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Determination of Antibacterial Action of Silver Nanoparticles Against Bacteria Isolated from Diabetic Foot Ulcers and Some Blood Biomarkers

A thesis submitted to the Council of the College of women Science, University of Babylon. As a partial requirement of the fulfillment of the Degree of Master in Sciences\ Biology

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

«وَزَكَرِيَّا إِذْ نَادَىٰ رَبَّهُ رَبِّ لَا تَذَرْنِي فَرْدًا وَأَنتَ خَيْرُ الْوَارِثِينَ»

صدق الله العلي العظيم

سورة الانبياء (الآية ٨٩)

CERTIFICATE

We certify that this thesis, entitled (**Determination of Antibacterial Action of Silver Nanoparticles Against Bacteria Isolated from Diabetic Foot Ulcers and Some Blood Biomarkers**) was prepared by (**Sarah Kadhim Abbas**) under our supervision at the college of science for women, University of Babylon as a partial requirement for the degree of Master of Science in Biology.

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Dedication

To

My beloved country, with all it encompasses...

The one who illuminated my path with prayers, my dear mother,

My first pillar of support, my dear father,

My brothers and sisters... you are a beautiful gift in my life,

My foremost supporter and partner, my dear husband,

My precious children,

My friends and colleagues... and to all those I love.

Sara

Acknowledgment

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Finally, I would like to thank all diabetic patients who participated in this study.

Sara

Summary:

Diabetic foot ulcers (DFUs) are a significant complication of diabetes mellitus, affecting up to 25% of diabetic patients and often leading to lower limb amputations. The study aimed to investigate the effects of silver nanoparticle (AgNP) preparations on isolated pathogenic bacteria from DFUs, assess antibiotic resistance, and evaluate the correlations between biomarkers such as Complete blood count (CBC), Interleukin-6 (IL-6), Vascular endothelial growth factor (VEGF), and Platelet-derived growth factor (PDGF).

A total of 125 DFU swab and blood samples were collected from the patients that admitted to Imam Hussain teaching hospital in Karbala between September 2022 and January 2023, along with 30 blood samples from healthy participants as a control group. The gender distribution among patients with DFUs was 40% male and 60% female. The most frequently identified bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa*, accounting for 24% of cases each. Antibiotic susceptibility profiles showed high resistance rates for commonly used antibiotics, with multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates being prevalent.

AgNP treatment demonstrated antibacterial activity against both *S. aureus* and *P. aeruginosa*. Increasing concentrations of AgNPs resulted in larger inhibition zones, with the highest inhibition zones observed at 125µg/ml for some isolates and up to 500µg/ml for others. Notably, AgNP treatment was effective against both MDR and XDR bacterial isolates, highlighting its potential as an alternative treatment for DFU infections.

Hematological analysis revealed that patients with DFUs had significantly higher white blood cell (WBC) counts compared to the

control group, with mean WBC counts of 8.77±0.9 and 6.35±1.3, respectively (P=0.042). This elevated WBC count was observed across all age groups and both genders. Additionally, patients with DFUs had significantly lower levels of Hb (9.81±0.13 g/dL) and higher levels of HbA1c (11.56±0.20%) compared to the control group (12.05±0.12 g/dL and 5.22±0.05%, respectively), with a p-value of (<0.001) for both parameters.

The biomarker analysis revealed significantly higher levels of VEGF, IL-6, and PDGF in the patient group (163.59±5.6, 19.76±0.45, and 116.62±3.40 respectively) compared to the control group (81.86±3.5, 11.35±0.36, and 82.85±3.30 respectively), with a p-value of <0.001 for all parameters. The Pearson correlation coefficient showed an inverse relationship between IL-6 and VEGF ($r=-0.279^{**}$) and a negative correlation between HbA1c and VEGF ($r=-0.236^{*}$). A strong positive correlation was observed between VEGF and PDGF ($r=0.302^{**}$), indicating a potential link between these biomarkers in DFU patients.

In conclusion, this study highlights the challenges posed by antibiotic-resistant bacteria in the management of DFUs and demonstrates the potential of silver nanoparticles as an alternative treatment for bacterial infections in DFUs. Furthermore, the analysis of hematological parameters and biomarkers contributes to a better understanding of the disease progression and may aid in the development of targeted therapies. The significant correlations between various biomarkers, such as IL-6, VEGF, and PDGF, suggest that these factors may play a role in the pathogenesis of DFUs and could serve as potential therapeutic targets.

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List of Abbreviations

Abbreviation	Meanings
ADAPPC	American Diabetes Association Prevention Program Committee
AgNPs	Silver Nanoparticles
Ag-SD	Silver Sulfadiazine
AP	Acute Pancreatitis
ASAP solution	Silver sol solution
ATP	Adenosine Triphosphate
AuNPs	Gold Nanoparticles
B cells	Beta cells
BMI	Body Mass Index
CEAP	Clinical, Etiologic, Anatomic, and Pathophysiologic
CI	Confidence Interval
CRP	C-reactive Protein
Cu	Copper
D cells	Delta cells
DFI	Diabetic Foot Infection
DFU	Diabetic Foot Ulcer
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DR	Diabetic Retinopathy
DVT	Deep Vein Thrombosis
E cells	Epsilon cells
EDIN	Epidermal Cell Differentiation Inhibitor
EFRMD	Excessive Fat-Related Metabolic Diseases
EG-VEGF	Endocrine Gland-Derived Vascular Endothelial Growth Factor
F cells	Pancreatic Polypeptide cells
Flk-1	Fetal Liver Kinase 1 (VEGFR-2 in mice)
Flt-1	Fms-like Tyrosine Kinase 1 (VEGFR-1)
Flt-4	Fms-like Tyrosine Kinase 4 (VEGFR-3)
GDM	Gestational Diabetes Mellitus
HbA1c	Hemoglobin A1c
IDDM	Insulin-based Diabetes Mellitus
IDF	International Diabetes Federation
IL-6	Interleukin-6
KDR	Kinase Insert Domain Receptor (VEGFR-2)
LDS	Lipodermatosclerosis

LLA	Lower Limb Amputation
MCV	Mean Corpuscular Volume
MDR	Multi-drug Resistant
MLR	Monocyte-to-Lymphocyte Ratio
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NIDDM	Non-insulin-based Diabetes Mellitus
NLR	Neutrophil-to-Lymphocyte Ratio
NP-1	Neuropilin-1
NP-2	Neuropilin-2
NPDR	Non-Proliferative Diabetic Retinopathy
NS	Nanosilver
PAD	Peripheral Arterial Disease
PLR	Platelet-to-Lymphocyte Ratio
PP	Pancreatic Polypeptide
PVL	Panton-Valentine Leukocidin
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
SEl-X	*Staphylococcal* Enterotoxin-like X
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
T2DM	Type 2 Diabetes Mellitus
TSS	Toxic Shock Syndrome
TSST	Toxic Shock Syndrome Toxin
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
WBC	White Blood Cell
WHO	World Health Organization
XDR	Extensively drug-resistant

Chapter One
Introduction

1.1 Introduction:

Diabetes is a chronic disease with symptoms influenced by blood sugar levels. Type 1 diabetes symptoms develop quickly and are more severe, including increased thirst, frequent urination, unintended weight loss, and ketones in urine (Adeleye *et al*., 2020). The global diabetic population has increased fourfold since 1980 and is expected to continue rising (WHO, 2016).

Foot ulceration is a common complication for patients with diabetes mellitus (DM), with up to 25% developing foot ulcers during their lifetime. Diabetic individuals are 15-30 times more likely to undergo amputations than non-diabetics (Raghav *et al*., 2018). Diabetic foot ulcers (DFUs) often result from various microorganisms, such as gram-negative bacteria like *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli* (Sadeghpour *et al*., 2019). *Pseudomonas aeruginosa* plays a significant role in DFUs in under-resourced communities due to its high pathogenicity and virulence factors like biofilm formation (Qin *et al*., 2022).

Vascular endothelial growth factor (VEGF) significantly impacts endothelial cells by increasing vascular permeability, inducing mitogenic and anti-apoptotic effects. VEGF plays a key role in physiological and pathological angiogenesis processes such as tumor growth, diabetic retinopathy, ischemic processes, preeclampsia (Melincovici *et al*., 2018). Inflammation also has a critical role in diabetes pathogenesis, with elevated IL-6 levels observed in patients suffering from type 2 diabetes (Qu *et al*., 2014). Likewise, CRP and WBC count are correlated with insulin resistance and heightened risk of developing type 2 diabetes (Oda, 2015; Gu *et al*., 2018).

Nanotechnology offers promising antibacterial strategies through nanoparticles like silver, zinc oxide, and copper that demonstrate potent antimicrobial properties against various bacteria (Baranwal *et al*., 2018). Silver

nanoparticles could address persistent infections, limb amputations, and morbidity in DFU management due to the presence of MDR microorganisms (Salomoni *et al*., 2017).

This study aimed to isolation and identification of the most gram-positive and gram-negative bacterial species that cause DFUs and evaluate the activity of silver nanoparticle preparations on isolated multi-drug resistance pathogenic bacteria, and also determine some biomarkers associated with DFUs.

The main subject to perform our work:

1- Isolation and Identification of bacteria-contaminated foot ulcer wounds.

2- Following standard microscopic, biochemical test, and VITEK2 system for identifying isolated bacteria.

3- Evaluated the impact of many biomarkers of the human body, like CBC, and IL6, and correlated them. Vascular endothelial increase element (VEGF)

4- Evaluation of silver nanoparticles against some MDR bacteria.

Chapter two
Literature Review

Literature review:

2.1 Diabetic Disease:

Diabetes mellitus (DM) is a widespread endocrine disorder characterized by high blood glucose levels due to impaired insulin secretion, function, or both. DM is primarily classified into three types: Type 1 diabetes mellitus, Type 2 diabetes mellitus, and gestational diabetes mellitus (GDM) (Mukhtar *et al*., 2019; Al-Musawi *et al*., 2021a 7\SH '0 UHVXOWV IURP WKH ERG\ LQVXOLQ GXH-WR SDQFUHDWLF cell destruction and is mainly associated with genetic factors. In contrast, Type 2 DM accounts for the majority of cases (85%) and arises from insulin resistance or insufficient insulin production, predominantly linked to multi-hormonal disorders (Katsarou *et al*., 2017; Chatterjee *et al*., 2017). GDM occurs during pregnancy when hormones obstruct insulin action, resulting in insulin resistance and hyperglycemia; women with GDM history have an increased risk of developing Type 2 diabetes later in life (Coustan, 2013).

combined with monofilament testing for neuropathy and noninvasive tests for arterial insufficiency can identify at-risk patients (Armstrong and Lavery, 1998).

Venous ulcers are the most common leg ulcer type but lack a consistent definition (Abbade and Lastória, 2005). They are characterized by chronic venous hypertension resulting from venous reflux or obstruction without primary arterial or systemic causes (Labropoulos *et al*., 2007).

2.3 Risk Factors predispose Diabetic:

The development of type 2 diabetes is influenced by various risk factors, some unmodifiable (e.g., family history, age, race, or ethnicity) and others modifiable through maintaining a healthy weight and being physically active (American Diabetes Association, 2022). The risk increases with age, family history of diabetes, or gestational diabetes history (Knowler *et al*., 2002). Body weight is a significant risk factor; losing weight and increasing physical activity can prevent or delay its development. Body Mass Index (BMI) and waist circumference are used to evaluate body weight; most adults with a BMI of 25 or more are classified as overweight and at increased risk for type 2 diabetes (Hansen *et al*., 2020).

2.4 White blood cells and Diabetic disease:

Kheradmand *et al*. (2021) examined the relationship between white blood cell (WBC) count and type 2 diabetes, finding a significantly higher average WBC count in diabetic patients compared to controls. The odds of diabetes based on WBC count were significant even after adjusting for confounding factors. Despite numerous studies identifying a connection between WBC count and insulin resistance/type 2 diabetes, inconsistencies persist in the literature (Du *et al*., 2009; Oda, 2015). Infection markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) have also been linked to glucose dysregulation and

diabetes (Qu *et al*., 2014). Kashima *et al*. (2019) found WBCs as a separate risk factor for type 2 diabetes in Japan, while another study with a healthy Japanese population showed no correlation between WBC count and diabetes incidence (Oda, 2015).

2.4.1 Neutrophils, Eosinophils, basophil, monocyte, and lymphocyte:

Recent findings suggest that platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-lymphocyte ratio (NLR) could be potential indicators of inflammatory responses (Yue *et al*., 2015). One study found that average PLR and NLR were significantly higher in DR patients than in those without DR. The mean MLR was higher in the non-proliferative diabetic retinopathy (NPDR) group compared to patients without DR, but no significant differences were observed among the three groups. PLR and NLR showed a significant increase in DR patients. After adjusting for confounding factors, MLR 18 was identified as a risk factor for DR, although its predictive ability was limited despite its possible pathophysiological and clinical significance in DR (Yue *et al*., 2015).

2.5 Red blood cells, Platelet, and it physiology

Hyperglycemia, the primary marker for diabetes mellitus (DM), causes structural and functional modifications in red blood cells (RBCs) or erythrocytes, which are the most abundant circulating cells and highly sensitive to plasma composition changes (Wang *et al*., 2021). Investigating erythrocyte-related markers is essential in preventing, diagnosing, and treating DM and its complications.

Erythrocytes consume significant glucose amounts, with sustained hyperglycemia leading to changes in shape, metabolism, and function that can

impact hemorheology and microcirculation (Zhou *et al*., 2018). Exploring these alterations in association with diabetes progression is critical for understanding their role in diagnosis, treatment, and prognosis (Sprague *et al*., 2006).

As blood glucose levels increase, more glucose enters erythrocytes and activates metabolic pathways. Lacking mitochondria, glycolysis serves as HU\WKURF\WHV¶ SULPDU\ HQHUJ\ VRXUFH (Lee *et al*., 2019). Adenosine triphosphate (ATP), a glycolysis byproduct, is a vital energy molecule supporting regular functions like transmembrane ion/lipid exchange and erythrocyte deformability (Bali and Thomas, 2001).

2.6 Bacterial pathogenicity and Toxins:

Diabetic patients frequently experience soft tissue infections in the foot, interdigital spaces, and toenails, potentially resulting in severe complications such as sepsis, amputation, or death if left untreated. *Staphylococcus aureus* is the most commonly isolated pathogen causing these complications (Driscoll *et al*., 2007; Casqueiro *et al*., 2012).

Diabetic foot infections (DFI) are a prevalent complication of diabetes with significant healthcare costs. They are often caused by polymicrobial agents, with *S. aureus* being the most common pathogen identified. Unique characteristics of DFI include fragile granulation tissue, necrosis, malodorous secretions, non-purulent exudates, and prolonged healing despite proper care (Dunyach-Remy *et al*., 2016; Lavery *et al*., 2016).

S. aureus strains in DFI can be classified into toxinogenic and non-toxinogenic types (Wilson *et al*., 2011). Toxinogenic strains possess genes for toxins such as exfoliatin, EDIN, PVL, or TSST and are often linked to more severe infections. Non-toxinogenic strains mainly affect deep structures and bones associated with diabetic foot osteomyelitis (Becker *et al*., 2003).

The toxic shock syndrome toxin-1 (TSST-1) is the best-characterized *S. aureus* superantigen responsible for toxic shock syndrome. Additionally, SEl-X has been implicated in necrotizing pneumonia caused by the USA300 MRSA strain (Wilson *et al*., 2011). Around 88% of DFU isolates carry the SEl-X gene while a small percentage contain TSST-1 gene or both genes (Becker *et al*., 2003).

2.7 The main symptoms of skin in diabetic patients:

2.7.1 Pigmented Purpuric Dermatoses

The phenomenon of pigmented purpuric dermatoses is commonly observed in older patients with diabetes and often coexists with diabetic dermopathy. It is characterized by non-blanching copper-colored patches that predominantly affect the pretibial areas of the legs or dorsum of the feet. While these lesions are typically asymptomatic, they may sometimes cause itching (Liau *et al*., 2016). This dermatosis is more commonly seen in advanced stages of diabetes in patients with nephropathy and retinopathy. The underlying mechanism is believed to be microangiopathic damage to capillaries, leading to the deposition of sequential erythrocytes (Demirseren *et al*., 2014).

2.7.2 Palmar Erythema

Palmar erythema is a harmless condition characterized by symmetrical redness and warmth in the palms, particularly in the hypothenar and thenar eminences. It is usually asymptomatic and considered to be associated with microvascular complications of diabetes, which contribute to its pathogenesis (Serrao *et al*., 2007).

2.7.3 Periungual Telangiectasias

Periungual telangiectasias, identified by erythema and telangiectasias around the proximal nail folds, are frequently observed asymptomatic occurrences in nearly 50% of individuals with diabetes. These manifestations may coincide with irregular cuticles and sensitivity at the fingertips. The fundamental cause is believed to be venous capillary dilation resulting from diabetic microangiopathy, which can lead to capillary irregularities, such as venous capillary twisting. This represents an initial indication of microangiopathy related to diabetes (Van Hattem *et al*., 2008; Oumeish, 2008).

2.7.4 Yellow Skin and Nails

Often, individuals with diabetes, especially older patients with type 2 diabetes, display asymptomatic yellowish alterations in their skin or fingernails. These harmless transformations commonly impact the palms, soles, face, or the distal nail of the big toe. The pathogenesis of these changes in complexion remains controversial, although the accumulation of various substances in patients with diabetes is thought to be responsible. (Bristow, 2008).

2.7.5 Onychocryptosis

Patients with type 2 diabetes may develop onychocryptosis, commonly known as ingrown toenails, with the great toes being most affected. It is believed that this condition occurs in diabetic patients due to various factors such as increased body mass index, trauma, weakened vascular supply, nail plate dysfunction, and subungual hyperkeratosis. These factors lead to the abnormal growth of the nail, which subsequently leads to its penetration into the surrounding skin. Onychocryptosis in diabetes is usually painful and may lead to the formation of an abscess or cellulitis. Therefore, it is essential to promptly

diagnose and treat this condition to avoid further complications (Yesudian *et al*., 2022).

2.7.6 Skin tissue structure and diabetic:

Diabetes mellitus is a prevalent and incapacitating condition that impacts various organs, including the skin. Skin-related complications of diabetes mellitus can be found in 30-70% of patients with both type 1 and type 2 diabetes. The dermatological manifestations of diabetes differ in severity, ranging from benign to disfiguring and even potentially fatal (Behm *et al*., 2012).

Dermopathy, also referred to as pigmented pretibial patches or diabetic shin spots, is the most frequent dermatological manifestation of diabetes, appearing in up to 50% of patients (Bustan *et al*., 2017). Although its diagnostic significance is debated, dermopathy is often deemed pathognomonic for diabetes. It is more common in men and individuals over the age of 50 (Morgan and Schwartz, 2008). While it can occur before diabetes onset, dermopathy typically presents as a late complication and is frequently associated with microvascular complications like nephropathy, neuropathy, and retinopathy (Naik and Farrukh, 2020).

Xerosis, or abnormally dry skin, is among the most frequent skin manifestations in diabetic patients, affecting up to 40% of them (Goyal *et al*., 2010). The affected skin may display scaling, cracks, or a rough texture, with feet being the most affected area. In diabetic patients, xerosis often arises in conjunction with microvascular complications (Sawatkar *et al*., 2014). Emollients such as ammonium lactate can be employed to manage xerosis and prevent complications like fissures and secondary infections (Pavicic and Korting, 2006).

2.8 Chronic venous ulceration:

Venous ulcers are full-thickness skin disorders associated with chronic

Y H Q R X V G L V H D V H W K D W I D L O W R K H D O V S R Q W D Q
R I W K H S R S X O D W L R Q W K H V H X O F H U V F D X V H S

et al., 2014). Affecting (Phillips *et al*., 2018). Advanced venous disease affects over 2.5 million US patients annually with symptoms like edema and leg ulceration (Robertson and Fowkes, 2008). The CEAP (Clinical, Etiologic, Anatomic and Pathophysiologic) classification system ranks venous disease severity from telangiectasia/reticular veins (C1) to chronic venous ulceration (C6) through lipodermatosclerosis (C4) (Kistner and Masuda, 1996). Venous ulcers take six to 365 days to heal but may recur in up to 70% cases within five years resulting in pain, disability, and significant workdays loss (Lal, 2015).

2.9 Diabetic foot bacterial infection:

Diabetic foot ulcers are a common diabetes complication, affecting over one-third of individuals at some point in their lives. Among those with DFUs, 50% will experience diabetic foot infections (DFIs), with 15% of patients requiring lower limb amputations to halt infection progression (Commons *et al*., 2018). Addressing both intrinsic factors, such as enhanced glucose control, and extrinsic factors, primarily the eradication of bacterial infections, are essential in the treatment of DFUs/DFIs. However, the complex bacterial communities associated with DFUs/DFIs present challenges in selecting appropriate treatment approaches (Sadeghpour Heravi *et al*., 2019).

Various laboratory techniques exist for identifying the bacterial composition of DFUs/DFIs, each with different sensitivity and specificity levels. Fully characterizing the polymicrobial community across diverse severity levels remains a significant hurdle (Rhoads *et al*., 2012). Culture-based methods are the primary approach for bacterial identification but often yield false-negative results

in patients treated with antibiotics and fail to identify slow-growing, fastidious, anaerobic, and unknown pathogens. These methods are also time-consuming, hindering prompt and accurate detection of the bacterial community in DFUs/DFIs. Recent advancements in molecular technology have addressed some limitations and provided new insights into the bacterial diversity of DFUs/DFIs, significantly impacting the identification of previously undetected and uncultivable microorganisms (Sadeghpour Heravi *et al*., 2019).

2.9.1 Diabetic foot ulcer-associated bacterial spectrum:

Biofilms provide bacteria protection against external stressors, like immune responses and antimicrobial agents, and are linked to increased antibiotic resistance and recurrent infections in diabetic individuals (Swarna *et al*., 2012). Peripheral neuropathy has also been associated with ulcer development in diabetic patients. Infections in these ulcers often involve Gram-positive bacteria (e.g., *Enterococcus*, *Staphylococcus aureus*) and Gram-negative bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* species, *Klebsiella* species), as well as anaerobic bacteria like *Clostridium* (Shankar *et al*., 2005).

Biofilm formation is a crucial virulence factor that allows bacteria to persist in wounds and contributes to chronic infections in diabetic foot ulcers (DFUs) (Banu *et al*., 2015b). Identifying biofilm-producing isolates can enhance wound infection management in diabetic patients who do not respond to repeated antibiotic treatments (Hughes and Webber, 2017).

Banu *et al* ¶ V a) study found monomicrobial isolates, while other research reported the polymicrobial nature of DFUs (Bansal *et al*., 2008). Early antimicrobial therapy and wound care initiation could lead to a reduced incidence of polymicrobial infections; however, as the infection progresses, a polymicrobial state emerges (Simões *et al*., 2018).

Regarding bacterial isolates, Bansal *et al*. (2015) discovered that 75.6% were gram-negative and 24.4% were gram-positive. These results align with both Bansal *et al* ¶ V I L Q G L Q J V D Q G W K R V H U H S R U W L Q negative organisms.

2.9.2 Bacterial biodiversity associated with DFU:

Gram-positive bacteria such as *Streptococcus* spp., *Enterococcus* spp., and *Staphylococcus* spp. are commonly reported causative agents of diabetic foot infections (DFIs) (Tascini *et al*., 2011; Al Benwan *et al*., 2012; Jneid *et al*., 2018;). Gram-negative bacteria, especially members of the *Enterobacteriaceae* family (e.g., *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Morganella morganii*), have been identified as predominant microorganisms associated with DFUs (Prompers *et al*., 2007; Al Benwan *et al*., 2012).

Less common bacterial species, such as Coagulase-negative *Staphylococcus* species and other uncommon species, have been detected in DFUs through metagenomic analysis. Additional research is needed to determine the significance of these rare species in the development and persistence of DFIs (Abdulrazak *et al*., 2005; Jneid *et al*., 2017).

2.10 Human Biomarkers:

2.10.1 Hemoglobin A1c

Hemoglobin A1c (HbA1c) is a crucial diagnostic test for diabetes and high blood sugar management. It reflects glycation synthesis from the terminal J O \ F D W L R Q - X Q L W R I W K H chain of hemoglobin, which is proportionate to the plasma glucose levels over the past six to eight weeks (Adeoye *et al*., 2014). HbA1c is a widely used marker to evaluate metabolic control, predict the development of diabetic complications, and measure the quality of diabetes care (Hussain, 2015). However, anemia can significantly affect HbA1c levels. An increase in red blood

cells (RBC) due to blood loss, hemolysis, hemoglobinopathies, myelodysplastic diseases, or hemolytic anemia can lower HbA1c levels (Cohen *et al*., 2008). Conversely, a decrease in erythrocyte count, as observed in patients after splenectomy or with iron deficiency anemia, can raise glycation levels of Hb and result in high HbA1c levels (Huang *et al*., 2018). Thus, HbA1c is a valuable marker for diabetic patients, but the interpretation of its results should consider the patient's hemoglobin status. HbA1c is used to assess metabolic control, predict diabetic complications, and measure diabetes care quality (Cohen *et al*., 2008; Adeoye *et al*., 2014).

2.10.2 Vascular endothelia growth factor (VEGF):

Vascular endothelial growth factor (VEGF) is a crucial proangiogenic agent that influences endothelial cells, increases vascular permeability, and promotes cell movement (Ucuzian *et al*., 2010; Niu and Chen, 2010).

Angiogenesis is a complex process influenced by various factors acting as either proangiogenic agents or angiogenesis inhibitors (Rosen, 2002). Among the powerful proangiogenic agents is VEGF, which plays a role in the pathophysiology of Diabetic Foot Ulcer (DFU). It contributes to neuropathy protection by promoting neurogenesis and angiogenesis and has a critical role in microcirculatory system angiogenesis in peripheral arterial disease (PAD) related to atherosclerosis (Paraskevas *et al*., 2008).

DFU formation results from factors such as trauma, neuropathy, ischemia, and infection. Changes in muscles, skin, bones, and tendons due to neuropathy cause alterations in pressure distribution on foot soles leading to ulceration. Furthermore, recurrent trauma, inadequate limb vascularization, neuropathy, and infection contribute to DFU development and hinder wound healing in individuals with diabetes (Mahsa *et al*., 2011).

Managing diabetic foot ulcers is complicated due to poor blood flow and potential for sustained infection, specific to patients with diabetes (Boulton, 2008). Diabetic patients with neuropathy experience reduced VEGF-A gene expression, as observed through epidermal VEGF-A biopsy (Quattrini *et al*., 2008). However, VEGF actively stimulates angiogenesis and facilitates wound healing in DFU patients by inducing vasodilation, endothelial cell migration, capillary tubule formation, and basement membrane formation (Rico *et al*., 2009).

Histopathological analysis of patients with diabetic neuropathy (DN) reveals abnormalities such as axonal atrophy, nerve fiber damage, and altered nerve regeneration processes. DN pathogenesis is multifaceted and involves metabolic and vascular factors (Caldwell *et al*., 2003). Research on VEGF receptor mice has shown that the VEGFR-2 receptor is vital for differentiation while VEGFR-1 is necessary for blood vessel formation. VEGFR-1 mediates blood vessel hyper-permeability, while VEGFR-2 is involved in angiogenesis (Hoeben *et al*., 2004). Additionally, VEGF promotes collagen production and angiogenesis by clearing matrix proteins, enabling migration processes, and stimulating endothelial cell growth (Bao *et al*., 2009). In a study by Ropper *et al*. LQ DW %RVWRQ 8QLYHUVLW\¶V 1HXURORJ\ GH found that intramuscular VEGF plasmid gene transfer could alleviate symptomatic indications of diabetic neuropathy.

2.10.3 Interleukin 6 (IL-6):

Inflammation markers, such as interleukin-6 (IL-6) and C-reactive protein (CRP), have been linked to glucose intolerance and diabetes (Qu *et al*., 2014; Oda, 2015). The connection between white blood cells (WBC) as a subclinical inflammation marker and insulin resistance, as well as type 2 diabetes, has been explored in various studies with inconsistent findings (Du *et al*., 2009). A U-

shaped correlation between WBC count and diabetes risk was found among young Chinese workers, while no association was observed in a healthy Japanese cohort. In contrast, Kashima *et al*. identified WBC count as an independent risk factor for type 2 diabetes in Japan (Kashima *et al*., 2019).

IL-6 has been associated with multiple metabolic illnesses, including T2DM, obesity, and cardiovascular disease. In obesity, adipose tissue is a primary source of IL-6, with production correlating to adiposity extent and insulin resistance (Mohamed-Ali *et al*., 1997). IL-6 induces insulin resistance by inhibiting peripheral tissue insulin signaling and promoting hepatic gluconeogenesis (Nieto-Vazquez *et al*., 2008). Additionally, IL-6 plays a role in diabetic complications such as retinopathy and nephropathy (Moresco *et al*., 2018; Xu *et al*., 2019).

Clinical studies have demonstrated the importance of IL-6 in T2DM. Increased IL-6 levels were observed in T2DM patients compared to healthy controls (Fernandez-Real *et al*., 2001) and were positively associated with insulin resistance and fasting glucose levels across various populations (Kern *et al*., 2001; Spranger *et al*., 2003; Thorand *et al*., 2005). As IL-6 signaling blockade improves insulin sensitivity in animal models (Wallenius *et al*., 2002; Kim *et al*., 2004), it has been proposed as a potential therapeutic target for T2DM.

2.11 Silver Nanoparticles and Diabetic treatment:

Diabetic foot ulcer (DFU) patients often face multi-drug resistant (MDR) microorganisms, leading to prolonged wound healing, increased hospital stays, treatment expenses, and patient mortality (Cho *et al*., 2005). Silver has been widely used as an antimicrobial agent to manage bacterial infections and prevent wound sepsis due to its strong antimicrobial properties (Beer *et al*., 2012). However, topical silver antimicrobials and dressings face several challenges such as reduced release rates, insufficient tissue penetration, rapid depletion of silver

ions, and pro-inflammatory effects in cream-based products (Chen and Schluesener, 2008).

To address these issues, silver has been incorporated directly into dressings like Acticoat Œand Silverlon® for wound treatment. This approach minimizes the likelihood of nosocomial infections, healthcare costs, and patient suffering. Nevertheless, these dressings are typically applied before bacterial infection occurs, whereas in DFUs, wounds are already infected with various microorganisms including MDR strains. Therefore, it is essential to develop effective antibacterial treatments that promote rapid DFU healing without the adverse effects of silver ions while eliminating microbial infections (Salomoni *et al*., 2017).

Nanotechnology offers new possibilities for biomedicine and biotechnology through nanoparticle synthesis. Nanomaterials possess unique physicochemical properties due to their nanoscale size ranging from 1-100 nm (Kreytsberg *et al*., 2011). Among antimicrobial nanomaterials capable of combating infectious diseases, silver nanoparticles (AgNPs) have gained significant attention because of their remarkable microbicidal activity against wild-type and nosocomial strains of MDR microorganisms in medical products such as bandages, wound dressings, catheters, and textiles (Salomoni *et al*., 2017).

In DFUs, polymicrobial infections are common and MDR bacteria increase the chances of persistent infection, morbidity, and limb amputation. Considering $ J 1 3 V ¶ D Q W L P L F U R E L D O *et al*., 2014), this study aims to F . D \ investigate their potential use in treating DFUs, as evidenced by photographs documenting wound healing progression during treatment, showing a visible reduction in DFU size without subsequent infections following AgNP treatment (Chaloupka *et al*., 2010).

2.11.1 Estimation ulcer Recovery with nanoparticles:

Maldonado-Vega *et al*. (2021) examined the DFU wound healing process following AgNP treatment using photographs. The wound was cleansed, and necrotic tissue removed, leaving a 2 cm long and 1 cm deep open wound. Daily application of AgNPs (1.8 mg/ml) resulted in a decrease in WKH ZRXQG¶V GLD and depth after eight days, along with improved closure and reduced pain.

AgNPs were then administered twice weekly. A steady decrease in lesion volume was observed, with reduced diameter and closure without necrosis or infection. Tissue pigmentation indicated reepithelialization progress (Kreytsberg *et al*., 2011).

Nanotechnology has been explored for wound care material development, with nano-scaffolds and nanofibers promoting wound healing through various phases. Silver nanoparticles (AgNPs) have shown antibacterial and anti-inflammatory potential for diabetic wounds (UcKay *et al*., 2014; Choudhurya *et al* 7KLV UHYLHZ DLPV WR KLJKOLJKW $J13\ DGYDQFHG GUHVVLQJ IRU GLDEHWLF SDWLHQWV¶ ds and ulcers (Paladini & Pollini, 2019).

2.11.2 Uses silver as Nanoparticles:

Metal nanoparticles such as copper (Cu), gold (Au), and silver (Ag) are LQFUHDVLQJO\ XVHG WR FRPEDW LQIHFWLRXV G protein synthesis, cell membranes, and nucleic acids (Faúndez *et al*., 2004; Perelshtein *et al*., 2009). AgNPs have medical applications but demonstrate limited antibacterial activity against specific bacteria when incorporated into polycaprolactone nanofibers, suggesting their use alongside other antibacterial agents (Li *et al*., 2017; El-Naggar *et al*., 2021).

Silver plays a critical role in wound healing by modulating cytokines and growth factors, while copper possesses strong biocidal properties and is essential for skin regeneration and angiogenesis (Alizadeh *et al*., 2019; Salvo & Sandoval, 2022). Silver nanoparticles exhibit significant antibacterial activity through multiple mechanisms affecting bacterial cells (Shrivastava *et al*., 2007; Yang *et al*., 2009; Chaloupka *et al*., 2010). Revelli *et al* G H P R Q V W U D W H G broader antimicrobial spectrum compared to antibiotics.

Silver hydrogel containing Ag nanoparticles is used in dressings for various wounds, with in vitro studies showing rapid and potent antimicrobial activity against both Gram-positive and Gram-negative bacteria (Galdiero *et al*., 2011; Rai *et al*., 2012; Munger *et al*., 2014). Essa *et al*. (2021) found SilvrSTAT Gel beneficial for DFU healing but highlighted the need for further controlled studies. Identifying microorganisms associated with DFU is vital.

Traditional treatment methods have shortcomings, and innovative wound management techniques like negative pressure therapy, growth factor products, maggot therapy, hyperbaric oxygen therapy, and bioengineered tissue or skin replacements can aid the healing process (Sarvajnamurthy *et al*., 2013; Baltzis *et al*., 2014). One approach involves using silver nanoparticles (SilvrSTAT Gel).

Chapter three

Materials and Methods

3.1 Materials

3.1.1 Laboratory instruments and equipment

Table (3-1) Below provides a record of the laboratory equipment and instruments used in this study.

No	Laboratory Equipment and Instruments	Manufacturer/Origin
1	Autoclave	Hirayama (Japan)
2	Benze burner	Amal (Turkish)
3	Centrifuge	Hettich (Germany)
4	Light microscope	Olympus(Japan)
5	Densichek Turbidity meter	Biomerieux (France)
6	Disposable and glassware	Afco(Jordan)
7	Distillator	Deltalab(Spain)
8	Electric sensitive balance	Sarteorius ag (Germany)
9	ELISA	BioTeck (USA)
10	Cobas	Roche (switzerland)
11	Hematology Auto analyzer	Beckman (USA)
12	Hot plate with magnetic stirrer	IKA (Germany)
13	Incubator	Memmert (Germany)
14	Micropipette	Dragon (china)
15	Refrigerator	Concord (Lebanon)
16	Safety hood	EuroClone (Italy)
17	VITEK 2	Biomerieux (France)

3.1.2 Chemicals Utilized in the Study

The table below (3-2) displays a list of the biological and chemical materials utilized in the study.

Table (3-2) Biological and Chemicals Material

No	Biological and Chemical Materials	Manufacturer
1	Glycerol ($C_3H_8O_3$)	Merck(England)
2	Gram stain	Spectrum (Germany)
3	Silver nanoparticles (AgNPs),size (40nm), shape circular, chemical synthesized ,and characterization by electron micro scope (TEM,SEM).	US research nanomaterials Inc (USA)
4	Gordon-Mcleod reagent (Oxidase)	Himedia(India)
5	Coagulase Plasma	Himedia(India)

3.1.3 Commercial kit

The table (3-3) presents the commercial kits that were employed in this study.

Table (3-3): Commercial kit

No	Type of Kit	Origin
1	Human Vascular Endothelial cell Growth factor A (VEGF-A) ELISA	Shanghai YL biotech (China)
2	Human PDGF-BB(Platelet Derived Growth Factor BB) ELISA	Elabscience (USA)
3	Interleukin-6 ELISA	Elabscience (USA)
4	VITEK 2 GN CARD	BioMérieux (France)
5	VITEK 2 GP CARD	BioMérieux (France)
6	VITEK 2 AST CARD	BioMérieux (France)
7	Cobas HbA1c	Roche (switzerland)

3.1.4 Culture Media

Table (3-4) presents a comprehensive list of the culture media used in the current study.

Table(3-4): Culture Media used

No	Culture Media	Company(Origin)
1	Blood Agar	Himedia(India)
2	MacConkey Agar	Himedia(India)
3	Mueller Hinton Agar	Himedia(India)
4	Mannitol Salt Agar	Himedia(India)

3.1.5 Antibiotic

All antibiotic used in this study with their concentration illustrated in table (3-5).

Table (3-5): Antibiotic used

No	Antibiotic	Symbol	Company (Origin)
1	Ticarcillin	TI	BioMérieux (France)
2	Ticarcilline + clavulanic acid	TIM	
3	Piperacillin	PIP	
4	Piperacillin + tazobactam	TZP	
5	Ceftazidime	CAZ	
6	Cefepime	FEB	
7	Imipenem	IM	
8	Meropenem	ME	
9	Amikacin	AK	
10	Gentamicin	GM	
11	Tobramycin	TO	
12	Ciprofloxacin	CIP	
13	Colistin.	CST	
14	Benzylpenicillin	BENPEN	
15	Oxacillin	OXA	

16	Gentamicin	GEN	
17	Ciprofloxacin	CIP	
18	Levofloxacin	LEVO	
19	Moxifloxacin	MOX	
20	Erythromycin	ERY	
21	Clindamycin	CLIN	
22	Quinupristin/Dalfopristin	Q-D	
23	Linezolid	LIN	
24	Teicoplanin	TEC	
25	Vancomycin	VAN	
26	Tetracycline	TET	
27	Tigecycline	TIG	
28	Nitrofurantoin	NIT	
29	Fusidic Acid	FUS	
30	Rifampicin	RIM	
31	Trimethoprim/Sulfamethoxazole	TMP	

3.2 Study design

The study design flow chart showed in figure 3-1

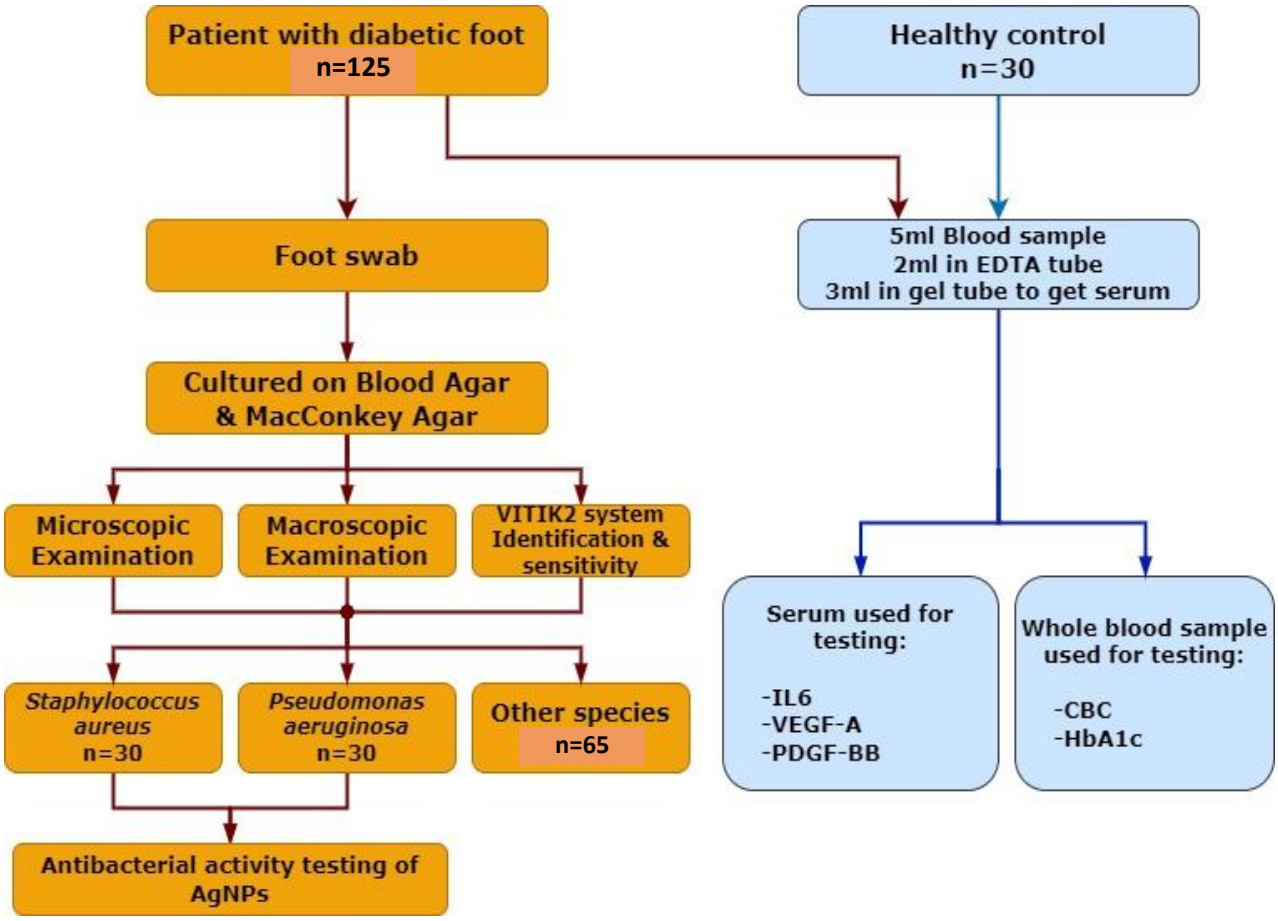


Figure (3-1): study design

A total of 125 swab and blood samples were collected from patients with diabetic foot infections. Of these, 30 were diagnosed as *Staphylococcus aureus*, 30 as *Pseudomonas aeruginosa*, and the remaining 65 were other species. The blood samples of patients diagnosed with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, along with 30 healthy controls, were tested for hematological and biochemical parameters.

3.3 Methods

3.3.1 Samples Collection

During the period from September 2022 to January 2023, 125 diabetic foot infection swab and blood samples were collected from diabetic foot infection patients admitted to Imam Hussain teaching hospital in Karbala, the swab was collected using sterile cotton swab, and 5 ml of blood collected, 2 ml in EDTA tube to get a whole blood sample, and 3 ml in gel tube, leaved to clot then centrifuged to get serum. Thirty blood samples were collected from heathy participant as control group, 2 ml in EDTA tube to get a whole blood sample, and 3 ml in gel tube, leaved to clot then centrifuged to get serum.

3.3.2 Diagnosis criteria

The diagnostic criteria for diabetic foot infection were based on the guidelines provided by the International Working Group on the Diabetic Foot (IWGDF), which encompassed:

7KH SUHVHQFH RI D ZRXQG ORFDWHG EHORZ WK
7KH ZRXQG LV SUHVHQW IRU DW OHDVW ZHHN
ZLWKLQ DIWHQW WUHDWPHQW
7KH ZRXQG WKLFNQHVV ORVV RI VNLQ H[WHQGL
VXEFXWDQHRXV WLVVXH RU EH\RQG DQG PD\ H
7KH ZRXQG LV QRW KHDOLQJ GHVSLWH DSSURS
ORFDO ZRXQG DQG FDUH DWPHQW RI XQGHU/LSLQJ\ LQ

et al

3.3.3 Culture Media Preparation and Sterilization Technique

3.3.3.1 Blood agar

The blood agar was prepared in accordance with the manufacturer's guidelines using the subsequent procedure: Forty grams of the medium were dissolved in 1 L of purified or distilled water. The solution was heated until boiling to ensure complete dissolution of the medium. Next, it was sterilized by autoclaving at 15 lbs pressure (121°C) for a duration of 15 minutes. Once sterilized, the mixture was cooled to a temperature between 45-50°C before adding 5% v/v of sterile defibrinated blood aseptically. The mixture was thoroughly combined and then poured into sterile Petri dishes. It was used as an enhancement medium to cultivate the bacterial isolates and to conclude their potential for hemolysis in the blood (Buxton, 2005).

3.3.3.2 MacConkey Agar

In order to prepare the medium, 49.53 grams of the dehydrated substance were mixed into 1 L of purified or distilled water. The solution was heated to a boil with continuous agitation for a duration of 1 minute. The medium was then sterilized using autoclaving at 15 lbs pressure (121°C) for 15 minutes. Upon completion of sterilization, the medium was allowed to cool down to a temperature between 45-50°C, thoroughly mixed, and subsequently poured into sterile Petri dishes. It was used to isolate furthermost (G-ve) bacteria primarily, in addition to distinguish between lactose and non-lactose fermentation (Jung *et al*., 2022)

3.3.3.3 Mueller Hinton Agar

For medium preparation, 38.0 grams were combined with 1 L of purified or distilled water, and the mixture was heated until boiling and completely dissolved. Next, the medium underwent sterilization via autoclaving at 15 lbs

pressure (121°C) for a period of 15 minutes. Once cooled to a temperature range of 45-50°C, the medium was thoroughly mixed and subsequently poured into sterile Petri dishes. Muller-Hinton agar medium was capable to hand over and used in antimicrobial testing.

3.3.3.4 Mannitol Salt Agar

In the process of medium preparation, 111.02 grams were combined with 1000 ml of distilled water. The mixture was subsequently heated until boiling, ensuring the medium had fully dissolved. The medium then sterilized through autoclaving at 15 lbs pressure (121°C) for a duration of 15 minutes. Upon cooling to a temperature between 45-50°C, the medium was thoroughly mixed before being poured into sterile Petri dishes. It is a differential medium for mannitol-fermenting *staphylococci* (Abd, 2022).

3.3.4 Laboratory diagnosis

3.3.4.1 Isolation and Identification of bacterial isolate

Swabs from all diabetic foot infections were cultivated on both blood agar and MacConkey agar, followed by incubation in aerobic conditions at 37°C for a 24-hour period. Colonies that appeared were identified based on their morphological characteristics, such as colony form, shape, color, boundaries, type of pigment, elevations, and textures. If it was golden colonies with beta hemolysis on blood agar, it was cultured on Mannitol Salt Agar and coagulase test done, then finally diagnosed with VITIK2 system. If it was yellow colonies and non-lactose ferment, an oxidase test done and finally diagnosed with VITIK2 system.

3.3.4.2 Identification using VITEK2

3.3.4.2.1 Preparation of bacterial inoculum

A 3.0 mL of sterile saline was transferred to a test tube, after which a sufficient quantity of morphologically similar colonies were moved to the saline tube using a sterile stick or swab. A microbial suspension with a density comparable to McFarland No. 0.50 to 0.63 was created using a calibrated VITEK 2 DENSICHEK Plus.

3.3.4.2.2 VITEK2 assay

Bacterial isolates that were diagnosed as *Staphylococcus aureus* or *Pseudomonas aeruginosa* were confirmed using VITEK GP card and VITEK GN card respectively, the suspension tube, along with the GP or GN card, was placed into the cassette, which was then loaded into the instrument.

3.3.4.2.3 Antibiotic susceptibility using VITIK 2

Antibiotic susceptibility of bacterial isolates that confirmed by VITIK2 as *Staphylococcus aureus* or *Pseudomonas aeruginosa* was done using VITIK2, the suspension tube, along with the AST card, was placed into the cassette, which was then loaded into the instrument.

3.3.4.3 Maintenance and preservation of isolated microorganism

The procedure was carried out in the following manner, as stated by Harley and Prescott (2002):

3.3.4.3.1 Short-term preservations

Isolates were preserved for periods of few weeks were sub cultured on the surface of appropriate. The plates were tightly wrapped with Parafilm and stored at 4 °C.

3.3.4.3.2 Long-term preservations

The isolate was preserved form long-term storage in brain heart infusion broth (BHI) medium containing 20% glycerol at low temperature without significant loss of viability, by adding 0.4 ml of sterile glycerol to 2 ml of exponentially growing bacteria in a sterile microcentrifuge tube and storing the mixture at -20 °C.

3.3.5 The evaluation of the antibacterial properties of silver nanoparticles using agar disk diffusion technique

This study followed the standard inhibition diameters as recommended by Okoli and Iroegbu (2004) and CLSI (2019).

3.3.5.1 Preparation of bacterial inoculum

In this study, the inoculum was prepared by transferring three to five isolated colonies grown on a nutrient agar plate to 5 ml of sterile normal saline, which was adjusted to correspond to the standard McFarland tube (1.5 x 10^8 cells/ml).

3.3.5.2 Agar disk diffusion technique

$ VWHULOH VZDE ZDV HPSOR\HG WR FROOHF
VXVSHQVLRQ ZKLFK ZHUH VXEVHTXHQWO\ VWUI
WR GU\
7KH H[SHULPHQW XVLQJ RYLQHGHVKHUWLFOHV LQGL
ZLWK FRQFHQWUDWLRQV RI J PO IROORZH
E\ VHWWOLQJ LQ WKH PHGLXP DW URRP WHPSH
SODWH ZDV LQFXEDWHG DW *f* & IRU KRXUV
,QKLELWLRQ]RQH XVLQJ VHQHXVLHUHDPHXOVHU RU FDOL
HVWDEOLVKHG YDOXHV WR DVFHUWDLQ ZKHWKH
UHVLVWDQFH WR WKH $J13V

3.3.6 Human Vascular Endothelial Cell Growth Factor-A (VEGF-A) ELISA assay

3.3.6.1 Principle

This kit includes the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Human VEGF-A antibody has been used to precoat the plate. If VEGF-A is present in the sample, it is introduced to the wells where it binds to the coated antibodies. The biotinylated Human VEGF-A Antibody is then added and binds to the VEGF-A in then sample. The Biotinylated VEGF-A antibody is subsequently conjugated to Streptavidin-HRP, which binds to streptavidin. In the washing phase that follows incubation, unbound Streptavidin-HRP is eliminated. After adding a substrate solution, the color develops in response to then amount of Human VEGF-A present. The reaction is ended by adding an acidic stop solution, and the absorbance at 450 nm is measured.

3.3.6.2 Kit components

The component of the kit mentioned in the table (3-5)

Table (3-6): VEGF-A kit components

Reagent	Specification	Reagent	Specification
Coated ELISA plate	12-Well * 8	Washing concentrate (30X)	20ml
Standard dilution	3ml	Instructions	1
Chromogen solution A	6ml	Seal plate membrane	2
Chromogen solution B	6ml	Hermetic bag	1
Streptavidin HRP	6ml	Stop solution	6ml
Standard solution	0.5ml	Antibodies labeled with biotin	1ml

3.3.6.3 Assay procedure

The assay procedure was performed as follow:

1. Everything, including the reagents, the samples, and the standards, was prepared.
2. The addition of prepared samples, standards, and ELISA solutions. They were given 60 minutes to react at 37°C.
3. The plate was washed five times. A and B chromogen solutions were added. The sample was incubated for 10 minutes at 37°C to produce color.
4. Stop solution was added.
5. The OD value was measured in less than 10 minutes.
6. Calculations were performed.

3.3.6.4 Standard curve and result calculation

Microsoft Excel was utilized to plot standard curves by graphing concentration (ng/l) on the horizontal axis (X-axis) and absorbance on the vertical axis (Y-axis) as depicted in Figure 3.2.

The concentration of unknown samples was determined by locating their absorbance on the vertical axis and finding the corresponding concentration on the horizontal axis.

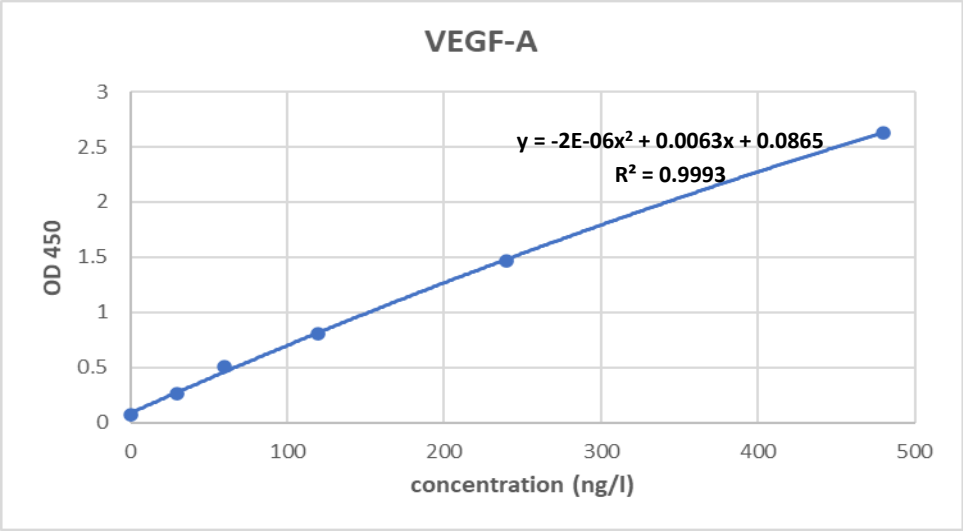


Figure (3-2): VEGF-A standard curve

3.3.7 Human PDGF-BB(Platelet Derived Growth Factor BB) ELISA assay

3.3.7.1 Principle

This Sandwich-ELISA kit employs a pre-coated microplate with a human PDGF-BB-specific antibody. Sample interactions occur in the wells, followed by treatment with biotinylated detection antibody and Avidin-HRP conjugate. After washing, a substrate solution is added, yielding a blue color in wells containing the target components. The enzyme-substrate reaction is halted using a stop solution, changing the color to yellow. Optical density (OD) is measured at 450 ± 2 nm, with OD values correlating to human PDGF-BB concentrations.

3.3.7.2 Kit components

The components of the kit described in table 3-6

Table (3-7): PDGF kit components

Reagent	Quantity
Micro ELISA Plate	8 wells ×12 strips
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	Y L D O /
Concentrated HRP Conjugate (100×)	Y L D O /
Reference Standard & Sample Diluent	1 vial,20mL
Biotinylated Detection Ab Diluent	1 vial,14mL
HRP Conjugate Diluent	1 vial,14mL
Concentrated Wash Buffer (25×)	1 vial, 30mL
Substrate Reagent	1 vial, 10mL
Stop Solution	1 vial, 10mL
Plate Sealer	5 pieces

3.3.7.3 Assay procedure

The procedure was performed as follow:

1. :HOOV ZHUH GHVLJQDWHG IRU GLOXWHG VWDQ of each dilution was added, and the plate was sealed and incubated for 90 minutes at 37°C.
2. 7KH OLTXLG ZDV UHPRYHG &Biotinylated Detection Ab solution was added, and the plate was resealed and incubated for 1 hour at 37°C.
3. The solution was decanted, and wells were washed thrice ZLWK / RI wash buffer.
4. 100 L of HRP Conjugate solution was added, and the plate was resealed and incubated for 30 minutes at 37°C.
5. The solution was removed, and wells were washed five times as in step 3.
6. 90 L of Substrate Reagent was added, and the plate was sealed and incubated for 15 minutes at 37°C, protected from OLJKW 7KH Stop / R Solution was added.
7. OD values were immediately determined at 450 nm using a microplate reader.

3.3.7.4 Standard curve and result calculation

The standard curves were created using Microsoft Excel by plotting the concentration (pg/ml) on the horizontal axis (X-axis) and absorbance on the vertical axis (Y-axis) as shown in Figure 3.3.

To determine the concentration of unknown samples, their absorbance was identified on the vertical axis, and the corresponding concentration was found on the horizontal axis.

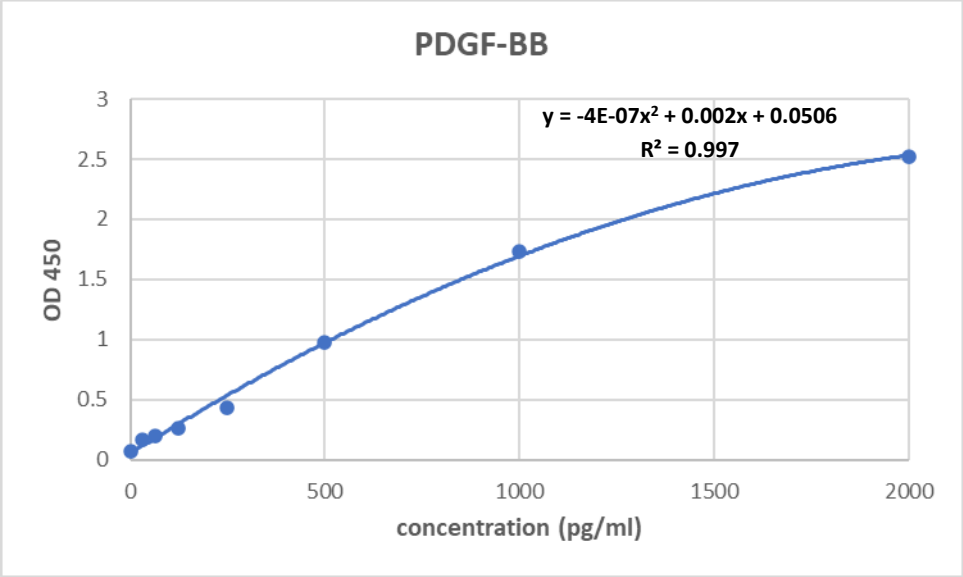


Figure (3-3): PDGF-BB standard curve

3.3.8 Interleukin-6 ELISA assay

3.3.8.1 Principle

This Sandwich-ELISA kit employs a pre-coated microplate with a human IL-6-specific antibody. Sample interactions occur in the wells, followed by treatment with biotinylated detection antibody and Avidin-HRP conjugate. After washing, a substrate solution is added, yielding a blue color in wells containing the target components. The enzyme-substrate reaction is halted using a stop solution, changing the color to yellow. Optical density (OD) is measured at 450 ± 2 nm, with OD values correlating to human IL-6 concentrations.

3.3.8.2 Kit components

The components of the kit described in table 3-7

Table (3-8): IL-6 kit components

Reagent	Quantity
Micro ELISA Plate	8 wells×12 strips
References Standard	2 vials
Concentrated Biotinylated Detection Ab	YLDO /
Concentrated HRP Conjugate	YLDO /
References Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial,14 mL
Concentrated Wash Buffer(25×)	1 vial,30 mL
Substrate Reagent	1 vial,10 mL
Stop Solution	1 vial,10 mL
Plate Sealer	5 pieces

3.3.8.3 Assay procedure

The procedure was performed as follow:

1. :HOOV ZHUH GHVLJQDWHG IRU GLOXWHG VWDQ of each dilution was added, and the plate was sealed and incubated for 90 minutes at 37.°C
2. 7KH OLTXLG ZDV UHPRYHG &Biotinylated Detection Ab solution was added, and the plate was resealed and incubated for 1 hour at 37°C.

3. The solution was G H F D Q W H G D Q G Z H O O V Z H U H Z D V K H wash buffer.
4. 100 L of HRP Conjugate solution was added, and the plate was resealed and incubated for 30 minutes at 37°C.
5. The solution was removed, and wells were washed five times as in step 3.
6. 90 L of Substrate Reagent was added, and the plate was sealed and incubated for 15 minutes at 37°C, protected from light.
7. 50 L of Stop Solution was added to each well in the same order as the substrate solution.
8. OD values were immediately determined at 450 nm using a microplate reader.

3.3.8.4 Standard curve and result calculation

Standard curves were generated using Microsoft Excel, with concentration (pg/ml) plotted on the horizontal X-axis and absorbance on the vertical Y-axis. The resulting graph is displayed in Figure 3.4

To ascertain the concentration of unknown samples, their absorbance was located on the vertical axis, and the corresponding concentration was identified on the horizontal axis.

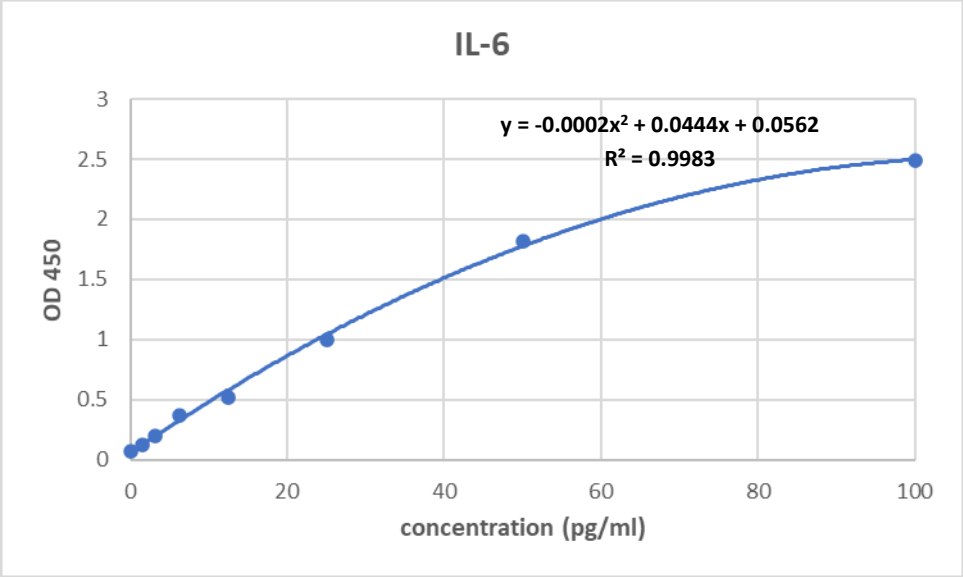


Figure (3-4): Interleukin-6 standard curve

3.3.9 Complete Blood Count (CBC) assay

The EDTA tube containing whole blood samples was immediately utilized to perform a complete blood count using an automated 3-part hematology autoanalyzer. The samples were mixed by swirling them several times and then processed in the autoanalyzer, which provided results within 60 seconds. The results were printed out and recorded.

3.3.10 HbA1c assay

The HbA1c test done using automated Cobas b 101 system, the test was done according to manufacturer instruction:

The Cobas b 101 module was loaded with these samples. Instructions on the Cobas c 501 module were adhered to, initiating the examination of the samples.

After the examination was finished, the HbA1c outcomes were shown on the Cobas b 101 module.

3.4 Statistical analysis

Data analysis was performed using SPSS (version 23, SPSS Inc., Chicago, Illinois, USA). Descriptive statistics (mean, standard deviation) were calculated, and a t-test was employed to compare the two groups. Differences were assessed using one-ZD\ 129 DW S ” XWLOL]LQJ WKH OHD (LSD) method. The correlation between studied parameters was determined using Pearson's correlation coefficient (r). Receiver-operating-characteristic (ROC) curves were analyzed to evaluate disease detection performance (Daniel, 1999).

3.5 Ethical approval

7KH VWXG\ ZDV FRQGXFWHG DIWHUSRE WLHQWLVQJ
7KH HWKLFDO FRPPLWWHH RI %DE\ORQ 6FLHQFI
VW.XG\
7KH.DUEDOHDDOWK 'HSDUWPHQW SURYLGHG DX
FRPPHQFHPHQWXGRI WKH

Chapter four

Results

4. Results

4.1 General demographical data of patients

The results presented in Figure 4-1 show the gender distribution of the patients with diabetic foot ulcer in this study, with 24 participants (40%) being male and 36 participants (60%) being female.

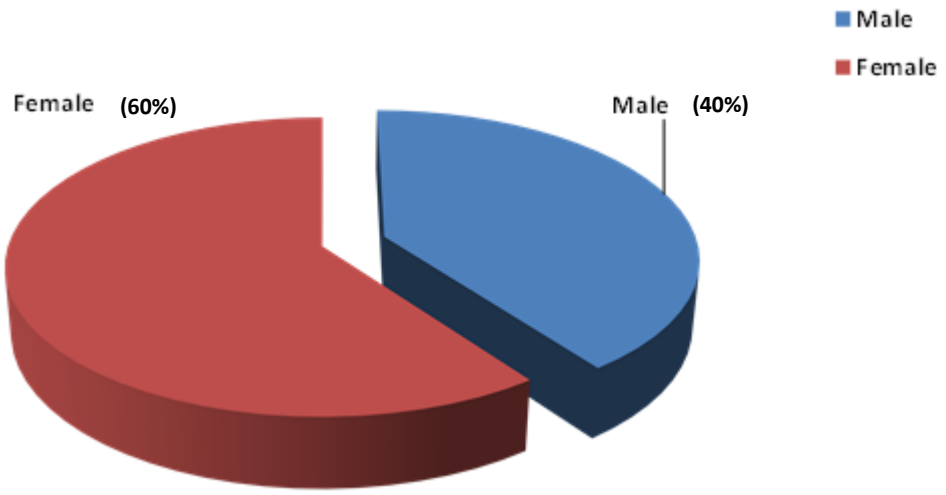


Figure (4-1): Percentage ratio (%) of males and females affected with diabetic foot ulcer.

The results presented in Figure 4-2 illustrate the age distribution of the patients with diabetic foot ulcer in this study, with 15 participants (25%) falling in the age group of 41-50 years, 20 participants (33.3%) falling in the age group of 51-60 years, and 25 participants (41.7%) falling in the age group of 61-70 years.

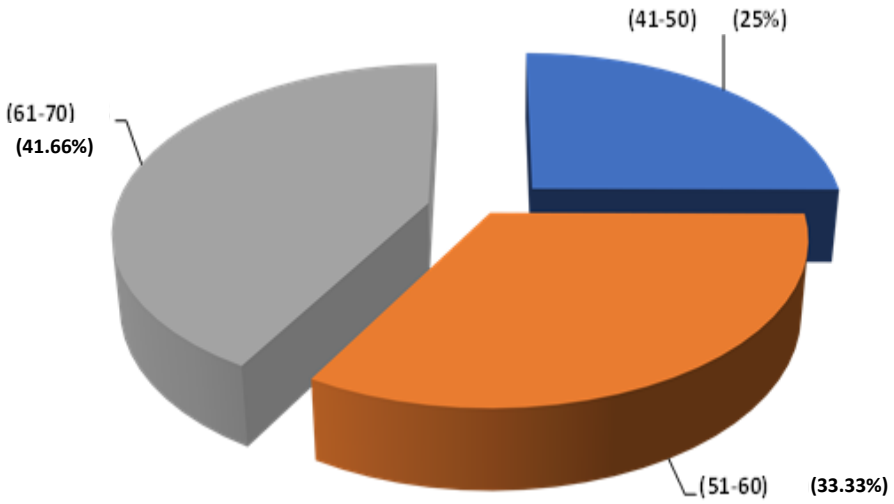


Figure (4-2): Percentage ratio (%) of age distribution of the patients with diabetic foot ulcer.

4.2 Bacteriological study results

4.2.1 Bacterial species isolated from diabetic foot ulcer

The distribution of bacterial species found in diabetic foot ulcers is shown in Figure 4-3. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most frequently identified bacteria, with each accounting for 24% of the cases. 12% of the cases had other bacterial species, while 40% of the cases had negative culture results.

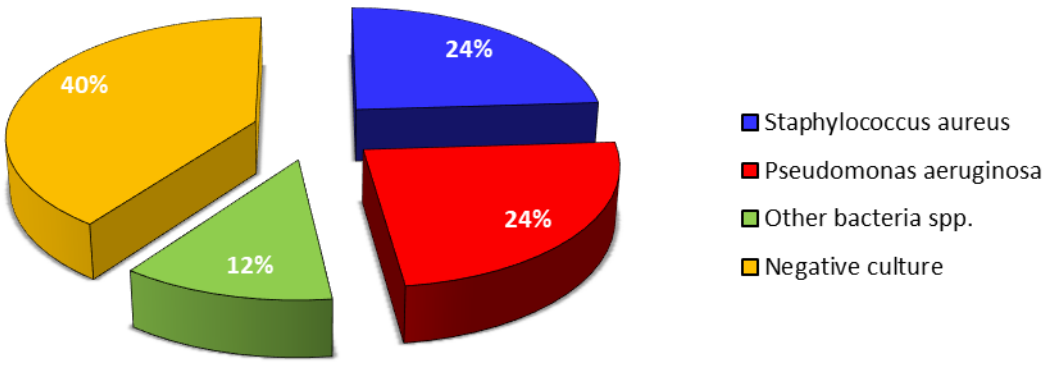


Figure (4-3): Isolation rate of bacterial species

4.2.1.1 Biochemical results of bacterial isolates

The results of biochemical tests used by VITEK2 system for diagnosis of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were described in tables 4-1 and 4-2 respectively.

Table (4-1): Identification of 30 *Pseudomonas aeruginosa* isolates by using VITEK 2 system.

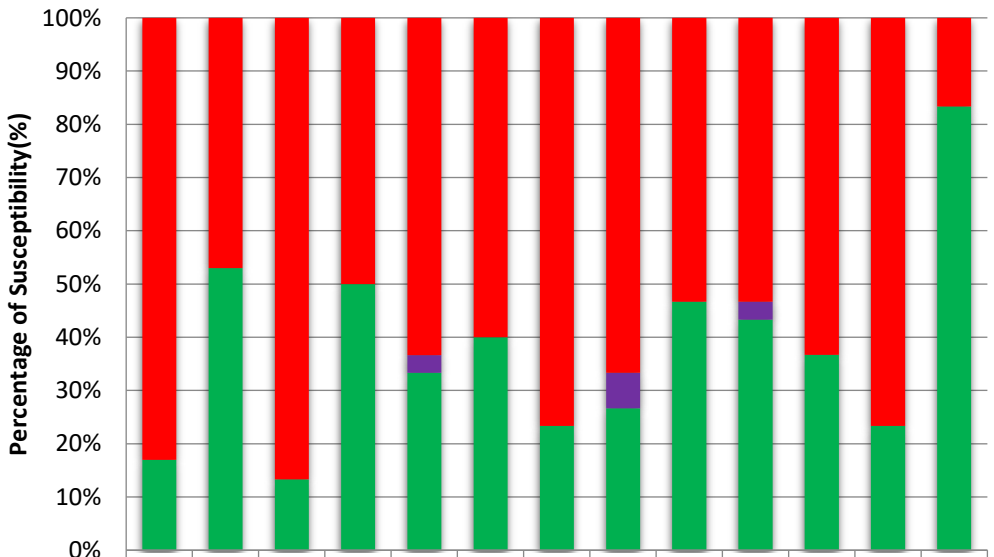
No. of well	Symbol of test	Result	No. of well	Symbol of test	Result
2	APPA	-	33	SAC	-
3	ADO	-	34	dTAG	-
4	PyrA	-	35	dTRE	-
5	lARL	-	36	CTT	+
7	dCEL	-	37	MNT	(+)
9	BGAL	-	39	5KG	-
10	H2S	-	40	ILATk	+
11	BNAG	-	41	AGLU	-
12	AGLTp	-	42	SUCT	+
13	dGLU	+	43	NAGA	-
14	GGT	+	44	AGAL	-
15	OFF	-	45	PHOS	-
17	BGLU	-	46	GlyA	-
18	dMAL	-	47	ODC	-
19	dMAN	-	48	LDC	-
20	dMNE	+	53	IHISa	-
21	BXYL	-	56	CMT	-
22	BAlap	+	57	BGUR	-
23	ProA	+	58	O129R	-
26	LIP	-	59	GGAA	-
27	PLE	-	61	IMLTa	-
29	TyrA	-	62	ELLM	-
31	URE	+	64	ILATa	-
32	dSOR	-			

Table (4-2): Identification of 30 *Staphylococcus aureus* isolates by using VITEK 2 system.

No. of well	Symbol of test	Result	No. of well	Symbol of test	Result
2	AMY	-	32	POLYB	+
4	PIPLC	-	37	dGAL	+
5	dXYL	-	38	dRIB	-
8	ADHI	+	39	ILATK	+
9	BGAL	+	42	LAC	-
11	AGLU	+	44	NAG	+
13	APPA	-	45	dMAL	+
14	CDEX	-	46	BACI	+
15	AspA	-	47	NOVO	+
16	BGAR	-	50	NC65	+
17	AMAN	-	52	dMAN	+
19	PHOS	+	53	dMNE	+
20	LeuA	-	54	MBdG	+
23	ProA	-	56	PUL	-
24	BGURr	-	57	dRAF	-
25	AGAL	-	58	O129R	+
26	PyrA	+	59	SAL	-
27	BGUR	-	60	SAC	+
28	AlaA	-	62	dTRE	+
29	TyrA	-	63	ADH2s	-
30	dSOR	-	64	OPTO	+
31	URE	-			

4.2.2 Antibiotic Susceptibility of *Pseudomonas aeruginosa*

The antibiotic susceptibility profile of 30 *Pseudomonas aeruginosa* isolates using the VITEK 2 system as shows in figure 4-4. The percent resistance and percent sensitivity for each antibiotic are provided in the figure. The results indicate that ticarcillin, piperacillin, and ciprofloxacin had a high percentage of resistance (83%, 86%, and 76.6%, respectively), while colistin had the lowest percentage of resistance (16.6%). Ticarcilline + clavulanic acid and piperacillin + tazobactam had intermediate resistance rates (47% and 50%, respectively). Imipenem and meropenem had moderate resistance rates of 76% and 66.6%, respectively. Ceftazidime, cefepime, amikacin, gentamicin, and tobramycin had resistance rates ranging from 33.3% to 63.3%.



	TI	TIM	PIP	TZP	CAZ	FEP	IM	ME	AK	GM	TO	CIP	CST
R %	83.0	47.0	86.6	50.0	63.3	60.0	76.6	66.6	53.3	53.3	63.3	76.6	16.6
I %	0.00	0.00	0.00	0.00	3.33	0.00	0.00	6.67	0.00	3.33	0.00	0.00	0.00
S %	17.0	53.0	13.3	50.0	33.3	40.0	23.3	26.6	46.6	43.3	36.6	23.3	83.3

TI:Ticarcillin; TIM:Ticarcilline + clavulanic acid; PIP:Piperacillin; TZP:Piperacillin + tazobactam; CAZ:Ceftazidime; FEB:Cefepime; IM:Imipenem; ME:Meropenem; AK:Amikacin; GM:Gentamicin; TO:Tobramycin; CIP:Ciprofloxacin; CST:Colistin.

Figure (4-4): Antibiotic Susceptibility profile for 30 isolates of *Pseudomonas aeruginosa* by VITEK 2 system.

The results of Antibiotic profile and MIC for 30 isolates of *Pseudomonas aeruginosa* by VITEK2 system illustrated in table 4-3.

Table (4-3): Antibiotic profile and MIC for 30 isolates of *Pseudomonas aeruginosa* by VITEK 2 system.

Antibiotic classes	Antibiotic	MIC	Interpretation		
			Resistance	Intermediate	Susceptible
Beta lactam	Ticarcillin	•	25 (83 %)	0 (0%)	5 (17 %)
	Ticarcillin/Clavulanic Acid	•	14 (47 %)	0 (0%)	16 (53 %
	Piperacillin	•	26 (86.67%)	0 (0%)	4 (13.33%)
	Piperacillin/Tazobactam	”	15(50%)	0 (0%)	15(50%)
Cephalosporin	Ceftazidime	•	19(63.34%)	1(3.33%)	10(33.33%)
	Cefepime	•	18 (60%)	0 (0%)	12 (40%)
Carbapenems	Imipenem	•	23(76.67%)	0 (0%)	7(23.33%)
	Meropenem	•	20(66.66%)	2(6.67%)	8(26.67%)
Aminoglycosides	Amikacin	•	16(53.33%)	0 (0%)	14(46.67%)
	Gentamicin	•	16(53.33%)	1(3.34%)	13(43.33%)
	Tobramycin	•	19(63.33%)	0 (0%)	11(36.67%)
Quinolones	Ciprofloxacin	•	23(76.67%)	0 (0%)	7(23.33%)
Polymyxins	Colistin	”	5(16.67%)	0 (0%)	25(83.33%)

Table 4-4 displays information on the resistance of *Pseudomonas aeruginosa* isolates to antibiotics. The results indicate that 76.7% of the isolates were classified as multidrug-resistant (MDR), meaning they were resistant to three or more classes of antibiotics. Many of the isolates (3.3%) were classified as extensively drug-resistant (XDR), meaning they were resistant to at least one

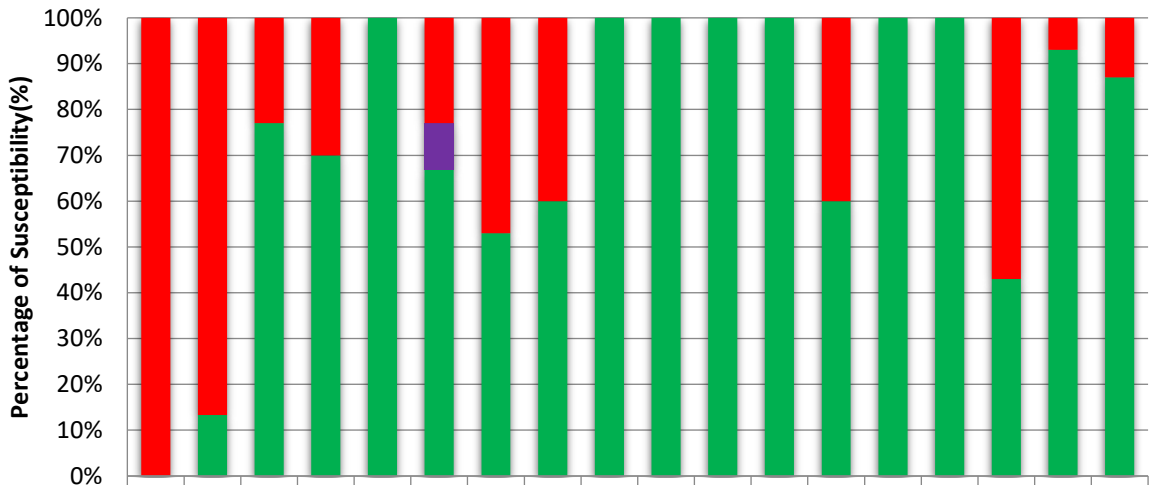
agent in all but two or fewer antimicrobial categories. Only 20% of the isolates were classified as non-resistant.

Table (4-4): Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* isolates.

Isolates	Antibiotic Resistance	Pattern Type
S1	IM; CIP; CST	**MDR**
S2	GEN; TOB; CIP	**Not R**
S3	TZP; FEP; IM; AK; GEN; CIP; LEV; TIG; TMP	**MDR**
S4	TI; PIP	**Not R**
S5	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S6	TI; TIM; PIP; CAZ; FEP; CIP	**MDR**
S7	TI; TIM; PIP; CAZ; FEP; IM; ME; GEN; TOB; CIP	**MDR**
S8	-	**Not R**
S9	TI; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S10	TI; PIP	**Not R**
S11	PIP; IM; CST	**MDR**
S12	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S13	TI; PIP	**Not R**
S14	TI; TIM; PIP; CAZ; FEP; IM; AK; GEN; TOB; CIP	**MDR**
S15	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; TOB; CIP	**MDR**
S16	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP; CST	**XDR**
S17	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; TOB; CIP	**MDR**
S18	TI; TIM; PIP; CAZ; FEP; IM; ME; TOB; CIP	**MDR**
S19	TI; TIM; PIP; CAZ; FEP; IM; ME; GEN; TOB; CIP	**MDR**
S20	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S21	TI; TIM; PIP; IM; ME; CIP	**MDR**
S22	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S23	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB	**MDR**
S24	PIP; IM; CST	**MDR**
S25	TI; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S26	CIP	**Not R**
S27	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S28	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S29	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
S30	TI; TIM; PIP; CAZ; FEP; IM; ME; AK; GEN; TOB; CIP	**MDR**
Antibiotic resistance Pattern%	**XDR (3.3%) and MDR (76.7%)** **Not Resistance (20 %)**	

4.2.3 Antibiotic Susceptibility of *Staphylococcus aureus*

Figure 4-5 shows the antibiotic susceptibility profile of 30 *Staphylococcus aureus* isolates using the VITEK 2 system. The percent resistance for each antibiotic is provided in the figure. The results indicate that benzylpenicillin had the highest resistance rate of 100%, followed by oxacillin at 87%. The fluoroquinolones, ciprofloxacin and moxifloxacin, had resistance rates of 30% and 23%, respectively. Erythromycin and clindamycin had resistance rates of 47% and 40%, respectively. Tetracycline and fusidic acid had resistance rates of 40% and 57%, respectively. Trimethoprim/sulfamethoxazole had a resistance rate of 13%. Notably, the glycopeptides, vancomycin and teicoplanin, as well as tigecycline and nitrofurantoin, had no resistance among the tested isolates.



	BEN PEN	OXA	GEN	CIP	LEV O	MO X	ERY	CLIN	Q-D	LIN	TEC	VAN	TET	TIG	NIT	FUS	RIM	TMP
R %	100	87%	23%	30%	0%	23%	47%	40%	0%	0%	0%	0%	40%	0%	0%	57%	7%	13%
I %	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
S %	0%	13%	77%	70%	100	67%	53%	60%	100	100	100	100	60%	100	100	43%	93%	87%

BENPEN: Benzylpenicillin; OXA: Oxacillin; GEN: Gentamicin; CIP: Ciprofloxacin; LEVO:Levofloxacin; MOX: Moxifloxacin; ERY: Erythromycin; CLIN: Clindamycin; Q-D:Quinupristin/Dalfopristin; LIN: Linezolid; TEC:Teicoplanin; VAN: Vancomycin; TET:Tetracycline; TIG:Tigecycline; NIT:Nitrofurantoin; FUS: Fusidic Acid; RIM:Rifampicin; TMP: Trimethoprim/Sulfamethoxazole

Figure (4-5): Antibiotic Susceptibility profile for 30 isolates of *Staphylococcus aureus* by VITEK 2 system.

The results of Antibiotic profile and MIC for 30 isolates of *Pseudomonas aeruginosa* by VITEK2 system illustrated in table 4-5.

Table (4-5): Antibiotic profile and MIC for 30 isolates of *Staphylococcus aureus* by VITEK 2 system.

Antibiotic classes	Antibiotic	MIC	Interpretation		
			Resistance	Intermediate	Susceptible
Pencillins	Benzylpenicillin	•	30 (100%)	0 (0%)	0 (0%)
	Oxacillin	•	26(87%)	0 (0%)	4(13%)
Aminoglycosides	Gentamicin	•	7(23%)	0 (0%)	23(77%)
Quinolones	Ciprofloxacin	”	9(30%)	0 (0%)	21(70%)
	Levofloxacin	”	0 (0%)	0 (0%)	30(100%)
	Moxifloxacin	”	7(32%)	3(10%)	20(67%)
Macrolide	Erythromycin	•	14(47%)	0 (0%)	16(53%)
Lincosamide	Clindamycin	”	12(40%)	0 (0%)	18(60%)
Macrolide - Lincosamide	Quinupristin/Dalfopristin	”	0 (0%)	0 (0%)	30(100%)
oxazolidinone	Linezolid	2	0 (0%)	0 (0%)	30(100%)
Glycopeptide	Teicoplanin	”	0 (0%)	0 (0%)	30(100%)
	Vancomycin	”	0 (0%)	0 (0%)	30(100%)
Tetracyclines	Tetracycline	”	12(40%)	0 (0%)	18(60%)
glycylcycline	Tigecycline	”	0 (0%)	0 (0%)	30(100%)
Nitrofuratoins	Nitrofurantoin	”	0 (0%)	0 (0%)	30(100%)
Fusidanes	Fusidic Acid	”	17(57%)	0 (0%)	13(43%)
Refamicin	Rifampicin	”	2(7%)	0 (0%)	28(93%)
Sulfonamide	Trimethoprim/Sulfamethoxazole	”	4(13%)	0 (0%)	26(87%)

Table 4-6 provides the antibiotic resistance patterns of *Staphylococcus aureus* isolates. The results indicate that 63.3% of the isolates were multidrug-

resistant, furthermore, 36.7% of the isolates did not exhibit any resistance to the tested antibiotics.

Table (4-6): Antibiotic Resistance Patterns of *Staphylococcus aureus* isolates.

Isolates	Antibiotic Resistance	Pattern Type
S1	BENPEN; OXA; ERY; TET; FUS	**MDR**
S2	BENPEN; OXA	**Not R**
S3	BENPEN; CIP; MOX; ERY; CLIN; TET; TMP	**MDR**
S4	BENPEN; OXA	**Not R**
S5	BENPEN; TMP	**Not R**
S6	BENPEN; OXA	**Not R**
S7	BENPEN; OXA; ERY; CLIN	**MDR**
S8	BENPEN; OXA	**Not R**
S9	BENPEN; OXA; GEN; CIP; MOX; ERY; TET;FUS	**MDR**
S10	BENPEN; OXA	**Not R**
S11	BENPEN; OXA; ERY; CLIN; TET; FUS	**MDR**
S12	BENPEN; OXA; GEN; CIP; ERY	**MDR**
S13	BENPEN; OXA; GEN; FUS	**MDR**
S14	BENPEN; OXA; ERY; CLIN	**MDR**
S15	BENPEN; OXA; ERY; CLIN; TET; FUS	**MDR**
S16	BENPEN; OXA; ERY; CLIN; FUS	**MDR**
S17	BENPEN; OXA; CLIN; TET; FUS; RIM	**MDR**
S18	BENPEN; OXA	**Not R**
S19	BENPEN; OXA; GEN; CIP; ERY; CLIN; TET; FUS	**MDR**
S20	BENPEN; OXA	**Not R**
S21	BENPEN; OXA; FUS	**Not R**
S22	BENPEN; OXA; CIP; MOX; ERY; CLIN; TET; FUS	**MDR**
S23	BENPEN; OXA; CIP; MOX; ERY; CLIN; TET; FUS	**MDR**
S24	BENPEN; OXA; GEN; CIP; MOX; ERY; TET; FUS	**MDR**
S25	BENPEN; OXA	**Not R**
S26	BENPEN; OXA; GEN; CIP; MOX; ERY; TET; FUS	**MDR**
S27	BENPEN; OXA; CLIN; TET; FUS; RIM	**MDR**
S28	BENPEN; OXA	**Not R**
S29	BENPEN; OXA; ERY; CLIN; TET; FUS	**MDR**
S30	BENPEN; OXA; GEN; CIP; ERY	**MDR**
Antibiotic resistance Pattern%	**MDR(63.3%)** **Not R(36.7%)**	

4.2.4 Antibacterial activity of AgNPs

The antibacterial activity of silver nanoparticles (AgNPs) against five *Pseudomonas aeruginosa* isolates at varying AgNP concentrations (31.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml) is presented in figures 4-6 and 4-7. The results shows the diameter of inhibition zones (mm) increase when AgNPs concentration increase. Increasing concentrations of AgNPs resulted in larger inhibition zone diameters for all isolates. The highest inhibition zone diameters were observed at 125µg/ml for the fourth and fifth isolates, 250µg/ml for the third isolate, and 500µg/ml for the first isolate.

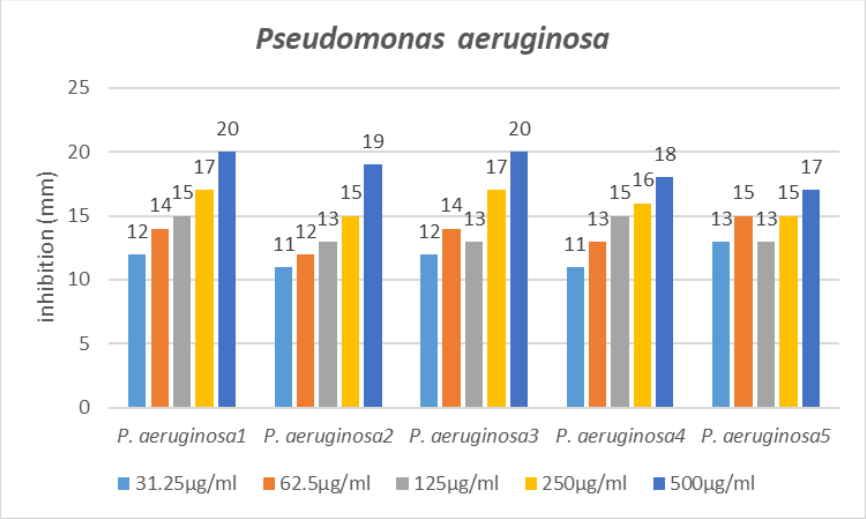


Figure (4-6): Antibacterial activity of AgNPs against *P. aeruginosa* isolates

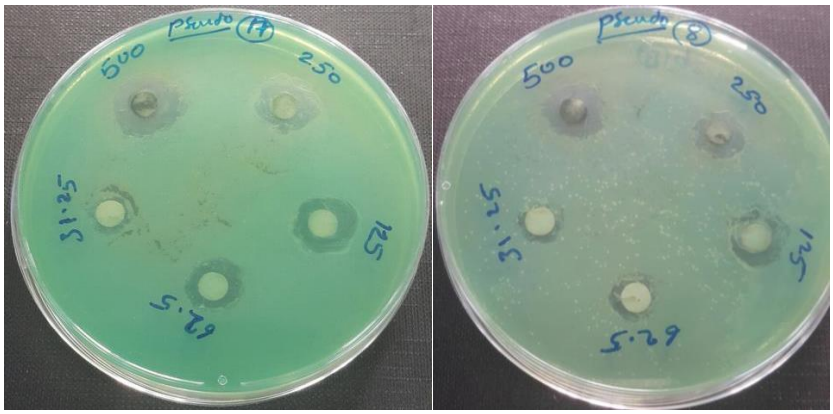


Figure (4-7): Antibacterial activity of AgNPs against *P. aeruginosa* isolates on Muller-Hinton Agar

AgNPs' antibacterial activity against 5 *Staphylococcus aureus* isolates at various concentrations (31.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml) presented in figures 4-8 and 4-9. The results show the diameter of inhibition zones (mm) increase when AgNPs concentration increase. The first isolate had diameters ranging from 15mm to 24mm, with higher concentrations showing larger diameters. The second isolate had diameters ranging from 15mm to 22mm. The third isolate showed diameters from 16mm to 23mm, with the highest at 250µg/ml. The fourth isolate had diameters ranging from 17mm to 23mm, with the highest at 250µg/ml. The fifth isolate had diameters ranging from 15mm to 21mm.

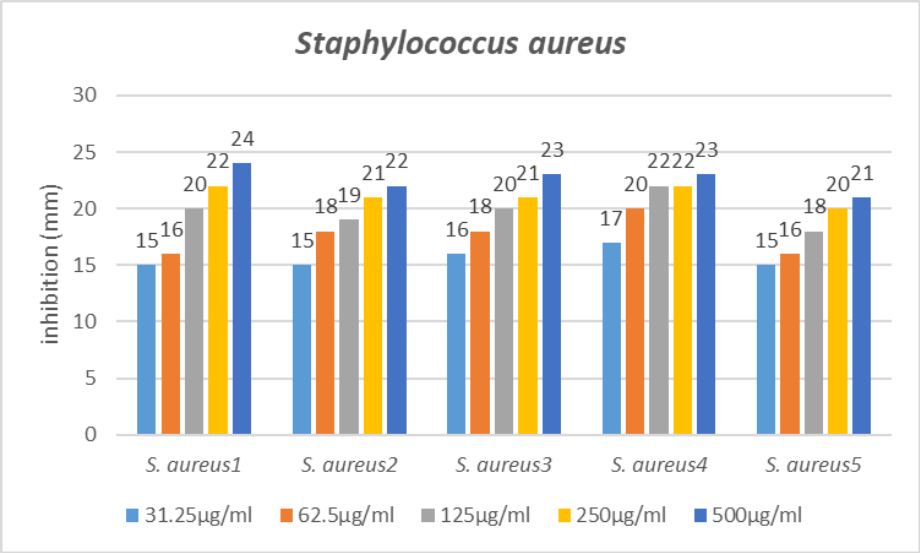


Figure (4-8): Antibacterial activity of AgNPs against *S. aureus* isolates

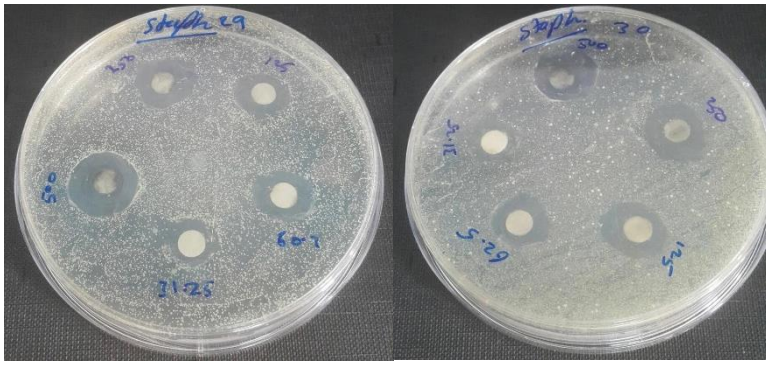


Figure (4-9): Antibacterial activity of AgNPs against *S. aureus* isolates on Muller-Hinton Agar

4.3 Hematological parameters and biomarkers

4.3.1 Complete blood count parameters

Table 4-7 presents a comprehensive comparison of complete blood count parameters between two groups: a patient group consisting of 60 individuals and a control group of 30 individuals. Each parameter is reported as an average value accompanied by the standard deviation.

In this comparison, the patient group demonstrated a significantly higher ZKLWH EORRG FHOO :%& FRXQW RI " LQ FR count of 6.35±1.3, which is statistically significant with a P-value of 0.042. However, the lymphocyte count showed no significant difference between the patient group and the control group, with respective counts of 26.75±4.2 and 27.67±3.4, and a P-value of 0.691.

Interestingly, the monocyte count was significantly lower in the patient group, 8.67±1.1, than in the control group, 10.66±1.9, with a P-value of 0.041. While the neutrophil count was slightly higher in the patient group at 62.31±5.6, WKLV GLIIHUHQFH ZDV VWDWLVWLFDOO\ VLJQLILF 58.45±4.4 count, as evidenced by a P-value of 0.054.

In terms of the eosinophil count, the patient group had a significantly lower FRXQW RI " FRPSDUHG WR WKH FRQWUROJU value of 0.031. The basophil count was also significantly lower in the patient group, 0.43±0.01, compared to the control group, 0.72±0.03, with a highly significant P-value of 0.001.

Furthermore, the hemoglobin (Hb) levels were noticeably lower in the patient group, 9.81±0.13 g/dL, in comparison to the control group, 12.05±0.12 g/dL, with a P-YDOXH RI " LQGLFDWLQJ D YHU\ VL platelet (PLT) count was significantly higher in the patient group at 241.21±4.2

P PP FRPSDUHG WR WKH FRQWURO JURXS¶V highly significant P- YDOXH RI ”

Table (4-7): Results of Complete blood count parameters of patients affected with diabetic foot ulcer and control group

Groups / Parameters	Patient (n=60)	Control (n=30)	P-value
	Mean ±S.D		
WBCs × 10^9/L	8.77±0.9	6.35±1.3	**0.042***
Lymphocytes %	26.75±4.2	27.67±3.4	**0.691**
Monocytes %	8.67±1.1	10.66±1.9	**0.041***
Neutrophils %	62.31±5.6	58.45±4.4	**0.054***
Eosinophils %	1.84±0.2	2.50±0.6	**0.031***
Basophils %	0.43±0.01	0.72±0.03	**0.001****
Hb (g/dL)	9.81±0.13	12.05±0.12	**≤0.0001**
PLT (m/mm^3)	241.21±4.2	194.17±2.20	**≤0.0001**

-All values are mean ±SD
-Results with two strikes DUH VLJQLILFDQWO\ GLIIHUHQW DW 3”

Table 4-8 provides a detailed comparison of various blood parameters across different age groups (41-50, 51-60, 61-70 years) and gender (male, female) in both patient and control categories.

For WBCs, in the 41-50 age bracket, male patients recorded 8.15±0.5, while female patients showed 7.27±0.6. The control group, however, had lower counts with males at 6.45±0.3 and females at 6.43±0.8. This pattern of higher WBCs in patients than controls continues in the 51-60 and 61-70 age brackets.

Lymphocyte counts reveal a mixed trend. In the 41-50 age group, both male and female patients had higher counts (31.69±3.6, 32.77±1.6 respectively) than their control counterparts (27.64±3.6, 30.78±7.2 respectively). However, in the 51-60 age group, both male and female patients recorded lower lymphocyte counts than the control group.

Monocyte numbers among patients in the 41-50 age group were slightly lower in males (8.45±1.0) and higher in females (9.71±0.2) compared to the control group. The trend varies in the 51-60 and 61-70 age groups.

Neutrophil counts in the patient group were generally higher than the control group across all age groups, with the exception of the 41-50 age group where female patients had slightly lower counts (54.96±6.9) than the control group (54.55±4.4).

Eosinophil counts among patients were generally lower than the control group across all age brackets. However, the difference between the patient and control groups was more pronounced in the 51-60 age group.

Basophil numbers showed a mixed trend across different age groups and gender. The patient group generally had lower counts than the control group.

The patient group, regardless of age and gender, consistently showed lower hemoglobin (Hb) levels than the control group. The difference was more pronounced in the 41-50 age group where male patients recorded 9.79±1.2 g/dL DQG IHPDOH SDWLHQWV " J G/ FRPSDUHG W g/dL for males and 11.69±0.6 g/dL for females.

Finally, platelet (PLT) counts were generally higher in the patient group across all age groups and genders.

Table (4-8): Complete blood count parameters of patients affected with diabetic foot ulcer and control according to gender and age.

Groups / Parameters	Age (year)	Male		Female		LSD (0.05)
		Patient	Control	Patient	Control	
		Mean ±S.D				
WBcs × 10^9/L	41-50	8.15±0.5	6.45±0.3	7.27±0.6	6.43±0.8	2.527
	51-60	9.41±1.1	6.26±0.4	11.40±1.4	5.84±1.3	
	61-70	8.13±0.2	6.71±0.1	8.23±1.3	6.39±0.7	
Lymphocytes %	41-50	31.69±3.6	27.64±3.6	32.77±1.6	30.78±7.2	5.142
	51-60	23.42±2.8	25.44±2.9	22.04±2.2	34.54±4.1	
	61-70	25.14±2.2	29.92±6.7	29.06±1.3	32.92±3.0	
Monocytes %	41-50	8.45±1.0	8.67±1.8	9.71±0.2	11.54±1.2	3.115
	51-60	8.76±1.3	14.70±2.1	6.90±0.8	9.12±0.4	
	61-70	8.79±0.9	8.62±1.3	9.48±1.1	8.08±1.3	
Neutrophils %	41-50	57.03±3.6	59.89±5.3	54.96±6.9	54.55±4.4	3.882
	51-60	66.17±5.2	57.17±4.7	69.64±4.3	52.56±3.8	
	61-70	63.74±4.1	58.29±3.1	59.20±2.8	56.56±4.6	
Eosinophils %	41-50	2.31±0.4	3.04±0.6	2.13±0.2	2.47±0.7	0.527
	51-60	1.26±0.1	2.06±0.2	0.93±0.1	3.20±0.5	
	61-70	1.94±0.2	2.39±0.1	1.66±0.3	1.82±0.9	
Basophils%	41-50	0.52±0.03	0.76±0.01	0.44±0.01	0.66±0.04	0.259
	51-60	0.38±0.01	0.62±0.02	0.49±0.02	0.59±0.01	
	61-70	0.39±0.02	0.77±0.14	0.61±0.05	0.62±0.04	
Hb (g/dL)	41-50	9.79±1.2	12.63±1.2	9.19±0.3	11.69±0.6	0.661
	51-60	11.11±1.6	13.13±2.6	9.67±0.7	11.28±0.7	
	61-70	9.65±0.8	12.07±0.6	9.44±1.2	11.49±1.2	
PLT (m/mm^3)	41-50	244.47±15.6	196.27±11.3	262.00±10.5	197.07±11.7	19.055
	51-60	232.67±11.1	201.00±10.7	247.07±9.7	192.80±7.9	
	61-70	229.07±10.3	192.53±7.9	232.00±7.2	185.33±5.4	

-All values are mean ±SD

4.3.2 Hematological and biochemical parameters

Table (4-9) presents the results of hematological and biomarker parameters in the patient group and control group. The patient group had significantly higher levels of HbA1c (11.56±0.20%) compared to the control group (12.05±0.12 g/dL and 5.22±0.05%, respectively), with a p- Y D O X H R I " 7 K H O H Y H O endothelial growth factor (163.59±5.6 Pg/m) and IL-6 (19.76±0.45 pg/ml) were also significantly higher in the patient group compared to the control group (81.86±3.5 Pg/m and 11.35±0.36 pg/ml, respectively), with a p- Y D O X H R I " for both parameters. Similarly, the patient group had significantly higher levels of platelet-derived growth factor (116.62±3.40 pg/ml) compared to the control group (82.85±3.30 pg/ml), with a p- Y D O X H R I "

Table (4-9): Shows the results of hematological and biomarkers parameters (IL-6, platelets HbA1c%, VEGF, Hb, and PDGF) of patient affected with diabetic foot ulcer of both sexes and control.

Groups / Parameters	Patient	Control	P≤0.05
	Mean ±S.D		
HbA1c (%)	11.56±0.20	5.22±0.05	**≤0.0001**
vascular endothelial growth factor (Pg/m)	163.59±5.6	81.86±3.5	**≤0.0001**
IL-6 (pg/ml)	19.76±0.45	11.35±0.36	**≤0.0001**
platelet derived growth factor (pg/ml)	116.62±3.40	82.85±3.30	**≤0.0001**

-All values are mean ±SD

Table (4-10) This table shows the hematological and biochemical markers of patients with diabetic foot ulcer compared to controls based on gender and age. The markers measured are IL-6, HbA1c%, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF).

For HbA1c%, the patients had higher levels compared to controls, with the difference being significant for all age and gender groups. For VEGF, the patients had higher levels compared to controls, with the difference being significant for all age and gender groups. For IL-6, the patients had higher levels compared to controls, with the difference being significant for all age and gender groups. For PDGF, the patients had higher levels compared to controls, with the difference being significant for males aged 41-50 and females aged 41-50. Overall, the results suggest that patients with diabetic foot ulcers have alterations in their hematological and biochemical markers compared to controls, which could contribute to the development and progression of the disease.

Table (4-10): Illustrates the hematological and biochemical markers (IL-6, platelets HbA1c%, VEGF, Hb, and PDGF) of patients affected with diabetic foot ulcer according to gender and age and control.

Groups / Parameters	Age (year)	Male		Female		LSD(0.05)
		Patient	Control	Patient	Control	
		Mean ±S.D				
HbA1c (%)	41-50	9.73±0.7	5.12±0.6	11.65±1.3	4.93±0.3	0.791
	51-60	11.53±1.0	5.21±0.8	12.31±0.6	5.29±1.2	
	61-70	11.91±1.6	5.43±0.3	12.23±4.8	5.36±0.8	
vascular endothelial growth factor (pg/mL)	41-50	230.83±9.8	101.59±1.6	216.66±9.4	100.68±10.3	14.761
	51-60	148.75±5.7	101.48±5.7	163.19±7.1	97.81±9.7	
	61-70	111.29±4.9	48.53±7.8	110.81±1.6	41.08±8.4	
IL-6 (pg/ml)	41-50	15.95±1.7	9.97±0.8	18.20±2.6	9.60±2.2	2.196
	51-60	20.34±2.3	11.95±1.1	22.30±1.7	12.50±3.7	
	61-70	20.75±2.1	12.10±0.5	21.02±3.3	11.96±1.2	
platelet derived growth factor (pg/ml)	41-50	137.79±12.8	84.94±4.3	114.97±11.3	110.91±7.7	17.780
	51-60	120.41±9.5	81.31±5.1	123.45±8.4	81.51±2.6	
	61-70	107.65±6.3	79.52±4.7	95.45±6.6	58.93±8.4	

-All values are mean ±SD

This table (4-16) shows the Pearson correlation coefficient among different markers of patients affected with diabetic foot ulcer. The table presents the correlation coefficient (r) and the significance level (Sig.) for each pair of markers.

The off-diagonal elements of the table present the correlation coefficients between pairs of markers. For example, the correlation coefficient between IL6 and VEGF is -0.236 (significant at the 0.01 level), while the correlation coefficient between VEGF and PDGF is 0.302** (significant at the 0.01 level).

Table (4-11): Pearson Correlation coefficient among different markers of patients affected with diabetic foot ulcer.

		PLT	HbA1c %	VEGF	Hb	PDGF
IL6	**r**	-.014	.182	-.279**	.046	-.123
	Sig.	.896	.086	.008	.667	.248
PLT	**r**	1	-.097	.156	-.091	.130
	Sig.		.365	.142	.392	.222
HbA1c%	**r**		1	-.236*	-.048	-.152
	Sig.			.025	.656	.153
VEGF	**r**			1	-.060	.302**
	Sig.				.575	.004
Hb	**r**				1	.090
	Sig.					.398

****. Correlation is significant at the 0.01 level (2-tailed).**
***. Correlation is significant at the 0.05 level (2-tailed).**

The figure (4-10) shows a scatter plot of the correlation between IL6 and VEGF of patients, with a Pearson correlation coefficient of r = -0.279**. The negative value of the correlation coefficient indicates an inverse relationship between the two variables, meaning that as the levels of IL6 increase, the levels of VEGF decrease. The ** symbol indicates that the correlation is significant at the 0.01 level.

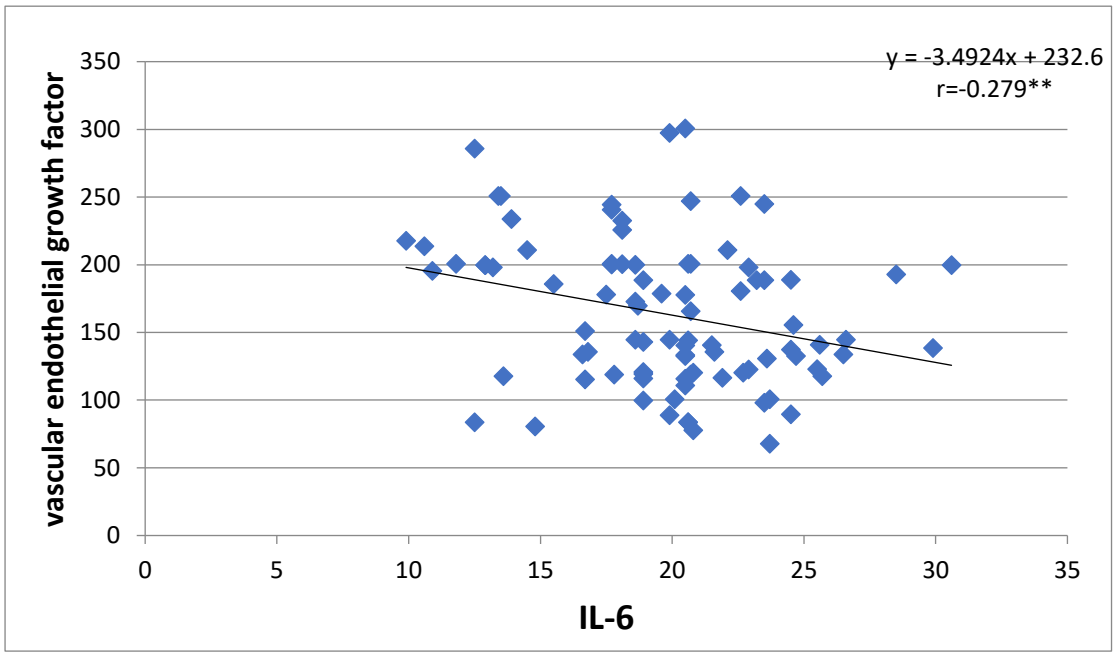


Figure (4-10): Correlation coefficient between IL6 and VEGF of patients.

In figure 4-11 there is a statistically significant negative correlation (r=-0.236*) between HbA1c and VEGF in patients with diabetic foot ulcers. This means that as the levels of HbA1c increase, the levels of VEGF tend to decrease.

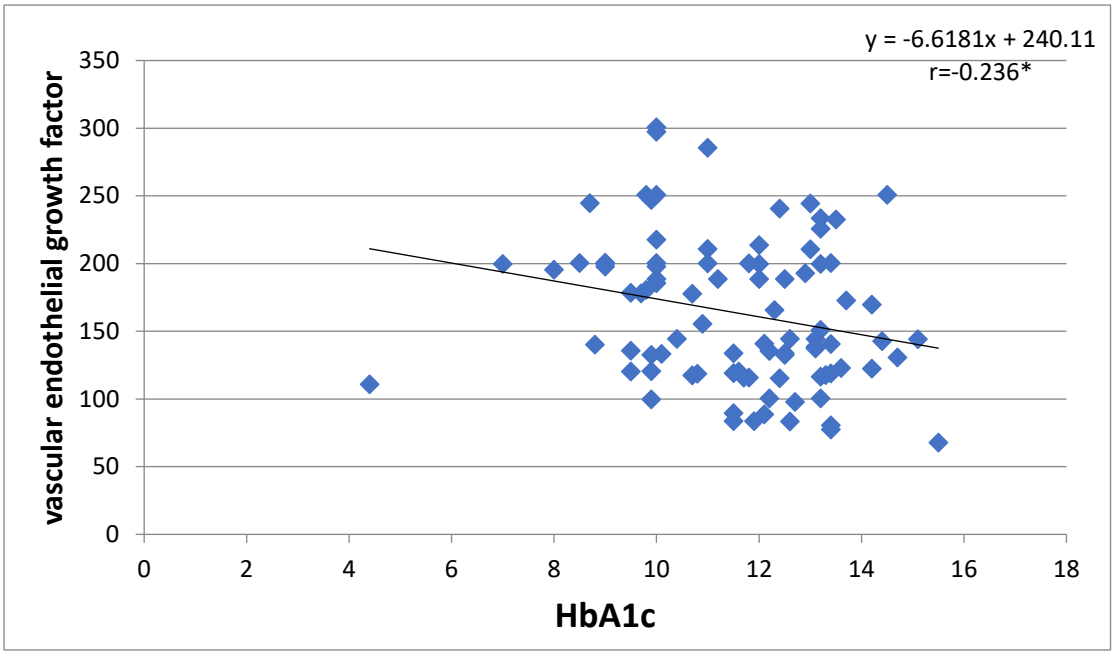


Figure (4-11): Correlation coefficient between HbA1c and VEGF of patients.

Figure (4-12) show Correlation coefficient between VEGF and PDGF of patients, the correlation coefficient between VEGF and PDGF of patients is r=0.302**, which indicates a strong positive correlation between these two variables.

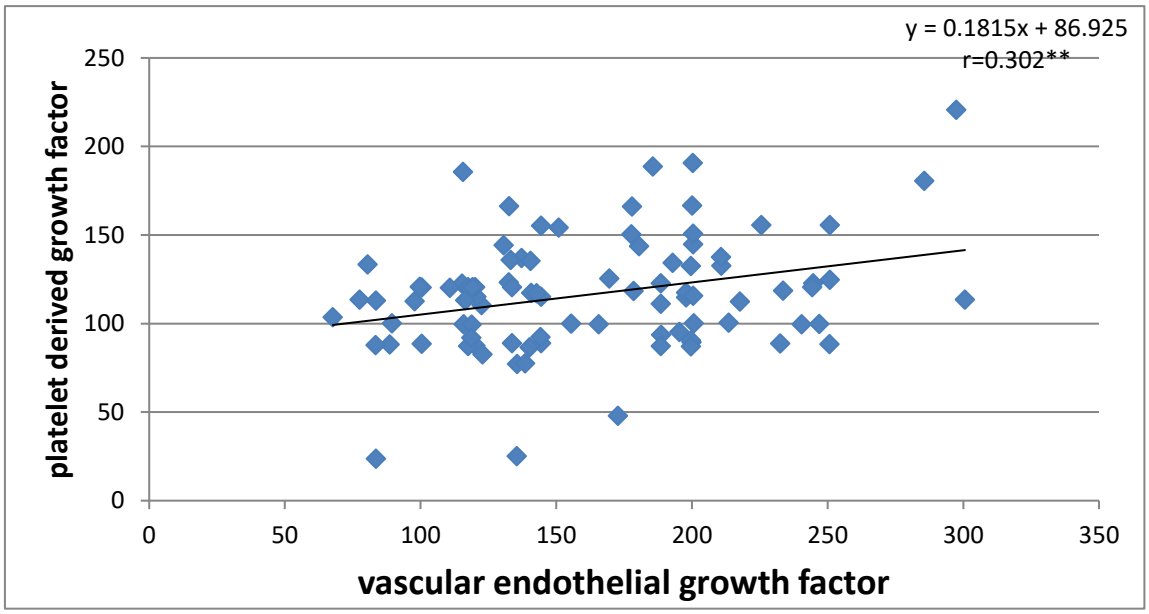


Figure (4-12): Correlation coefficient between VEGF and PDGF of patients.

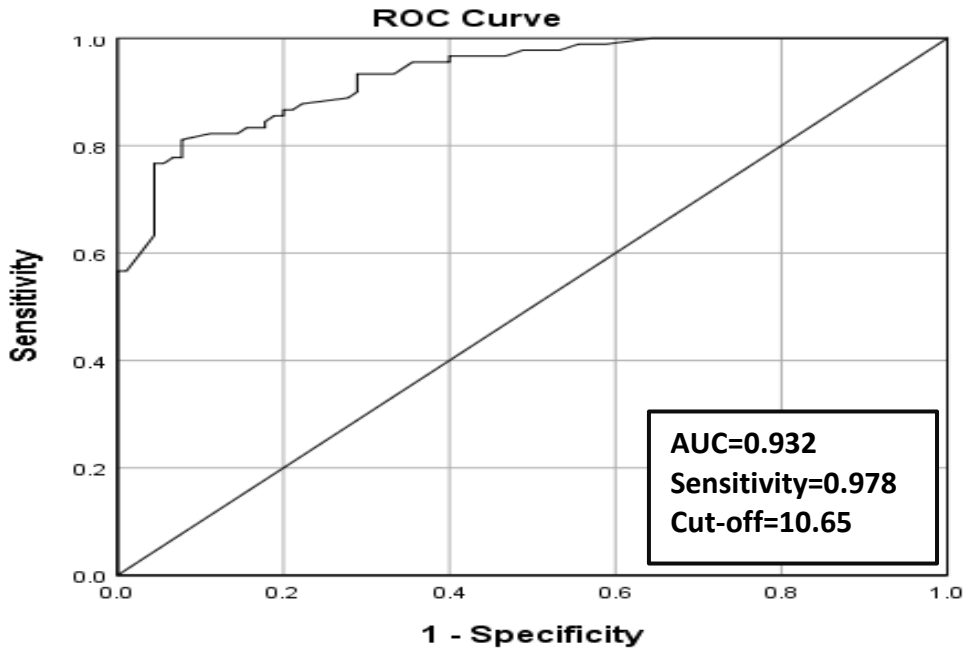


Figure (4-13): ROC curve for prediction of the disease activity by IL-6

Table 4-12: ROC curve of IL-6

IL-6	AUC	Sensitivity	Cut-off	P-value
	0.932	0.978	10.65 pg/ml	<0.001

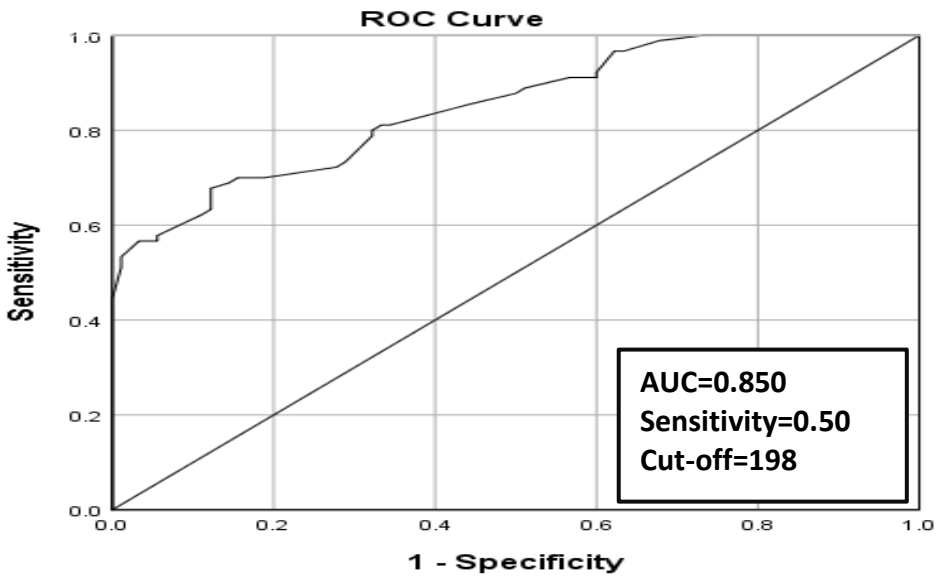


Figure (4-14): ROC curve for prediction of the disease activity by PLT

Table 4-13: ROC curve of PLT

PLT	AUC	Sensitivity	Cut-off	P-value
	0.850	0.50	198 m/mm^3	<0.001

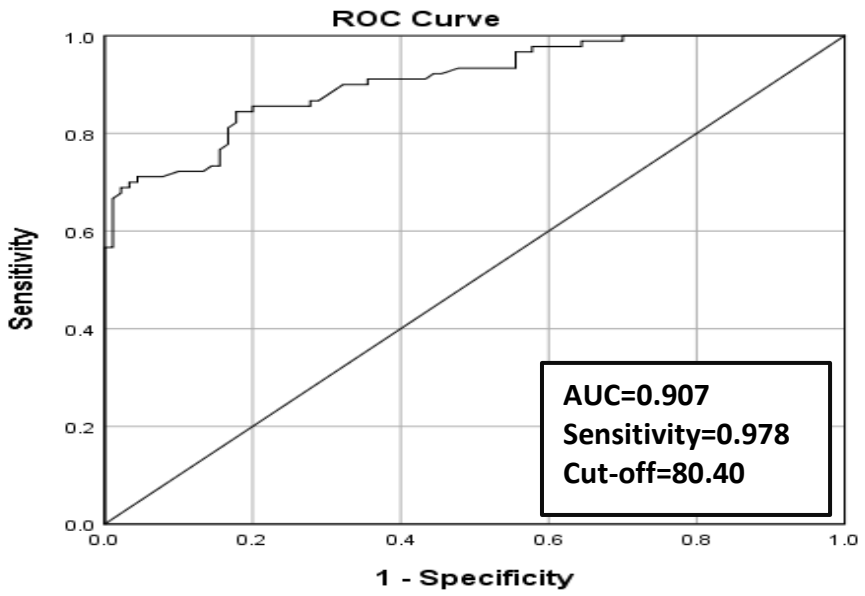


Figure (4-15): ROC curve for prediction of the disease activity by vascular endothelial growth factor

Table 4-14: ROC curve of VEGF

VEGF	AUC	Sensitivity	Cut-off	P-value
	0.907	0.978	80.4 pg/ml	<0.001

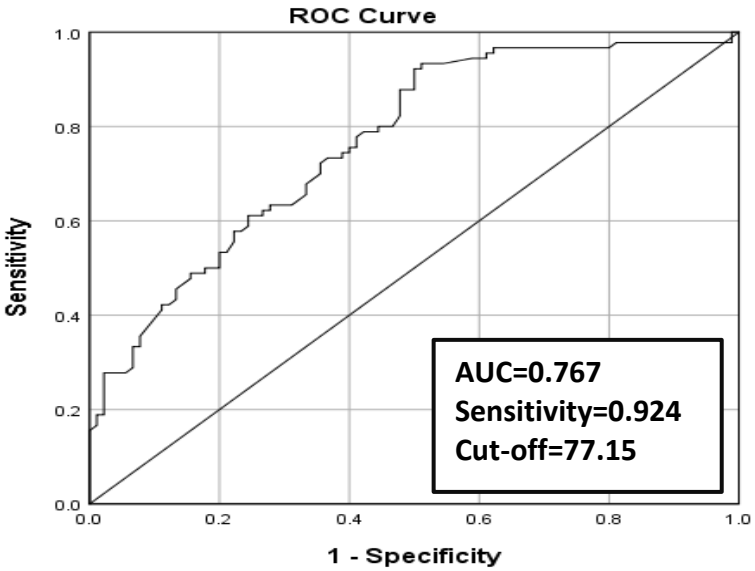


Figure (4-16): ROC curve for prediction of the disease activity by platelet derived growth factor

Table 4OC curve of PDGF

VEGF	AUC	Sensitivity	Cut-off	P-value
	0.767	0.924	77.15 pg/ml	<0.001

Based on figures (4-13, 4-14, 4-15, and 4-16), it can be observed that each marker has a varying ability to predict the disease activity in patients with diabetic foot ulcer.

Chapter Five
Discussion

5. Discussion

5.1 General demographical data of patients

The general demographical data of patients with diabetic foot ulcer presented in this study provide valuable insights into the gender and age distribution of this patient population. Understanding these demographics is crucial to better comprehend the prevalence and management of diabetic foot ulcers in different population groups.

The results presented in Figure 4-1 indicate that there is a higher prevalence of diabetic foot ulcers among female patients (60%) as compared to male patients (40%). The reasons for this gender disparity may be attributed to a combination of factors, such as hormonal differences, lifestyle choices, and the overall prevalence of diabetes among women.

The age distribution of the patients with diabetic foot ulcer in this study, as shown in Figure 4-2, reveals that the majority of patients (75%) are aged between 51 and 70 years. This finding is in line with previous research, which has demonstrated that the risk of developing diabetic foot ulcers increases with age (Yazdanpanah *et al*., 2018). The age-related risk factors may include a longer duration of diabetes, a higher likelihood of complications associated with diabetes, and an increased susceptibility to peripheral vascular diseases and neuropathy.

The results also show that 25% of the patients are aged between 41 and 50 years, indicating that diabetic foot ulcers can affect relatively younger individuals as well. This finding underscores the importance of early screening and intervention strategies to prevent the development and progression of diabetic foot ulcers in patients across all age groups.

5.2 Bacteriological study

In diabetic foot cases, infections that are preceded by ulcers or trauma constitute a prevalent clinical issue, frequently remaining undetected as a result of impaired sensation, which subsequently leads to unnecessary amputations. The factors contributing to this grave condition encompass inadequate treatment approaches, inappropriate antimicrobial therapy, protracted wound healing processes, and an inaccurate assessment of wound bioburden. A diminution in lower extremity amputations associated with diabetes can be accomplished through the precise diagnosis and efficacious management of such infections (Noor *et al*., 2018).

5.2.1 Bacterial species identified in diabetic foot ulcer

The prevalence of bacterial species identified by current study were two major species *S. aureus* and *P. aeruginosa* both of them were distributed as 24% of total patients with DFUs included in this study, while other bacterial species isolated from only 12% of total patients included, on the other hand 40% of total patients showed negative bacterial growth results.

The results of the current study consistent with results of previous studies, a meta-analysis study conducted on 57 previous study concluded that *S. aureus* and *P. aeruginosa*, were the most common species isolated from DFUs patients (23.4% and 11.1% respectively), while other bacterial species constitute less frequency (Macdonald *et al*., 2021). *S. aureus* was also identified to be the most prevalent if DFUs (52.8%) in a French study conducted in 2018, while other *Staphylococcus* spp. such as *S. lugdunensis* and *S. epidermidis* showed to be less frequent (18.7% and 11.3% respectively) (Jneid *et al*., 2018)

On the other hand, several studies showed that other bacterial species were more frequent in DFUs, study of Noor *et al* (2018) identify Klebsiella pneumonia as the most frequent bacterial species isolated from DFUs, while *Staphylococcus*

aureus and *Pseudomonas aeruginosa* were less frequent (27.2% and 18.1% respectively). The study of Shahi and Kumar (2016), identified that majority of DFUs patients were infected with Enterococcus spp. (47.61%), while *Staphylococcus* spp. and Pseudomonas spp. frequency were 33.33% and 30.95% respectively.

The differences in the prevalence of bacterial species in diabetic foot ulcers (DFUs) between current study and other previous studies may be due to several factors, such as geographical location, patient population, variations in sample collection and processing methods, use of antibiotics prior to specimen collection, and the duration and severity of DFUs. These factors can influence the microbial profile of DFUs and contribute to differences in bacterial identification and prevalence rates. Understanding the potential factors that can influence the prevalence of bacterial species in DFUs is crucial for developing effective treatment strategies for these infections.

5.2.2 Antibiotic Susceptibility of *Pseudomonas aeruginosa*

Antibiotic susceptibility testing is an essential tool for guiding the selection of appropriate antibiotics for treatment of DFU infections that caused by *Pseudomonas aeruginosa*. In the current study, antibiotic susceptibility testing conducted on 30 isolates of *P. aeruginosa* obtained from DFUs.

Antibiotic susceptibility test results (Figure 4-4) revealed a concerning trend of high resistance to commonly used antibiotics. The highest levels of resistance were observed for Piperacillin (86.6%), Ticarcillin (83%), Imipenem (76.6%), and Ciprofloxacin (76.6%). These antibiotics belong to the classes of penicillins, carbapenems, and fluoroquinolones, which are frequently used to treat *P. aeruginosa* infections. Our results suggest that alternative antibiotics from different classes may be more effective in treating DFU infections caused by *P. aeruginosa*.

The antibiotics with the lowest levels of resistance were Amikacin (53.3%), Gentamicin (53.3%), and Tobramycin (63.3%), which belong to the aminoglycoside class. These antibiotics are commonly used in combination with other classes of antibiotics to treat *P. aeruginosa* infections, and their lower resistance rates make them viable treatment options for DFU infections caused by this pathogen.

Colistin, which belongs to the polymyxin class, showed the lowest level of resistance at 16.6%. However, colistin is an antibiotic of last resort and is generally reserved for treating multidrug-resistant infections. The low resistance rate observed in this study suggests that colistin may be an effective treatment option for DFU infections caused by *P. aeruginosa*.

In addition to the high levels of resistance observed in the previous analysis, it is important to note that a significant proportion of the *Pseudomonas aeruginosa* isolates showed multidrug resistance (MDR) and extensively drug-resistant (XDR) phenotypes. Of the 30 isolates tested, 20% were classified as MDR, meaning they were resistance to three or more antibiotics classes. Additionally, 63.3% of the isolates were classified as XDR, indicating they were resistance to almost all available antibiotics classes, that including those commonly used as a last resort. The presence of XDR and MDR phenotypes in *P. aeruginosa* isolates is particularly concerning, as it limits treatment options and increases the risk of treatment failure and disease spread. The high prevalence of MDR and XDR isolates in this study highlights the need for continued surveillance and monitoring of antibiotic resistance in *P. aeruginosa*. 16.7% of the isolates showed no resistance to any of the antibiotics tested. This highlights the importance of identifying susceptible isolates, which can guide the selection of appropriate antibiotics and improve treatment outcomes.

Previous studies reported that *P. aeruginosa* isolates were MDR, the study of Mahmoud *et al*. (2013) reported that MDR percent among *P. aeruginosa* was 52%, study of Abd El-Baky *et al*. (2020) demonstrated that MDR constituted 96% of isolated *P. aeruginosa,* while XDR constitute 87% of total isolates. de Almeida *et al*. (2017) classify 71.4% of total *P. aeruginosa* isolates in study as MDR.

Several studies have explored the mechanisms underlying antibiotic resistance in this bacterium. Pang *et al*. (2019) reported that the primary mechanisms of antibiotic resistance in *P. aeruginosa* include efflux pumps, alterations in antibiotic targets, and the production of antibiotic-degrading enzymes. Efflux pumps are one of the major contributors to antibiotic resistance in *P. aeruginosa* and are involved in expelling antibiotics from the bacterial cell. Altered antibiotic targets, such as mutations in the penicillin-binding proteins, FDQ OHDG WR -lactam antibiotics. The production of antibiotic-GHJUDGLQJ HQ]\PHV VXFK DV -lactamases, can hydrolyze and inactivate antibiotics. Additionally, Botelho *et al*. (2019) reported that the emergence and spread of antibiotic resistance in *P. aeruginosa* are facilitated by horizontal gene transfer, biofilm formation, and the presence of mobile genetic elements. The evolution of resistance in this bacterium is complex and can be attributed to the acquisition of resistance genes via mobile genetic elements, such as plasmids and transposons. Biofilm formation also plays a significant role in antibiotic resistance, as it can protect bacteria from the effects of antibiotics.

The alternative therapeutic strategies suggested for the management of *P. aeruginosa* infections include the use of antimicrobial peptides, combination therapy, and the use of adjuvants to enhance the activity of antibiotics. Understanding the mechanisms of antibiotic resistance in *P. aeruginosa* is crucial for the development of effective treatment strategies to combat infections caused by this pathogen (Pang *et al*., 2019).

5.2.3 Antibiotic Susceptibility of *Staphylococcus aureus*

Staphylococcus aureus is one of the most common pathogens isolated from DFUs, and its antibiotic susceptibility profile is of utmost importance in guiding appropriate antimicrobial therapy. In the current study, the antibiotic susceptibility profile of 30 *S. aureus* isolates obtained from DFUs were analyzed. The results showed that all isolates were resistant to Benzylpenicillin (BENPEN) and 87% of the isolates were resistant to Oxacillin (OXA), indicating a high prevalence of methicillin-resistant *S. aureus* (MRSA) in DFUs. The high prevalence of MRSA in DFUs is a growing concern, as it limits the choice of antimicrobial agents available for treating these infections.

Among the tested antibiotics, Gentamicin (GEN) and Moxifloxacin (MOX) exhibited moderate susceptibility, with resistance rates of 23%. The fluoroquinolones Ciprofloxacin (CIP) and Levofloxacin (LEVO) showed variable susceptibility, with resistance rates of 30% and 0%, respectively. Erythromycin (ERY) and Clindamycin (CLIN) showed resistance rates of 47% and 40%, respectively, indicating limited utility of these antibiotics in treating DFUs caused by S. aureus.

Interestingly, Linezolid (LIN), Teicoplanin (TEC), and Vancomycin (VAN) showed 100% susceptibility, indicating their potential as alternative agents for MRSA infections treatment. However, these antibiotics should be used judiciously to prevent the emergence of resistance.

Tetracycline (TET) and Fusidic Acid (FUS) showed moderate resistance rates of 40% and 57%, respectively, indicating a limited role in treating *S. aureus* infections in DFUs. Rifampicin (RIM) showed a low resistance rate of 7%, while Trimethoprim/Sulfamethoxazole (TMP) showed a resistance rate of 13%, suggesting potential use as an adjunct therapy in combination with other antibiotics.

Furthermore, the study identified a significant proportion of the isolates as MDR (43.3%) and XDR (20%). This highlights the urgent need for the development of new antibiotics and alternative treatment strategies for DFUs caused by S. aureus, as the options for effective treatment are becoming increasingly limited. In addition, the study showed that a considerable percentage of the isolates were not resistant (36.7%), indicating that some antibiotics may still be effective in treating DFUs caused by S. aureus.

The study conducted by Al-Zoubi *et al*. (2015) examined the antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in the Northern area of Jordan. The analysis of the results showed that a significant proportion of the *S. aureus* infections were resistant to multiple antibiotics, with 31.6% of infections being Methicillin-resistant *Staphylococcus aureus* (MRSA), 31% being MDR, and 42.7% being Oxacillin-resistant (ORSA). The majority of these resistant strains were isolated from specimens of wounds. The study also found that all isolates were susceptible to vancomycin, which is a drug of last resort for MRSA infections, and several other antibiotics including nitrofurantoin, chloramphenicol, rifampicin, linezolid, and teicoplanin with susceptibility rates of over 80%. However, the isolates were found to be resistant to erythromycin and penicillin.

Another study conducted by Dilnessa and Bitew (2016) fount that 17.5% of *S. aureus* isolated were MRSA, whereas 50.5% were MDR, with the highest percent of resistance for penicillin (96.4%). They reported that MRSA strains were 100 % resistant to penicillin G, erythromycin, trimethoprim-sulfamethoxazole and least resistant to vancomycin (29.4 %).

The resistance of *S. aureus* to antibiotics is complex and multifactorial, with various mechanisms involved, such as the acquisition of resistance genes on mobile genetic elements, alterations of the target site of antibiotics, production of

enzymes that inactivate antibiotics, or modification of the antibiotic molecule itself. The resistance mechanisms in *S. aureus* include the production of beta-lactamases that hydrolyze beta-lactam antibiotics, efflux pumps that pump out antibiotics, alteration of the target site of antibiotics, and modification of the antibiotic molecule itself (Guo *et al*. 2020). In addition, *S. aureus* can form biofilms that protect the bacteria from the action of antibiotics and the host immune system. MRSA is a major healthcare-associated pathogen that is resistant to all beta-lactam antibiotics, including methicillin, oxacillin, and cephalosporins. The resistance exhibited by MRSA can be attributed to the procurement of a mobile genetic element known as staphylococcal cassette chromosome mec (SCCmec). This element encompasses the mecA gene, responsible for encoding an alternative penicillin-binding protein (PBP2a) characterized by its diminished affinity for beta-lactam antibiotics. As a result, beta-lactam antibiotics cannot bind to PBP2a and inhibit cell wall synthesis, leading to antibiotic resistance (Peacock and Paterson, 2015; < Õ O P D] D Q G $ V O D Q W D ú, 2017).

5.2.4 Antibacterial activity of AgNPs

The emergence of antibiotic-resistant bacterial strains has increased the demand for alternative antibacterial agents. One of the promising candidates is silver nanoparticles (AgNPs) due to their broad-spectrum antibacterial activity and low toxicity to human cells (Markowska *et al*., 2013). In this study, the antibacterial activity of AgNPs against five *Pseudomonas aeruginosa* isolates was investigated at varying concentrations (31.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml), and the results were evaluated based on the inhibition zones of the isolates. The results showed that AgNPs had concentration-dependent antibacterial activity against *P. aeruginosa* isolates, with higher AgNP concentrations resulting in larger inhibition zones. The smallest inhibition zones were observed at the lowest concentration of AgNPs (31.25µg/ml), with the isolates showing only mild susceptibility to the nanoparticles. However, as the

concentration of AgNPs increased, the inhibition zones became more prominent, with the highest concentration of AgNPs (500µg/ml) showing the largest inhibition zones against all the tested isolates.

These results are consistent with previous studies, Hussein and Muslim (2019) found that the biosynthesized AgNPs demonstrated antibacterial efficacy against both Gram-positive and Gram-negative bacterial species, including *P. aeruginosa.*

Salomoni *et al*. (2017) also investigated the antibacterial effect of AgNPs in *P. aeruginosa*. However, their study used a single concentration of AgNPs (50 µg/ml) and evaluated their antibacterial activity over different exposure times (2, 4, 6, and 24 hours), The findings indicated the potential utility of silver nanoparticles (AgNPs) as a substitute for traditional antimicrobial agents in current use, as well as the prospect of integrating nanosilver with antibiotics in order to augment antimicrobial effectiveness.

De Lacerda Coriolano *et al*. (2021) investigated the antibacterial and antibiofilm potential of AgNPs against both antibiotic-sensitive and multidrug-resistant *P. aeruginosa* strains. Their results showed that AgNPs had strong antibacterial and antibiofilm activity against all the tested *P. aeruginosa* strains, which is consistent with our results. However, their study used a single concentration of AgNPs (10 µg/ml) and evaluated their antibacterial and antibiofilm activity using different assays, such as the colony-forming unit (CFU) count and crystal violet assay, respectively.

The results of the antibacterial activity of AgNPs against five *Staphylococcus aureus* isolates at varying AgNP concentrations indicate that the AgNPs have significant antibacterial activity against S. aureus. As the concentration of AgNPs increased, the inhibition zones also increased, indicating that the antibacterial activity of AgNPs is dose-dependent. At the lowest

concentration of 31.25µg/ml, the inhibition zones were relatively smaller, ranging from 15-17 mm. However, at the highest concentration of 500µg/ml, the inhibition zones increased up to 24 mm. These results suggest that higher concentrations of AgNPs could effectively inhibit the growth of S. aureus.

These findings are consistent with the findings of a study conducted by Ananda *et al*. (2019), which found that AgNPs had concentration-dependent antibacterial activity against *Staphylococcus aureus*. However, our study found higher inhibition zones than the Ananda *et al*. study at lower concentrations of AgNPs, which may be due to differences in the type and size of AgNPs used, as well as the method of synthesis.

The study by Salomoni *et al*. (2015) also investigated the antibacterial activity of AgNPs against *Staphylococcus aureus*. Although their study did not use the same concentrations of AgNPs as our study, they found that AgNPs had significant antibacterial activity against *Staphylococcus aureus*. However, their study also found that AgNPs had cytotoxic effects on mammalian cells, which is an important consideration when evaluating the potential use of AgNPs as antibacterial agents.

The mechanisms of the antibacterial activity of AgNPs have been studied extensively in recent years. One mechanism is the physical damage caused by the nanoparticles to the bacterial cell membrane, leading to leakage of intracellular contents and ultimately cell death. This mechanism has been observed in studies on *Staphylococcus aureus* and *Escherichia coli* (Tang and Zheng, 2018). Another mechanism involves the generation of reactive oxygen species (ROS) by AgNPs, which can cause oxidative stress in bacterial cells, leading to DNA damage, protein denaturation, and ultimately cell death (Patil and Kim, 2017; Zawadzka *et al*., 2016). In addition, AgNPs have been shown to disrupt bacterial biofilms by inhibiting the formation of extracellular polymeric substances (EPS) and

interfering with quorum sensing, a process by which bacteria communicate and coordinate biofilm formation (Tang and Zheng, 2018). Furthermore, AgNPs have been shown to have synergistic effects with antibiotics, enhancing their antibacterial activity (Patil and Kim, 2017).

5.3 Hematological parameters and biomarkers

5.3.1 White blood cells

Statistical analysis that are obtained from the present study indicated a significant increase ($p<0.05$) in the levels of total WBCs and all studied groups compared to control. Concerning the differential WBCs there are a significant increase in percentage of neutrophils and lymphocytes in all patient groups.

Results of present study are consistent with previous study of Lee *et al*. (2013) who found increase of WBCs and C-reactive protein in diabetic patients affected with DFUs and these changes occur as a response to infection and inflammatory evens associated with impairment of wounds.

There is an evidence suggested that up-regulation of WBC give an indicator of inflammation and infection of diabetic foot ulcers, so this is a good dependent risk factor (Eneroth *et al*, 1997).

Moreover, it has been found that there is a positive correlation between levels of phagocytic activity of neutrophils and grade of diabetic foot ulcer (Hameed and Baras, 2020).

Inflammatory cytokines have ability to bring neutrophils and monocytes to the area of wound and initiate inflammatory processes (Falanga, 2005).

Another study showed inhibition of leukocyte activities because of chronic hyperglycemia (Nolan *et al*., 1999).

Further study gives more explanation that is increase count of WBCs and progression of endothelial cells in those patients affecting with diabetes mellitus (Dinh *et al*., 2012).

In order to evaluate the relationship between incidence of foot ulcer healing and neutrophil, lymphocyte ratio, it has found remarkable increase in the neutrophil, lymphocytes, and ratio of neutrophils to lymphocytes, so from these documents it appears that these cells are considers as predisposition factor for non-healing of ulcers and recorded poor prognosis (Sathvik *et al*., 2023).

The acceptable and prominent factor that explain the inflammatory status and progression of diabetic ulcer is neutrophil/lymphocyte ratio (NLR) and ratio of platelet to lymphocyte (P/L ratio), these mentioned parameters were found to be increased in ulcers of type 2 diabetes mellitus (Serban *et al*., 2021).

The prognosis of ulcerative wound is associated with neutrophilia, lymphopenia, and different inflammatory infections (Howard *et al*., 2019).

5.3.2 Hemoglobin (Hb)

Results of hemoglobin (Hb) that are obtained from the present study showed a significant decrease ($p<0.05$) in all patient groups affected with DFU compared to the control.

In fact, diabetes mellitus is commonly associated with anemia that is manifested itself by lowering Hb concentration (Kwon and Ahn, 2012).

Another study was confirmed that hypoxia (low PO2) causing beta-cell dysfunction that is low oxygen (hypoxia) can damage to insulin secreting cells (Rhodes, 2005).

Study of yang *et al*. (2017) who investigated the relationship between the diabetic peripheral neuropathy and hemoglobin levels, and they concluded that

Hb concentrations were decreased in patients with diabetic peripheral neuropathy. There is evidence confirmed that HPV abnormalities could be implicated in development of diabetic peripheral neuropathy this is resulted from bilirubin metabolism and RBC deformities (Wagner *et al*., 2015).

According to physiological point of view, hemoglobin is a protein contained with RBCs responsible for carrying oxygen to peripheral tissues and particular in nervous tissues, there are many neuro-vascular markers contribute to disruption of blood supply to nervous causing progression of diabetic peripheral neuropathy (Javed *et al*., 2015).

In addition, Hb considerers essential components in RBC deformability by which O2 deliver and can accelerated and reduce flow resistance during stress, lowering Hb in RBC lead to decreased viscosity of cytoplasm and effect RBC deformability that causing damage to endothelium of microvasculation, this is prominent predisposing factor take part in developing microvascular events in diabetic patients (Popel and Johnson, 2005).

Cross-sectional study performed by Taderegew *et al*. (2020) revealed that patients affected with diabetes mellitus are commonly complaint from anemia.

The present observations are agreed with previous study expressed that anemia are more prevalence in patients with diabetic foot ulcers (Gezawa *et al*., 2019).

Interestingly, high sugar level in blood for long time can affect RBCs at a level of their structure and function, so, there are as many morphological changes affecting red cells, it was found the cells become spherocytic or echinocytes that is produced due to damage of plasma membrane by oxidative stress, the abnormal cells eventually are removed by reticulo-endothelial system and anemia resulted (Wang *et al*., 2021).

5.3.3 Blood platelets

another previous study concluded the same results that is increased platelet count in patient with DM. Results that are yield from the present study give clear evidence on significant increase in the levels of platelets in all tested patients group compared to those healthy groups. In regard ROC test, the data of platelets recorded the following, AUC 0.850, cutoff 198, sensitivity 0.500.

The present study agrees with study of Adane *et al*. (2012) who concluded the same results that is increased platelet count in patients with DM. Obviously, another previous study conducted by Rodriguez and Johnson (2020) who documented increase platelet count and their indices especially mean platelet volume as well as an increased tendency of platelets to aggregate.

Study of Ferreiro *et al*. (2010) confirm that prothrombotic event are predisposing factor because of platelet hyperactivity and there is platelet dysregulation of several cellular signaling pathway causing substantial adhesion, platelet activation, and their aggregation of patients with DM.

In contrast, previous study explained that there is no significant correlation between platelet indices in particular MPV, platelet distribution width in those patient affected with DM (Jabir, 2019).

Another retrospective study pointed out elevation of blood platelet count and it is related with early clinical signs of neuropathy and these observation are clearly documented and female gender (Starner *et al*., 2004).

A substantial evidence supports the present results who indicate elevation of platelet levels are generally noted in infectious and inflammatory events (Williams, 1999).

There is substantial evidence suggested up-regulation of reactivity and activity of blood platelets in patients with DM compared to those control (Kakoures *et al*., 2011).

Previous report confirmed increased thrombosis that dependent on increased platelet count and activities in patients with DM and concluded increased cardiovascular risk factor because of high levels of glucose (Shechter *et al*., 2000).

5.3.4 Hemoglobin A1c (HbA1c)

Data obtained from the present study indicated a significant increase ($p<0.01$) in the percentage of HbA1c in all patients groups compared to those healthy subjects. It's levels showed a significant negative correlation with PDGF, and VEGF in patients with DFU.

The present results was consistent with study of Casadei *et al*. (2021) who confirmed that a higher levels of HbA1c is good and risk factor in developing of DFU and it is responsible for it is responsible for incidence of neuropathy and vascular abnormalities.

There are two previous studies have examined the effects of HbA1c on DF peripheral neuropathy and they get a high relationship occurring between HbA1c and threshold of vibration perception maybe evidence for progressive complication affecting foot after DPN (Maiya *et al*., 2019).

Inspite of the most research confirmed that elevation of HbA1c fluctuation is more related with incidence of DPN in diabetic patients (Mayeda *et al*., 2020).

It has been documented that there is a strong positive association among DFU, age, HbA1c, and duration of disease but there was no relation with gender and BMI (Yonis *et al*., 2018).

The hypoglycemia can affect the activity of polymorphonuclear cells that empire the defense mechanism against infection and predispose onychomycosis (Gupta and Humk, 2000).

Another study of Pastore *et al*. (2022) indicated that the ROC analysis of HbA1c in patients with DM affected with DFU give strong indicator for worsening of wounds and these analysis of HbA1c AUC was 0.75.

However, another author confirmed that there was no relation between hba1c level and wound healing (Fesscha *et al*., 2018).

The toxic effects of higher levels of hba1c on the pulmonary function was investigated and the results showed a significant negative inverse correlation between hba1c and vital capacity that can decrease spo2 in diabetic patients and impair wound healing (Maan *et al*., 2021).

Furthermore, chronic hyperglycemia induce oxidative stress through production excessive free radicals that can upset and in impair mitochondrial function (Dludla *et al*., 2017).

In addition, overproduction of free radicals during hypoglycemia affect many physiological functions at level of cellular and molecular activities (Pasupuleti *et al*., 2020).

Paul *et al*. (2020) suggested that chronic hyperglycemia is a accompanied with increasing vascular complications.

In fact, there is a clear evidence confirmed that hyperglycemia is responsible for several micro and macro vascular abnormalities, I'm on these effects induced oxidative stress by generation excessive free radicals within cells through and product of advanced glycation such as protein kinase, hexosamine, and diacylglycerol processes, as well as compensatory hyperinsulinemia can also

induce Pro-etherogenic processes within smooth muscle of blood vessels (Madonna and Catesina, 2011)

From observations and facts mentioned above it appears clearly that hba1c represents essential indicator to explain prognosis of wound and healing in patients with DFU.

5.3.5 Interleukin-6 (IL-6)

Results of IL-6 indicates a significant elevation ($p<0.0001$) in all patients groups compared to those healthy subjects. Also values of IL-6 explained negative correlation with VEGF. The cut of in ROC 10.65 and the sensitivity was 0.978, and AUC was 0.932.

The present study was consistent with previous data yield by Kheiralla *et al* (2012) who indicated increase the levels of inflammatory mediator, in particular IL-6, CRP, and TNF.

It's well known that the mechanisms involved in pro and inflammatory are related with different events of healing processes and homeostasis of tissue, so, these inflammatory processes can disrupt the healing and causing chronic non-healing wound (Zubair *et al*., 2012)

Kartika *et al*. (2021) were also obtained the same results that involved increase IL-6, VEGF, and PDGF in those patients affected with diabetic foot ulcer. Korkmaz and his colleagues (2018), they evaluates the levels and role of IL-6 in diabetic foot ulcer and they found that IL-6 with other inflammatory markers are increased in infected and non-infected diabetic foot ulcer.

Any infectious state always is related with increase levels of ILs as pro inflammatory markers and for this reason consider the diagnostic parameters in inflammatory processes, IL-6 enhances synthesis of C-reactive protein by

hepatocytes and fibrinogen especially during stage of inflammation (Mosesson *et al*., 2001).

It's well documented and agreement with present study that the levels of IL-6 is accompanied with a higher concentration of glucose (hyperglycemia) in patients in diabetes mellitus type 2 and consider the early diagnostic marker for prediction the incidence of diabetic foot ulcer (Al-Salih and Ali, 2021)

The process of diabetic foot ulcer is progressive due to lowering of growth factors such as VEGF and PDGF and continuous inflammatory condition (Muller *et al*., 2008).

The inflammatory processes are exaggerated by inflammatory cells releasing inflammatory mediator (Kany *et al*., 2019).

From the above documents, it appear clearly that the levels of IL-6 is associated with progressive inflammatory processes in diabetic foot ulcer.

5.3.6 Platelet derived growth factor (PDGF)

A significant increase ($p<0.05$) was found in the level of PDGF in all studied patients group and tend to be decreased with advanced ages but remain higher than those of control.

It showed a positive correlation ($r^2=0.091$) with VEGF. The ROC analysis indicated cut off (77.15) with area under curve equals to 0.767.

It is well found that PDGF conduct powerful activator for many perforation cells, expression of gene in inflammatory cells, and chemotaxis in wounds affected the body especially soft tissues (Pierce *et al*., 1991)

Previous study revealed there are changes in the levels of different cytokines especially those growth factor, in diabetic patient suffering from DFU and other diabetic complication such as nephropathy, retinopathy, and neuropathy and this

study concluded the wound healing can be increased if the levels of cytokines are maintained (Zubair and Ahmed, 2019).

That is consistent idea with the present results this idea suggest that as long as injury presented there's multiples cytokines in particular growth factors are released from the cells in the affected areas or those secreted by blood cells especially blood platelets or inflammatory cells those secreted cytokines cooperate to accelerate angiogenesis processes and metabolic activities (Liu *et al*., 2021)

PDGF is a cytokine to be essential growth factor reach to affected site following injury, this is released from alpha granules of blood platelet (Delair, 1999).

Previous study indicated increase several growth factors to enhance wound healing and diabetic foot ulcers (Demireci *et al*., 2016).

Of those growth factor connective tissue growth factor with interactions with other growth factors to improve food ulcer through the aggregation of collagen fibers (Henshaw *et al*., 2015).

Regarding to management of DFU it has been found that topical application of growth factor can accelerate the healing of ulcers (Sridharan and Sivaramakrishnan, 2017).

Another previous study confirmed an increase blood platelets indices such as platetcrit (PCT), platelet count, mean platelet width, and mean platelet volume in diabetic patients with food ulcer (Mardia *et al*., 2018).

It's well documented that presence of lesion can induce capillary leakage and adhesion of blood platelets to collagen fiber within matrix, this is in turn enhance the secretion of bioactive molecules from platelets especially that are contained

in alpha granules, of those bioactive PDGF, prostocyclin, serotonin, thromboxan, and histamine (Simao *et al*., 2022)

from data obtained from this study it can suggest the increase level of PDGF have maybe a result from elevation of a platelet count and increase capillary permeability which lead to increase PDGF.

5.3.7 Vascular endothelial growth factor (VEGF)

Results which are obtained from present study indicated as significant elevation in the levels of VEGF at $P < 0.05$ in all studied groups of patients compared to control and it's concentration recorded a negative significant correlation with IL-6 and HbA1c in patient males. In addition there is a positive significant correlation with PDGF. ROC analysis of VEGF pointed out a sensitivity 0.978, AUC= 0.907 and cut off equals 80.40.

It is well recognized that insufficient blood supply in affected areas (wound) in diabetic patients may cause degeneration, ischemia, and necrotic tissues that are diagnosed as diabetic ulcers especially in far sites of the body lower extremities (Prompers *et al*., 2007).

In patients with diabetic foot ulcer there is decrease in levels of VEGF (Mi *et al*., 2018) and this study disagree with the present study especially with patients at age four decade.

Another experimental study was performed on induced diabetic ulcer in rods indicated lowering of VEGF compared to control (Lin *et al*., 2019).

The data documented from the present study showed up regulation of VEGF in patients with four decade and then the levels of VEGF become decreased with the progress of age but it's level remains higher than that of healthy one on this may be resulted from ability of the body to increase stimulation of gene responsible for encoding VEGF in middle ages higher than of advanced ages.

Previous study of Barc *et al*. (2022) revealed an improvement of diabetic patients ulcer who have therapeutic preparation having VEGF.

It's had been found that administration of patients with hyperbaric oxygen therapy (HBOT) led to increase concentration of VEGF (Semadi, 2019).

A progressive decrease levels of VEGF protein is related with advanced states of diabetic foot ulcer and this is accompanied with regression of granulation tissues of wound and type 2 diabetes mellitus, also there was no difference of VEGF level in patient with or without diabetic food ulcer (Bassar *et al*., 2022).

Another study was focused on the effects of sexual hormone levels on the expression of VEGF and it found that estrogen enhances expression of VEGF in ovariectomized animals treated with VEGF in compared to those control or administrated testosterone (Berciano Guerrero *et al*., 2010).

According to effect of age, the impairment of the angiogenesis processes and endothelial roks is demonstrated in old ages, also it was found that hypoxia and increased metabolic pathway is associated with increase levels of VEGF in order to elevate angiogenesis processes in that injured sites (Lahteenvuo *et al*., 2012).

Moreover, levels of mRNA encoding VEGF and its proteins are downregulated in old man whether after physical activity or after ischemic lesion in matching with those young healthy subjects (Croley *et al*., 2005).

Conclusions

From the data previously mentioned, one can be conclude the following:

1- *Pseudomonas aeruginosa* (G-ve) and *Staphylococcus aureus* (G+ve) bacteria considered as the most common bacterial species isolated from infections of DFUs.

2- There are many bacterial species have high resistance for used antibiotics.

3- AgNPs have the ability to inhibit MDR bacterial isolates.

4- Increase in inflammatory response in DFUs may be associated with infectious pathogen.

5- The increase in Vascular Endothelial Growth Factor and Interleukin levels indicating and ongoing inflammatory response.

6- Increase levels of growth factors but it is not sufficient for wound healing because of decreased blood supply to affected areas.

Recommendations

1- Treatment with effective antibiotic to prevent infection.

2- An attempt to combine AgNPs with antibiotics to be more effective against MDR isolates.

3- An attempt to use alternative treatments (like nanoparticle, plant extracts, and essential oils) and test it in inhibition of MDR bacteria and adopt it as alternative for traditional treatment.

4- Treatment of affected areas with topical growth Factor preparations.

5- Blood transfusion to patients suffering from anemia.

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الخلاصة

تعتبر تقرحات القدم السكري (DFUs) مضاعفة كبيرة لمرض السكري، حيث تؤثر على ما يصل إلى ٢٥٪ من المرضى المصابين بالسكري وغالباً ما تؤدي إلى بتر الأطراف السفلية. هدفت الدراسة إلى اختبار تأثير جزيئات الفضة النانوية (AgNP) على البكتيريا الممرضة المعزولة من تقرحات القدم السكري، وتقييم مقاومة المضادات الحيوية، وتقييم العلاقات بين المؤشرات البيولوجية مثل CBC و-IL 6و VEGFو .PDGF

تم جمع ١٢٥ عينة مسحة لتقرحات القدم السكرية وعينة دم من المرضى الذين أُدخِلوا إلى مستشفى الحسيني بين ايلول ٢٠٢٢ وكانون الثاني ٢٠٢٣، إلى جانب ٣٠ عينة دم من المشاركين الأصحاء كمجموعة سيطرة. كان توزيع الجنس بين المرضى الذين يعانون من تقرحات القدم السكري ٤٠٪ من الذكور و ٦٠٪ من الإناث. أكثر البكتيريا التي تم التعرف عليها من البكتريا الموجبة لصبغة كَرام كانت العنقودية الذهبية (*Staphylococcus aureus*) والسالبة لصبغة كَرام الزائفة الزنجارية (*Pseudomonas aeruginosa*)، بمعدل ٣٠ عزلة لكل نوع حيث شكلت كل منهما ٢٤٪ من الحالات. أظهر الفحص لحساسية المضادات الحيوية معدلات مقاومة للمضادات الحيوية المستخدمة بشكل شائع، مع وجود عزلات متعددة المقاومة للأدوية (MDR) وعزلات شديدة المقاومة للأدوية (XDR) .

أظهر اختبار معالجة تأثير جزيئات الفضة النانوية نشاطًا مضادًا للبكتيريا ضد كل من العنقودية الذهبية وجرثومة الزائفة الزنجارية. أدى زيادة تركيزات جزيئات الفضة النانوية إلى مناطق تثبيط أكبر، حيث لوحظت ان مناطق التثبيط تبدأ عند ٥٠٠ ميكروغرام/مل لبعض العزلات وحتى ١٢٥ ميكروغرام/مل للعزلات الأخرى. يُذكر أن جزيئات الفضة النانوية كانت فعالة ضد العزلات البكتيرية المتعددة المقاومة للأدوية وشديدة المقاومة للأدوية، مما يُبرز إمكاناته كعلاج بديل لعدوى تقرحات القدم السكرية.

كشف تحليل الدم أن المرضى الذين يعانون من تقرحات القدم السكري كان لديهم عدد خلايا الدم البيضاء (WBC) أعلى بشكل ملحوظ مقارنة بمجموعة السيطرة، مع متوسط عدد خلايا الدم البيضاء ٨,٧٧±٠,٩ و٦,٣٥±١,٣ على التوالي .(P=0.042) لوحظ ارتفاع عدد خلايا الدم البيضاء هذا عبر جميع الفئات العمرية وكلا الجنسين. بالإضافة إلى ذلك، كان لدى المرضى الذين يعانون من تقرحات القدم السكري مستويات أقل بشكل ملحوظ من الهيموغلوبين (Hb) (٩,٨١±٠,١٣ جم/دل) ومستويات أعلى من الهيموغلوبين A1c (HbA1c) (١١,٥٦ ± ٠,٢٠٪) مقارنة بمجموعة السيطرة (١٢,٠٥±٠,١٢ جم/دل و ٥,٢٢±٠,٠٥٪ على التوالي)، مع قيمة p <0.001 لكلا المعلمين.

أظهر تحليل المؤشرات البيوكيميائية مستويات أعلى بشكل ملحوظ من VEGF و IL-6و PDGF في مجموعة المرضى (١٦٣,٥٩±٥,٦ و١٩,٧٦±٠,٤٥ و ١١٦,٦٢±٣,٤٠ على التوالي) مقارنة بمجموعة السيطرة (٨١,٨٦±٣,٥ و ١١,٣٥±٠,٣٦ و ٨٢,٨٥±٣,٣٠ على التوالي)، مع قيمة p <0.001لجميع المعلمات. أظهر معامل الارتباط بيرسون علاقة عكسية بين IL-6 و VEGF (r=-0.279**) وارتباطاً سلبيًا بين HbA1c و VEGF (r=-0.236*). لوحظ وجود ارتباط إيجابي قوي بين VEGF و(r=0.302**) PDGF، مما يشير إلى وجود علاقة محتملة بين هذه المؤشرات لدى مرضى تقرحات القدم السكري.

استنتاجاً، تسلط هذه الدراسة الضوء على التحديات التي تواجهها البكتيريا المقاومة للمضادات الحيوية في إدارة تقرحات القدم السكري وتوضح إمكانات جزيئات الفضة النانوية كعلاج بديل للعدوى البكتيرية في تقرحات القدم السكري. بالإضافة الى ذلك، يساهم تحليل المعلمين الدمويين والمؤشرات البيوكيميائية في فهم أفضل لتطور المرض وقد يساعد في تطوير العلاجات المستهدفة. تشير الارتباطات الهامة بين مختلف المؤشرات البيولوجية، مثل IL-6 و VEGFوPDGF، إلى أن هذه العوامل قد تلعب دورًا في تطور قرحات القدم السكرية ويمكن أن تكون أهدافًا علاجية محتملة.

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات
قسم علوم الحياة

تحديد الفعالية المضادة للبكتريا لدقائق الفضة النانوية ضد البكتريا المعزولة من تقرحات السكري وبعد المعايير الدموية

رسالة مقدمة
الى مجلس كلية العلوم للبنات/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستيرفي علوم الحياة

من قبل الطالبة
سارة كاظم عباس
بكالوريوس علوم حياة كلية العلوم للبنات/ جامعة بابل (٢٠١٥)

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٢٠٢٣ م ١٤٤٤ هـ