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Department of Biology

Association Gene Polymorphism of Some Biomarkers with Bacterial Infections and Severity of Diabetic Foot Ulcer

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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Dedication

To the one who led the hearts and minds of humanity to safety, the first teacher of humanity, Muhammad (peace and blessings of God be upon him and his family).

To the righteous martyrs, the valiant prisoners, and the blessed wounded

To my dear mother and father

To the one who was my shadow when fatigue scorched my husband, my support me.

To the seed of the heart and the hope of tomorrow, my beloved children

To my brothers, the source of my pride and honor

To every hand and heart that walked with me on the path of achievement so that I could be

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Summary

Diabetes is a common disease all over the world, diabetic foot ulcer is an open wound that may be occurs in patients with diabetes and represent that the most important complications in these type 2 diabetes patients , there are few studies on the relationship between genetic polymorphisms and type 2 diabetes patients therefore, this study addressed the genetic polymorphisms of the *Monocyte chemoattractant protein-1(MCP-1*) (I\D) and *C-reactive protein (CRP*) (A\G) genes with the severity of the disease for patients with and without foot ulcers.

This study is a cross section study, one hundred blood samples were collected in an EDTA tube from both sexes (males and females) aged between (33-76) years old of patients have been attended to the AL-Imam Al-Hasan center for endocrinology and diabetes during the period from August to December 2023. Two groups of patients have been formed: 50 type 2 diabetic patients with foot ulcers and 50 type 2 diabetic patients without foot ulcers, each group underwent testing and a clinical examination, and information about the patients was collected. The genetic polymorphism of the *MCP-1* and *CRP* genes were determined by amplification refractory mutational system (ARMS) using polymerase chain reaction (PCR) technique. In addition, swabs with media were taken from the each foot ulcer patient, also aerobic bacteria were isolated and diagnosed by culturing them on appropriate cultural media using several biochemical tests and the VITEK2 compact system. Blood samples were collected in a sodium citrate tubes to determine the fibrinogen level for all patients.

This study was showed Most of descriptive data for the study groups of diabetic patients with and without foot ulcer showed a significant differences ($p<0.05$). About the duration of disease, there were a significant result in diabetic patients with foot ulcer group in period (>1-3 week) and (1-3 month) compared with

other period, while (1-5year) and (<5years) periods significantly affect diabetic without foot ulcer patients more than other groups. Also, about the distribution of other disease the majority of diabetes with foot ulcer was affected with hypertension, while majority of diabetes without foot ulcer were affecting with both hypertension and cardiovascular diseases with a significant differences. In the context of dividing patients according to obesity, a significant difference was recorded only in DFU group.

Moreover, there were a significant increase in fibrinogen levels of diabetic patients with foot ulcer mean (596.08) comparing to diabetic patients without ulcer mean (305.66). About the bacterial growth, the majority (76%) of samples with positive culture, while 24% with no growth. The bacterial type revealed that the majority 78% of bacterial growth were gram-negative bacteria, while only 22% gram-positive bacteria, in context of type of bacterial species isolated from ulcer, the highest percentage was for the *Staphylococcus aureus* (34%), *Klebsiella pneumonia* (28%) and *Proteus mirabilis* (26%).

Concerning the *MCP-1* gene, the significant difference were recorded in context of allele frequency; I allele showed a significant increase in both groups compared with D allele, in addition, *CRP* gene showed a significant difference in context of allele frequency, where G allele significantly appeared in both patients groups as compared with A allele ,

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List of Abbreviations

Abbreviation	Description Full from
ARMS	Amplification-refractory mutation system
AGEs	advanced glycation end products
BMI	Body mass index
CRP	C-reactive protein
CCL2	Chemokine C Ligand 2
CVD	Cardio Vascular Disease
D.W.	Distilled water
DFI	Diabetic foot infection
DFU	Diabetic foot ulcer
DM	Diabetes mellitus
DKA	Diabetic ketoacidosis
DNA	Deoxy ribonucleic acid
dNTPs	Deoxy nucleotide triphosphates

EDTA	Ethylene diamine tetra acetic acid
E. coli	Escherichia coli
ESR	erythrocyte sedimentation rate
F	Forward
Gram+ve	Gram negative
Gram_ve	Gram positive
HHS	Hyperglycemic Hyperosmolar State
hsCRP	high sensitivity C-reactive protein
IR	insulin resistance
IWGDF	International Working Group on the Diabetic Foot
ID	Identification
MCP-1	Monocyte chemoattractant protein-1
PAD	Peripheral arterial disease
PCR	Polymerase chain reaction
P. aeruginosa	Pseudomonas aeruginosa
R	Reverse
ROC	receiver operating characteristic
SBP	spontaneous bacterial peritonitis
S. aureus	Staphylococcus aureus
SNP	Single Nucleotide Polymorphisms
TBE	Tris-Borate EDTA
T2DM	Type 2 diabetes mellitus
UV	Ultra violet
WHO	World Health Organization
WBC	white blood cells

Chapter One

Introduction and Literatures Review

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic condition that affects the way the body metabolizes glucose, the primary source of energy for cells , people with T2DM typically produce insulin but their cells become resistant to it or relative insulin deficiency , leading to high blood glucose levels (Robertson *et al*., 2024) . T2DM is associated with the disruption in the metabolism of fat, proteins and carbohydrates, different complications that are associated with T2DM includes the retinopathy, neuropathy, nephropathy and danger and other issues, due to the loss of the function of the insulin, the metabolism is disturbed. Also the effects of inflammation and aging on the diabetes mellitus (Halim , 2019).

Classification method for diabetic foot ulcers was the most widely used Wagner's classification, IWGDF/IDSA classification, etc. Wagner's, most widely accepted, and most straightforward one worldwide (Hobizal *et al*., 2012). Determining the bacterial spectrum of diabetic foot infections (DFI) is essential for managing the illness and preventing lower limb amputation, patients with severe DFIs reported a higher range of microorganisms, referred to as poly microbial infection (Radzieta *et al*., 2021).

Diabetic foot ulceration (DFU) is defined as localized findings of inflammation or purulence at the site where a diabetic patient's malleoli are located (Paulson *et al.,* 2018) .The patients with diabetes are most susceptible to foot infections due to neuropathy, vascular insufficiency and decreased neutrophil function (Bader, 2008).

Bacteria can enter the ulcer and cause an infection, which increases inflammation in the surrounding tissue, this worsens the condition and delays healing, the presence of bacteria stimulates the immune system’s response, which increases inflammation.

Sometimes, the body's response may not be enough to fight the infection; high blood sugar levels promote the growth of bacteria, increasing the risk of infection. Diabetes can also weaken the immune system. If the infection is not treated, the ulcer can develop into more serious conditions, such as gangrene. Appropriate assessment of the severity of the wound is necessary to determine if hospitalization, the best course of antibiotic to take, whether surgery is required, and if multiple staging systems involving physical examination findings, symptoms of systemic inflammation, and ischemia are required (Thurber *et al*., 2017).

Fibrinogen or acute-phase protein is glycoprotein that essential for blood clotting , it is produced by the liver and it another indicator of inflammation elevated , levels of fibrinogen can be observed in patients with DFUs due to the inflammatory response, it is involved in the formation of a fibrin matrix, which is essential for wound healing, monitoring fibrinogen levels can help in assessing the inflammatory status and guiding treatment strategies in DFU management (Li *et al*., 2016).

In addition, DFU has a complex etiology that includes both environmental and genetic components. The role of the monocyte chemoattractant protein-1 gene Polymorphism (rs1024611) in diabetic foot ulcers plays a key part in the inflammatory process and is a pro-inflammatory cytokine. Few studies have examined the impact of the *MCP-1* polymorphism and its correlation with *MCP-1* expression levels in DFU patients (Obied *et al*., 2019). Furthermore combined effects of C-reactive protein (*CRP*) gene, is an important component of the human immune response, it part of the acute-phase response and is produced by the liver in response to inflammation. It plays a critical role in the immune system by binding to dead cells and certain bacteria, which promotes their clearance by immune cells, elevated levels of CRP in the blood are associated with inflammation, infection, and chronic diseases, making it a useful biomarker for various conditions, including

DFU ,cardiovascular diseases, autoimmune disorders, and infections (Brull *et al.*, 2003) .

1.1 Aims of Study:

This study aims to investigate the role of polymorphisms of *MCP-1* and *CRP* genes in determining the severity of the disease in among patients with and without diabetic foot ulcers; this was achieved by following objectives:

1- Study the association of some demographic factors in all diabetic patients with and without foot ulcer.

2- Isolation and identification of aerobic pathogenic bacteria in diabetic foot ulcers using culture media, biochemical tests and the VITEK 2 system.

3- Estimation the fibrinogen level for all patients with and without ulcers by Fibrinogen Reagent Kit (clotting).

4- Determination the polymorphism of *MCP-1 and CRP* genes by ARMS method using the PCR technique

1.2 Literatures Review

1.2.1 Diabetes Mellitus (DM)

Diabetes Mellitus is a disease of metabolism marked via increased blood glucose levels brought by deficiencies in insulin action, secretion, or both, along with varying degrees of impairment in the metabolism of proteins, lipids, and carbohydrates (Schleicher *et al*., 2022). Type 1 and type 2 diabetes are two primary forms of diabetes mellitus identified by the etiological classification type 2 diabetes makes up more than 90% of cases of diabetes mellitus worldwide. Type 1 occurs in young people when they secrete little or no insulin at all, while type 2 diabetes occurs in older people who suffer from obesity because the beta cells have become functionally weak and cannot secrete insulin sufficiently and naturally, or because the body's cells do not respond to insulin and are resistant to it. The most common metabolic abnormality lead to type 2 diabetes through eating large amounts of sugar, protein, carbohydrates, and fats can lead to high blood sugar levels, which puts pressure on the pancreas to produce more insulin, making it difficult for the body to use insulin effectively (Forouhi *et al.*, 2022).

Insulin, a polypeptide hormone responsible for sugars, is secreted by the β-cells in the pancreatic islets; appears in the Langerhans islets. It speeds up the blood glucose's entry into body cells so that it may be turned into energy, a vital component in controlling blood glucose levels is the hormone insulin, which is generated by the pancreas (Ahmad, 2014) .

The role of insulin is important in the body through action, as it carries glucose to the body's cells that depend on it as a source of energy after insulin binds to the receptor proteins on the surface of the cells to allow glucose to enter. However, in the case of disease, the cells are weakly responsive to insulin or resistant to it, and thus do not allow glucose to enter, or insulin is weakly able to carry glucose, which

leads to the accumulation of large amounts of glucose, which causes beta cells to secrete an increase in insulin, which causes Increased urination because the kidneys are trying to excrete an excess amount of glucose that the body does not need with water, and increased hunger because the cells are losing glucose and need to compensate. All of this causes weight loss in the long term due to the disease (Petersen *et al.*, 2018).

1.2.1.1 Symptoms of Diabetics

Many symptoms of type 2 diabetes appear gradually but they differ in severity that initially appear gradually before progressing to one of the complications leading to the majority of cases (Chamberlain *et al.*, 2016). There are wide range of typical symptoms include increased thirst, dry lips, urging more frequently of the need to urinate the reason is that the kidneys are trying to remove the high amount of glucose in the body through urination and enhanced hungry feelings even after eating food (Beale, 2013).

Additions The patient may not realize small wounds or injuries until they become severe and slow healing wounds back to high glucose levels have a negative impact on blood circulation reducing the transfer of oxygen and nutrients to tissues. Diabetes can weaken the immune system making the body less able to resistance infection, high levels of sugars, fat and cholesterol in the blood can contribute to nerve damage which reduces the sensation of pain in the extremities as a result (Zhuang *et al*., 2022). Lack of fluids in the body can lead to dry skin, making it more susceptible to cracks, skin infections, infections such as fungi and bacteria, changes in skin color, especially in areas exposed to friction and ulcers or sores on the feet due to poor blood circulation or neuropathy (Begic *et al*., 2016).

1.2.1.2 Epidemiology of Diabetes

Diabetes continues to be a major global source of morbidity and death and is a serious health problem, according to the newest estimates; there are 537 million adults worldwide with diabetes in 2021, in the following years that number is predicted to increase, because of the unequal distribution of this burden 75% of adult diabetics reside in low- and middle-income nations. Diabetes is a disease that affects thousands of people in Iraq and ranks as the fifteenth most common cause of mortality in the country as a whole the Iraqi, type 2 diabetes is a significant public health concern , estimates suggesting that around 10-15% of the adult population may be affected by diabetes , disease was the cause of 2.9% of fatalities in world , 3.2% of cases of diabetes in Iraq (Neama, 2020 ; Forouhi and Wareham, 2022) .

It is widely recognized that adults and older people are susceptible to type-2 diabetes that the risk of type-2 diabetes rises with age. However, the number of pediatric patients reporting type-2 diabetes is rising. According to estimates, 8–45% of anew-diagnosed cases of diabetes are the pediatric and adolescent age groups (Almahfoodh *et al*., 2017). In light of the increasing rate of childhood obesity, it is anticipated the frequency of type-2 diabetes would continue to climb (Copeland *et al*., 2011).

One of the reasons for the increased spread of diabetes in different environments is that in high-income countries that obesity and a lack of activity are frequently associated with higher prevalence rate but in low- and middle-income, countries rising prevalence type 2 some individuals in low income countries lack awareness of the risks of consuming excess sugar, leading to increased consumption (Abusaib *et al*. 2020 ;Forouhi and Wareham, 2022).

1.2.1.3 Pathogenesis of diabetic

Insulin resistance and a relative decrease in insulin production are two characteristics of type-2 diabetes, which is a term used to describe chronic, worsening metabolic problems it is a major pathophysiological event in type-2 diabetes, according to multiple investigations (Ma *et al*., 2018; Petersen and Shulman, 2018) .

Diabetic is a complex illness influenced by resistance to insulin causes body cells especially those in the liver, fat, and muscle when the body does not respond well to insulin; glucose is not transported from the blood into the cells effectively, leading to high blood sugar levels، to combat insulin resistance, the pancreas secretes larger amounts of insulin but over time, the pancreas may not be able to continue producing this much, as the stress on the pancreas increases, the beta cells responsible for producing insulin can deteriorate, leading to decreased insulin production (Zhao *et al*., 2019).

Blood sugar levels and insulin resistance are impacted by fat cells, which are an important contributor in type 2 diabetes, when fat accumulates in the body, especially in the abdominal area, insulin resistance increases, it can effectively store glucose, which leads to high blood sugar levels, then release more glucose into the bloodstream, which further exacerbates the problem. Genetics diabetes may run in families, suggesting a hereditary propensity, physical inactivity due to insulin resistance and weight gain might result from irregular activity (Solis-Herrera *et al*., 2021).

Genes play an important role in regulating how insulin works in the body. They affect how the body's cells respond to insulin; some people have genes that make their cells less sensitive to insulin, which can lead to insulin resistance and genetic

changes are linked to an increased risk of diabetes. In addition problems using tobacco is linked to a higher incidence of type 2 diabetes (Imam, 2013) .

Diabetes type 2 is caused by a complex pathological sequence that involves numerous components that work together to generate the disease as diets high in sugar, refined carbohydrates, and unhealthy fats can promote insulin resistance, family history can play a role in the likelihood of developing insulin resistance, which may contribute to insulin resistance and insulin resistance tends to increase with age. A sedentary lifestyle through the availability of unhealthy behaviors and the recent surge in obesity, lack of cultural, living and economic awareness are keys variables contributing to the global diabetic epidemic (Leahy, 2005).

1-2-1-4 Diagnosis of Diabetic

There are several reports of changes to the diabetes diagnostic criteria, diabetes and the co-morbidities it causes in fact, duration dependent. Therefore, in order to lessen the existing load of the disease, it is essential to diagnose diabetic cases as soon as feasible ((Schleicher et al. 2022).

Diagnosis is made through presence of symptoms including common symptoms include Increased thirst, frequent urination, increased hunger, fatigue blurred vision slow healing sores or frequent infections(Committee, 2006). In addition, tests on blood as fasting test for blood sugar determines blood sugar levels following an overnight fast normal is less than 100, prediabetes is100–125 mg/dL, diabetes is 126 mg/dL or above. Also the oral glucose tolerance test OGTT, takes blood sugar readings both before and two hours after consuming a sugary beverage is normal less than140 mg/dL is the diagnosis,140–199 mg/dL is prediabetes, diabetes at least 200 mg/d. The hemoglobin A1c test shows the average

blood sugar levels for the previous two to three months, diagnosis standard is under 5.7%, have prediabetes 5.7% to 6.4%, diabetes at least 6.5% (Abdulsaid *et al*., 2023).

A chronic illness with a high treatment difficulty rate, treatment of type 2 diabetes involves a combination of strategies aimed at controlling blood sugar levels it is usually treated with a mix of medication, lifestyle modifications, and frequent monitoring (Of and Mellitus, 2014). Getting medical care is essential to reduce the possibility of negative results, maintaining normal blood glucose levels during fasting and following periods requires patients to know two important things are exercise and self-blood glucose monitoring and support from doctors and health groups can help in understanding and managing the disease in order to lower the likelihood of complications from DM (Doshi and Friedman, 2017). In adding to taking an oral medication and insulin injections for peoples with long term diabetes who have diabetes and have not responded to oral therapy. One must also control food as eat balanced meals that contain adequate amounts of carbohydrates, proteins, and healthy fats; it is preferable to eat fiber from fruits, vegetables, and whole grains , exercise and weight loss in order to achieve blood sugar regulation (Magkos *et al* ., 2020).

1.2.1.5 Risk Factor of Diabetics

A number of risk factors, such as obesity, age, family history, sedentary lifestyle, influences the pathogenic process of type-2 diabetes. Unhealthy diets, inactivity, and smoking are the most prominent risk factors for rising diabetes epidemiology, these variables also lead to obesity, dyslipidemia, hypertension, CVD, and impaired glucose tolerance (Bellou *et al*., 2018)

Obesity is the primary risk factor for type 2 diabetes, about 80% of the risk associated with DM is thought be related to body fat, thus, even with there is a strong

association between obesity and T2DM, with insulin resistance, blood sugar levels may rise, increasing the risk of diabetes complications, importantly, the only treatable risk factor is obesity, as a result, reducing body weight and controlling obesity greatly lower the risk of type-2 diabetes, it promotes greater glycemic control with little medication use (Malone and Hansen, 2019). And almost all type-2 diabetic patients have BMI > 25, the sharp rise in the rate of the obesity and type2 diabetes can be caused by a person's genetic or family history are strongly associated. In addition, obesity has been related to the morbidities of diabetes, specifically hypertension and the problems related to the cardiovascular system, supporting by the fact that losing weight is strongly linked to a decreased risk of complications (Mertens and Gaal, 2002).

There is a direct relationship between blood pressure and blood sugar; high blood pressure can exacerbate insulin resistance, which raises blood sugar levels, some diabetes medications can affect blood pressure, and vice versa. Blood pressure medications can affect blood sugar levels because some medications may alter the body's response to insulin, increasing insulin resistance, type 2 DM can cause blood vessel problems, which can raise blood pressure. Where the percentage of those suffering was found 30–50% of people with type 2 diabetes have hypertension, which is common in diabetics and a significant risk factor for coronary artery disease and stroke (Schaper *et al.,* 2020) .

Moreover, type-2 diabetes commonly occurs among middle-aged and older adults. The risk of having type-2 diabetes increases with aging, indeed, type-2 diabetes is a long-term process, remains asymptomatic years after induction of the disease. Consequently, ages 45 to 64 is the most common age group diagnosed by type-2 diabetes, nevertheless, it is increasingly common among the pediatric and

adolescent population. This may be attributed to the increasing prevalence of obesity among the pediatric population (Campagna *et al*., 2019).

Men may be more likely to develop diabetes than women, they tend to store fat in their abdomen (visceral fat), a type of fat that is more strongly associated with insulin resistance, women are less physically active than men, increasing their risk of obesity and insulin resistance and they are less likely to seek health care or psychological support, which can lead to delays in detection and management of the disease (Jayalakshmi *et al*., 2020). One of the most risk factors for DM is smoking cigarettes and there is evidence linking smoking to an increased risk of type 2 diabetes so smoking can increase insulin resistance, making it harder for the body to use insulin effectively, smoking can lead to poor blood circulation causing serious diabetic complications (Schleicher *et al* ., 2022).

1.2.1.6 Complication of Diabetics

It contains both chronic and acute complications (Muneer and Akbar, 2021)

Acute complication the immediate consequences are Hyperglycemic Hyperosmolar State (HHS) and Diabetic ketoacidosis (DKA) is more common in people with DM are the acute complications, lowering insulin levels stimulates ketone body synthesis in the liver, which in turn promotes lipolysis in adipose tissue then acidosis is caused by a decrease in blood pH brought on by an increase in ketone bodies (Dhatariya *et al*., 2020).

Hyperglycemic Hyperosmolar State (HHS) is more common when a diabetic patient's blood glucose level is extremely high. Water is drawn osmotically from cells into the bloodstream and renal system, where it is subsequently eliminated via urine, the blood's osmolality increases due to the kidneys' loss of water (Dhatariya and Vellanki, 2017).

Chronic complication some of long-term effects of diabetes include peripheral neuropathy which can result in foot ulcers, amputations, and retinopathy which can cause vision loss, nephropathy which can cause renal failure and autonomic neuropathy which can cause gastrointestinal, genitourinary and cardiovascular symptoms as well as sexual dysfunction (Punthakee, 2018; Balaji, 2019). Diabetes can cause a number of complications for a person, but none is as severe as those that affect the feet, diabetic foot ulcers account for more than 20% of hospital entry related to diabetes (Ahmadishooli *et al*., 2020) .

1.2.2 Diabetic Foot Ulcer (DFU)

Diabetic foot ulcers are injuries to the all layers of skin that typically affect the soles of the feet; these injuries can include ulcerations, infections, and gangrene. They can arise from peripheral neuropathy or peripheral arterial disease, poor glycemic control, and foot deformities in people with diabetes mellitus (McDermott *et al.,* 2023) . One of the most prevalent metabolic disorders that impedes wound healing is diabetes mellitus, according to reports. The inflammatory and regenerative phases of wound healing are prolonged in diabetes people, moreover, 15% of people with diabetes develop to (DFU), which is the cause of 85% of related lower extremity amputations , it is a major risk factor for plantar ulceration and one of the most dangerous effects of DM type 2 (Smith-Strøm *et al*., 2017). DFU has the power to change blood vessels, skin, tendons, neurons, and even bone, the rise in DM prevalence is accompanied by an increase in DFU morbidity, most foot ulcers (60–80%) heal in 6–18 months after the initial inspection, although 10-15% remain active and 5–24% result in limb amputation (Paul and Das, 2019).Other numberous risk factors, such as sex, advanced age, long-term diabetes (more than 10 years), and a high body mass index, are associated with diabetic foot ulcers (Shahbazian *et al*., 2013).

Development of diabetic foot ulcers through peripheral neuropathy, bacterial infection, cell dysfunction, and peripheral arterial disease (Lim et al., 2017). Which is the greatest important factor because there is insufficient blood flow to ulcerated tissues, severe ischemia of the lower limb skin can result in necrosis, peripheral neuropathy may result in dysfunctions of the lower limbs' skin, including sensory, motor, and secretory functions, these pathological changes not only affect the feet's defensive sense and physical systems, but they also cause dry skin, which inhibits healing of diabetic wounds (Hunt, 2011; Alavi *et al.,* 2014) . Bacterial infections wounds cause a delay in the healing process, then cell dysfunction the quality of healing is directly regulated by the functional state of wound cells (Zubair and Ahmad, 2019).

1.2.2.2 Epidemiology of Diabetic Foot Ulcer

Diabetic foot ulcers are a serious health risk, especially for people with peripheral vascular disease and diabetes, for prevention, early detection, and efficient management. Their epidemiology is essential, according to global estimation the prevalence of diabetes in 2019 was 9.3% (463 million people) it will be rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045, the prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%) (Omar *et al*., 2023). The yearly rate has generally ranged from less than from 0.2% to 11% in hospitals treated specifically for diabetes an estimated 4.2 million deaths among 20–79-year-old adults are attributable to diabetes, diabetes is estimated to contribute to 11.3% of deaths globally (Rasmussen *et al*., 2017) . According to estimations from the International Diabetes Foundation (IDF) 40 million to 60 million people worldwide suffer from DFU similar frequency estimates of prevalence exhibit significant variability and are

impacted by variations in DFU classifications, and monitoring methodologies (Margolis and Jeffcoate, 2013).

The estimates that about 33 million adults with diabetes, or 6.3% of the global population, have DFU, though DFU was previously believed to be more common in North America, modern cohort studies show rates as high as 15% in communities of individuals with diabetes as in many countries, which is affected by several factors such as the level of healthcare and awareness about diabetes and some studies suggest that the prevalence of diabetic foot ulcers may range from 4% to 10% among diabetics in Iraq. However, there may be variation depending on the region and the type of healthcare available (Rasheed and Salih, 2018). Despite developments in wound care approaches over the past 15–20 years, longer healing durations and one-year recurrence rates of 20% still exist, the lifetime risk of DFU has been reported to vary from 15% to 25% , while a recent study by Armstrong *et al*.(2017*)* indicated that because expected life expectancy has increased lifetime risk may be higher between 19% and 34%.

1.2.2.3 Classification of Diabetic Foot Ulcer

Several classification techniques for diabetic foot ulcers have been developed, numerous wound classification systems are based on characteristics such the degree of infection, neuropathy, ischemia, depth of tissue injury, and location. When identifying ulcer features, a suitable classification system is crucial as it will facilitate the development of management strategies for DFUs (Ghotaslou *et al*., 2018). Diabetic foot ulcers able to be classify to multiple systems including the Wagner, IWGDF/IDSA systems, and others, where Wagner's classification system is the most simple, well known, extensively applied, and generally acknowledged classification system for the diagnosis and organization of ulcers in diabetic feet. It

uses six basic wound grades (grades 0-5) to determine the depth of ulcers (Mehraj and Shah, 2018). As shown in the table (1-1).

Table (1-1): Wagner's DFU classification (Mehraj and Shah, 2018)

Grade	Description
0	Do not yet have DFU, but are at high risk to develop one
1	Superficial ulcer
2	Deeper, full thickness extension
3	Deep ulcer that involves abscess formation, and osteomyelitis (infection of the muscle, tendon, joint, or bone)
4	Partial Gangrene of forefoot
5	severe gangrene all through the whole foot

This classification does not include situation to loss of protective sensation, infection and ischemia and thus its efficacy may differ between countries. While the IWGDF/IDSA classification was first published in 2004, other studies worldwide have since demonstrated a connection between the severity of an infection and elevated levels of inflammatory markers (Ozer *et al*., 2019).

The IWGDF/IDSA categorization has several advantages, including assisting physicians in making direct medical decisions regarding infections, having no discernible adverse effects, and being quite easy for physicians to employ based solely on a clinical examination. Additionally, academics and working clinicians now generally accept it (Monteiro-soares *et al*., 2020). This category was displayed in table (1-2).

Table (1-2) IWGDF/IDSA classification (Monteiro-soares *et al*., 2020)

Grade	Infection Severity	Manifestations
1	Uninfected Infected	No localized or systemic symptoms of disease There are a minimum of two of these items: - Localize drigidity or swelling -Erythema > 0.5 cm* around the wound -Localized pain and sensitivity -Localized elevation in temperature -Purulent discharge and not at all other reason(s) of an inflammatory reaction of the skin (e.g. trauma, breakage, thrombosis) -osteoarthropathy, clotting, break.
2	Mild	infection without any symptoms of systemic manifestations involving: -just the superficial layer of skin or subcutaneous tissue; no deeper tissues - any erythema existing does not extend >2 cm** round the wound
3	Moderate	infection including the following that shows no systemic symptoms: -erythema extending ≥ 2 cm* from edge of the wound -tissue deeper than skin and subcutaneous tissues, such as tendon, muscle, joint and bone
4	Severe	A foot infection accompanied by ≥ 2 of the following systemic symptoms (of the systemic inflammatory response syndrome [SIRS]) - Temperature >38°C - Respiratory rate > 20 times per minute
Add (O)after 3 or 4 ***	Highly severe	Infection concerning bone (osteomyelitis)

Note: * Infection refers to any part of the foot, not just of a wound or an ulcer; ** In any direction, from the rim of the wound. The presence of clinically significant foot ischemia makes both diagnosis and treatment of infection considerably more difficult; *** If osteomyelitis is demonstrated in the absence of ≥ 2 symptoms of local or systemic inflammation, classify the foot as either grade 3(O) (if ≥ 2 SIRS criteria)

1.2.2.4 Pathogeneses of Diabetic Foot Ulcer

The cause of DFUs is complicated and different, including a number of interrelated factors, the following are some of the main DFU pathogeneses (Syafril , 2018) .

- Neuropathy refers to a condition resulting from damage to the peripheral nervous system, which is plays a main role in the pathogenic process leading to foot ulcers and poor wound healing, sensory and motor peripheral neuropathy is an essential factor in the development of DFU (Savelieff *et al.*, 2024). Decreased sensation in the foot may result in damages, infections, and wounds being untreated, muscle atrophy and changed foot biomechanics brought on by motor neuropathy can result in wrong pressure distribution (Kucera *et al*., 2016) . In addition neuropathy symptoms are injuries, tingling, or numbness in the damaged nerves, weakness in muscles or absence of reflexes, reduced sense or balance blood pressure drops when standing, it is estimated that around 15% of people with diabetic neuropathy have a higher risk of developing DFUs and suffering one or more lower limb amputations (Shahbazian., 2013) .

- Peripheral arterial disease (PAD) is a condition characterized by the narrowing or blockage of the peripheral, the building up of fatty deposits in the artery walls, known as atherosclerosis, can obstruct blood flow, diabetes the risk of PAD may increase if diabetes is not well managed (Chun *et al*., 2019) . One of the main risk factors for PAD influenced by obesity, high blood pressure, and lack of exercise, in addition symptoms of PAD are ischemic rest pain prolonged pain in the feet or toes, caused by insufficient blood flow, skin changes discoloration, hair loss, thickened toenails; wounds or ulcers that heal slowly and claudication, cramping, pain, or discomfort in the leg muscles during physical activity, which is relieved by rest (Ikem *et al*., 2010) .

- Foot infection the consequences include severe diabetic foot ulcers, surgical intervention is frequently required for infections with severe grades, particularly in individuals with diabetes foot infections Cause DFUs by loss of sensation because diabetes can cause peripheral neuropathy, leading to reduced sensation in the feet, injuries or infections may go unnoticed, allowing them to worsen and vascular issues often affects blood flow impairing the body's ability to fight infections and heal wounds this can lead to the development of ulcers (Du *et al.*, 2022). When infections occur bacterial or fungal infections can break down the skin, leading to open sores, if not treated promptly, these sores can evolve into ulcers, increased pressure, foot deformities conditions such as Charcot foot (is a condition that affects the bones, joints, and soft tissues of the foot and ankle and characterized by progressive damage to the foot) or bunions can create pressure points. the body's inflammatory response to infection can lead to tissue damage and ulceration, particularly in already compromised areas of the foot (Richard *et al.*, 2011).

- Osteomyelitis is an infection and inflammation of the bone marrow that due to the infectious spread from the surrounding inflammatory soft tissues, ulcers that develop over bearing weight bones are more susceptible to ongoing stress and osteomyelitis. On the other hand, osteomyelitis often affects the first and fifth metatarsal heads, it is, in all severity, increases the chance of amputation four times more than soft tissue infection alone, (Sharma *et al*., 2022) . It is typically caused by bacteria, often from skin flora, that enter the bone through an open wound, poor blood circulation and neuropathy in diabetes can contribute to the development and progression of DFUs and subsequent infections. It is in fact more probable to occur in ulcers larger than 2 cm (Giurato *et al*., 2017). Furthermore, the existence of osteomyelitis lowers quality of life due to frequent admissions to hospitals and extended hospital stays. It is one of the most common consequences of DFUs and infection, it is responsible for 15%

of people with diabetic foot ulcers and 20% of individuals with diabetic foot infections (Jeffcoate and Harding, 2003).

- Impaired wound healing the stages of wound healing that hyperglycemia affects include granulation, re-epithelialization, and inflammation and impaired wound healing is a result of oxidative stress, aging and chronic diseases such as high blood pressure, family history, heart disease and diabetes. Van Nguyen, (2006) ; Paulson *et al.*, (2018)

1.2.2.5 Diagnosis of Diabetic Foot Ulcer

Diabetes patients often develop diabetic foot ulcers (DFUs), which are frequently brought on by infection, peripheral vascular dysfunction, and neuropathy (Hinchliffe *et al*., 2020).

Diagnosis of diabetic ulcers on the foot through history of the patient of review for diabetes care by system, timing, and drugs, examination of earlier infections or ulcers on the feet, besides, it discovers out about the tingling and numbness associated with neuropathy, physical assessment to check for wounds, nodules, redness, or swelling on both feet, examines for abnormalities such as bunions or hammertoes, , uses a tuning fork to assess feeling, also to measure blood flow and feels for pulses in the feet, as well as evaluation of ulcer by ulcer size, depth, and appearance and presence of secretions or necrotic tissue with surrounding state of the skin (Alavi *et al*. 2014 ; Tuttolomondo *et al*. 2015).

In addition, laboratory examinations by blood glucose levels for evaluation of diabetes, cultures of wounds to detect infection, perform a complete blood count (CBC) to look for a systemic disease (Kareem and Mohammed, 2024). Determine the degree of inflammation, evaluate inflammatory markers such as erythrocyte

sedimentation rate (ESR) and CRP, and then use X-ray imaging investigations to rule out osteomyelitis or bone disease (Das *et al*., 2023).

Determining the wound's vascular and infectious state is essential to treatment DFU. The mainstay of care is giving sufficient antibiotics, hyperglycemia management, foot cleanliness, and, when necessary, surgical debridement or amputation, Necrotomic debridement (Yesil *et al*.,2009). Which is a medical procedure that involves removing dead, infected or damaged tissue from wounds or ulcers in order to help speed up the healing process by removing obstacles that may be an environment for microorganisms as bacteria and fungi growth that prevent wound healing and reduce the risk of infection, in addition, it helps improve the overall appearance of the wound and facilitates subsequent repair processes, it is an important and crucial step in the treatment of DFUs (Baal , 2004). The DFI treatment guidelines state that, depending on the severity of the infection, empirical antibiotic treatment should be selected for clinically infected wounds (Lipsky *et al* ., 2012). Decreasing the load/pressure on the wounded region, managing the infection by identifying the kind of bacteria, and treating, clean, moist wounds due to DFU therapy (Lipsky *et al*., 2004 ;Rebolledo *et al* ., 2011)

1.2.2.7 Bacteriological Spectrum of DFU

The best definition of infection is the invasion and growth of microorganisms in the tissues of the host, which causes an inflammatory response in the host and typically results in damage to tissues (Lipsky *et al* ., 2020) . One of the causes of bacterial infection is nerve damage, which leads to loss of sensation, making the patient unable to feel any injury, poor blood circulation. Because it reduces the body's ability to fight infection, and the presence of sugar in the blood, which increases bacterial growth, DFIs are mostly caused by skin ulcers, wounds ,certain types of trauma, and peripheral neuropathy, a lack of protective sensation (Kwon

and Armstrong, 2018; Idrees *et al*. 2024). Even though bacteria colonize all wounds, at least two of the standard indicators of inflammation or purulence indicates the existence of infection, the next step is to categorize the infections as light (superficial), moderate (deeper) or severe (supplemented by systemic signs), this classification system together with a vascular valuation helps to determine which patients need to be hospitalized, which ones might benefit from surgery, and which ones will need amputation (Lipsky *et al* ., 2012).

While the majority of DFUs appear to be rather superficial, microorganisms can extend near to subcutaneous tissues, such as muscles, tendons, joints, fascia, and bones (Aragón-Sánchez *et al*., 2012). The microbial infection patterns in DFU are not stable, the bacterial profile indicated a poly microbial pattern, with cocci being the most frequently identified pathogen (Chalya *et al*., 2011). It has been determined that the majority of DFIs include poly microbial organisms the most prevalent of which aerobic gram-positive cocci are the most common, aerobic gram-negative bacteria that were commonly responsible for persistent infections that continued after antibiotic therapy, while obligate anaerobes microbiological may be pathogens in ischemia or necrotic lesions, according to a study on the spread of microorganisms and bacteria including both anaerobes and aerobes, were found to be growing in all diabetic foot wounds (Aldhfyan *et al*., 2018).

Bacterial species obtained from DFI were used to isolate the microorganisms from different depths of ulcers. The results by Mohsin and Jasim . (2023) of the isolation procedure included isolates of Gram-positive bacteria as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus,* and *Enterococcus faecalis,* while *Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli* were isolated from Gram-negative bacteria, *Klebsiella pneumoniae.* The ulcer microbiota was dominated by gram-positive species, primary

Staphylococcus aureus. The gram-negative sector was mainly formed by Pseudomonas aeruginosa and Enterobacteriaceae (Proteus spp., Enterobacter spp., Escherichia coli and Klebsiella spp.). With increase in age, P. aeruginosa and S. aureus became more frequent, while Streptococci decreased. Ischemic and/or deep wounds were more likely to bear gram-negative species (Dörr *et al.*, 2021).

1.2.3 Role of Molecular Markers in Diabetic Patients

Many genes in the body are affected by diseases and lead to changes in them such as Monocyte Chemoattractant Protein (*MCP*)-1 and C-reactive protein (*CRP*) genes, where *MCP-1* is small proteins that are a part of the cytokine family are called chemokines, the *MCP-1* gene, also identified as CCL2 (C-C motif chemokine ligand 2. The protein-encoding gene is located on chromosome 17, the 76 amino acids that make up human *MCP-1* (Harvanová *et al*., 2023)). It job is to attach to the right receptors and control the migration of cells to areas of tissue damage or infection, particularly leukocytes, it is one of the most important chemokines that regulates monocyte and macrophage migration and infiltration throughout the immune response, Monocyte migration via the vascular endothelium into the circulatory system is essential for both routine immunological tissue surveillance and infection response (Saoud *et al*., 2019).

In addition, *MCP-1* polymorphisms influence, and it has been suggested that increased *MCP-1* expression has existed to a number of health disorders, such as diabetes, heart illness, multiple sclerosis, and cancer, elevated MCP-1 levels are often linked to chronic inflammation, for several reasons high levels of MCP-1 continuously attract monocytes and other immune cells to the site of injury, leading to persistent inflammation, this chronic influx can perpetuate inflammation and creating a cycle that inhibits the healing process the immune cells drawn to the site by MCP-1 release various inflammatory mediators (cytokines, enzymes) that can

damage surrounding tissues, this tissue damage can slow down the natural repair mechanisms, making it harder for wounds to heal, it requires new blood vessels (angiogenesis) to supply nutrients and oxygen to the wound and chronic inflammation can disrupt the integrity of the skin and surrounding tissues, making it easier for pathogens to enter and cause infections. which can hinder wound healing and contribute to conditions like diabetic foot ulcers (DFU) (Mohammed and Jasim, 2020; Singh *et al.*, 2021). It is primarily secreted from several cell types as macrophages response to inflammatory stimuli, Adipocytes (fat cells) secrete MCP-1 linking it to obesity and related inflammatory conditions, endothelial cells (cells lining blood vessels) response to injury or inflammation (Saoud *et al*., 2019).

While the human C-reactive protein gene (*CRP*) is a substance produced by the liver in response to inflammation, it plays a starring role in the immune system, helping to identify and strong present dead cells and pathogens such as arthritis, respiratory infections ,DM , and heart disease, also it can be used to monitor the effectiveness of treatment in some medical conditions, it is located on chromosome 23, encodes the C-reactive protein, (Hu *et al*., 2022).

Elevated levels of CRP are associated with chronic inflammation, which is a contributing factor to insulin resistance, this resistance can lead to the development of type 2 DM ,then blood sugar levels are high, and the body may increase CRP production as part of its inflammatory response, this can create a cycle where elevated glucose levels lead to increased inflammation and further insulin resistance. Adipose tissue secretes pro-inflammatory cytokines, further stimulating *CRP* production. Poor glycemic control has been linked to higher *CRP* levels, managing blood sugar levels can help reduce inflammation and lower *CRP* expression and genetic variations in the CRP gene itself can influence individual responses to diabetes and susceptibility to complications (Amirian *et al*., 2020). In DFU, tissue

damage gets worse by the proliferation of polymorph nuclear neutrophils, which further impedes the healing process and leads to the development of chronic wounds, one feature of DFU is an acute-phase response lengthening due to CRP reactants in the acute phase (CRP) (Li *et al*., 2016) . Additionally, recent clinical and experimental studies have demonstrated a connection between *CRP* and insulin resistance with beta-cell dysfunction of T2DM patients because chronic inflammation can impair pancreatic beta-cell function elevated *CRP* and other inflammatory markers affecting insulin secretion (Zee *et al*., 2008).

1-2-4 Fibrinogen as Physiological Parameters

Fibrinogen is a glycoprotein found in the blood that serves as a physiological marker , it is composed of three pairs of unique polypeptide chains and it is a protein created in the liver and is a main component of blood. It acting a key role in blood clotting and fibrinogen services inflammatory indication that elevated in cases of inflammation or infection and stimulates wound healing (Oo *et al*., 2020).

when occur a damage or bleeding after arterial injury it is transformed to fibrin by thrombin , when a blood vessel is injured or damaged, thromboplastin is activated to help start the clotting process, the thromboplastin protein is produced mainly in cells known as platelets and in other tissues such as the liver, it combines with calcium, which acts as a clotting factor, converting inactive prothrombin into thrombin. Which cleaves fibrinogen to produce fibrin, it is the material that forms a blood clot which stop bleeding the most frequent component to prevent blood loss , fibrinogen is the main coagulation agent because bleeding is more likely to occur when plasma fibrinogen concentration is low (*Afrah et al*., 2020).

Diabetes is associated with elevated levels of fibrinogen, DM particularly type 2 is characterized by a state of chronic inflammation, increased production of

inflammatory cytokines stimulates the liver to produce more fibrinogen, leading to elevated levels in the blood stream . Prolonged high blood sugar levels can lead to glycation of proteins including fibrinogen which can increase its levels and activity in the blood, And many individuals with type 2 diabetes are overweight or obese, adipose tissue can secrete inflammatory cytokines further promoting the production of fibrinogen, higher fibrinogen levels indicate a tendency toward increased clot formation, this is particularly concerning in diabetes, where the risk of thrombosis (blood clots) is already elevated (Lowe *et al*., 2004; Sola *et al*., 2007) .

In addition, it is create in the liver highly when vascular issues in people with type 2 diabetes arise because high blood sugar levels cause glucose to build up in the blood. Which can damage blood vessel cells and surrounding tissues and high blood sugar contributes to increased inflammation in the body, which leads to tissue damage and increases the risk of atherosclerosis, this monomer rapidly aggregates to connect to surrounding molecules, forming the core of the blood clot leads to PAD, CVD, and stroke are among the consequences (Li et al., 2016). High levels of fibrinogen increase the likelihood of blood clots forming, which can lead to pulmonary embolism. Fibrinogen is link to the development of atherosclerosis, where it can contribute to the buildup of fat and plaque inside the arteries, increasing the risk of heart attacks and strokes (Khudder *et al*., 2019)

According to estimates people with DFU had greater amounts levels of fibrinogen than people without ulcers because individuals with DFU the inflammatory response is heightened, leading to elevated fibrinogen levels and often microvascular damage which can exacerbate inflammation and further increase fibrinogen levels in individuals with DFU which can complicate the healing process of ulcers (Pase *et al*., 2018).

Blood fibrinogen levels typically range from 200 to 400 mg/dl, High fibrinogen levels are usually those greater than 400 mg/dL elevations in blood، the reasons for its increased concentration are pneumonia, kidney infections, and cancers. Its levels linked to sedentary lifestyle, and numerous variables, both modifiable and non-modifiable, affect fibrinogen itself, age, sex, smoking, body mass index (BMI), hypertension, glycemic management, lipid profile, and hypertension. There are a number of factors that affect the level of fibrinogen, including infections, medications, poor nutrition and chronic diseases (Pieters and Wolberg, 2019).

Chapter Two

Patients, Material, and Method

2.1 Patients

Present investigation as a case study design involved 100 participants: 50 subjects having type 2 diabetes patients with foot ulcers, 50 subjects Type 2 diabetes patients without foot ulcer. Samples have been obtained from the Al-Imam Al-Hassan center for diabetes and endocrinology. In addition, age range (33-76 year). The samples were collected between August 2023 and December 2023 from individuals of both sexes, a case information form was taken for each patient, it had some inquiries about: sex, age, and other information was completed (appendix -1).

2.1.1 Patient Inclusion Criteria

Clinical symptoms and further testing identified diabetes mellitus type 2 without a foot ulcer and patient with diabetic foot ulcer.

2.1.2 Exclusion Criteria

Patients without diabetes develop foot ulcers, aged less than 33 years, diabetic mellitus type 1, pregnant women, endocrine diseases, kidney diseases ،liver diseases and skin diseases such as psoriasis**.**

2.2 Ethical Approval:

The College of Science/University of Kerbala's Ethical Committee accepted this study. Before the sample was collected, all participants in this study were informed and their verbal consent was acquired.

2.3 Body Mass Index (BMI):

It is a number that is calculated using a person's height and weight. BMI = weight (kg) / (height (m)) ^2, When is BMI < 18.5 (Underweight), 18.5 – 24.9 (Normal weight), 25 – 29.9 (Overweight) and BMI 30 or greater (Obesity).

2.4 Study design:

The study design approach was displayed in figure (2-1). The current study was designed to be cross-sectional.

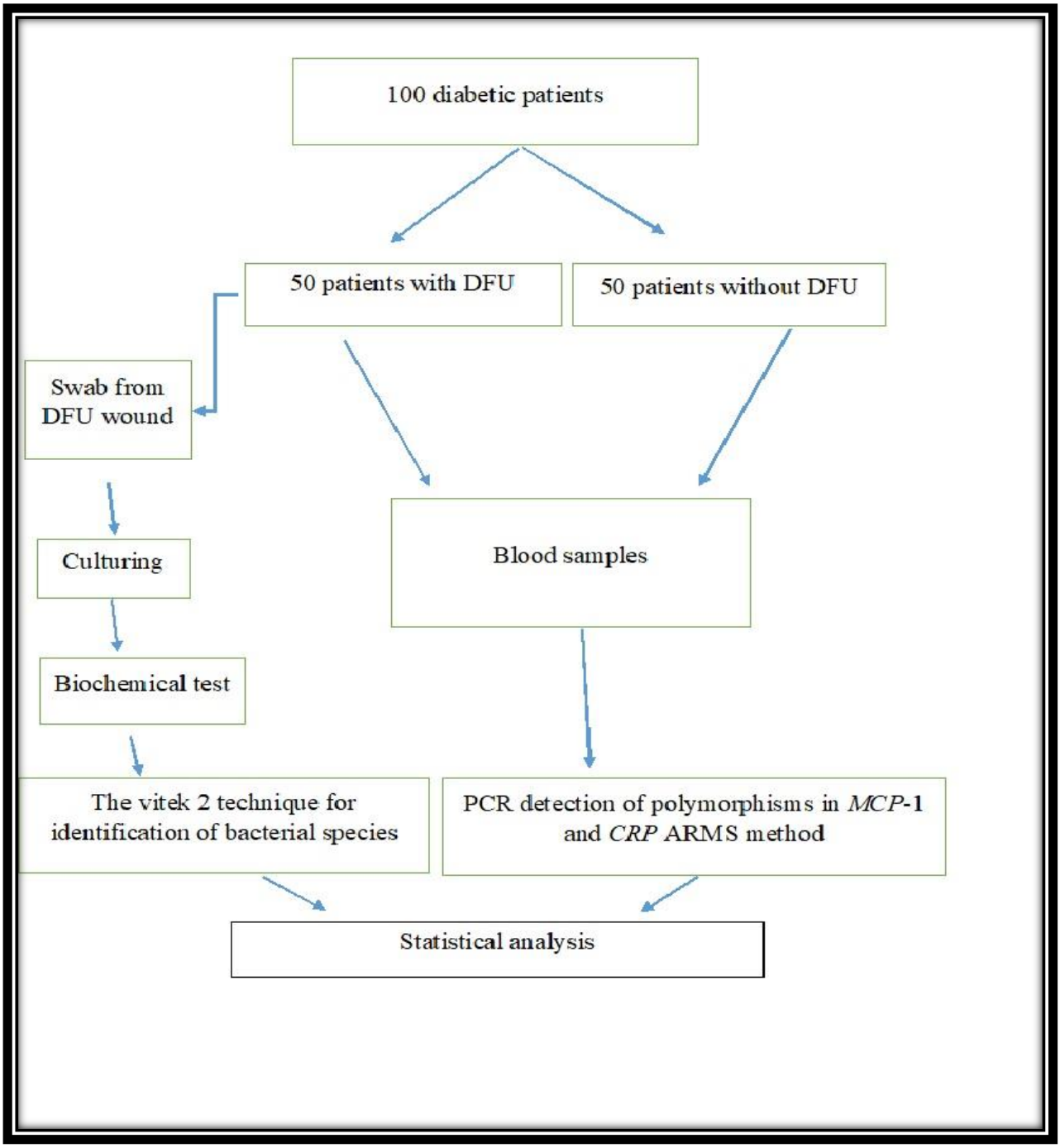

Figure (2-1): study design scheme.

2.5 Materials

2.5.1 Apparatus and Equipment Used in Laboratories

All apparatus and equipment utilized for this investigation were illustrated in table (2-1).

Table (2-1): Names of all apparatus and equipment with their nation of origin and manufacturing company.

Names of laboratory Equipment and instruments	Company/ Origin
Autoclave	Hirayama /Japan
Benzene burner	Amal /Turkey
Centrifuge	Hitachi/Japan
Compound light microscope	Medline /England
Conventional PCR system	Biobase/China
Cooling centrifuge	Biobase/Cina
Cotton	Almodawa/Iraq
Deep freeze refrigerator	Concord /Lebanon
DensiChek	Biomerieux / France
EDTA tube	Zhongfan medical/china
Eppendrof tubes	Geneaid/korea
Fibrinogen Reagent Kit	P.R/ China
Filter paper	Himedia /India
Gel electrophoresis	Biobase/China
Glass Beaker	Iwaki glass- Japan

Glass graduated cylinder	Jlassco - India
Gloves	Marco Medical SDN/Malaysia
Incubator	Memmert (Germany)
Inoculation Loop sterile	Himedia (India)
Micro centrifuge tube	Geneaid/Korea
Microbiologic safety cabinet	Nuve (turkey)
Micropipette (different sizes)	Eppendorf/ Germany
Micropipette tips (different size)	Human/ Germany
Microwave oven	Samsung /Korea
Oven	BioTek- England
Petri dishes	Himedia/India
Plane tube (10 ml)	AFCO(Jordan)
Plastic rack	Meheco -China
Polymerase chain reaction PCR	Biobase -China
Sodium citrate tube	Concord /Lebanon
Sensitive electrical balance	WTW (Germany)
Slide and cover slide	Super star/India
Spectrophotometer	Tuder -Korea
Sterile cotton swab	AFCO/Jordan
Syringes 3 ml	DMK/China
UV Transilluminator	Biobase/China
Vitek 2 compact	Biomerieux -France
Vortex mixer	IKA (Germany)
Water distiller	GFL(Germany)

2.5.2 The Culture Media

Culture media that utilized for current investigation were displays in table (2-2)

Table (2-2): Culture media with their manufacturer company.

Name	Purpose of use	Company (Origin)
Brain-heart agar	grow and preserve bacterial isolates for long periods	Liofilchem (Italy)
Mannitol salt agar	medium ferments mannitol and is specific for isolating staphylococci	
MacConkey agar	medium distinguishes between bacteria that ferments lactose and those that do not, making it selective for Gram-negative bacteria	

2.5.3 Chemical and Biological Materials

Chemical and biological materials used in this study listed in table (2-3).

Table (2-3).The country and manufacturing use in companies biological and chemical materials.

Name of material	Company(Origin)
Absolute Ethanol	Bioneer/ Korea
Agarose	Conda/Aspain
Ethanol 70%	MIRNIA/Iraq
Ethidium bromide	Bioneer/ Korea

Glycerol	BDH -England
Hydrogen peroxide	UN/Germany
Normal saline	Choueifa/ Lebanon
Nuclease free water	Add bio -Korea
Oxidase	Himedia (India)
Potassium hydroxide	BDH -England

2.5.4 The VITEK2 kits Utilized in this Study

The VITEK2 kits of current study were displayed in table (2-4)

Table (2-4): Lists VITEK2 kits utilized in this study research along with the origin and company.

Kits	Company /origin
AST-GN222	Biomerieux (France)
ID- GP Card	
ID- GN Card	

2.5.5 Materials to Amplify DNA

2.5.5.1 DNA Extraction

The components of the DNA extraction kit used in this investigation are shown in tables (2–5).

Table (2-5): Components of DNA extraction kit

DNA Extraction Kit	Company /origin
collection tubes 2ml	Geneaid/Korea
Elution Buffer 30 ml	
GB Buffer 40	
GD Colum 2 ml	
Wash1 Buffer 45 ml	
Wash2 Buffer 45 ml	
Proteinase k	

2.5.5.2 Master Mix

Master Mix components that was utilized in PCR proceeding are displaying in table (2-6).

Table (2-6): Master mix compositions

PCR Master Mix composition	Company /origin
Taq DNA polymerase	Bioneer/korea
dNTPs (dATP, dCTP, dGTP, dTTP)	
Reaction buffer with 1.5 mM MgCl2	
Stabilizer and tracking dye	

2.5.5.3 PCR Constituents

The contents of the PCR constituents have been illustrated in table (2-7).

Table (2-7): PCR constituents

PCR constituents	Company /origin
DNA ladder	Bioneer/Korea
Master mix	Bioneer/ Korea
Free nuclease water	Promega /USA
TBE (Tris-Borate EDTA) buffer	MarLiJu/ Korea

2.5.5.4 Primer

The *CRP* gene primers were designed in accordance with the nucleotide sequence gene bank website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). While, the *MCP-1* gene polymorphism was found in this investigation using the following primers by Saoud *et al*. (2019), The *MCP-1* and *CRP* genes polymorphisms listed in table (2-8)

Table (2-8): Lists the primer sequences for the *CRP* and *MCP-1* genes (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)

Genes	**Primers sequences (5′-3′)**	**Amplicon (bp)**
MCP-1 (rs3917887)	P1:5-GCTGATCTTCCCTGGTGCTGAT-3 P2:5-CATTAAATCCCAGTGCTTCTGCCTA-3	IN/202 De/188
CRP rs1130864	F-G:5CCTCAAATTCTGATTCTTTTGGACCG F-A:5CCTCAAATTCTGATTCTTTTGGACCA R- 5CCCTTCAGTCCTAATGTCCTGAACT	375

2.5.6 Materials for Determining the Patient's Fibrinogen Levels:

The fibrinogen testing is prescribed as part of an investigation of a possible bleeding disorder or inappropriate blood clot formation. Each box contained:

- A desiccant pouch and a test strip are supplied in each of the 24 separate sealed bags.
- Optical coagulation Analyzer.
- ID chip.
- 0.109 mol/l trisodium citrate anticoagulant.
- Transfer pipette or capillary tube. Fibrinogen Reagent Kit (clotting) has been stored at 2-30°C; the strip was used within 15 minutes.

2.6 Methods

2.6.1 Samples Collection

2.6.1.1 Swab Samples of DFU:

The swab was used to collect the specimens; first, the ulcer was cleaned with saline to remove any surface contaminants, and the ulcer was scraped to obtain exudate before sampling, then the swabs were applied in a zigzag pattern over the wound area, torsion the swab to ensure that head of the swab was perfectly came in touch with the wound surfaces. Finally, the swabs were applied from wound center outwardly to edges of the lesion (Travis., 2023). The swabs were placed in a transport medium. The swabs were cultured on blood agar and macConkey agar and incubated for a 24 hour at 37°C.

2.6.1.2 Blood Samples

Disposable syringes have been employed to get 5 milliliters of blood from all individuals participated in current research, It has been placed three milliliters of blood are inserted in a sodium citrate tube for measuring each patient's fibrinogen level, and two milliliters of blood are deposited in an EDTA tube was employed to extract the DNA from the genome for genetic study (MacFadden, 2000 b).

2.6.2 Sterilizing Method:

2.6.2.1 Ethanol 70%

Ethanol 70% was used for cleaning the workbench's surface and some study aids (Harrigan and McCance 2014) .

2.6.2.2 Autoclave (Moist Heating)

Using moist heat sterilization, the culture medium was sterilised for 15 to 20 mins at 121°C as well 1.5 bar of pressure (Harrigan and McCance, 2014)

2.6.2.3 Oven (Dried Heating).

The glass wares have been sterilized for two hours at 160 °C by dry heat using the electrically operated oven (Harrigan and McCance, 2014)

2.6.3 Preparing of Media (Blood Agar)

Prepare this culture medium according to the manufacturer's instructions (Liofilchem/Italy) by dissolving 40.0 gm of blood agar base in one liter of D.W. The medium was heated until completely dissolved, and then sterilized by autoclave cooled to 45 °C, and 5% of fresh human blood was added. It was used as an enrichment medium for the bacterial isolates and to determine their ability to hemolysis RBCs (MacFaddin, 2000 a) ..

2.6.4 Culturing of Samples

The swabs were streaked on blood agar ,macConkey agar and mannitol agar , then it incubated for overnight at 37°C under aerobics conditions. The plates had checked to determine the growth and the isolated bacteria were diagnosed by using morphological colonies and biochemical characteristics (Dubey and Maheshwari, 2023).

2.6.4.1 Oxidase Reagent

The manufacturer states that the oxidase reagent is made by dissolving 0.1 gm of tetramethyl-para-phenylenediamino dihydrochloride in 10 ml of distilled water at

a concentration of 1%. Microorganisms that might produce the cytochrome oxidase enzyme can be identified using the oxidase test (Green and Goldman, 2021) .

2.6.4.2 Catalase Reagent

The catalase reagent was made at a concentration of 3%, according to the manufacturer's company, by mixing 1 milliliter of 30% hydrogen peroxide with 9 milliliters of pure water; it has been employed to identify bacteria that are capable of producing the catalase enzyme (Green and Goldman, 2021).

2.6.4.3 Coagulase Reagent

For performing tube test, the bacterial isolates were adding to the plasma in the test tube, coagulation of plasma(including any thickening or formation of fibrin threads) point out to a positive result, the plasma is ordinarily exanimated for clotting (without shake) subsequent to approximately 4 hours, as the coagulation may happen too soon and get back to liquid within 24 hours (Benson, 2002) .

2.6.5 Bacterial Identification with the VITEK2 System

Identification of bacterial isolates are performed by using the automated Vitek- system, which is produced in France by BioMerieux, it depends on the medium in the VITEK-2 Identification Cards and the biochemical reactions between the bacterial colonies suspended in their solutions (Pincus *et al*., 2006). The identification of bacteria in according to instruction of manufacturer is the next stages.

1. In a sterile tube, one pure colony of the isolated bacteria was suspended in three milliliters of physiological saline.

2. The DensiChek VITEK instrument was used to measure the turbidity of the bacterial suspension, and the results showed that it was between 5.0 and 0.63.

3. Each tube examination included the installation of a VITEK 2 cassette, and the tubes were then arranged in their own racks.

4. After transferring the rack (with the tubes as well cassette) to the system, the rack was putted in the first filler field, which automatically filling the cassette with bacterial suspension. When procedure was done, the appliance sent the finish signal.

5. The proffer order is a digital signal that was held, and second field reader (reader), of which the first was to cut the tapes, had been moved to it, on a PC linked to the VITEK2 system, the rack containing the tube slides out of the apparatus to show the data for every sample.

6. After the taps were left running at 37 °C for a full day, the findings were analyzed to determine whether bacteria were present, the results showed that 96% to 100% of the isolates had excellent identifying, 93-95% had very good identifying, 89-92% had good identifying, 85- 88% had acceptable identifying, and there was no identification in the other isolate.

2.6.6 Genetic Analysis :

2.6.6.1 Procedure for Extracting DNA

The steps involved in extracting DNA were as follows:

The blood was collected in EDTA tubes.

1. Two hundred µl of whole blood had been transferred to a 1.5 ml micro centrifuge tube.
2. Two hundred µl of Proteinase K had been added to the sample tube and gently mixed.
3. The mixture had been placed in incubation at 60 °C for 10 minutes. During incubation, the tube was inverted every 3 minutes.

4. Two hundred µl of GB buffer had been added to the sample and mixed by vortex for 10 seconds.
5. The tube was incubated for at least 10 minutes at 70°C to ensure that the sample lysate had been cleared. During incubation, the tube was inverted every 3 minutes.
6. Two hundred µl of absolute ethanol had been added and immediately mixed by shaking vigorously for 10 seconds.
7. Carefully apply the mixture from step 7 to the GD Column (in a 2 ml collection tube), close the cap, and centrifuge at 14,000 rpm for 1 minute. Discard the filtrate and place the column in a new 2 ml collection tube.
8. Four hundred µl of wash1 buffer had been added to the GD column. The centrifugation process was achieved at 14000 rpm for 30 seconds.
9. The flow-through had been discarded, and the GD column was placed back in the GD column. A volume of 400µl of wash2 buffer had been added to the GD column. The centrifugation process was achieved at 14000 rpm for 30 seconds
10. The centrifugation had been done at 14000 rpm for 3 minute to dry the column matrix.
11. The dried GD column had been transferred to a clean 1.5 micro centrifuge tube.
12. One hundred µl of pre-heated elution buffer had been added to the center of the column matrix and left to stand for at least 3 minutes to ensure the elution buffer was absorbed by the matrix.
13. The centrifugation process had been achieved at 14000 rpm for 30 seconds to elute the purified DNA.
14. The purifier DNA was stored at -20 °C until used for PCR.
15. The purity and concentration of the DNA obtained were determined through 260/280 nm absorbance measures using the NanoDrop.

2.6.6.2 Preparation of solution

- **Buffer Triose Borate-EDTA (TBE)**

This solution was prepared according to the manufacturing company, MarLiJu/Korea, manufactured this solution by diluting it from (10X) to get (1X) and adding 100ml of triose borate EDTA buffer (10XTBE) to 900 ml of steriled D.W. The mixture was then stored at room temperature (Sambrook and Russell, 2001)

- **Primer preparation**

The primers have been prepared according to instructions of manufacturer, where the lyophilize primers had been dissolved in deionize distilled water for creating a stock solution with a concentricity of 100 pmol/µl. The solution was prepared separately for each primer concentration 10 pmol/µl by taking 10 µl of the primer with 90 of deionize distilled water and mixing the mixture by vortex Store solutions at 20°C.

2.6.6.3 Mix the Polymerase Chain Reaction (PCR) method

All the necessary components for performing the polymerase chain reaction were added to the tubes in the PCR kit for *MCP-1* and *CRP* genes are listed in tables (2-9) and (2-10), respectively.

Table (2-9): Components of the PCR product for *MCP-1* gene

Ingredients	**Amount (µL)**
Master mix	10
rs3917887-p1/ F	2
rs3917887-p2/ R	2
Nuclease-Free Water	7
DNA sample	4
Total	25

Table (2-10): Components of the PCR product for CRP

Ingredients	**Amount (µL)**
Master mix	10
Rs1130864- F/ G	2
Rs1130864 –F/ A	2
Rs1130864 / R	2
Nuclease free water	7
DNA sample	4
Total	27

The PCR mixture's ingredients were added to a PCR tube, which then neatly mixed using the vortex for 5 second. These tubes had then moved to PCR thermal cycle appliance, where they were heated to the optimum temperature for DNA replication.

2.6.6.4 Amplification-Refractory Mutation System Technique for PCR Conditions

Amplification Refractory Mutation System method for genotyping is a technique used to detect specific mutations in genes, this method is particularly useful for identifying single nucleotide polymorphisms (SNPs) and can be applied in various fields as commonly applied in research to study genetic variations associated with diseases. ARMS uses allele-specific primers that can amplify only the target sequence, Components of ARMS from two sets of primer forward and reverse, the method is based on the principle that DNA polymerase requires a perfect match between the primer and the template to initiate amplification. PCR (Polymerase Chain Reaction) products can be analyzed using method gel electrophoresis, where the presence of bands corresponding to the mutant genotype. Advantage ARMS high specificity for detecting specific mutations. In addition, it relatively simple and cost-effective compared to other genotyping methods and rapid results often within a few hours (Moon, 2024).

Specific primer pairs and the traditional PCR have been utilized for amplifying the target DNA, the PCR products are created by amplification-refractory mutation systems (ARMS) procedure. The standard method consisted of three stages: denaturation, annealing, and elongation, each stage was repeated cycle after cycle, cycling conditions of *MCP-1* and *CRP* are listed in table (2-11) and (2-12).

Table (2-11): Cycling conditions of *MCP-1* gene

Step	Temperature	Time	Number of cycles
Initial Denaturation	95 °C	5 Minutes	1 cycle
Denaturation	95°C	30 Seconds	29 cycles
Annealing	60°C	60 Second	
Extension	72°C	60 Seconds	
Final Extension	72°C	5 Minutes	1 cycle

Table (2-12): Cycling conditions of *CRP* gene

Step	Temperature	Time	Number of cycles
Initial Denaturation	95 °C	5 Minutes	1 cycle
Denaturation	95°C	30 Seconds	30 cycles
Annealing	65°C	65 Second	
Extension	72°C	60 Seconds	
Final Extension	72°C	5 Minutes	1 cycle

2.6.6.5 Electrophoresis on Agarose Gel

This procedure was followed in accordance with (Krieger *et al*. 2004). Agarose gel electrophoresis has been used to prove that PCR amplification was successful. 0.75 g of agarose powder and 50 ml of previously made TBE buffer (X1) were combined to create an agarose, the liquid had heated until boiling and then cooling it to 50°C , adding 3 µl of ethidium bromide before to use, the agarose was put in a stabilize gel tray, closed at both ends, and a comb was fastened to the end, it was let to consolidate at room temperature for 30 mins. Then both seal and comb were gently taken out of the tray, the comb made wells were filled with the PCR product, subsequently filled one agarose gel well was filled with a ladder of DNA markers (3 µl) and electrophoresis chamber has been filled with DNA samples (3 µl), DNA has been transferred for 35 minutes, electricity had a 60 volt current, and examine the gel by exposing it to AUV trans illuminator was employed to observe the DNA bands.

2.3.7 A determination of Fibrinogen levels in-Patients

The level of fibrinogen in DFUs cases and diabetics with no foot ulcer is determined in a number of steps as follows:

1- The ID chip was removed from the kit and inserted into the ID chip slot after the device was turned on.

2- The test strip was removed from its foil pouch.

3- The device was operated by heating the test strip to the required temperature.

4- 20 ml of sample was loaded into the sample container.

5- The result was displayed on the main screen.

6- The used test strip was removed from the device.

7- The fibrinogen concentration in the blood sample was measured in g/L, which is the reference range determined using an apparently healthy individual from the fibrinogen (clotting) reagent kit.

2.3.8 Statistics Analysis

The current study data have processed using Statistical Package for the Social Sciences software, version 26. Data was analyzed for means, and standard deviations (SD) were calculated for the continuous variables, while frequencies were calculated for qualitative data. Correlation between analyzed parameters had assessed using Pearson regression, the mean of the investigated biomarkers were comparing between studied groups by t-Test. Chi-Square analysis was employed to significant compare between percentages; differences among groups were analyzed using one-way ANOVA analysis of variance. Duncan's test was used to detect critical values for comparisons between means. The results of all hypothesis tests with p-values smaller than 0.05 (two-side) were deem as statistically significant. A receiver operating characteristic (ROC) is effective statistical technique for assessing the effectiveness of binary classification models is (ROC) analysis, a single scalar value that summarizes (AUC), the model's performance across all thresholds , curve had analyzed for assessing the research indicator for predicting the disease activity by fibrinogen (Duncan ,1983 ; Basher, 2003). In addition, SPSS program and Microsoft Excel 2010 program applied to draw the figures in the present investigation.

Chapter Three

Results and discussion

3.1 Demographic Data for Study Groups

The descriptive data for the study groups are displayed in table (3-1), these data including: age, sex, diet, residency, economic status, and education status, relied on the statistical outcomes of current study. Most of parameters compared between the study populations of diabetic patients with and without foot ulcer showed significant differences ($p<0.05$).

Table (3-1): Descriptive data of the study groups

Age group (year)						***P value*** **(P ≤ 0.05).**
Study Group	**33-43**	**44-54**	**55-65**	**66-76**	**Total**	
Diabetic with foot ulcer	3 (6.0%)	8 (16.0%)	19 (38.0%)*	20 (40.0%)*	50 (50%)	0.045
Diabetic without foot ulcer	5 (10.0%)	13 (26.0%)	16 (32.0%)*	16 (32.0%)*	50 (50%)	0.0047
Total	8 (8%)	21(21%)	35 (35%)*	36 (36%)*	100 (100%)	0.0001
Sex						
Study Group	**Male**	**Female**		**Total**	***P value*** **(P ≤ 0.05).**	**ODD (CI95%)**
Diabetic with foot ulcer	32 (64.0%)*	18 (36.0%)		50	0.005	2.087 (.936 – 4.653)
Diabetic without foot ulcer	23 (46.0%)	27 (54.0%)		50	.٤٢٣NS	
Total	55 (55.0%)	45 (45.0%)		100 (100.0%)	.٣١٧NS	
Diet						
Study Group	**Vegetarian**	**Non vegetarian**		**Total**	***P value*** **(P ≤ 0.05).**	**ODD (CI95%)**
Diabetic with foot ulcer	7 (14.0%)	43 (86.0%)*		50	0.0001	1.000 (.323-3.095)

Diabetic without foot ulcer	7 (14.0%)	43 (86.0%)*	50	0.0001	
Total	14 (14.0%)	86 (86.0%)*	100 (100%)	0.0001	
Residency					
Study Group	**Urban**	**Sub-urban**	**Rural**	**Total**	***P value* (P ≤ 0.05).**
Diabetic with foot ulcer	36 (72.0%)*	10 (20.0%)	4 (8.0%)	50	0.0001
Diabetic without foot ulcer	35 (70.0%)*	10 (20.0%)	5 (10.0%)	50	0.0001
Total	71 (71%)	20 (20%)	9 (9%)	100 (100%)	0.0001
Economic status					
Study Group	**Lower class**	**Middle class**	**Higher class**	**Total**	***P value* (P ≤ 0.05).**
Diabetic with foot ulcer	22 (44.0%)*	21 (42.0%)*	7 (14.0%)	50	0.0001
Diabetic without foot ulcer	18 (36.0%)*	29 (58.0%)*	3 (6.0%)	50	0.0001
Total	40 (40%)	50 (50%)	10 (10%)	100 (100%)	0.0001
Education status					
Study Group	**Illiterate**	**Secondary school or less**	**Graduate or above**	**Total**	***P value* (P ≤ 0.05).**
Diabetic with foot ulcer	19 (38.0%)*	21 (42.0%)*	10 (20.0%)	50	0.032*
Diabetic without foot ulcer	9 (18.0%)	21 (42.0%)*	20 (40.0%)*	50	0.029*
Total	28 (28%)	42 (42%)*	30 (30%)	100 (100%)	0.009*
*Mean significant differences at level of 0.05 by χ2-test NS: Non-significant					

3.1.1 Study Groups Distribution Based on Age:

The findings of current work showed a significant increase diabetic with foot ulcer in 55-65 was (38.0%) and 66-76 was (40.0%) age groups compared with other ages, and diabetic without foot ulcer 55-65 and 66-76 were (32.0%) compared with other groups in both diabetic's groups according to previous investigations by Manda *et al*. (2012) and Al-Rubean *et al*. (2015) who found a same trend in diabetic without foot ulcer (35%) and diabetic with foot ulcer (39%),the prevalence increases with age because, it is more common in older individuals, and older diabetic patients had a higher frequency of DFU than younger diabetic patients. Foot ulcers are among the diabetic complications that are impacted by aging, because adults age their risk of foot ulcers increases and they having chronic health illnesses that might make managing diabetes and ulcer healing more difficult, older persons may have diabetes for a longer period of time, which can lead to more serious problems according to McDermott *et al*. (2023).

However, a second study done by Kafrawy *et al.* (2014) who discovered no relationship between age and individuals with diabetes type2 with or without foot ulcer, the reason for the contradiction was differences in study groups and average age taken by the researcher.

3.1.2 Study Groups Distribution Based on Sex:

The results of the current investigation showed that males were more affected by DFUs that were significantly higher (64.0%) than females (36.0%) in diabetic with foot ulcer group while sex did not shown significant differences in diabetic without foot ulcer group, this result was consistent with other studies such as Manda *et al*. (2012) as well as Alam *et al.* (2017), which demonstrated that males were more likely than females to acquire diabetic foot infections. Furthermore, Jayalakshmi *et*

al. (2020) assessed the quality of life of DFUs subjects and demonstrated that, (81.40%) were of those subjects were male, while only (18.60%) were female and this result in the same line of present study. This may be due to factors such as lifestyle, health behaviors, and personal cleanliness, men tend to have higher rates of uncontrolled diabetes, high blood pressure and women may be better protected from some complications that increase the risk of developing foot ulcers, a reason differences in how men and women approach health care with men being more likely to seek medical help later. In addition, the demographics of people with diabetes may influence rates, with communities with a higher male population being more likely to have DFU. Similar findings were found a previous research, where researchers have suggested that males work outside for a longer period of time, which in the end increases the possibility of foot injuries and damage (Patil and Mane, 2017 ; Vanherwegen *et al*., 2023) .

In contrast, additional studies have indicated no statistically significant variation between males and females, and this is inconsistent with the results of current study. Shahbazian, (2013) and Saleem *et al*., (2017) whose suggested that eating habits and physical activity are may similar between the sexes and that men and women have similar medical histories also the reason for the difference is due to the geographical distribution of sex which the samples were taken.

3.1.3 Study Group Distribution Based on Diet

The results of the current investigation showed the context of dividing patients according to the nature of the diet, which included a vegetarian group and a non-vegetarian group, where the percentage of non-vegetarian was (86.0%) significantly ($p<0.05$) higher, while the percentage of vegetarian was (14.0%) in both diabetic patients .

Following a plant-based diet can help prevent onset of diabetes as well as obesity, antioxidants and fiber included in plant meals can help lower inflammation and increase healing and helps improve blood sugar levels by slowing down the digestion and absorption of sugar (Basiri *et al*., 2022). Plant foods, often lower in calories, it is provide a wide range of vitamins and minerals that support overall health which can help by maintain a healthy weight being overweight is a major risk factor for diabetes and contain healthy fats such as unsaturated fatty acids ,while animal foods may contain saturated fats that can lead to insulin resistance and meat foods that is processed and other non-vegetarian foods may include additional sugars or chemicals that raise blood sugar levels (Agrawal *et al.* 2013 ; Apergi *et al.* 2023).

The present study's findings are consistent with the investigations conceded by Jiang *et al.* (2015) and Huang *et al*. (2022) whose discovered that the type and quantity of food have an immediate effect on people who have diabetes mellitus, raising their risk of developing DFU, also BMI values of meat eaters were highest, that of vegans was lowest . Conversely, current study's results did not align with the data published by Bechara *et al*. (2021) and Donnelly *et al.* (2022), those suggested that there is no relationship with the type of food, even healthy foods can lead to weight gain or high blood sugar if eaten in large quantities, and an unbalanced vegetarian diet may lack some nutrients such as proteins.

3.1.4 Study Group Distribution Based on Residency

The current study's results indicated 72% of DFU and 70% of diabetic without foot ulcers resided in urban areas, and these percentages were significantly higher compared to Sub-urban and Rural , urban areas and sub urban are more prone to diabetes and its complications compared to rural areas and districts because city dwellers usually live a more sedentary lifestyle, preferring modern means of transportation such as cars instead of walking, which leads to less physical activity,

as cities may lack green spaces, urban have more unhealthy food options, such as fast food and sugary drinks, which leads to increased consumption of calories and unhealthy fats (Mainous *et al.,* 2004) . In addition, stress and psychological pressure associated with the work environment and urban life can contribute to the development of diabetes, high levels of pollution in cities. this results alignment with results done by Azimi-Nezhad *et al*. (2008) and Pourkazemi *et al*. (2020).

On the other hand the findings of the present investigation did not match the details released by Bragg *et al. (* 2017), who found that there were no behavioral or socioeconomic risk factors that might account for the variation in the prevalence of diabetes mellitus, which was found to be 7.1% in rural and 12.1% in urban area because the level of awareness about diabetes and its management may have a greater impact than where they live, individuals who are better informed about the disease may make healthier choices

3.1.5 Study Group Distribution Based on Economic Status

The current study's results indicated, the percentage of both diabetic groups (with and without ulcers) was significantly lower in the higher class (14.0%) compared with lower class (44.0%), and middle class (42.0%) , this result aligns with studies accomplished by Suwannaphant *et al.* (2017) and Lo *et al.* (2021), that associated diabetes prevalence has increased more quickly in countries with low or middle incomes during the last ten years than in countries with high incomes.

Compared to wealthier people, low-income populations may have up to twice as many cases of diabetes. Low being poor is linked to a higher hospitalization rate for acute diabetes-related complications in persons with diabetes (Rabi *et al*., 2006). It is accepted as one of the health issues that has a direct correlation with an individual's economic status, People with poor incomes may have difficulty getting

quality medical care, which can delay diabetes diagnosis and treatment and raise the risk of foot ulcers, and the kind of food that people can eat depends on their income level. People with diabetes are more susceptible to ulcers due to the increased pressure on their feet caused by difficult work environments or activities that require standing for a long amount of time; the medical materials required for foot care may be out of reach for those with low incomes (Hashempour *et al*., 2024).

On the other hand, this study's findings challenged those of previously published study by Ha *et al.* (2021) who suggested there are no significant differences between the economic status and diabetes patients, and the reason is that the researcher collected the samples size small and from one class.

3.1.6 Study Group Distribution Based on Education Status

The current study's results indicated the significantly highest percentages of DFU were in illiterate (38.0%) and secondary school or less (42.0%) respectively, compared to graduate or above (20.0%), this result aligns with studies carried out by Yazdanpanah *et al.* (2018) and Midhin (2022). They finding it was clear that no patient with a greater educational background suffered from a higher-grade ulcer, it was demonstrated that there was a statistically significant correlation between the patients' work position and reading level and the severity of DFU, because patients with medium and high levels of education and skilled occupations had decreased rates of major amputations.

Higher educated people are more likely to be aware of the dangers of complications, and education helps patients had better understand how to take care of their feet by wearing the right shoes, checking them frequently, and maintaining them clean. People with higher levels of education might find it simpler to communicate with medical doctors, which would facilitate advice and appropriate

treatment. Healthy behaviors that assist regulate blood sugar levels and lower the risk of problems (Malik *et al*., 2023).

Conversely, the results of this study that indicating that there wasn't correlation between education and DM2 by Azimi-Nezhad *et al.(* 2008), the reason for the difference in results was that the sample was taken from a community with one cultural level.

3.2 Clinical Characteristics of Study Groups

The clinical characteristics of the study groups, a significant ($p<0.05$) effect in duration of disease were recorded, there are a significant increase in diabetic patients with foot ulcer group in period (>1-3 week) 46.0% and (1-3 month) 36.0% compared with other period, while (1-5year) 34.0% and (<5years) 50.0% periods significantly affect more than others in diabetic without foot ulcer patients, the clinical characteristics are displayed in table (3-2)

With regard to distribution of other disease between diabetes with and without foot ulcer, the majority of diabetes with foot ulcer was affected with hypertension 27 (54.0%), while majority of diabetes without foot ulcer were affecting with both hypertension and cardiovascular diseases 29 (58.0%) with a significant differences.

In the context of dividing patients according to obesity, a significant difference was recorded only in DFU group, where the patients with DFU were non-obese 30 (60.0%) and non-significant difference with the patients with DFU were obese 20 (40.0%), while diabetic without foot ulcer was obese 23 (46.0%) and non-obese 27 (54.0%).

Table (3-2): Clinical characteristic of study groups**.**

Duration of disease								
Study Group	**>1-3 week**	**1-3 months**	**4-7 months**	**8-11 months**	**1-5 year**	<5years	**Total**	***P value* (P ≤ 0.05).**
Diabetic with foot ulcer	23 (46.0%)*	18 (36.0%)*	6 (12.0%)	2 (4.0%)	1 (2.0%)	0 (0.0%)	50	0.000*
Diabetic without foot ulcer	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (16.0%)	17 (34.0%)*	25 (50.0%)*	50	0.000*
Total	23 (23%)	18 (18%)	6 (6%)	10 (10%)	18 (18%)	25 (25%)	100	0.000*

Other disease						
Study Group	**No disease**	**hypertension**	**cardiovascular**	**Both disease**	**Total**	***P value* (P ≤ 0.05).**
Diabetic with foot ulcer	2 (4.0%)	27 (54.0%)*	6 (12.0%)	15 (30.0%)	50	0.016*
Diabetic without foot ulcer	2 (4.0%)	12 (24.0%)	7 (14.0%)	29 (58.0%)*	50	0.015*
Total	4 (4%)	39 (39%)	13 (13%)	44 (44%)	100 (100%)	0.004*

Obesity					
Study Group	**Yes**	**No**	**Total**	***P value* (P ≤ 0.05).**	**ODD (CI95%)**
Diabetic with foot ulcer	20 (40.0%)	30 (60.0%)*	50	0.0455*	.783 (.354-1.730)
Diabetic without foot ulcer	23(46.0%)	27 (54.0%)	50	٠.٤٢٣NS	
Total	43 (43%)	57 (57%)	100 (100.0%)	٠.١٦١NS	

*Mean significant differences at level of 0.05 by - $\chi 2$ test

NS: Non-significant

3.2.1 Study Group Distribution Based on Duration of Disease

Diabetes duration was significantly related with diabetic foot ulcer development, the period of the ulcer is one factor that affects healing time, the likelihood of complications like foot ulcers increases with the length of time a person has diabetes those who have type 2 diabetes are particularly affected by this. Diabetes can harm nerves over time, impairing foot sensation and raising the possibility of injuries that might go undetected, furthermore, diabetes can damage blood arteries, decreasing blood flow to the limbs and slowing the healing of wounds, treatment for diabetic foot ulcers is difficult and taking time. Therefore, estimating patient outcomes for diabetic foot ulcer patients enables healthcare professionals to use efficient management techniques (Smith-Strøm *et al*., 2017). According to results of the present study a significant effect in duration of disease was recorded, there are significantly increase of DFU patients in period (>1-3 week) and (1-3 month) compared with other period, while (1-5year) and (<5years) periods significantly affect more than others in diabetic without foot ulcer patients, this finding is in line with the result of previous studies done by Nisar *et al*. (2015) and Caruso *et al*. (2020) who found (>3 week) and (1-5 month) significantly increase of DFU patients, while significantly increase (<6 years) periods with diabetic without foot ulcer patients.

On the other hand, this study findings inconsistence with a study of Gazzaruso *et al*. (2021) who showed non-significant result with duration of disease. The reason for the difference in results obtained in the current study is expected to be the close intervals in the duration of the disease in the patients from whom the samples were taken.

3.2.2 Study Group Distribution Based on Other Disease

Numerous studies have examined the connection between incident cardiovascular disease (CVD) and hypertension. These conditions can exacerbate the complications of diabetes, such as circulation issues that can impair wound healing and blood flow to tissues, which can hinder the normal healing of ulcers and infections. Additionally, individuals with high blood pressure may experience elevated levels of inflammation (Jeyaraman *et al*., 2019).

Through regard to distribution of other disease between diabetes with and without foot ulcer, the majority of diabetes with foot ulcer was affected with highly significant differences that hypertension (54.0%), while majority of diabetes without foot ulcer were affecting with both hypertension and cardiovascular diseases (58.0%).The present finding is similar to the results of Qiu *et al.(* 2015) who founded highly significant differences with hypertension (50.0 %) in DFU, while diabetes without foot ulcer were affecting with both hypertension and cardiovascular diseases (70.0 %) which indicated diabetics are at greater risk of cardiovascular disease, high blood sugar can damage blood vessels and nerves, increasing the risk of heart attacks and strokes. Contrary to the results of current study, (Brownrigg *et al.,* 2012) reported that diabetes without foot ulcer were affecting with cardiovascular disease about (70%) . The reason for the differences may be that all of the researcher's samples were suffering from heart disease and high blood pressure.

Many diabetics also have high blood pressure, which puts additional stress on the heart and blood vessels, high blood pressure can worsen heart problems cause mortality that is around twice as high as risk of non-diabetics, individuals with DFU may have an even greater death risk (Chin *et al.,* 2024)

3.2.3 Study Group Distribution Based on Obesity

Obesity is a critical risk factor for diabetes, which is related to insulin resistance (IR), the effect of obesity on the gradual deterioration in insulin secretion, accompanied by its role in insulin resistance, is what leads to the development of diabetes in obese people (Wondmkun, 2020).

When fat accumulates in the abdominal area, it leads to increased insulin resistance; this means that the body needs more insulin to regulate blood sugar levels, excess body fat can cause chronic inflammation, which negatively affects the body's ability to use insulin effectively, as insulin resistance increases blood sugar levels can rise leading to the development of diabetes. In addition to genetic factors, individuals with a family history of obesity or diabetes are more likely to develop diabetes if they are obese. (Golay and Ybarra, 2005; Chen *et al.*2023).

Although the current study did not record significant differences regarding the relationship between diabetes and obesity, 60% of DFUs were not obese, and there were significant differences, as similar result were reported by Freemantle *et al.* (2008) ; Mariam *et al.* (2017) and Wondmkun (2020) . This result explains that the long period of diabetes causes a lot of weight loss, and after years, diabetes complications lead to diabetic foot ulcers, so patients appear thin.

While the result of (Guo *et al*., 2023) indicated that there is a statistically significant correlation between obesity and DFUs because obese or overweight can significantly disrupt the natural blood circulation pattern in the lower limbs, which boost the risks of progression DFUs.

3.3 Concentrations of Fibrinogen in Patients According to Other Disease

The statistics analysis recorded a significant rising of fibrinogen levels of diabetic patient's foot ulcers hypertension, cardiovascular and both disease (595.85, 609.67, and 590.27) comparing diabetic patients without ulcers (282.83, 312.43, 311.97) according to other disease, except for regarding to no disease group showed insignificant differences, as shown in table (3-3).

Table (3-3): The Concentration of fibrinogen in cases according to other disease

Other Disease	**Con. of fibrinogen in patients Mean ± Std. Deviation**		***P value*** **(P ≤ 0.05).**
	Diabetic with foot ulcer	**Diabetic without foot ulcer**	
No disease	602.00±79.196	327.50± 104.500	0.147^{NS}
hypertension	595.85±48.357	282.83± 60.558	**0.000***
cardiovascular	609.67± 18.522	312.43± 20.057	**0.000***
Both disease	590.27± 30.837	311.97± 73.600	**0.000***
Total	596.08±41.351	305.66± 67.755	**0.000***
P value **(P ≤ 0.05)**	0.815^{NS}	0.609^{NS}	
*mean significant differences at p ≤ 0.05. NS: Non-significant			

Fibrinogen is one of important inflammatory parameters, it is one of crucial protein that implicated in blood coagulation, a key determinant for blood viscosity

and aggregation of platelets (Danesh *et al*., 2001). Regardless of inflammatory processes, it is formation the low permeability fibrin clots, disturbances blood flow, along with platelet hyperactivity and engaged with the progression atherosclerosis (Li *et al.,* 2016).

Elevated fibrinogen is linked to diabetes mellitus, it is produced in the liver and, after the coagulation pathway is activated and is changed into a fibrin monomer (by thrombin). This monomer then quickly aggregates to bind to nearby molecules, producing the main of the blood clot. The statistics analysis recorded a significant higher levels of fibrinogen in diabetic patients with foot ulcer comparing to diabetic patients without ulcer in patients have both hypertension and cardiovascular diseases, these results are agreement with the study done by Kotbi *et al*., (2016); Yao *et al*.,(2016) and Khudder (2019) .

Patients with other diseases have a significant effect on fibrinogen, so it is critical for determining the level of fibrinogen in type 2 diabetic patients, besides the usefulness of these indicators in the prediction of vascular lesions in people with newly diagnosed hypertension or diabetes. Previous investigations have addressed the significance of therapeutic regulation of fibrinogen levels in high-risk patients for cardiovascular (Corrado *et al*., 2006). Fibrinogen, which is elevated in DFU, given the fact that patients with DFU have been shown to have greater levels of fibrinogen than people without ulcers. Therefore, the statistical analysis demonstrated that diabetic patients with foot ulcers had significantly higher fibrinogen levels. (Pase *et al* ., 2018 ; Afrah *et al*., 2020a).

Constantly there was not a significant difference in patient studies by (Sechi *et al.,* 2000). The reason for the discrepancy is due to the possibility of a delay or

mistake in examining the samples for the fibrinogen level after they are taken directly from the patients, which leads to incorrect results.

3.4 Bacteriological Study

The bacterial culture results are displayed in figure (3-1), the majority 76% of samples culture have a bacterial growth, while 24% only presented with no growth.

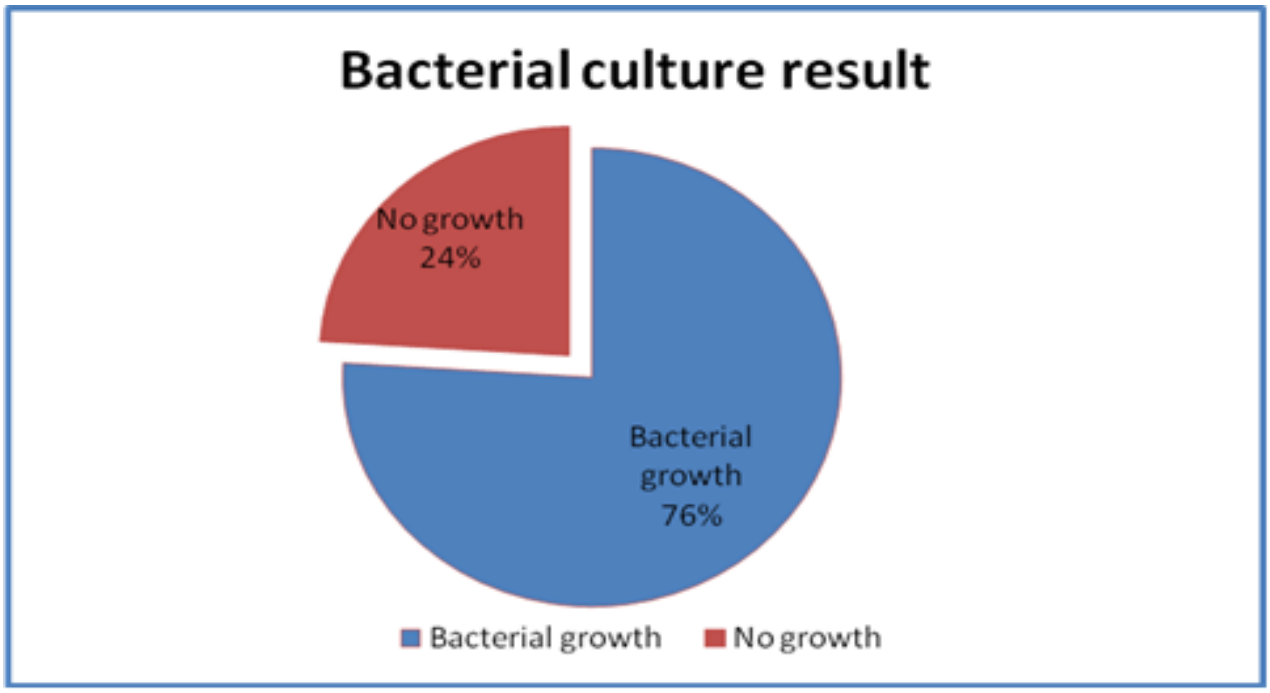

Figure (3-1): Percentage of bacterial culture

Bacterial growth in diabetic foot ulcers is an important topic in health care, it occurs when the tissues in the foot become damaged due to high blood sugar levels, which increases the growth of bacteria. In addition to poor circulation which reduces the body's ability to fight infection and neuropathy which reduces the sensation of pain, which worsens the injury. Present study statistics match aligns with multiple studies, including those done by Hurlow *et al*., (2018) and Dörr *et al.* (2021). While these results doesn't aligns with study performed by Du *et al.* (2022), where the

majority (95%) of samples culture having a bacterial growth, whereas, (5%) only presented with no growth. This variation in bacterial growth is due to the duration of infection and the severity of the diabetic foot ulcer, the more advanced the infection, the higher the bacterial rate.

Regarding to bacterial type, figure (3-2) revealed that the majority (78%) of bacterial growth were gram-negative bacteria, while only (22%) gram-positive bacteria.

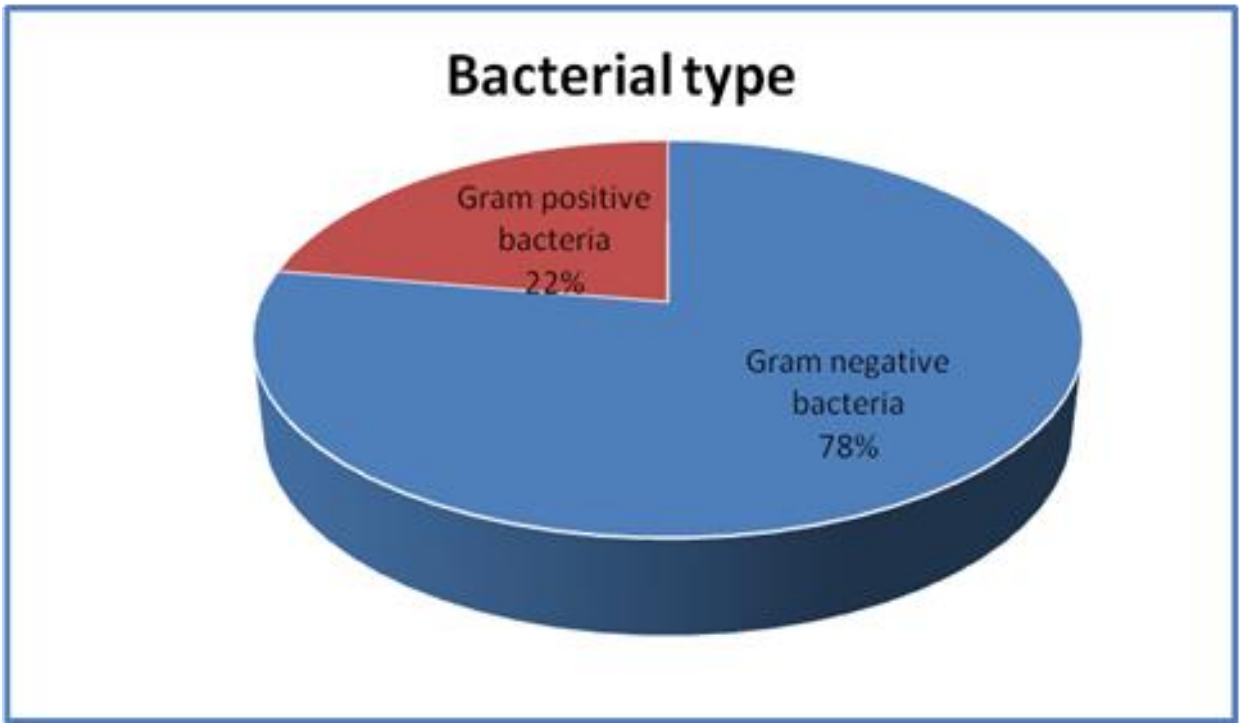

Figure (3-2): Percentage of bacterial type

The present finding compatible with a number of studies, including those of (Palomo *et al*., 2022) and (Shi *et al*., 2023). However, in their studies that conducted, respectively, the percentages of incidences of Gram^{+}ve bacteria were (52.3%) and (68.1%) respectively. The present results were disagree with those of multiple investigations, that Gram –ve bacteria were found to be the most common type (52.4%, 75.9%, and 59.2%) respectively (Adeyemo *et al*., 2019 ; Rahman *et al*.,

2021; Du *et al*., 2022). This variance may be related to more frequent diabetic foot and the improper use of antibiotics in the developing countries. It was documented that Gram positive bacteria were prevalent in acute DFIs, whereas patients who had chronic wounds or had recently undertaken antibiotic therapy were at an enlarged risk of infection with Gram-negative bacteria (Lipsky *et al*., 2004).

In context of type of bacterial species isolated from ulcer, the highest percentage was for the bacterial species of *Staphylococcus aureus* (34%) , *Klebsiella pneumonia (*28%) and *Proteus mirabilis* (26%) , as shown in figure (3-3).

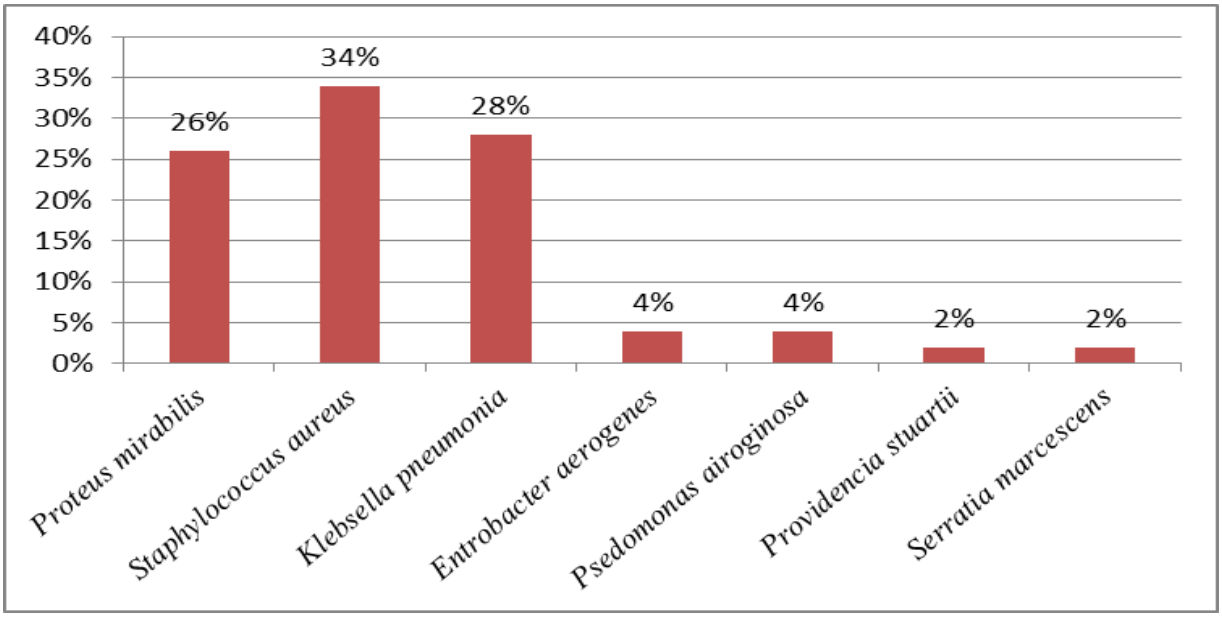

Figure (3-3): Types of bacterial species isolated from foot ulcer

Although variations in the distribution of bacteria , *S. aureus* continues to be the most common in numerous investigations conducted in many different countries , diabetes patients' skin as well as mucosal surfaces are frequently colonized by *S. aureus*, it appear as clusters, gathering in groups resembling grapes. They are often yellow or golden in color, which distinguishes them from other types of bacteria, which secretes a wide range of toxins and enzymes, including collagenase, lipases,

proteases, hyaluronidases, and hemolysis, these substances make the host tissue exceedingly conducive to bacterial infection and tissues invasion, including (71%) (Shettigar and Murali 2020) . *Klebsiella pneumonia* (15%) it primarily affects people who have weak resistances, which might lead to serious consequences, for people with diabetes mellitus, a significant problem can result in osteomyelitis and "diabetic foot" infections, As infection being started, *K. pneumoniae* creates a biofilms enable it to avoid the host defense (Du *et al*., 2022) and *Proteus mirabilis* (14%) as present in a study conducted, when *P. mirabilis* colonies were cultivated on blood agar, swarming colonies became visible. Initially, bacterial isolate got from clinical specimens were categorized accord to their cultures morphology, microscopic features, as well the biochemical examinations. When *P. mirabilis,* a gram-negative *bacillus,* was observed under a microscope, its colonial appearance was used to identify the species culturally by Du *et al*. (2022). Differences in the way samples are collected; the location, the type of treatment used, and the degree of the infection could all be contributing factors to the diversity in bacterial profiles found in DFU patients. Previous investigations had shown that ulcer's period and the use of antibiotics in the past are related to the bacterial type of DFU (Al-Rubeaan *et al*. ,2015; Banu *et al*. 2015 ; Shi *et al*. 2023)

Diabetes patient's skin as well as mucosal surfaces are frequently colonized by *Staphylococcus aureus*, it appears as clusters, often yellow or golden in color, which distinguishes them from other types of bacteria (Shettigar and Murali, 2020). Enterobacteriaceae is the family to which *Klebsiella pneumoniae* belongs. It primarily affects people who have weak resistances, which might lead to serious consequences, for people with diabetes mellitus, a significant problem can result in osteomyelitis and "diabetic foot" infections (Turkei and Al-Dulaimi, 2024). The infection being started, *K. pneumoniae* creates a biofilms enable it to avoid the host

defense (Akers *et al*., 2014). When *P. mirabilis* colonies were cultivated on blood agar, swarming colonies became visible (Al Fahadawi *et al*. 2019).

3.5 Association of Some parameters With Study Group

3.5.1 Smoking Effect

The distribution of study population according to smoking status, most of DFU patients and as well as diabetic with no foot ulcer were smokers (64%, 78%), respectively shown in figure (3-4).

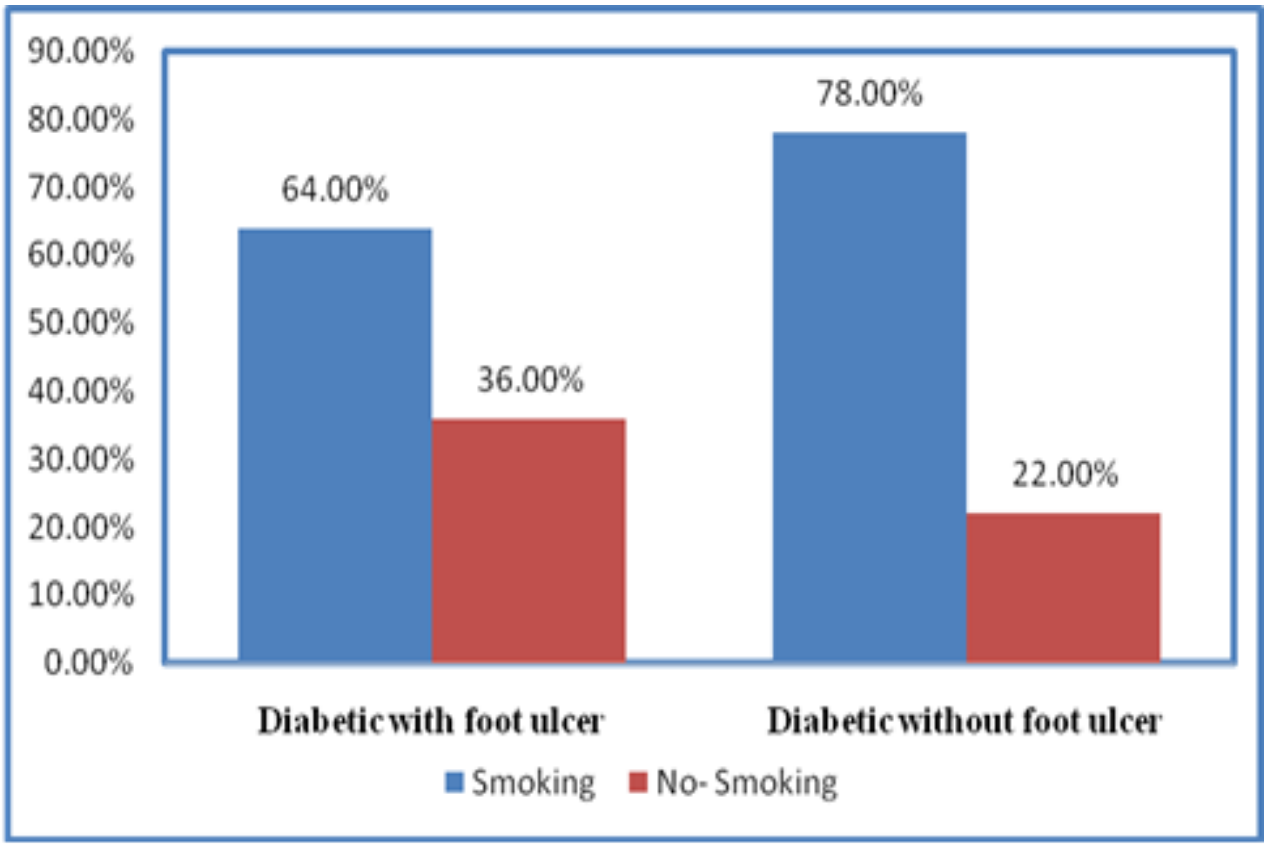

Figure (3-4): Distribution of Smoking in study groups

A wide range of studies conducted across many demographics have reported a favorable correlation between the development of diabetes mellitus and active smoking (Luo *et al*., 2013).

Smoking is known to raise the risk of DFUs and to slow down the healing process that numerous studies showed that the byproducts of cigarette smoke, including as carbon monoxide and cyanides, which impeded the normal metabolism of healing. In addition, nicotine have an impact on blood vessel contraction or spasms, which could result in tissue ischemia and poor ulcer healing (Zhong *et al.*, 2017). As like to effects smokers with diabetes are at higher risk for complications such as heart disease, stroke, and kidney disease, smoking increases an important danger of diabetic foot amputation, risk of blocked arteries it appears that giving up smoking lowers the chance of diabetic foot amputation. The current study is consistence with a study that recorded nearly similar distribution to smoking status; 68% of DFU patients and 80% of diabetic without foot ulcers were smokers (Liu *et al.*, 2018). Also, the percentage of smokers and diabetic foot ulcer patients was 95% as reported by Jalilian *et al* . (2020).

In contract the study by Obaid and Eljedi, (2015) revealed that patients smoking status showed not an important association to a higher risk of DFUs because everyday tissue hypoxia in diabetes patients can result in vascular and neuropathic diseases in their feet. The reason for the inconsistency with the current result is that the researcher's samples are very small or may be associated with sample collection method.

4.5.2 Obesity Effect

Table (3-4) displays the association of obesity with other disease in the studied population, according to the results of the statistical analysis, there are no significant differences recorded between obese and non-obese diabetic patients who were dividing accord to no disease or who have hypertension; the analysis demonstrated that the highest percentage of diabetic patients with cardiovascular complications who had foot ulcers were non-obese at 66.7%, while 33.3% were obese. As for

diabetic patients with cardiovascular complications who did not have foot ulcers, 71.4% of them were obese, while only 28.6% were not obese, with highly significant differences at ($p<0.01$).

As for diabetic individuals with and without foot ulcers and suffering from both disease (hypertension and cardiovascular), the highest percentage of both groups was not obese (73.3% and 65.5%, respectively) and only 26.7% and 34.5% were obese with highly significant differences recorded at ($p<0.01$).

Table (3-4): Association of obesity in study patients with other disease

Other Disease	Obesity					
	Diabetic with foot ulcer			Diabetic without foot ulcer		
	Yes	No	*P value*	Yes	No	*P value*
No disease	1 50.0%	1 50.0%	1.000^{NS}	1 50.0%	1 50.0%	1.000^{NS}
Hypertension	13 48.1%	14 51.9%	0.763 ^{NS}	7 58.3%	5 41.7%	0.0875^{NS}
Cardiovascular	2 33.3%	4 66.7%	0.0009*	5 71.4%	2 28.6%	0.0001*
Both disease	4 26.7%	11 73.3%	0.0001	10 34.5%	19 65.5%	0.0018*
Total	20 40.0%	30 60.0%	0.0455 *	23 46.0%	27 54.0%	٠.٤٢٣^{NS}

*significantly difference at the (0.05) level by chi-square test

NS: non- significant difference

Certain serious metabolic disorders, such as hypertension, are linked to obesity, which is also thought to be independently linked to an increased risk cardiovascular disease (CVD) also to some extent to hypertension and hyperglycemia. Diabetes and obesity are related to one another because obesity may lead to varying degrees of insulin resistance, which contributes to the consequences of diabetes (Hanefeld *et al*., 2007). Obese diabetic patients are more likely to experience long-term vascular consequences (Nguyen and Lau, 2012). There was a significant difference in the study populations when it came to the obesity subject when it was examined as an effector factor with other diseases (Resnick *et al*., 2004 ; Ikura *et al*., 2015).

The findings of current study are aligning with Anari *et al* (2017) who revealed that the rate of obesity among diabetic patients with other diseases was 68.8%. Also, findings of table (3-4) are consistent with result of Ouyang and Jin (2021), where the researcher found that the obesity frequency among DFU patients with other diseases was 37.76%. On the other hand (Lu *et al*., 2021) revealed that patients with DFU did not have a higher risk of hypertension or CVD and non-significant difference were reported their study populations. The reason for the contradiction in the results reached by the researcher is the choice of young ages as well as the choice not from different societies.

Regarding the low prevalence of obesity among DFU patients found in the current study may be attributed to the long duration of the disease, which may undoubtedly lead to weight loss in diabetic patients, as it is known that one of the most important complications of diabetes is weight loss with the progression of the disease, in addition to the appearance of accompanying complications such as heart disease and foot ulcers. What reinforces this opinion is that diabetic patients who do not suffer from foot ulcers had a higher rate of obesity, and this may be because the disease is still in its early stages in these patients.

3.5.3 Diet Effect

Type of diet in study population according to other disease, regarding diabetic without foot ulcer patients who followed a vegetarian diet had higher rates of other diseases percentage 71.40% and those who did not follow a vegetarian diet have 55.80%, while individuals who have DFU and followed a vegetarian diet they have hypertension a percentage 57.10% and they non-vegetarian have 53.50% , diet in study population was displayed in figure (3-5).

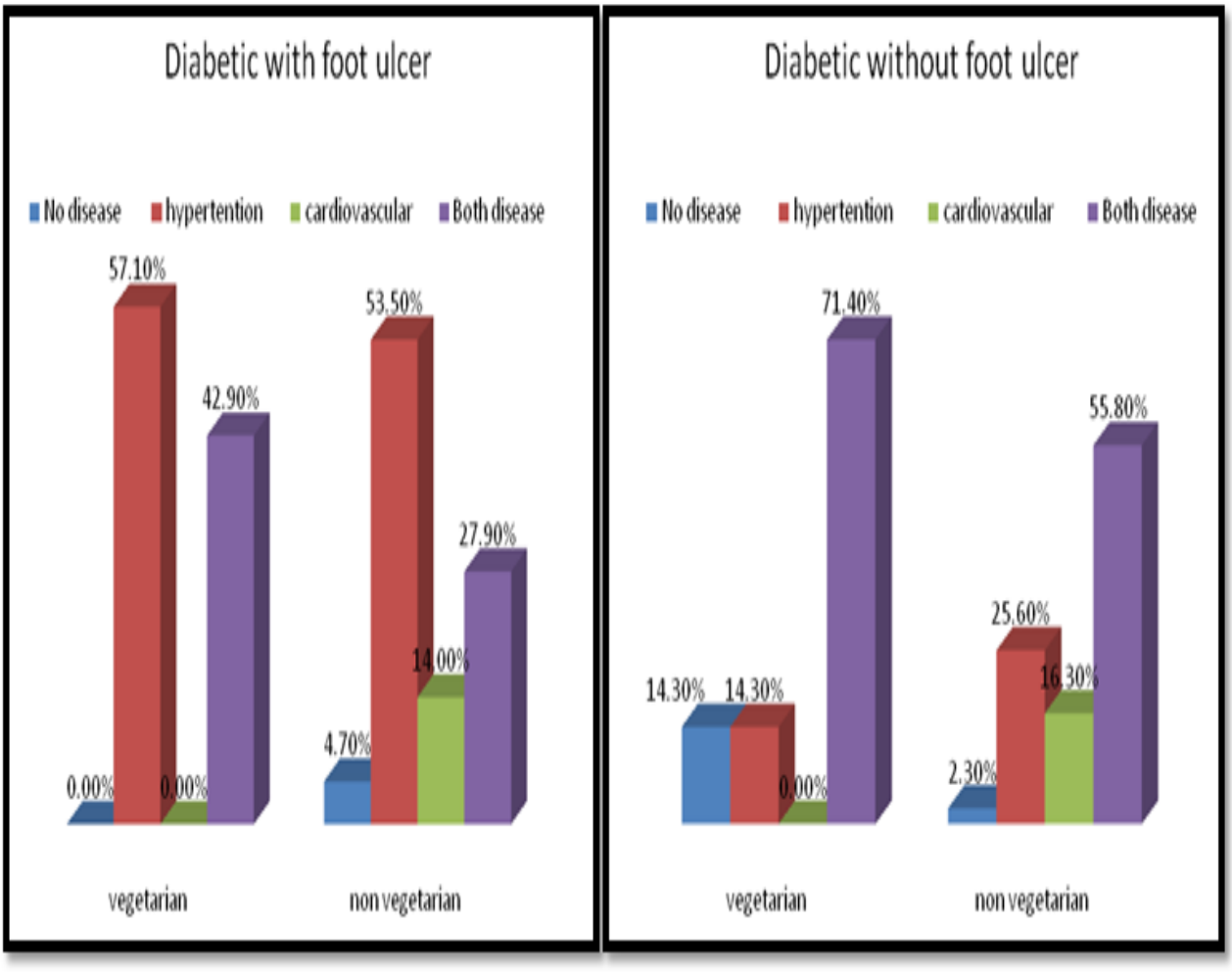

Figure (3-5): Association of Diet in patients with other disease

In previous studies, both vegetarian diet as well as no vegetarians transitioning to vegetarian diets are related with 50% lower risk of diabetes (Chiu *et al.*, 2018). Research has indicated that the prevalence of T2DM is lower in vegetarians than in no vegetarians. Diets that are vegetarian are linked to improvements in secondary outcomes like blood pressure, CVD, serum lipid profile, and weight loss. Vegetarian diets have been shown to be a generally effective strategy for preventing type 2 diabetes and for enhancing blood glucose control. It has been demonstrated that vegetarians are less likely to die from heart disease or require hospitalization (Pawlak, 2017). Additionally, studies on vegetarian diets have demonstrated a regression of vascular stenosis in heart disease patients. Moreover, these diets associated with a lower chance of developing other metabolic disorders, such as T2DM (Crowe *et al.,* 2013). Vegetarian diets are associated with improvements in secondary outcomes such as blood pressure and cardiovascular disease in DFU patients (Tripathy *et al.,* 2020). In contrast other study, found there was no difference between vegetarians and non-vegetarians in terms of body mass index, CVD, or the prevalence of diabetes or hypertension (Shridhar *et al.,* 2014). The reason for the contraction with these results are their study was conducted in India, were individuals in these regions differ in the nature and system of nutrition.

3.5.4 Concentration of Fibrinogen in Patients Affected to Smoking and Obesity

The results in table (3-5), showed highly significant difference in fibrinogen concentration in DFUs patients groups. The present results showed that the concentration of fibrinogen is very high in smokers and obese people who suffer from diabetic foot ulcers (611.67) compared to diabetes people without foot ulcer (292.35) , and the concentration of fibrinogen is high with smokers who have DFU (590.41), and concentration of fibrinogen is high in obese people who suffer from ulcers (611.20) compared to people who are neither smokers nor obese.

Table (3-5): Concentration of Fibrinogen in patients affected to smoking and obesity

Smoking	Obesity	Study population	Fibrinogen g/l concentration		CI (95%)		*P value*
			Mean	SD	Lower	Upper	
Yes	Yes	Diabetic with foot ulcer	611.67	44.974	586.76	636.57	0.000*
		Diabetic without foot ulcer	292.35	58.368	262.34	322.36	
	No	Diabetic with foot ulcer	590.41	31.992	573.96	606.86	0.000*
		Diabetic without foot ulcer	312.77	69.427	281.99	343.55	
No	Yes	Diabetic with foot ulcer	611.20	37.877	564.17	658.23	0.000*
		Diabetic without foot ulcer	326.00	98.618	222.51	429.49	
	No	Diabetic with foot ulcer	579.69	45.211	552.37	607.01	0.000*
		Diabetic without foot ulcer	295.20	57.954	223.24	367.16	

*Mean highly significant difference under $p \leq 0.05$

CI: Confidence interval

Smoking and diabetes can cause chronic inflammation in diabetic body. Fibrinogen is a protein that is involved in the clotting process and is produced more in response to inflammation, chemicals in cigarettes can damage blood vessels and increase inflammation, leading to higher levels of fibrinogen; people with diabetes often have insulin resistance, which can cause higher levels of fibrinogen, diabetic foot ulcers may indicate problems with circulation, which can lead to increased production of fibrinogen(Hunter *et al*., 2001; Anantha, 2010).

Obesity is often associated with a state of chronic inflammation in the body. Excess fat, especially visceral fat, produces inflammatory substances that lead to increased levels of fibrinogen (Han and Boyko, 2018). Obese people often have insulin resistance, which contributes to increased levels of fibrinogen in response to high blood sugar levels, diabetic foot ulcers indicate problems with blood flow, which may lead to increased production of fibrinogen as part of the body's response to try to heal (Nascetti *et al*., 2001; Bembde, 2012). Mertens and Gaal (2002) stated that obese DFUs patients expressed an increase fibrinogen concentration. Also this statement was confirmed by study of Sola *et al*. (2007). The group of diabetic patients foot ulcers is a significant affected by obesity factors this study done by Ali *et al*. (2021) and Hussein and Saleh, (2023). Therefore, one should think that the results of the obesity challenge should alter the present treatment suggestions about the significance of weight loss for patients group who are overweight or obese. A study by Belalcazar *et al*. (2011), was found that high levels of fibrinogen did not change when body weight was reduced and blood sugar levels were regulated when comparing the groups because they suffer from chronic inflammation that keeps fibrinogen high.

3.6 Genetic Study

The Genotypes and Allele frequency for *MCP-1* and *CRP* genes in study population were displayed in table (3-6). Regarding to *MCP-1* gene, the significant difference were recorded in context of allele frequency; I allele showed a significant high frequency in both diabetic patients groups versus D allele. In addition, *CRP* gene showed a significant difference in context of allele frequency, where G allele significantly appeared in both patients groups as compared with A allele.

Table (3-6): *MCP-1* and *CRP* genes in study population

***MCP-1* gene**					
Study Group	**Insertion (II)**	**Deletion (DD)**	**Insertion/ Deletion (ID)**	**ODD (CI95%)**	***P value***
Diabetic with foot ulcer	38 (76.0%)	10 (20.0%)	2(4.0%)	1.9709 (0.8176-4.6072)	0.1327^{NS}
Diabetic without foot ulcer	31 (62.0%)	8 (16.0%)	11 (22.0%)		
Total	69 (69%)	18 (18%)	13 (13%)		
Study Group	**I**	**D**	**ODD (CI95%)**		***P value***
Diabetic with foot ulcer	78	22	0.9427 (1.0552-3.5767)		0.0330^{*}
Diabetic without foot ulcer	73	49			
CRP gene					
Study Group	**AA**	**GG**	**GA**	**ODD (CI95%)**	***P value***
Diabetic with foot ulcer	10 (20.0%)	35 (70.0%)	5 (10.0%)	1.430 (0.6224-3.2858)	0.399^{NS}
Diabetic without foot ulcer	10 (20.0%)	31 (62.0%)	9 (18.0%)		
Total	20 (20%)	66 (66%)	14 (14%)		
Study Group	**A**	**G**	**ODD (CI95%)**		***P value***
Diabetic with foot ulcer	15	75	0.4828(0.2389-0.9754)		0.0424^{*}
Diabetic without foot ulcer	29	70			

*Mean significantly differences ($p< 0.05$)

NS: No-significant; CI: confidence interval

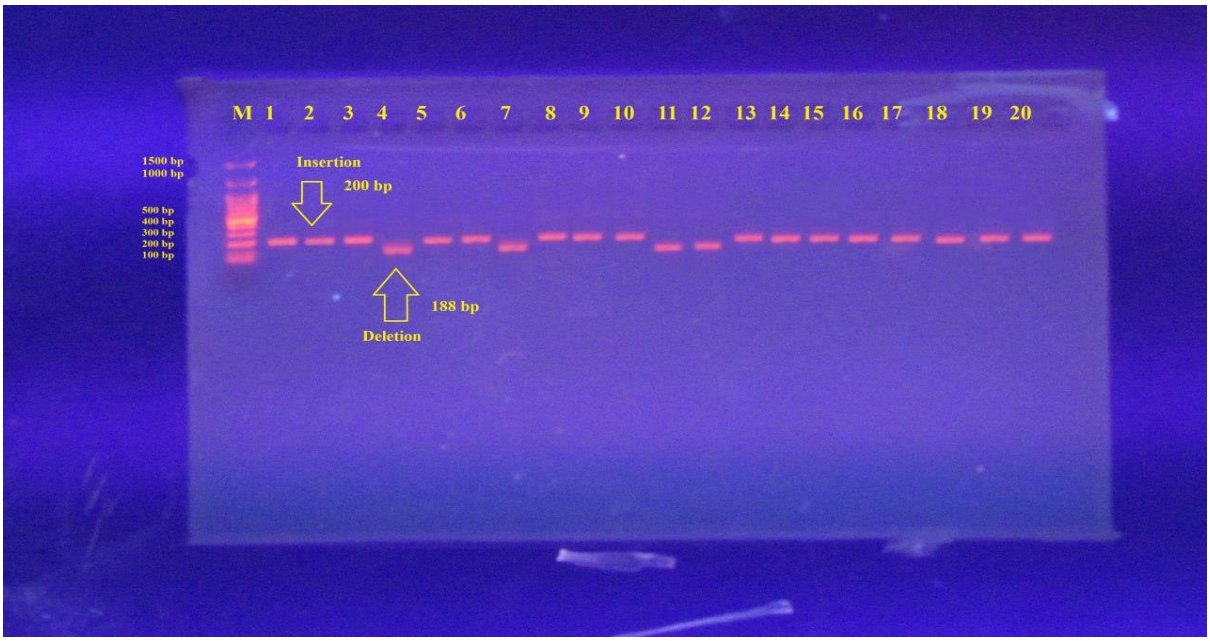

Figure (3-6): Electrophoresis of PCR products of I/D allele isolated from DFU infections using the *MCP-1* gene-specific primer (Insertion 200bp, Deletion 188bp), 1% Agarose, 60V, for 35 min. (10µl in each well), stained with Ethidium bromide.

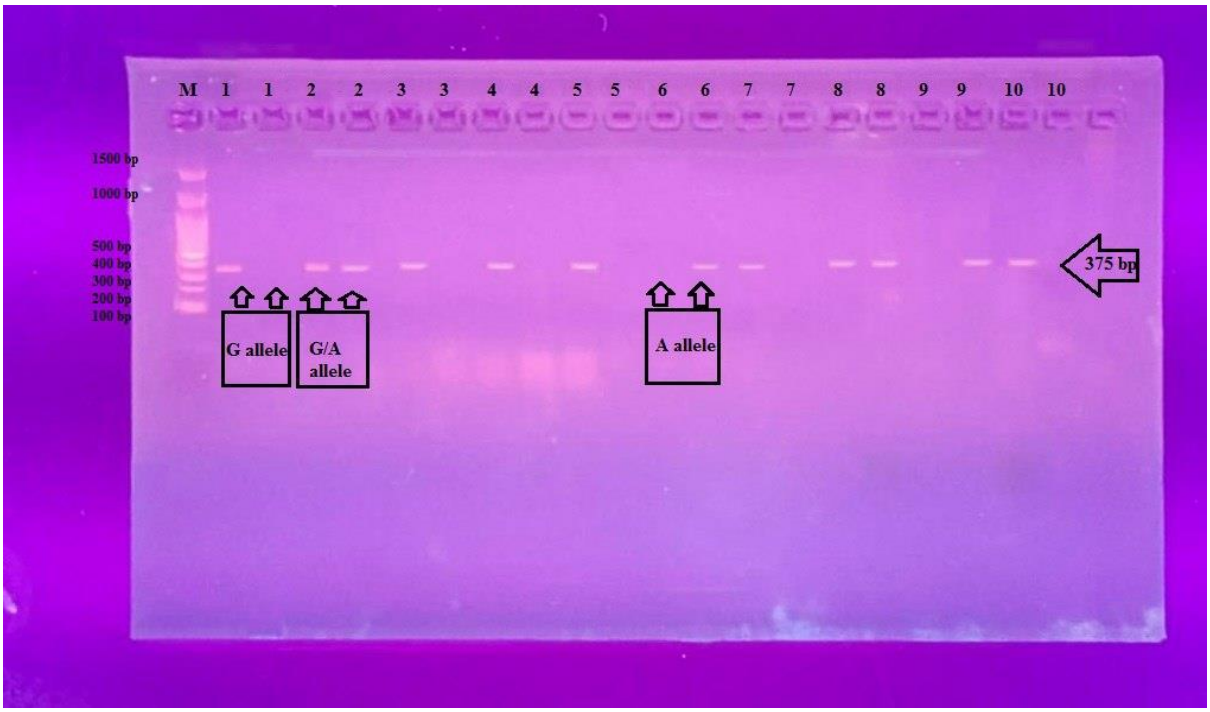

Figure (3-7) : Electrophoresis of PCR products of A/G allele isolated from DFU infections using the *CRP* gene-specific primer (A 375 bp, G 375 bp), 1% Agarose, 60V, for 35 min. (10µl in each well), stained with Ethidium bromide.

DFU patients are frequently linked to chronic inflammation, and elevated levels of MCP-1 can result in increased monocyte infiltration, which may worsen tissue damage and impede healing. Inflammatory gene single nucleotide polymorphisms (SNPs) are helpful DFU candidates. Also, monocyte chemoattractant protein1 (MCP-1) is one of the key proinflammatory cytokines in inflammatory proceedings.

Individuals with certain alleles may have a higher risk of developing DFUs due to heightened inflammatory responses. Some studies suggest that patients with specific *MCP-1* polymorphisms are more susceptible to DFUs, possibly due to impaired wound healing and increased inflammation (Obied *et al*., 2019).

The current study was concerned with the genetic analysis for *MCP-1* gene and showed its genotypes and allele frequencies in the patients under study, and the statistics analysis revealed only significant differences in regard of alleles frequencies; the I allele demonstrated a significant increase relative to the D allele.

The *MCP-1* gene variation was found to have a strong negative correlation with both plasma *MCP-1* levels and the incidence of T2DM and insulin resistance in a large Caucasian sample by Inan Sarikaya *et al*. (2024).

The finding of current study recoded that no significant association between study groups regards the frequencies of *MCP*-1 genotypes and this result agrees with a previous study of Raina *et al*. (2021), who also recorded a same fact for *MCp-1* rs1024611 genotypes. A similar result was also documented by Chang *et al*. (2023), who pointed out that no significant changes in *MCP-1* genotypes distribution between patients with T2DM and DFU.

Regarding to *CRP* A\G SNP, the present investigation revealed significant differences in the frequencies of *CRP* alleles, with the G allele appearing in both

patient groups more frequently than the A allele. It is well-characterized it is associated with diabetes and its complications such as DFU (Dhamodharan *et al*., 2015). A similar to results were found, where the GG genotype was more frequent in DFU patients than in those with the AA genotype (Hu *et al*., 2022).

Conversely, results of current study contradict the study by Simeoni *et al*. (2004), who recorded in-significant differences between alleles and genotypes frequency of *CRP* in diabetic patients. The reason for the difference is due to the difference in the duration of the disease between the patients and the difference in the community from which the samples were taken, along with the varied in procedure that used in these studies.

3.6.1 Phenotype of Bacterial Isolate According to Genotypes of *MCP-1* Gene Polymorphism.

Phenotype for bacterial isolate according to *MCP-1* genotypes, the results of statistical analysis showed that insertion genotype has significantly higher percentages in *Entrobacter aerogenes (*100%), co-infection with *S. aureus / K. pneumonia (*87.5%), co-infection with *S. aureus/Proteus mirabilis* (85.7%). Deletion genotypes showed no significant bacterial distribution. The insertion/deletion genotype showed significantly higher percentages in both *P. mirabilis (*20%), *and K. pneumonia (*40%), phenotype for bacterial isolate according to *MCP-1* genotypes was illustrated in table (3-7).

Table (3-7): Phenotype for bacterial isolate according to MCP-1 genotypes

Bacterial isolate		*MCP-1* genotypes			Total
		Insertion	Deletion	Insertion /Deletion	
No growth	Count	10	2	0	12
	%	83.3%	16.7%	0.0%	100.0%
Proteus mirabilis	Count	3	1	1*	5
	%	60.0%	20.0%	20.0%	100.0%
Staphylococcus aureus / Klebsella pneumonia	Count	7*	1	0	8
	%	87.5%	12.5%	0.0%	100.0%
Staphylococcus aureus	Count	2	1	0	3
	%	66.7%	33.3%	0.0%	100.0%
Staphylococcus aureus/ Proteus mirabilis	Count	6*	1	0	7
	%	85.7%	14.3%	0.0%	100.0%
Entrobacter aerogenes	Count	2	0	0	2
	%	100.0%	0.0%	0.0%	100.0%
Pseudomonas aeruginosa	Count	0	2	0	2
	%	0.0%	100.0%	0.0%	100.0%
Klebsella pneumonia	Count	2	1	2*	5
	%	40.0%	20.0%	40.0%	100.0%
Proteus mirabilis/ Klebsella pneumonia	Count	1	3	0	4
	%	25.0%	75.0%	0.0%	100.0%
Providencia stuartii	Count	1	0	0	1
	%	100.0%	0.0%	0.0%	100.0%
Serratia marcescens	Count	0	1	0	1
	%	0.0%	100.0%	0.0%	100.0%
P value		0.050*	0.130NS	0.016*	/

*Mean significantly differences at level of probability 0.05 using $\chi 2$-test

NS: No-significant

The *MCP-1* gene is involved in attracting immune cells, such as neutrophils and macrophages, to sites of inflammation, in the case of diabetic foot ulcers, isolated bacteria may play a role in increasing the expression of *MCP-1*, when bacteria are present in the ulcer, the body responds by secreting *MCP-1*, which leads to the recruitment of immune cells to the area to fight the infection.

However, chronic inflammation resulting from infection worsen the condition of the ulcer, and thus the role of *MCP-1* become dual on the one hand, it helps fight the infection, and on the other hand, it may contribute to worsening the inflammation and slowing the healing process .Overall, the relationship between *MCP-1* and bacteria isolated from diabetic foot ulcers suggests a complex interaction between the immune response and inflammation resulting from bacterial infection.

There are several studies that have looked at the relationship between certain types of bacteria and increased expression of the gene, where *S. aureus* was found to be linked to increased expression of *MCP-1* in skin infections by Mohammad *et al.* (2021) .*P. aeruginosa* is a common bacteria in wound infections by Sweere *et al.* (2020). Studies have shown that it can stimulate increased levels of *MCP-1*, reflecting the body's response to infection; these studies are important for understanding how bacterial infections affect inflammation and wound healing, especially in the context of diabetic foot ulcers.

However, Hassen *et al*. (2022) was proven that there were significant differences between the genotypes of the *MCP-1* gene among patients who have spontaneous bacterial peritonitis (SBP) and the risk of this disease is increased.

3.6.2. Phenotype of Bacterial Isolate According to Genotypes of *CRP* Gene

. Phenotype for bacterial isolate according to CRP and the results of statistical analysis revealed significant bacterial distribution according to genotypes. The AA

genotype showed significant high percentage in *Entrobacter aerogenes, P. airoginosa* (50%) and *P. mirabilis (*40%), respectively. The GG genotype showed significant high percentage in *Staphylococcus aureus, Providencia stuartii, Serratia marcescens* (100%), *K. pneumonia (*80%), *P. mirabilis/K. pneumonia* and patients with no growth culture (75%), phenotype for bacterial isolate according to *CRP* was showed in table (3-8).

Table (3-8): Phenotype for bacterial isolate according to *CRP* genotypes

Bacterial isolate		*CRP* gene			Total
		AA	GG	GA	
No growth	Count	3	9*	0	12
	%	25.0%	75.0%	0.0%	100.0%
Proteus mirabilis	Count	2*	2	1	5
	%	40.0%	40.0%	20.0%	100.0%
Staphylococcus aureus / klebseilla pneumonia	Count	2	5	1	8
	%	25.0%	62.5%	12.5%	100.0%
Staphylococcus aureus	Count	0	3*	0	3
	%	0.0%	100.0%	0.0%	100.0%
Staphylococcus aureus/ proteus mirabilis	Count	0	4	3*	7
	%	0.0%	57.1%	42.9%	100.0%
Entrobacter aerogenes	Count	1*	1	0	2
	%	50.0%	50.0%	0.0%	100.0%
Psedomonas airoginosa	Count	1*	1	0	2
	%	50.0%	50.0%	0.0%	100.0%
Klebsella pneumonia	Count	1	4*	0	5
	%	20.0%	80.0%	0.0%	100.0%
Proteus mirabilis/ Klebsella pneumonia	Count	1	3*	0	4
	%	25.0%	75.0%	0.0%	100.0%
Providencia stuartii	Count	0	1	0	1

	%	0.0%	100.0%	0.0%	100.0%
Serratia marcescens	Count	0	1	0	1
	%	0.0%	100.0%	0.0%	100.0%
P value		0.0001	0.0001	0.0001	
*Significantly differences at level of probability 0.05 using χ2-test					

The present investigation dialed with association between *MCP-1* and *CRP* genes polymorphisms and type of bacterial species that isolated from DFU patients, but did not find any previous investigations on this relationship .Therefore, this study represents the first study regard the relation between *MCP-1 or CRP* genotypes and bacterial types in diabetic foot ulcer patients.

Recognition of genetic determinants such as *MCP-1* and *CRP* can promote the prediction of diabetic foot ulcers' risk and the administration of individualized dealings for therapy. On the other hand, linking these determinants to other risk factors associated with the disease, such as bacterial infection, types of bacteria invading tissues, and their severity, can facilitate the causes of the development of the disease in some and its faster recovery in others. Thus direct patients to the danger of the interaction between genetic determinants and bacterial infection, which can be reduced by sterilization and personal hygiene methods.

In the current study, showed the distribution of both of *MCP-1* and *CRP* genotypes among bacterial isolates with a significant difference. Linking the polymorphism of a specific gene with the nature of the bacteria isolated from a specific disease can provide a logical picture of the causes of these mutations and pave the way for understanding the mechanism of interaction between the phenomenon of polymorphism due to the effect of bacteria or their metabolic

products, which provides future prospects for reducing these effects, which may lead to broader genetic or epigenetic changes.

3.6.3 Concentration of Fibrinogen in Study Populations According to Genotypes of *MCP-1* Gene Polymorphism

Table (3-9) displays the levels of fibrinogen with diabetes patients with and without foot ulcer; the results of statistical analysis revealed a significant increase in levels of fibrinogen of diabetes patients with foot ulcer in all genotypes of *MCP-1* gene (Insertion, Deletion, and Insertion/Deletion). On the other hand, no significant differences were recorded within-group comparison (within group with DFU or in-group without DFU) according to genotypes of *MCP-1* gene.

Table (3-9): Concentration of fibrinogen in study populations according to *MCP-1* genotypes.

***MCP-1* genotypes**	**Con. of Fibrinogen g/l in patients**						***P value***
	Diabetic with foot ulcer			**Diabetic without foot ulcer**			
	NO.	Mean	SD	NO.	Mean	SD	
Insertion	38	600.34	35.898	31	305.45	61.113	0.000*
Deletion	10	596.00	40.972	8	279.50	71.738	0.000*
Insertion/deletion	2	515.50	85.560	11	325.27	81.957	0.012*
Total	50	596.08	41.351	50	305.66	67.755	
P value	0.015^{NS}			0.355^{NS}			

*Significantly differences at level of probability 0.05 using t-test

NS: no significant using ANOVA test

According to the data, persons with diabetic foot disease appear to have greater amounts of fibrinogen compared to those with no ulcer. Present study displayed a strong correlation between fibrinogen and the degree of DFU illness. The increase in fibrinogen levels in diabetic patients with foot ulcers compared to those with no ulcer that recorded is in line with the findings of previous studies of Li *et al*. (2016).

All genotypes of the *MCP-1* gene polymorphism showed elevated fibrinogen levels in diabetic patients with foot ulcers, and this finding is agree with those of Obied *et al.* (2019), who found that *MCP-1* gene polymorphism rs1024611 significantly related with greater propensity to DFU patients compared to T2DM without foot ulcers.

3.6.4 Relationship of Fibrinogen Concentration with *MCP-1* Genotypes According to Bacterial Isolated

The association of Fibrinogen in diabetes patients with foot ulcer with *MCP-1* genotypes and according to bacterial isolated; the results of statistical analysis revealed non-significant differences in the levels of fibrinogen. The association of fibrinogen in diabetes patients as shown in table (3-10).

Table (3-10): Association of fibrinogen concentration with *MCP-1* genotypes according to bacterial isolated

***MCP-1* genotypes**	**Bacterial isolated**	No.	**Con. of Fibrinogen g/l in Diabetic with foot ulcer**		***P value***
			Mean	**SD**	
Insertion	No growth	10	504.90	166.584	0.784^{NS}
	Proteus mirabilis	3	507.67	156.465	
	Staphylococcus aureus / Klebsiella pneumonia	7	543.00	152.668	
	Staphylococcus aureus	2	551.00	38.184	
	Staphylococcus aureus/ proteus mirabilis	6	505.83	195.851	

	Entrobacter aerogenes	2	568.50	13.435	
	Klebsiella pneumonia	2	593.00	55.154	
	Proteus mirabilis/ Klebsiella Pneumonia	1	577.00	.	
	Providencia stuartii	1	220.00	.	
	Total	34	518.53	151.070	
Deletion	No growth	2	505.00	103.238	0.312^{NS}
	Proteus mirabilis	1	546.00	.	
	Staphylococcus aureus / Klebsiella pneumonia	1	576.00	.	
	Staphylococcus aureus	1	258.00	.	
	Staphylococcus aureus/ Proteus mirabilis	1	687.00	.	
	Psedomonas airoginosa	2	466.50	219.910	
	Klebsiella pneumonia	1	587.00	.	
	Proteus mirabilis/ Klebsiella pneumonia	3	595.33	35.501	
	Serratiamarcescens	1	225.00	.	
	Total	13	508.31	151.333	
Insertion/deletion	*Proteus mirabilis*	1	223.00	.	0.117^{NS}
	Klebsiella pneumonia	2	432.50	31.820	
	Total	3	362.67	123.030	
NS: No-significant difference at level of probability 0.05 using t-test					

The findings of current study compatible with the previous investigation, which also reported non-significant differences in the levels of fibrinogen in all genotypes of *MCP-1* gene polymorphism in study population among patients with- diabetic foot ulcer (Li, 2018). Fibrinogen is considered as significant risk factor of vascular proceedings (Danesh *et al.*, 2005). Regardless of inflammatory processes, its fibrinogen also be engaged with endothelial injury, formation the low permeability

fibrin clots, disturbances of blood flow, in addition to platelet hyperactivity and aid in the development of subclinical atherosclerosis (Li *et al*., 2016).

Fibrinogen has been generally investigated in coronary arterial disease and peripheral artery disease in people with diabetes (*Kotbi et al*., 2016; Yao *et al*., 2016). The current study's findings about the higher levels of fibrinogen in diabetic patients with foot ulcers as opposed to those without ulcers are in line with previous studies findings. (Li *et al*., 2016). Different aerobes and anaerobes bacteria have the ability to colonize DFUs, based on evidence from molecular methods and bacterial culture. Shorter-duration diabetic foot infections (DFIs) are mostly inhabited by *Staphylococcus* and *Streptococcus species* are examples of gram-positive cocci that seem to have a more basic microbiota. Conversely, through polymicrobial infections, various aerobic bacterial species, such as *Pseudomonas species, Staphylococcus, Streptococcus, Enterococcus*, and anaerobic pathogens, may inhabit chronic DFIs (Sadeghpour *et al*,. 2019). The current study reported a non-statistical significant regarding the association of fibrinogen in diabetes foot ulcer patients with *MCP-1* genotypes according to bacterial isolated and this result compatible with the results of (Hu et al. 2022) who demonstrated a non-significant differences in the levels of fibrinogen in all genotypes of *MCP-1* gene polymorphism in study population among patients with- diabetic foot ulcer.

3.6.5 Concentration of Fibrinogen in Study Groups According to Genotypes of *CRP* Gene Polymorphism

The concentrations of fibrinogen in diabetes cases with and without foot ulcer according to genotypes of *CRP* gene displayed in table (3-11). However, all genotypes of the *CRP* gene showed a statistically significant increase in fibrinogen levels in diabetic individuals (AA, GG, and GA). On the other hand, no significant differences were recorded within-group comparison according to genotypes of *CRP*.

Table (3-11): Concentration of Fibrinogen in patients according to *CRP* genotypes

***CRP* genotypes**	**Con. of Fibrinogen g/l in patients**						***P value***
	Diabetic with foot ulcer			**Diabetic without foot ulcer**			
	NO.	Mean	SD	NO.	Mean	SD	
AA	10	600.20	34.483	10	311.50	59.936	0.000*
GG	35	595.11	42.726	31	304.94	71.208	0.000*
GA	5	594.60	51.969	9	301.67	70.709	0.000*
Total	50	596.08	41.351	50	305.66	67.755	
P value	0.942^{NS}			0.949^{NS}			
* Significantly differences at level of probability 0.05 using t-test NS: no significant							

As the finding in table (3-11) explained that DFU individuals had statistically significant higher levels of fibrinogen than individuals without foot ulcers and these findings are comparable to (Afrah *et al.*, 2020); but all genotypes of the *CRP* gene (AA, GG, and GA) showed nearly a same concentrations of fibrinogen in DFU patients with as well patients without foot ulcers; so no significant differences were found within-groups comparisons according to genotypes of the *CRP* gene.

3.6.6 Relationship of Fibrinogen Concentration with *CRP* Genotypes According to Bacterial Isolated

The association of fibrinogen in diabetes patients with foot ulcer with *CRP* genotypes and according to bacterial isolated; the results of statistical analysis revealed non-significant differences in the levels of fibrinogen in all genotypes of *CRP* according to bacterial**.** (AA) showed *P. mirabilis, S. aureus / k. pneumonia* with fibrinogen (598.00, 624.00), showed in table (3-12).

Table (3-12): Association of fibrinogen with *CRP* according to bacterial isolated

***CRP* genotypes**	**Bacterial isolated**	NO.	**Con. of Fibrinogen g/l in Diabetic with foot ulcer patients**		***P value***
			Mean	**SD**	
AA	No growth	3	505.33	183.413	0.833^{NS}
	Proteus mirabilis	2	598.00	1.414	
	Staphylococcus aureus / klebsella pneumonia	2	624.00	76.368	
	Entrobacter aerogenes	1	559.00	.	
	Psedomonas airoginosa	1	622.00	.	
	Klebsella pneumonia	1	410.00	.	
	Proteus mirabilis/ klebsella pneumonia	1	577.00	.	
	Total	11	557.09	109.408	
GG	No growth	9	504.78	155.374	0.121^{NS}
	Proteus mirabilis	2	436.50	154.856	
	Staphylococcus aureus / klebsella pneumonia	5	510.60	170.032	
	Staphylococcus aureus	3	453.33	171.305	
	Staphylococcus aureus / Protus mirabilis	4	631.75	15.586	
	Entrobacter aerogenes	1	578.00	.	
	Pseudomonas airoginosa	1	311.00	.	
	Klebsella pneumonia	4	557.00	75.140	
	Proteus mirabilis/ klebsella pneumonia	3	595.33	35.501	
	Providenc iastuartii	1	220.00	.	
	Serratia marcescens	1	225.00	.	
	Total	34	506.00	148.864	
AG	*Proteus mirabilis*	1	223.00	.	0.669^{NS}
	Staphylococcus aureus / klebsella pneumonia	1	576.00	.	
	Staphylococcus aureus/ Proteus mirabilis	3	398.33	250.791	
	Total	5	398.80	216.852	
NS: No-significant difference at level of probability 0.05 using t-test					

Genotypes (GG) showed *S. aureus / k. pneumonia*, *S. aureus/ P. mirabilis* and *K. pneumonia* with fibrinogen concentration (510.60 , 631.75 , 557.00) and genotypes (AG) showed *S. aureus/ P. mirabilis* with fibrinogen (398.33) ,the relationship between fibrinogen and the gene according to the bacteria isolated from diabetic foot ulcers is not significant.

3.6.7. Distribution of Sex According to *MCP-1* and *CRP* Genes in Study Group

Table (3-13) displays the association of sex with *MCP-1* and *CRP* genes, both males and females showed a significant increase in relation to insertion genotype *MCP-1* gene in diabetic with foot ulcer, while *CRP* gene showed non-significant differences.

Table (3-13): Association of sex with *MCP-1* and *CRP* genes in study groups

Genes		Sex					
		Diabetic with foot ulcer			Diabetic without foot ulcer		
		Male	Female	Total	Male	Female	Total
***MCP-1* genotypes**	**Insertion**	29*	9*	38*	15	16	31
		90.6%	50.0%	76.0%	65.2%	59.3%	62.0%
	Deletion	3	7	10	3	5	8
		9.4%	38.9%	20.0%	13.0%	18.5%	16.0%
	insertion/deletion	0	2	2	5	6	11
		0.0%	11.1%	4.0%	21.7%	22.2%	22.0%
	Total	32	18	50	23	27	50
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
P value		0.004*	0.003*	0.001*	0.859NS	0.857NS	0.785NS

***CRP* genotypes**	**AA**	5	5	10	6	4	10
		15.6%	27.8%	20.0%	26.1%	14.8%	20.0%
	GG	24	11	35	13	18	31
		75.0%	61.1%	70.0%	56.5%	66.7%	62.0%
	GA	3	2	5	4	5	9
		9.4%	11.1%	10.0%	17.4%	18.5%	18.0%
	Total	32	18	50	23	27	50
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
P value		0.548^{NS}	0.556^{NS}	0.516^{NS}	0.605^{NS}	0.605^{NS}	0.483^{NS}
*Mean significantly differences at level of probability 0.05 using χ2-test NS: No significant							

This aspect of the current study was concerned with analyzing the proportions of genotypes of *MCP-1* and *CRP* in one sex and whether there is a bias in the appearance of a certain genotype in a certain sex, rather than comparing the sexes with each other. According to results of the current study, insertion genotype of *MCP-1* showed a significant increase in both males and females patients with foot ulcer, while the diabetic patient without ulcer showed non-significant differences in the distribution of MCP-1 genotypes in males as well females.

A previous studies analyzed the distribution of genotypes between males and females, and noted that significant increase females more than males in relation to insertion genotype of *MCP-1* gene in diabetic with foot ulcer (Simeoni *et al.*, 2004; Yang *et al.*, 2004) . While the study done by Obied *et al* ., (2020) whose revealed that no visible change in gender or age between group (DFU and non-DFU) these differences could be justified according to the difference in population between the

two studies, the difference in the number of samples used for analysis, as well as the difference in molecular and statistical analysis techniques.

The *CRP* gene showed insignificant differences with sex in study population and these results are similar to the study carried out by Jie *et al*. (2023); Rashad *et al*. (2024) whose reported the distribution of *CRP* genotypes in male and female diabetes patients did not differ significantly. Whereas the results of the study carried out by Reynoso-Villalpando *et al*. (2021) whose reported the distribution of *CRP* genotypes exhibited a significant increase, the reason for the disagreement is that the researcher used a limited number of samples sexes.

3.7 Receiver Operative Characteristic Curve Analysis

The Receiver Operative Characteristic Curve (ROC) analysis yielded a cut off value of fibrinogen (495.5) of disease activity by biomarkers for diabetic disease in two cases with and without foot ulcer. The overall area under curve (AUC), specificity and sensitivity for fibrinogen was as follows: (0.980, 1.000 and 0.98) as displayed in figure (3-8) and table (3-14).

Table (3-14): ROC curve of fibrinogen in diabetic with foot ulcer patients

Fibrinogen	AUC	Specificity	Sensitivity	Cut-off	P-value
	0.980	1.000	0.98	495.5	<0.001

It is essential to establish a consistent fibrinogen cut-off value when measuring the severity of diabetic foot ulcers. The optimal cut-off point of fibrinogen was calculated depending on the greatest sum of sensitivity and specificity of the ROC-coordinate points. The current study revealed that the cut-off fibrinogen level for

estimating diabetic foot ulcers is (495.5) with AUC of (0.980) (sensitivity 0.98, specificity 1.000).

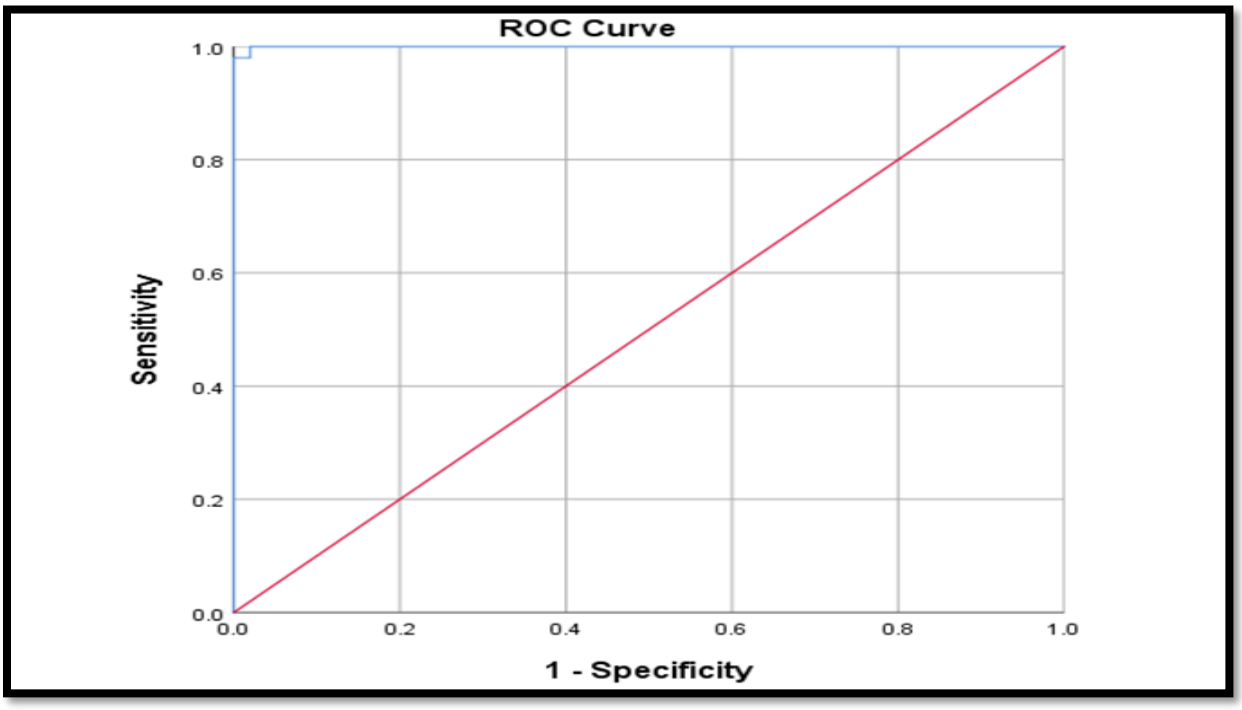

Figure (3-9): ROC curve for prediction of the disease activity (DFU) by fibrinogen

The current study found that AUC for fibrinogen (0.980), which higher than that obtained in previous studies, which were (0.858) in the study of Li *et al*. (2016), and about (0.86) in the study of Shi *et al.* (2021). Given that those patients need to start an optimal curing as soon as possible, so the increased levels of fibrinogen seems to give a considerable clue to the physicians about the disease severity.

According to the study done by Pan *et al.* (2018), fibrinogen is a strong predictor of DFU although hemostatic measures have a modest predictive value for T2DM. The sensitivity of DM's area under the ROC curve (AUC) to predict T2DM (0.592) was poor. At an AUC of (0.669), the best onset for angle α values was determined to be there (60°), yielding a sensitivity of (41.0%) and a specificity (95.6%) (Zhuang *et al.*, 2022).

Conclusions and Recommendations

Conclusion

1- In the current study, there was a significant effect of some demographic factors on diabetic patients suffering from diabetic foot ulcers compared to diabetic patients without ulcers.

2- Many aerobic bacteria that infect ulcers, species are classified in the foot which lead to amputation of limbs were diagnosed.

3- Increased fibrinogen levels in patients suffering from foot ulcers compared to patients who do not suffer from them, levels indicate active inflammation in the body, which helps assess the severity of the ulcer, fibrinogen levels can help doctors monitor healing by measuring fibrinogen levels periodically; doctors can monitor the patient's response to treatment. A decrease in fibrinogen levels may indicate improvement in the condition.

4- Genetic polymorphism of *MCP-1*: I allele showed a significant increase in both groups compared with D allele, *CRP* genes showed a significant difference in context of allele frequency, where G allele significantly appeared in both patients groups as compared with A allele

Recommendations

1- Further studies are needed to investigate the aerobic and anaerobic bacterial species and their association with severity DFU.
2- Some genes that play a role in the development of diabetic foot ulcers, such as genes associated with insulin sensitivity.
3- Additional physiological markers to study their association with type 2 diabetes in people with and without foot ulcers for example, kidney functions and blood oxygen level.
4- Monitoring fibrinogen levels is a valuable tool in the management of diabetic foot ulcers.
5- More genetics SNPs of MCP-1 and CRP to study for to know its connection link with type 2 diabetes.

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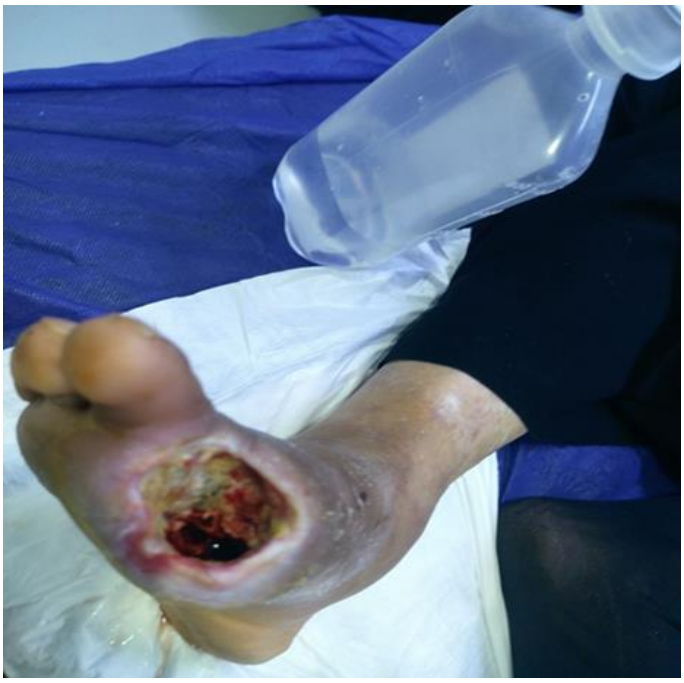
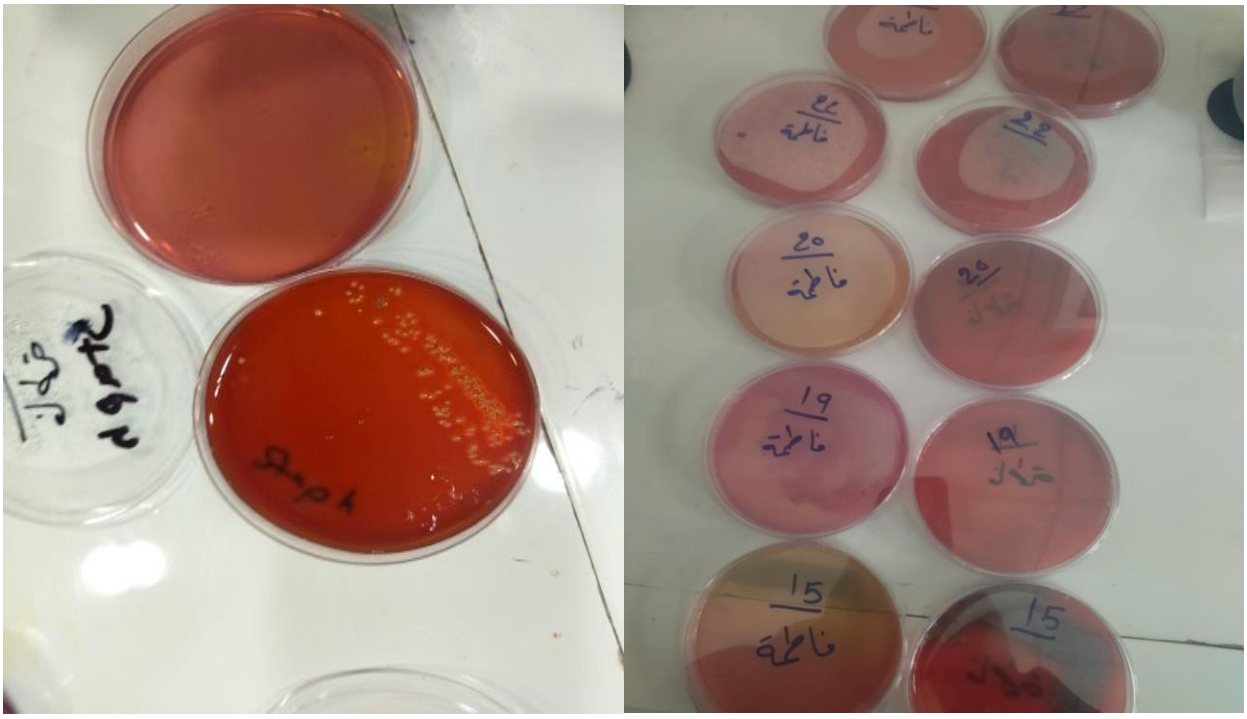
Appendices

Appendix I: diabetes mellitus type 2 with and without foot ulcer patients' questionnaires

1- Age
2- Sex (male – female)
3- Body mass (BMI)
4- Duration of disease
5- Other disease (hypertension – cardiovascular)
6- Diet (vegetarian – non vegetarian)
7- Smoking (yes – no)
8- Obesity (yes – no)
9- Education status (graduate or above - secondary school or less –literate)
10- Economic status (higher – middle – lower) class
11- Habitat status (urban – sub urban – rural)





Appendix II: Taking samples from foot ulcers and culturing them on different media

Appendix III: VITEK2 System

Appendix IV: Conventional PCR

الخلاصة

السكري هو مرض شائع انحاء العالم ، وقرحه القدم السكريه هي جرح مفتوح قد يحدث لمرضى السكري وتمثل اهم المضاعفات لدى مرضى السكري من النوع الثاني وهناك دراسات قليله حول العلاقه بين تعدد الاشكال الجينيه ومرضى السكري من النوع الثاني وهناك دراسات قليلة حول العلاقة بين تعدد الاشكال الجينية ومرضى السكري من النوع الثاني ، لذلك تناولت هذه الدراسه تعدد الاشكال الجينيه لجين بروتين جاذب للخلايا الوحيده (*MCP-1*) وبروتين (*CRP*) مع شده المرض للمرضى الذين يعانون من قرحه القدم وممن لا يعانون منها .

في هذه الدراسة تم جمع ١٠٠عينة دم في انبوب (EDTA) من كلا الجنسين ذكور واناث تتراوح اعمارهم بين (٣٣-٧٦) عاما من المرضى الذين تم استقبالهم في مركز الامام الحسن للغدد الصماء والسكري خلال الفترة من اغسطس الى ديسمبر ٢٠٢٣. تم تشكيل مجموعتين: ٥٠ مريضا مصابا بالسكري من النوع الثاني ويعانون من قرحه القدم و٥٠ مريضا مصابا بالسكري من النوع الثاني بدون قرحه القدم ،خضعت كل مجموعه للاختبار والفحص السريري، وتم جمع المعلومات عن المرضى فتم تحديد تعدد الاشكال الجينية للجينات (*CRP* and *MCP-1*) بواسطه نظام الطفرة المقاومة للتضخيم (ARMS) باستخدام تقنيه (PCR) بالإضافة الى ذلك تم اخذ عينات من كل مريض يعاني من قرحه القدم حيث تم عزل البكتيريا الهوائية وتشخيصها عن طريق زراعتها على وسائط زرعيه مناسبه باستخدام العديد من الاختبارات الكيميائية الحيوية .بالإضافة تم جمع عينات الدم في انابيب سترات الصوديوم لتحديد مستوى الفايبرينوجين لجميع المرضى.

.

أظهرت هذه الدراسة ان معظم البينات الوصفية لمجموعات الدراسة من مرضى السكري المصابين بقرحه القدم وغير المصابين بها اظهرت فروقا معنويا وفيما يتعلق بمده المرض كانت هناك نتائج معنويه في مجموعه مرضى السكري المصابين بقرحه القدم من الفترة اقل من (3-1اسبوع)و (3-1شهر) مقارنه بالفترة الاخرى بينما تؤثر الفترتان من (٥-١ سنه) واقل من (٥ سنه) بشكل كبير على مرضى السكري غير المصابين بقرحه القدم اكثر من المجاميع الأخرى. فيما يتعلق بتوزيع الامراض الاخرى تؤثر غالبيه مرضى السكري المصابين بقرحة القدم يعانون من ارتفاع ضغط الدم بينما تؤثر غالبيه مرضى غير المصابين بقرحه القدم بمتوسط (٥٩٦,٠٨)مقارنه بمتوسط مرضى السكري غير المصابين بقرحه القدم (٣٠٥,٦٦).اما يتعلق بنمو البكتيريا، فكانت غالبيه (٧٦%) من العينات ذات زراعة ايجابيه، بينما لم يكن هناك نمو في (٢٤%) منها ،واظهر نوع البكتيريا ان الغالبية (٧٨%) من نمو البكتيريا كانت بكتيريا سالبيه جرام بينما (٢٢%)فقط بكتيريا ايجابيه جرام، وفي سياق تنوع انواع البكتيريا المعزولة من القرحة كانت اعلى نسبه بكتيريا مكونات (*Staphylococcus aureus* ٣٤%) وبكتيريا (*Klebsiella pneumonia* 28%) وبكتيريا (*Proteus mirabilis* 26%) .

وفيما يتعلق بجين (*MCP-1*) فقد تم تسجيل الاختلاف الكبير في سياق تردد الاليل(I/D) وقد اظهر الاليل(I) زياده كبيره في كلتا المجموعتين للمرضى مقارنه بالاليل (D) بالإضافة الى ذلك اظهر الجين

(*CRP*) فرقا كبيرا في ســياق تردد الاليل (G) بشــكل ملحوظ في كلت المجموعتين لمرضـــى مقارنه بالليل (A).

جامعة كربلاء

كلية العلوم

قسم علوم الحياة

ارتباط تعدد الأشكال الجيني لبعض المؤشرات الحيوية مع العدوى البكتيرية وشدة قرحة القدم السكرية

رسالة مقدمة

الى مجلس كلية العلوم/ جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

فاطمة عدنان حسين السلامي

بكالوريوس علوم حياة – جامعة كربلاء (٢٠١٠)

بإشراف

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ا.د عبير ظاهر ناجي الحسناوي

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