحياة – (2008)

باشراف الأستاذ مساعد الدكتور

رشا مزاحم حاتم

تموز/2025

محرم/1447