University of Kerbela

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Department of Clinical laboratories

Evaluation of Procalcitonin, Cystatin C, Nephrin and Kidney Injury Molecule-1 in Patients with Diabetic Foot and Relation with chronic Kidney Disease

A thesis

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صدق الله العلي العظيم

(يوسف٧٦)

Supervisor's certification

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Dedication

To …Martyrs of Iraq

To... the person we take as a role model, my dead father

To...the source of success and success in this world, my dear mother

To... my creative brothers all

To... my dear wife

To... the delight of the eyes and the pleasure of my son (yossif)

To whom help me

To everyone I love

To every patient who is suffering from DFU and need our efforts

Special thanks to my close colleague.

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Ahmed Malik

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List of Abbreviation

Abbreviation	Details
ADA	American Diabetic Association
ADPKD	Autosomal dominant polycystic kidney disease (
AKI	Acute kidney injury
BUN	Blood urea nitrogen
CKD	Chronic kidney disease
CysC	Cystatin C
DFU	Diabetic foot ulceration
DKA	diabetic ketoacidosis
DM	Diabetes mellitus
DN	Diabetic nephropathy
DPN	diabetic peripheral neuropathy
ESR	erythrocyte sedimentation rate
FFAs	free fatty acids
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
HBA1C	Glycated hemoglobin
IL1	interleukin-1
IL6	interleukin-6
KIM1	Kidney molecule injury 1
LADA	Latent Autoimmune Diabetes in Adults
MODY	maturity-onset diabetes of the young
NKHS	non-ketotic hyperosmolar state
NPHS1	Nephritic syndrome gene 1
OGTT	Oral glucose tolerance test
PAD	peripheral artery disease (
PC	phosphocholine
PCT	Procalcitonin
PN	Peripheral neuropathy
PPG	Post prandial glucose
ROS	Reactive oxidative stress
T1DM	Type 1 diabetic mellitus
T2DM	Type 2 diabetic mellitus

Summary

Diabetes mellitus is a metabolic disorders characterized by high blood suger levels due to insulin deficiency or resistance ,one of the most important complication of it diabetic foot , that may be lead to foot amputation if not treated. Chronic hyperglycemia, peripheral arteries disease, ischemia and neuropathy are the main risk factor for diabetic foot. When the skin damage or break in foot , the infection complicates to ulcer. In their lifetime, about 34% of diabetics patients (Type 1 or Type 2) get a foot ulcer .It may lead to serious complications as gangrene and limb amputation if not recognized in the early stage.

This study aimed to investigated the serum level of Procalcitonin (PCT), Cystatin C, Nephrin and Kidney injury molecule -1(KIM-1) in patients with Diabetic foot and kidney disease , also determine the specificity and sensitivity, cut off value of this factors and there correlation with various clinical parameters.

This is a case-control study, including 120 individuals who were divided into four groups: control group, diabetes mellitus without complication group, diabetic foot ulcer group and (diabetic foot with chronic kidney disease) group .

All the diabetic patients were diagnosed by an endocrinologist conducted in the diabetic center at Marjan Teaching Hospital and ALHilla Teaching Hospital in AL Hilla City from October 2023 to March 2024.

Procalcitonin, Cystatin C, Nephrin ,and Kidney injury molecule-1 were determined by using the Enzyme-linked immunosorbent assay (ELISA) technique while C-reactive protein(CRP) and HbA1C were determined by using immune detection Method, Blood glucose, blood urea and serum creatinine were measured using a colorimetric method.

The results of the current study revealed increased a significantly in mean of serum Procalcitonin, Cystatin C, CRP, Nephrin, KIM-1 ,HbA1C ,fasting blood

sugar, blood urea and serum creatinine among diabetic foot patients when compared with control and a significant decrease in body mass index (BMI) and estimated glomerular filtration rate in diabetic foot patients compared with control. This study showed that PCT may be diagnostic performance for Diabetic foot , current results showed area under the curve(AUC) (0.96), sensitivity(91.1%) , specificity(99%), cut-off points were(0.231) at ($p < 0.001$) ,also the study showed that KIM-1 prognostic diabetic kidney disease current results showed area under the curve(AUC) (0.939), sensitivity(93.3%) ,specificity(99.3%), cut-off points were(0.66) at ($p < 0.001$).The correlation of PCT with Cystatin C, CRP, and WBC were positive correlation,

In conclusion the PCT, Cystatin c, increase in diabetic foot and may be used as parameters for early detection of infection in these disease. KIM-1 and Nephrin levels were significantly increased in Diabetic foot with kidney disease patients and can be used as prognostic parameters .

Chapter one

Introduction

Introduction:

Diabetes mellitus (DM) is a common chronic metabolic condition with increasing prevalence rate among adult populations worldwide. Diabetes currently affects 415 million people, It is a metabolic disorder with hyperglycemia and (relative or absolute)insulin deficiency , insulin resistant or both , (Khalid., *et el*., 2022).

Complication of Diabetes

Diabetes mellitus if uncontrolled over a prolonged period , is linked to the development of many consequences include macro and microvascular complications , microvascular complications can be primarily categorized into nephropathy, retinopathy, and peripheral neuropathy , diabetes is also accompanied by a substantial increase in atherosclerotic disease of large vessels , including cardiac , cerebral and peripheral vascular disease (Surowiec *et al.*, 2022) , although this macrovascular atherosclerotic disease causes serious morbidity and the largest fraction of excess mortality among people with diabetes , but also acute metabolic complications such as diabetic ketoacidosis and hyperglycemic hyperosmolar non-ketotic coma may considered as a complications of diabetes and hypoglycemia is complication of its treatment , control of hypertension and dyslipidemia is an important step in minimizing the risk of complications (Pop-Busui *et al.*, 2022).

Diabetic foot

Uncontrolled diabetes contributes to the development of neuropathy and peripheral arterial disease by complex metabolic pathways. Loss of sensation caused by peripheral neuropathy, ischemia due to peripheral arterial disease, or a combination of these may lead to foot ulcers (Lu *et al.*, 2021).Foot disease affects nearly 6% of people with diabetes and includes infection, ulceration or destruction of tissues in the foot. It can impair patients' quality of life and affect social participation and living. Between 0.03% and 1.5% of patients with diabetic foot require an amputation (Rigato et el.,2018) .

Diabetic kidney disease

Diabetic kidney disease is a serious complication of type 1 diabetes and type 2 diabetes. It's also called diabetic nephropathy. In the United States, about 1 in 3 people living with diabetes have diabetic nephropathy(Abbad *et al.*, 2022). Diabetic nephropathy affects the kidneys' usual work of removing waste products and extra fluid from the body. The best way to prevent or delay diabetic nephropathy is by living a healthy lifestyle and keeping diabetes and high blood pressure managed(Banfi *et el,* 2021) . Over years, diabetic nephropathy slowly damages the kidneys' filtering system. Early treatment may prevent this condition or slow it and lower the chance of complications. Diabetic kidney disease can lead to kidney failure. This also is called end-stage kidney disease. Kidney failure is a life-threatening condition (Vishnupriya, 2019).

Biomarkers in this study

Procalcitonin (PCT), a precursor hormone of calcitonin, is a 116 amino-acid peptide, member of the calcitonin superfamily of peptides. Procalcitonin (PCT) is a marker that can be used to diagnose bacterial infections and sepsis (Schuetz *et el*.,2022) . It is also a tool for assessing how effectively treatments are working. In diabetic patients, Procalcitonin is a useful a biomarker for the identification of foot infections(Karakas et el ., 2021)

Serum Cystatin C (Cys C) is a tiny molecular protein produced by human nucleated cells that belongs to the type 2 cystatin superfamily and works as a reversible competitive inhibitor of cysteine proteinase (Al-Nori and Ahmed, 2019).

Kidney molecule injury 1 (KIM-1) is a cell surface receptor in epithelial and lymphoid/myeloid cells, it behaves as a scavenger receptor for oxidized LDL and phosphatidylserine and as Ebola and Hepatitis A virus entry receptor in epithelial cells .Kim-1 binding to phosphatidylserine allows the phagocytosis of apoptotic cells. In addition, KIM-1 regulates Th2, Th1, and **Th17** differentiation (Hojs *et al.*, 2020). Kim-1 is markedly upregulated in proximal tubules in AKI and CKD. . KIM-1 is not expressed in the normal kidney but is expressed in a variety of human kidney diseases, predominantly in the apical membrane of proximal tubular cells while KIM-1 is primarily used in the setting of drug-induced acute kidney injury recent studies suggest that KIM-1 may be useful in predicting chronic kidney disease progression as well (Pei *et al.,* 2022).

Nephrin is a specialist glomerular adhesion protein that is expressed as the podocyte matures and begins to differentiate and form protrusions(Aljorani *et el,* 2023). In addition to its structural role, Nephrin is also involved in podocyte

signaling. Abnormalities of Nephrin caused by mutations in the *NPHS1* gene have been implicated in the autosomal recessive congenital nephritic syndrome of the Finnish type. The single gene mutations are responsible for the developmental failure of the podocyte foot processes and slit diaphragms, with extensive proteinuria present in urine (Xiong and Zhou., 2019) .

Aim of the Study

This study aims to investigate the relationship between diabetic kidney disease and diabetic foot ulcer in patients with diabetes mellitus. Specifically, it seeks to evaluate the diagnostic potential of inflammatory markers-Procalcitonin, Cystatin C (Cys C), Kidney Injury Molecule-1 (KIM-1), and nephrin-in detecting these complications at an early stage.

The main objectives of present study can be summarized as:

1-To evaluate the serum level of Procacitonin ,cystatin c,Kidney injury molecule -1 and Nephrin in study groups

2-To Determine the effect of disease severity on the serum levels of (Procalcitonin , Cystatin C, Kidney molecule injury-1 and Nephrin).

3-To Evaluate the correlation between these parameters: Procalcitonin , Cystatin C, Kidney molecule injury-1 and Nephrin with (Age, smoking, Complete blood count, C Reactive Protein , Random blood sugar, HBA1C, Blood urea and Serum creatinine

4- Measurements of sensitivity and specificity of Procalcitonin , Cystatin C, Kidney molecule injury-1 and Nephrin for diagnosing diabetic foot ulcer and diabetic kidney disease.

Chapter Two

literature review

2.1 Diabetes Mellitus

2.1.1 – Definition

Diabetes mellitus is one of the main chronic non- contagious diseases threatening the health of human around the world (Suk *et al.*, 2019), It is a group of common endocrine diseases that affect in metabolism of carbohydrate, lipid, and protein. It is expressed precisely by persistent hyperglycemia (elevation of blood glucose) due to either the pancreas not producing enough insulin or the cells of the body becoming unresponsive to the insulin effects (Chaudhury *et al.*, 2019). The disease if left untreated, can lead to various health complications, including disorders of the nerves ,cardiovascular system, eyes and kidney, untreated or poorly treated diabetes accounts for approximately 1.5 million deaths every year, therefore, diagnosis of disease and treatment in early stages is very essential (Lind *et al.*, 2021).DM remains one of the most public health challenges in the worldwide imposing emotional costs and significant financial on sufferers families as well as the community. In general, about 450 million people worldwide (8.8% of adults aged 20–79 years) are expected having DM. According to the World Health Organization (WHO), DM will be the 7th main cause of death in 2030 (Balakumar *et el*., 2022).

2.1.2. Clinical Presentation

Patients with diabetes most commonly present with increased thirst, lack of energy and fatigue, bacterial and fungal infections, delayed wound healing, polyuria, polyphagia, some patients can also complain of numbness or tingling in their hands and feet or with blurred vision and gradually loss of weight (Chawla *et el*.,2020). Diabetic ketoacidosis is a medical emergency that occurs most

commonly in type 1, but may also occur in type 2 if it has been longstanding or if the individual has significant β-cell dysfunction. Excessive production of ketone bodies leads to signs and symptoms including nausea, vomiting, abdominal pain, the smell of acetone in the breath, deep breathing known as Kussmaul breathing and in severe cases decreased level of consciousness and in case of inconvenience therapy that may cause death, hyperosmolar hyperglycemic state is another emergency characterized by dehydration secondary to severe hyperglycemia, with resultant hypernatremia leading to an altered mental state and possibly coma (Balaji *et el*., 2019).

2.1.3. Etiology and Pathogenesis

In T1DM, there is cellular-mediated, autoimmune destruction of pancreatic beta cells. T1DM has a strong genetic predisposition. The major histocompatibility complex (MHC), also known as human leukocyte antigens (HLA), is reported to account for approximately 40 to 50% of the familial aggregation of T1DM(Ozougwu *et al.*, 2021). The significant determinants are polymorphisms of class II HLA genes encoding DQ and DR4-DQ8, with DR3-DQ2, found in 90% of T1DM patients. Another form of T1DM is latent autoimmune diabetes of adults (LADA). It occurs in adulthood, often with a slower course of onset (Gomes *et al.*, 2018).

The rate of islets cells destruction is generally rapid in children and faster in adults. Autoantibodies against islet cells, insulin, glutamic acid decarboxylase-65 (GAD-65), and zinc transporter 8 (Zn T8) may be detected in the serum of such patients. These antibodies wane over time and do not have sufficient diagnostic accuracy to be used routinely for diagnosis, especially after the first year . With the progressive destruction of beta cells, there is little or no secretion of insulin. These

patients are generally not obese. They are more prone to develop other autoimmune disorders such as Addison disease, Graves disease, Hashimoto thyroiditis, and celiac disease(Wu *et al.*, 2022). A subset of T1DM not associated with insulin autoimmunity and not associated with the above HLA is termed idiopathic T1DM. It is more common in African and Asians and presents with episodic diabetic ketoacidosis (DKA) (JA *et el*., 2020).

In Type 2 diabetes Reduced insulin secretion or weaker effect of insulin on its receptor leads to high glucose content in the blood (Karalliedde and Gnudi, 2014). The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. Many people with type 2 diabetes have evidence of prediabetes (impaired fasting glucose and/or impaired glucose tolerance) before meeting the criteria for type 2 diabetes (Ozougwu, 2021).

The progression of prediabetes to overt type 2 diabetes can be slowed or reversed by lifestyle changes or medications that improve insulin sensitivity or reduce the liver's glucose production. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress and urbanization (Tosur and Louis H Philipson, 2022).

The DM may be secondary consequence of other diseases, such as pancreatic diseases (pancreatitis or haemochromatosis) , some endocrine disorders like acromegaly or Cushing’s syndrome, there is an antagonism of insulin action by abnormal secretion of hormones with opposing activity and several drugs antagonistically affect glucose tolerance (Karalliedde and Gnudi, 2022).

2.1.4. Prevalence of DM

The prevalence of diabetes mellitus is gradually elevated in the world ,a global report on diabetes released by WHO on 6th April 2016 stated that the prevalence of DM in adult population worldwide was 8.5%(Pham and Eggleston, 2021) . In 2019, which is nearly two fold of that (4.7%) in 1980 ,the same report also referred that about five million people died because of DM in 2015. As of 2021, an estimated 537 million people had diabetes worldwide accounting for 10.5% of the adult population, with type 2 making up about 90% of all cases (Ohishi *et el,* 2022). Imposing emotional costs and significant financial on sufferers families as well as the community, the global expenditure on diabetes-related healthcare is an estimated US$760 billion a year. In general It is estimated that by 2045, approximately 783 million adults, or 1 in 8, will be living with diabetes, representing a 46% increase from the current figure. The prevalence of the disease continues to increase, most dramatically in low- and middle-income nations (Wu *et al.*, 2022). Additionally, countries that have not been generally associated with high prevalence of DM have recorded a significant increases in prevalence recently, one of these regions in the world with high prevalence of DM is middle east with about 10% of adults while about twenty million people in Africa have DM with predicting this number to double by 2035 (Atlas, 2021).

2.1.5 Diagnosis of Diabetes Mellitus

The patient's blood glucose levels are used to diagnose and to monitor diabetes. Four glucose tests give a snapshot of a patient's current ability to regulate blood glucose levels Fasting plasma glucose (FPG) is taken at least 8 hours after the patient has had any food . Diabetes is characterized by an FPG > 126 mg/dl (7 mmol/l) (Rios-Arce *et al.*, 2022).

- Postprandial glucose level (PPG) is taken 1 to 2 hours after a meal . Diabetes is characterized by any random PPG > 200 mg/dl with symptoms (11.1 mmol/l).
- Oral glucose tolerance test (OGTT) is a standardized postprandial glucose test . The OGTT is taken serially every one hour after the patient had taken 75 g of oral glucose. DM is diagnosed if the plasma glucose (PG) level in the 2-hour sample is more than 200 mg/dL (11.1 mmol/L).
- Glycated hemoglobin (HbA1c) test reflects the average glucose saturation over three months' time and is strongly predictive of diabetic complications at higher levels. In 2019 the American Diabetic Association(ADA) adopted standards recommending the use of the A1c test to diagnose diabetes with a threshold set at .6.5% (Beran *et al.*, 2022).

2.1.6 Classification of Diabetes

Diabetes mellitus can be classified according to ADA into the following general categories:

2.1.6.1 Type 1 Diabetes

Type 1 diabetes mellitus accounts for 5 to 10% of diabetes cases and is the most common type diagnosed in patients less than 20 years, however, the older term "juvenile-onset diabetes" is no longer used as the disease not uncommonly has onset in adulthood. The disease is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to severe insulin deficiency and can be further classified as immune-mediated or idiopathic (without known cause). The majority of cases are immune-mediated, in which cytotoxic CD8-T lymphocytes attack and destroy the pancreatic beta islands causes insulin deficiency (Alvarenga *et al.*, 2022) . Scientists understand type 1 diabetes causes

the body to attack the cells that make insulin. Patients often have irregular and unpredictable blood sugar levels due to very low insulin and an impaired counter-response to hypoglycemia (Rios-Arce *et al.*, 2022).

Type 1diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors . In contrast to the tremendous amount of data about the role of genetic factors in T1DM pathogenesis, there is much less information about the role of environmental factors because of the complexity of environmental parameters and their mechanisms of actions (Katsarou *et al.*, 2017) .

Type 1 diabetes can occur at any age, and a significant proportion is diagnosed during adulthood. Latent autoimmune diabetes of adults (LADA) is the diagnostic term applied when type 1 diabetes develops in adults; it has a slower onset than the same condition in children. Given this difference, some use the unofficial term "type 1.5 diabetes" for this condition. Adults with LADA are frequently initially misdiagnosed as having type 2 diabetes, based on age rather than a cause. LADA leaves adults with higher levels of insulin production than type 1 diabetes, but not enough insulin production for healthy blood sugar levels (Lind *et al.*, 2021)

2.1.6.2 Type 2 Diabetes

Type 2 diabetes (T2DM) is the most common form of diabetes, it represents 90% of all people with diabetes , it is an insulin-resistance condition in which many cells in the body become less responsive to insulin with associated beta-cell dysfunction and for many individuals up to 50% of beta cell function is lost by the time the diagnosis is made , an additional 3% to 5% may be lost in each subsequent year Initially, there is a compensatory increase in insulin secretion, which maintains glucose levels in the normal range (Ciardullo *et el*.,2022). As the disease

progresses, beta cells change, and insulin secretion is unable to maintain glucose homeostasis, producing hyperglycemia (Dettmer *et al.*, 2022) . Even before the disease shows clinical signs and symptoms , mildly elevated blood glucose levels can be detected in tests at this stage , the condition is called prediabetes (Farag *et el*., 2022) .

The progression of type 2 diabetes is gradual, over the years , the prediabetes individuals worsens , especially if patients are in active and overweight or have a higher body fat percentage, distributed predominantly in the abdominal region. This adipose tissue itself promotes insulin resistance through various inflammatory mechanisms, including increased free fatty acids (FFA) release and adipokine dysregulation. Lack of physical activity, prior Gestational DM (GDM) in those with hypertension or dyslipidemia also increases the risk of developing T2DM (Steinbrenner *et el*., 2022). Evolving data suggest a role for adipokine dysregulation, inflammation, abnormal incretin biology with decreased incretins such as glucagon-like peptide-1 (GLP-I) or incretin resistance, hyperglucagonemia, increased renal glucose reabsorption, and abnormalities in gut microbiota (Forsblom *et al.*, 2019).

2.1.6.3. Gestational Diabetes

Gestational diabetes is seen as persistent hyperglycemia during pregnancy due to the overall stress of pregnancy and other additional risk factors it resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. It is most often diagnosed in the second or third trimester because of the increase in insulin-antagonist hormone levels that occurs at this time. However, after pregnancy

approximately 5–10% of women with gestational diabetes are found to have another form of diabetes, most commonly type 2 (Tourkmani *et al.*, 2022)

2.1.6.4 Other Types of Diabetes

A) **Maturity-onset diabetes of the young(MODY**) is a rare autosomal dominant inherited form of diabetes, due to one of several single-gene mutations causing defects in insulin production, It is significantly less common than the three main types (type 1, type 2 and GDM), constituting 1–2% of all cases. The name of this disease refers to early hypotheses as to its nature, the disease varies in age at presentation and in severity according to the specific gene defect , there are at least 13 subtypes of MODY (Tosur and Louis H. Philipson, 2022).The patients still produce some insulin and are clinically closer to a type 2 (Mushtaq *et al.*, 2022).

B) **Latent Autoimmune Diabetes in Adults (LADA)** presents in young adults in their twenties and may be confused as type 2 because of age , however , they do not produce any insulin and are clinically similar to type 1 requiring insulin . They have often been labeled as “diabetes 1.5” because they are clinically between type 1 and type 2 (Rak and Bronkowska, 2022) .

C) **Endocrinopathies** may include polycystic ovarian syndrome ,pancreatic cancer or tumors and other hormonal disruptions in insulin production (Alam *et al.*, 2021)

2.1.7. Complications of Diabetes Mellitus

Persistent hyperglycemia in uncontrolled diabetes mellitus can cause several complications, both acute and chronic. Diabetes mellitus is one of the leading causes of cardiovascular disease (CVD), blindness, kidney failure, and amputation of lower limbs. (Balaji *et el* .,2022)

2.1.7.1 Acute Complications

Acute complications include hypoglycemia, diabetic ketoacidosis, hyperglycemic hyperosmolar state, and hyperglycemic diabetic coma. Chronic microvascular complications are nephropathy, neuropathy, and retinopathy, whereas chronic macrovascular complications are coronary artery disease (CAD), peripheral artery disease (PAD), and cerebrovascular disease. It is estimated that every year 1.4 to 4.7% of middle-aged people with diabetes have a CVD event (Asmat *et el* ., 2016) (Meshram., 2021).

Diabetic ketoacidosis is a complex metabolic condition known by hyperglycemia, ketonaemia, and acidosis. DKA usually happens due to relative or absolute insulin shortage that is joined with elevation in counter regulatory hormones such as glucagon and cortisol. This kind of hormonal instability enhances glycogenolysis and gluconeogenesis causing acute hyperglycemia. Raised lipolysis increases free fatty acids (FFAs) in serum as an alternative energy source, which is then metabolised in the process of ketogenesis. This consequence resulting from agglomeration of large amounts of ketone bodies is accompanied by subsequent metabolic acidosis. Ketone bodies include acetoacetate, 3-beta-hydroxybutyrate, and acetone, the prevalent ketone in DKA is 3-betahydroxybutyrate (Dhatariya and Umpierrez, 2019).

Non-ketotic hyperosmolar state is characterised by extreme elevations in concentrations of serum glucose and hyperosmolality without significant ketosis. It has been infrequent in children. The NKHS is a syndrome recognised by severe hyperglycemia, hyperosmolarity and dehydration in the absence of ketoacidosis (Zhuravlyova *et al.*, 2019).

2.1.7.2. Chronic Complications of Diabetes Mellitus

The major long-term complications of diabetes relate to damage to blood vessels at both macrovascular and microvascular levels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in people with diabetes are due to coronary artery disease(Chawla and Jaggi, 2020).Other macrovascular morbidities include stroke and peripheral artery disease. The blood vessels damaged lead to microangiopathy and as a result of this damage appear one or more of the following: diabetic neuropathy, nephropathy, and retinopathy (Madonna *et al.*, 2022).

Diabetic retinopathy

Diabetic retinopathy is one of the most common micro vascular complications of diabetes , it affects up to 80 percent of those who have had both type 1 and type 2 for 20 years or more of diabetic patients , it is the leading cause of blindness in people of working age between 20 to 64 years. And it is an important cause of visual impairment in patients with diabetes , in the United States, diabetic retinopathy accounts for 12% of all new cases of blindness each year, The longer a person has diabetes, the higher chances of developing diabetic retinopathy, In at least 90% of new cases, progression to more aggressive forms of sight threatening retinopathy and maculopathy could be seen , The eyes can also be affected in other ways, including development of cataract and glaucoma (Farag *et el*, 2022), the biochemical pathways related to hyperglycemia such as oxidative stress, polyol , hexosamine pathway activation , advanced glycosylation end product formation and activation of protein kinase C isoforms are related to the pathogenesis of DR(Bilal *et al.*, 2022) .

Diabetic neuropathy

Diabetic neuropathy can affect any peripheral nerves including sensory neurons, motor neurons, and the autonomic nervous system. Therefore, diabetic neuropathy has the potential to affect essentially any organ system and can cause a range of symptoms. Diabetic peripheral neuropathy (DPN) is known to predict foot ulceration, lower-extremity amputation and mortality. Patients with diabetes mellitus have a predisposition toward developing chronic inflammatory demyelinating polyneuropathy, and this may also facilitate the formation of diabetic foot and cutaneous impairment, which are considered one of the most serious impairments of diabetes mellitus, with a prevalence of 4–10% in this population(Monteiro *et al.,* 2022) .

Approximately half of all people with DM suffer from neuropathy, which can be mono-neuropathy, polyneuropathy and autonomic neuropathy. Mono-neuropathy is less common than polyneuropathy and involved dysfunction of isolated cranial or peripheral nerves, in polyneuropathy patients suffer from weakness of peripheral sensation, when together with impaired microvascular and macrovascular junction in the periphery, can contribute to difficulty in healing ulcers, the main cause non-traumatic amputation (Vishnupriya, 2019). Diabetic autonomic neuropathy is a common and severe complication of diabetes. It is a form of peripheral neuropathy; it can affect body temperature, blood pressure, digestion, bladder function, control and even sexual function (Balaji *et el*, 2022).

Diabetic Nephropathy

Diabetic nephropathy (DN) or diabetic Kidney disease in patients with diabetes can be a result of micro vascular complications from diabetes , a concomitant kidney disease of other origin or a combination of both , in type 1

diabetes patients, micro vascular disease secondary been to diabetes and is the most common etiology to chronic kidney disease usually affects young and middle-aged patients a condition which has been referred to as diabetic nephropathy or 'diabetic kidney disease' in literatures while there is a whole spectrum of chronic kidney disease etiologies can cause kidney disease in type 2 diabetes patients (Feng *et al.*, 2022) .

Type 2 diabetes patients are often older at the time of diagnosis and kidney disease due to other causes than diabetes is likely to occur , several studies have verified that kidney disease in type 2 diabetes may be a more compounded entity than what is seen in type 1 , The disease progression of diabetic nephropathy involves various clinical stages: hyperfiltration, microalbuminuria, macroalbuminuria, nephrotic proteinuria to progressive chronic kidney disease leading to end-stage renal disease (ESRD) (Abbad *et el*.,2022). The damage is exerted on all compartments of the kidney: the glomerulus, the renal tubules, the vasculature (afferent and efferent renal arterioles) and the interstitium. Renal fibrosis is the final common pathway of DN, however, not all patients with diabetes go on to develop diabetic nephropathy (Elsayed *et el* .,2019). The pathophysiology of diabetic nephropathy is thought to involve an interaction between hemodynamic and metabolic factors. Interestingly, one study from the United States which examined kidney biopsies in patients with type 2 diabetes and kidney disease found that typical diabetic micro vascular disease were present in 37% of the cases , non-diabetic kidney disease in 36% of the cases , such as nephrosclerosis or immunological kidney disease while mixed forms of diabetic and non-diabetic kidney disease were found in 27% of the cases (Tong *et al.*, 2020). Interestingly, one study has found different insulin resistance phenotypes in diabetes to be associated with different risks for chronic kidney disease (Banfi *et*

el, 2021).Regardless of kidney disease etiology, strict blood glucose control is most important to prevent kidney disease to develop in patients with type 1 and type 2 diabetes , normalization of blood glucose might act reno-protective through different mechanisms (Abbad *et al.,* 2022) .

2.2. Diabetic foot

A diabetic foot disease is any condition that results directly from peripheral artery disease(PAD),vascular compromise ,repetitive trauma and sensory neuropathy affecting the feet of people living with diabetes, it can be acute or chronic complications of diabetes(Wang *et al.*, 2022). Presence of several characteristic diabetic foot pathologies such as infection, diabetic foot ulcer (DFU) and neuropathic osteoarthropathy is called diabetic foot syndrome (DFS) and as defined by the World Health Organization it is an "ulceration of the foot (distally from the ankle and including the ankle) associated with neuropathy and different grades of ischemia and infection". It affect high numbers of patients with diabetes mellitus(Pichu *et al.*, 2020).

Approximately 34% of people with diabetes (Type 1 or Type 2) will develop a foot ulcer in their lifetime.A foot ulcer will develop in 18.6 million people worldwide each year. About 15-20% of moderately to severely infected foot ulcers eventually lead to amputation and the mortality rate of diabetic foot ulcers is 30% at 5 years with a mortality rate of 70% in those with a foot ulcer who receive an above the foot amputation (El-Kafrawy *et al.*, 2019) .

Diabetic foot disease is the leading cause of non-traumatic lower limb amputations. Pathogenic events able to cause diabetic foot ulcers are multifactorial. Among the commonest causes of this pathogenic pathway it's possible to consider peripheral neuropathy, foot deformity, abnormal foot pressures, abnormal joint

mobility, trauma, peripheral artery disease. The refractory nature of foot ulcer is reflected in that even after healing there is still a high recurrence rate and amputation rate which means that management and nursing plans need to be considered carefully (Akyüz et el.,2023) . diabetic foot ulcer as a set of symptoms secondary to current or previous diabetes including skin chapping, ulceration, infection or destruction of foot tissue which partly reflects the fuzzy and imprecise nature of this concept. DFU invariably with neuropathy and/or peripheral artery disease (PAD) that disrupt the foot epidermis and dermis, breach the skin envelope, expose sterile structures and finally form full-thickness lesions (Atlaw *et al.*, 2022).

2.2.1 Classification of diabetic foot

The multiple factors associated with the development of DFU, such as the complex process and complications of diabetes, may all lead to various degrees of neurological abnormalities and vascular damage (known as neuropathy and Preverbal arterial disease PAD). Once the ulcer is formed, the factors affecting healing may be more complex and different factors may dominate at different stages over time (Monteiro *et al.*, 2022) ,thus these related factors play different roles depending on the severity of disease and duration of recovery, necessitating different diagnoses and treatments for seemingly the same symptoms and causing differences in the curative effect (Jeon *et al.*, 2017). It's possible to classify a diabetic foot in a pathophysiological and clinical way in: ischemic diabetic foot, neuropathic ischemic foot and infected diabetic foot, but this type of classification in clinical practice may appear too simple owing to the fact that it's possible to distinguish more frequent mixed clinical variants called neuro-ischemic diabetic foot. All these clinical variants of DFS have typical morphologic and clinical

findings (Cruz *et al.*, 2020) , thus the classification and scoring criteria for describing lesions of DFU should be formatted in a manner that is clinically recognized and widely used, which will allow characterization of DFU on the basis of differences and facilitate suggestions for treatment or care programs. Considering the different audiences and objectives of the classification and scoring systems, no universally accepted system has been published to date. Various systems are used to describe and assess the severity of DFU, and three types of key factors contributing to the scoring system have been proposed, namely, patient-related, limb-related, and ulcer-related factors, which reflect end-stage renal failure, PAD, and loss of protective sensation, along with classification of the wound grade (Barutta *et el*, 2022).

Most systems set scoring criteria based on the size and characteristics of the wound, such as size, depth, ischemia, and infection, allowing characterization of the lesion, while risk factors such as neuropathy and peripheral arterial occlusive disease are incorporated when clinical interventions or preventive guidance are required . Doctors also use the Wagner Grades to describe the severity of an ulcer. The purpose of the Wagner Grades is to allow specialists to better monitor and treat diabetic foot ulcers. This grading system classifies Diabetic foot ulcers using numbers, from 0 to 5 as in table (2-1) (Wang *et al.*, 2022).

Table (2-1): Wagner Grades from 0 to 5 Grade classification of diabetic foot ulcer

Grade	**Description**
0	No diabetic foot ulcer is present, but there is a high risk of developing one
1	A surface ulcer involves full skin thickness, but does not yet involve the underlying tissues
2	A deep ulcer penetrates past the surface, down to the ligaments and muscle. There is no abscess or bone involved yet
3	A deep ulcer occurs with inflammation of subcutaneous connective tissue or an abscess. This can include infections in the muscle, tendon, joint, and/or bone.
4	The tissue around the area of the ulcer (limited to the toes and forefoot) has begun to decay. This condition is called gangrene
5	Gangrene has spread from the localized area of the ulcer to become extensive. This involves the whole foot

2.2.2 Etiology of Diabetic Foot

Diabetic foot ulcer is a break of the epidermis and at least part of the dermis in a person with diabetes. Among the commonest causes of this pathogenic pathway it's possible to consider peripheral neuropathy, foot deformity, abnormal foot pressures, abnormal joint mobility, trauma and peripheral artery disease. More superficial or closed lesions that do not penetrate to dermis (e.g., callous, blister, warmth, or erythema) are characterized as preulcerative but are at high risk of progression to ulcer. Repetitive minor trauma causes ulcer formation in most cases typically as a result of elevated pressure at Plantar weight bearing sites, friction and shearing due to poorly fitting shoes or gait abnormalities or an unrecognized injury sustained on an insensate foot (e.g puncture wounds, burns or ingrown toe nails)(McDermott., *et el*,2023) . Structural deformities such as charcot neuroarthropathy confer additional risk of DFU . Some studies classified the underlying etiology of DFU into three types: purelyneuropathic (35%), purely ischemic (15%), and mixed neuroischemic (50%).These classifications are based on the presence or absence of peripheral neuropathy (PN)and associated sensory loss (neuropathic), peripheral artery disease (PAD) ischemic), or both (neuroischemic) *(* Mariadoss., *et el*, 2022) .

2.2.3 Pathogenesis of Diabetic foot

There is many different mechanisms that proposed for the pathogenesis of DFU and precise mechanism underlying its unclear. Neuropathic changes of the foot leading to foot deformities and loss of protective sensation predispose persons with diabetes to DFU. Diabetic neuropathy can affect the sensory, motor and autonomic functions to varying degrees. The insidious nature of neuropathy may go unnoticed by the patient this predisposes the foot to repetitive trauma until ulceration ensues,

thus emphasizing the importance of regular assessment of the diabetic foot (Goyal *et el*.,2018)

Motor neuropathy leads to muscle atrophy, foot deformity, altered foot biomechanics, and redistribution of foot pressures the presence of foot deformities causes' uneven pressure distribution and areas of abnormally high pressure with movement. These high-pressure areas are then prone to damage from repetitive stress and trauma that occur with routine activities of daily living which eventually predispose the foot to ulcerate(Cardoso *et el* .,2022).

Sensory neuropathy renders the foot 'deaf and blind' to stimuli, which would normally elicit pain or discomfort (Boulton.,*et el* ,2018). The loss of protective sensation diminishes the ability of a person to discern or mitigate foot trauma and prevent further injury. Autonomic neuropathy results in loss of sweating, with the resultant dry skin being predisposed to cracks and fissures. The altered autonomic regulation of cutaneous blood flow also contributes. Charcot neuroarthropathy is a non-infective process occurring in a well-perfused and insensitive foot. It is characterized by bone and joint destruction, fragmentation and remodeling. Although Charcot's neuroarthropathy was first described as a complication of tabes dorsalis, it can develop with any type of sensory neuropathy and currently diabetes is the commonest cause (Al-Shammaree *et el*.,2017) .

The neurotraumatic theory attributes bony destruction to the loss of pain and proprioception, combined with repetitive mechanical trauma to the foot, which is largely unperceived by the patient who continues to weight bear . The result is eccentric loading of the foot and excessive plantar pressures promoting the development of micro fractures and progressive bony destruction. This insensitive

deformed foot is at an increased risk of ulceration (Doğruel *et al.*, 2022). Subjects with peripheral vascular disease have one or more of the following criteria:

Neuropathy ,Peripheral vascular disease, Trauma , Infection ,Poor glycemic control, Improper footwear , Others: old age, smoking, low socioeconomic status, psychological factor (Giurato *et el*.,2017) .

2.2.4. Diagnosis of diabetic foot

Clinical examination includes inspection of statue, gait, foot (integrity of skin, muscular condition and bone structure, deformities of the feet such as claw toe, hallux valgus, hollow foot, skew foot and flat foot) and footwear. Prominent features are dry and fissured skin with hyperkeratosis as a sign of polyneuropathy. Another visual diagnosis is Charcot's foot (diabetic neuronal-osteoarthropathy). Charcot's foot is characterized by reactive hyperemia with significant swelling and destruction of osseous structure which consequently causes sintering of the metatarsus region (Sanz *et al*.,2018).

Classic neuropathic ulcers present as painless, "punched out" round ulceration on the weight bearing surfaces of the foot with raised, macerated, or undermined margins and thick surrounding callous (ASM *et el*.,2018). Ischemic or neuroischemic ulcers are characteristically irregular lesion often with a pale or necrotic base, sometimes presenting as gangrene, or round ulcerations at points of ischemia and friction, such as the dorsal surfaces of toe joints. Ischemic and neuroischemic ulcers are more likely than purely neuropathic ulcers to present as larger ulcers, mid foot ulcers, or hind foot ulcers and to present with cellulitis, abscess, or osteomyelitis (Appil *et el*.,2022).

The prevalence of both PN and PAD increases with age, duration of diabetes, and higher HbA1C (Lauwers *et el* .,2022). Increased glucose levels in the body end up in uncontrolled covalent bonding of aldose sugars to a protein or lipid without any normal glycosylation enzymes. These stable products then accumulate over the surface of cell membranes, structural proteins and circulating proteins. These products are called advanced glycation endproducts (AGEs) or Amadori products. Formation of AGEs occurs on extracellular matrix proteins with slow turnover rate. AGEs alter the properties of matrix proteins such as collagen, vitronectin, and laminin through AGE-AGE intermolecular covalent bonds or cross-linking. AGE cross-linking on type I collagen and elastin results in increased stiffness and comprise an increasing proportion of DFU as the longevity of diabetes patients increases (Cardoso *et al.*, 2019).

2.3 Parameters in this study

2.3.1 Procalcitonin (PCT)

Procalcitonin is a polypeptide consisting of 116 amino acids and is the prohormone of calcitonin. It is synthesized in thyroid C-cells or (parafollicular cells), lungs, and pancreas. Generally, the level in blood is very low or undetectable in healthy people because it is not released in blood in the absence of inflammation. It is significantly elevated in bacterial infection as it is released by all parenchymal tissue under the influence of endotoxins and pro-inflammatory cytokines, It can be used as a suitable and specific biomarker of bacterial infection to replace conventional markers (Arti *et el* ,2019).

Diabetic foot ulcers (DFUs) are frequent in diabetic patients with 15–25% estimated to experience such an ulcer during their lifetime and are responsible for 85% of amputations having a high morbidity and mortality rate so early diagnosis and adequate treatment are essential to prevent amputation especially in the presence of infection (Pichu *et al.*, 2019). The distinction between infected and non-infected DFU remains a very challenging task for clinicians in everyday practice. Even when infection is documented, the spectrum of diabetic foot infection is wide, ranging from cellulitis and soft tissue infection to osteomyelitis(Massaria *et al.*, 2017).

Procalcitonin , a well-established sepsis biomarker, has been used in the diagnosis of several infections including osteomyelitis in patients with diabetes mellitus (El-Kafrawy *et al.*, 2019). Current evidence suggests that PCT levels could aid clinicians in distinguishing infected from non-infected DFUs as well as in the distinction between soft tissue infection and bone involvement The diagnosis of diabetic foot ulcer infection is essentially based on clinical evaluation, but laboratory parameters such as erythrocyte sedimentation rate (ESR), white blood count (WBC), C-reactive protein (CRP) and, more recently, Procalcitonin could aid the diagnosis, especially when clinical signs are misleading. Biochemical parameters such as erythrocyte sedimentation rate (ESR), leukocytosis and circulating inflammatory proteins are known to be of poor value for diagnosing diabetic foot infections as, even in the most severe cases, there are few systemic manifestations(Rothenbacher *et el*.,2020) . Procalcitonin has recently gained acceptance as a marker for diagnosing infection. Some authors claim that its accuracy as a predictor of bacterial infection is higher than that of C-reactive protein (CRP). PCT remains fairly low in viral infections and non-specific inflammatory diseases (Jeandrot *et el* .,2018).

2.3.2 Cystatin-C

Cystatin C (Cys C) is a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. Recently, it has been studied for its role in predicting new-onset or deteriorating cardiovascular disease. It also seems to play a role in brain disorders involving amyloid (a specific type of protein deposition), such as Alzheimer's disease (An *et al.*, 2023). In humans, all cells with a nucleus (cell core containing the DNA) produce cystatin C as a chain of 120 amino acids. It is found in virtually all tissues and body fluids. It is a potent inhibitor of lysosomal proteinases(enzymes from a special subunit of the cell that break down proteins) and probably one of the most important extracellular inhibitors of cysteine proteases (it prevents the breakdown of proteins outside the cell by a specific type of protein degrading enzymes). Cystatin C belongs to the type 2 cystatin gene family. It is broadly distributed and found in most body fluids, including plasma. It is a member of the type 2 family of cysteine proteinase inhibitors(Elsayed *et al.*, 2019) .

Cysteine proteinases irreversibly hydrolyze a peptide bond in an amino acid sequence and have a significant role in cell regulation, cell proliferation , adhesion, apoptosis, lipid metabolism and immune response (Ohishi *et al.*, 2022).Interest in its role as a measure of kidney function stems from its physical and chemical properties as an endogenous marker. In addition to its constant rate of production, it is relatively freely filtered by glomeruli, is reabsorbed and catabolised by proximal renal tubular cells and is unaffected by muscle mass. Apart from renal function, CysC was found to have an associative relationship and strong potential predictors, with PAD and diabetic peripheral neuropathy (DPN) . Moreover, the underlying mechanism of DFU is poorly comprehended with few possible

biomarkers associating DFU prediction. A reliable predictive biomarker to identify and diagnose risk for DFU and its potential severity remains elusive yet. The severity and prognosis of DFU have a close relationship with renal insufficiency, peripheral artery disease (PAD), and neuropathy, consequently making it possible for early screening and identification of DFU biomarker related to above diseases(Zhang *et al.*, 2016).

Cystatin C can be used to measure estimated glomerular filtration rate (eGFR).it have stable production rates, absence of overall renal tubular effect on serum levels and stable concentrations which are not heavily influenced by race, sex, or lean body mass proportions . Recent studies showed that cystatin C strongly predicted the incidence of PAD events in the elderly. Apart from renal function, cystatin C recently has been found to be associated with cardiovascular events. In addition, previous studies have demonstrated that serum cystatin C was a strong marker for lower limb ischemia and diabetic peripheral neuropathy in Chinese type 2 diabetic patients. However, the association of this small molecular weight protein with the therapeutic DFU outcome remains undetermined and no ideal serum marker is available to predict the prognosis of DFU(Werner *et al.*, 2019)

2.3.3 Kidney Injury Molecule-1 (KIM-1)

Kidney Injury Molecule-1 is also named , hepatitis A virus cellular receptor HAVCR-1 , T-cell immunoglobulin and mucin domain-1 TIM-1). KIM-1 is a cell surface receptor in epithelial and lymphoid/myeloid cells**,** It behaves as a scavenger receptor for oxidized LDL and phosphatidylserine and as Ebola and Hepatitis A virus entry receptor in epithelial cells .Kim-1 binding to phosphatidylserine allows the phagocytosis of apoptotic cells. In addition, KIM-1

regulates Th2, Th1, and Th17 differentiation (Pei *et al.*, 2022). KIM-1 is markedly upregulated in proximal tubules in AKI and CKD. Indeed, the urinary excretion of the KIM-1 ectodomain was qualified by the Federal Drug Administration and by the European Medicines Agency for preclinical assessment of nephrotoxicity and on a case-by-case basis for clinical evaluation. is a transmembrane protein that is up regulated in renal tubular cells after ischemic injury(Khan *et al.*, 2019).

Kidney injury molecule-1 is not expressed in the normal kidney but is expressed in a variety of human kidney diseases, predominantly in the apical membrane of proximal tubular cells while KIM-1 is primarily used in the setting of drug-induced acute kidney injury (AKI), recent studies suggest that KIM-1 may be useful in predicting chronic kidney disease (CKD) progression as well .

Kidney injury molecule-1 which the same molecule, are relatively recently discovered transmembrane proteins with Ig-like and mucin domains in their ectodomain. KIM-1 modulates $CD4^+$ T-cell responses and is also expressed by damaged proximal tubules in the kidney. A chronic expression of KIM-1 leads to progressive renal fibrosis and chronic renal failure, which is speculated to be due to oxidized lipids.KIM-1 can activate signaling through the PI3K pathway. Which mediate phagocytotic functions down regulate the inflammation and innate immune responses in acute ischemic and toxic injury. It is thought that KIM-1 has a role in tubular interstitial damage. The expression of tubular KIM-1 is specific to ongoing tubular cell damage and de-differentiation, and urinary concentrations of KIM-1 are thought to reflect this expression. KIM-1 is also associated with renal

interstitial fibrosis and inflammation in certain types of renal disease (Siddiqui *et el*., 2022).

2.3.4 Nephrin

Nephrin is transmembrane protein of the immunoglobulin superfamily. It is a 180 KD and 1242- amino-acid and it is has been localized to the slit membrane between adjacent podocytes of the glomerulus. consists of 8 extracellular immunoglobulin-like modules, a fibronectin type III-like domain, and a cytosolic C-terminal tail. It is a single-pass, transmembrane protein that interacts with other nephrin proteins (both trans and cis) within and outside the cell, The link between permutations in nephrin expression and proteinuria has been shown in animal models by using neutralizing antibodies or studying mice with inactivation of the nephrin gene (Dumont *et el*.,2017). it a protein necessary for the proper functioning of the renal filtration barrier. The renal filtration barrier consists of fenestrated endothelial cells, the glomerular basement membrane and the podocytes of epithelial cells, it is a transmembrane receptor molecule located at the specialized podocyte cell-cell junction, termed the slit diaphragm .The expression of nephrin has been shown to be reduced in various animal models of proteinuric renal disease(Kandasamy *et al*., 2014).

The relationship between changes in nephrin expression and proteinuric renal disease in humans is not fully elucidated, with a reduction in expression of this protein reported in a range of renal diseases. Diabetic nephropathy, is one of the major causes of end-stage renal disease and associated with substantial proteinuria with reduction in slit pore density, nephrin expression has been described as being transiently increased in the first 8 weeks of diabetes, in longer-term studies show

reduced nephrin expression in association with increasing proteinuria.An angiotensin II-receptor blocker has been shown to prevent depletion in glomerular nephrin expression in the diabetic kidney(Jim *et al.*, 2012).

Human studies in both type 1 and type 2 diabetes suggest down regulation of Nephrin expression in the diabetic kidney and it has been postulated that these changes may play a role in the pathogenesis of diabetic nephropathy, specifically the development of proteinuria in this condition. Although there are other proteins involved in the structure of the epithelial podocyte and specifically the slit pore, nephrin seems to play a pivotal role in preventing passage of protein through the glomerular barrier(Aljorani *et el*., 2023) , Furthermore, it is suggested that the antiproteinuric effects of inhibition of the renin-angiotensin system may partly relate to the effects of these agents on nephrin expression.

Nephrin forms an integral part of podocytes, which together with endothelial cells and the basement form the glomerular filtration barrier. Mutations in the nephrin gene (*NPHS1*) lead to congenital nephrosis, suggesting that nephrin is essential for the glomerular filtration barrier (Kostovska *et al.*, 2020). In addition to its structural role, nephrin is also involved in podocyte signaling. Abnormalities of nephrin caused by mutations in the *NPHS1* gene have been implicated in the autosomal recessive congenital nephrotic syndrome of the Finnish type. The single gene mutations are responsible for the developmental failure of the podocyte foot processes and slit diaphragms, with extensive proteinuria present in utero. Children affected with this condition are nephrotic at birth. The majority of the *NPHS1* mutations are seen within the extracellular domain, with one recorded in the cytoplasmic domain, and only a few within the fibronectin domain (Kandasamy *et al.*, 2014) .

2.3.5 Glycated Haemoglobin (HbA1c)

Glycated haemoglobin is formed through the spontaneous attachment of glucose to haemoglobin A. Glycated haemoglobin (HbAlc) is widely accepted as a good indicator for blood glucose control. In 2019, the American Diabetic association(ADA) recommended that HbA1C of 6.5 % or higher is used for the diagnosis of DM. It is a marker of average plasma glucose through the preceding 2-3 months, the time period dictated by 120- day lifespan of the red blood cells (Ezegbogu *et el*.,2018) . The control of the diabetes mellitus relies both on individual actions for self-care and on medical treatments and surveillance Regarding medical surveillance, a common strategy to evaluate the effectiveness of DM treatment is the use of a biomarker (Pasupathi *et al.*, 2019). By definition, a biomarker is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention". Specifically, for the case of DM, the levels of glycated hemoglobin (HbA1C or hemoglobin A1c) are periodically measured, as glycemic variability has been recognized as the most important risk factor for foot ulcers (D'Emden, 2019). Early detection and good glycemic control be proven to prevent adverse outcomes associated with diabetic foot, HbA1C provides the better measure, as it reflects levels of blood glucose over several weeks, and it is the main method of monitoring glycaemia in diabetes. Glycemic variability is an important factor that contributes to axonal ion channel dysfunction (Casadei *et el*., 2021).

Hemoglobin A1c (HbA1c) reflects glycemia over two to three months, and according to the guidelines set forth by the American Diabetes Association, the goal of type 2 diabetes therapy is to reduce glycated hemoglobin A1c (HbA1c) to

7% or 6.5% (Lau *et al.*, 2020) .Elevated HbA1C levels would mostly be associated with poor wound healing, and HbA1c is a good biomarker for foot ulcer outcomes (wound healing time) in diabetic patients (Akyüz *et al.*, 2023) . The American diabetes association classified diabetes criteria and correlated it with glucose and HbA1c shown in table (2.2)

Table 2.2:Classification of American Diabetes Association Diabetes criteria in relation with HBA1C(Beran *et el* .,2022).

Condition	**HbA1C**	**Type of test**	**Plasma glucose concentration (mg/dl, mmol/l)**
Normal	< 5.7%	Fasting plasma glucose	< 100 mg/dl (< 5.6 mmol/l)
		Oral Glucose Tolerance	< 140 mg/dl < 7.8 mmol/l
Prediabetes	5.7%-6.4%	Fasting plasma glucose	100-125 mg/dl 5.6-6.9 mmol/l
		Oral Glucose Tolerance	140 - 199 mg/dl 7.8- 11.0 mmol/l
Diabetes Mellitus	≥ 6.5%	Fasting plasma glucose	≥ 126 mg/dl ≥7.0 mmol/l
		Oral Glucose Tolerance	≥200mg/dl ≥11.1mmol/l
		Random Plasma Glucose	≥200mg/dl ≥11.1 mmol/l

2.3.6. C- Reactive Protein (CRP)

C reactive protein is a cyclic pentameric protein comprised of five identical non-covalently attached subunits. Each subunit has an intra-disulfide bond and the molecular weight of each subunit is ~23 kDa (Hadavand *et al.*, 2019)

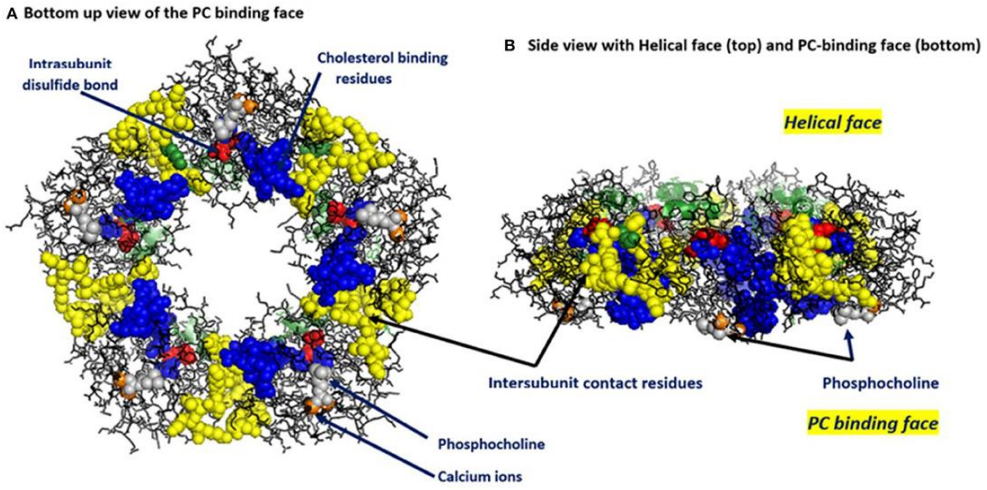


Figure (2-1) C-Reactive protein with its active binding sites (Kandelouei *et al.*, 2022)

C-reactive protein is found in blood plasma , it is an acute - phase protein mainly synthesized in the liver in response to factors released by macrophages and fat cells (adipocytes) upon stimulation by IL – 6 , it belongs to the pentraxin family of proteins and binds to lysophosphatidylcholine on the surface of dead cells and bacteria , an interaction that activates the complement system via C1q and promotes subsequent phagocytosis (Levinson and Wasserman, 2022) . Human CRP is the classical acute phase reactant , the circulating concentration of which rises rapidly and extensively in a cytokine-mediated response to tissue injury , infection and inflammation(Sahebkar *et al.*, 2022).

When patients with IDFU, under the regulation of interleukin-6 ,interleukin-1, and other cytokines, CRP is produced by hepatocytes in time, and rapidly increases, and even can be increased to 1000 times the normal value at the site of infection, which is a hot spot for research on biomarkers of inflammation or infection, Some *cis*-acting elements can also induce liver-specific expression of CRP. Serum CRP values are routinely measured , empirically , to detect and monitorany human diseases . However, CRP is likely to have important host defense, scavenging and metabolic functions through its capacity for calcium-dependent binding to exogenous and autologous molecules containing phosphocholine (PC) and then activating the classical complement pathway (Levinson and Wasserman, 2022) . High-sensitivity C-reactive protein (hs-CRP) is a systemic inflammation marker that has been revealed to be correlated with DKD development .CRP level increases sharply during the process of inflammation and recovers to normal level when inflammation has ceased , so hs-CRP levels were significantly and positively correlated with the presence of DKD which may provide predictive and diagnostic values in clinical practice (Stanimirovic *et al.*, 2022)

2.3. 7 RENAL FUNCTION TEST

2.3.7. 1 Blood Urea

Urea is major nitrogenous end product of protein and amino acid Catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water (Gowda *et al.*, 2019). Urea clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends on several non-renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, and shock and bleeding in the digestive tract. The high BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. If the BUN level is higher than 100 mg/dL it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use (Biekpe, 2019).

2.3.7.2 Serum Creatinine

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass (Chauhan and Malukar, 2019) . Creatinine is a commonly used as measure of kidney function. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the “normal” interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules (Ciardullo *et el*., 2022).

Creatinine values may alter as its Generation may not be simply a product of muscle mass but influenced by Muscle function, muscle composition, activity, diet and health status (Banfi and Del Fabbro, 2021).The increased tubular secretion of

creatinine in some patients with kidney dysfunction could give false negative value. The elevated values are also seen in muscular dystrophy paralysis, anemia, leukemia and hyperthyroidism. The decreased values are noticed with glomerulonephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease, and dehydration(Ostermann and Joannidis, 2020) .

2.3.7.3 Estimating Glomerular Filtration Rate

The equations based on creatinine and/or cystatin C levels have been developed to estimate glomerular filtration rate (eGFR) ; However , whether these equations accurately reflect renal function is still debated (Lin *et al.*, 2022) . eGFR is often different from measured glomerular rate (mGFR) by ± 30% or more , that eGFR values incorrectly staged CKD in 30-60% of patients and that eGFR and mGFR gave different rates of GFR decline (Gounden and Jialal, 2018) . Circulating blood is filtered across the glomerular barrier to form an ultrafiltrate of plasma in the Bowman's space. The volume of glomerular filtration adjusted by time is defined as the glomerular filtration rate (GFR) and the total GFR is the sum of all single-nephron GFRs(Hoefield *et al.*, 2021).

Evaluation of glomerular filtration rate is central to the assessment of kidney function in medical practice, research and public health. Measured GFR (mGFR) remains the reference standard but through the past 20 years a major advances in estimated GFR were seen , both eGFR and mGFR are associated with some error compared with the true GFR . EGFR is now recommended by clinical practice guidelines, regulatory agencies and public health agencies for the initial evaluation of GFR, with measured GFR (mGFR) typically considered an important confirmatory test depending on how accurate the assessment of GFR needs to be for application to the clinical, research or public health setting (Werner *et al.*,

2018). GFR is classified into the following stages based on the kidney disease according to: Improving Global Outcomes (KDIGO) stages of chronic kidney disease (CKD) (Nichols *et el* .,2020) .

- Stage 1 GFR greater than 90 ml/min/1.73 m2 , normal or high
- Stage 2 GFR-between 60 to 89 ml/min/1.73 m2, mildly decreased
- Stage 3a GFR 45 to 59 ml/min/1.73 m2, mildly to moderately decreased
- Stage 3b GFR 30 to 44 ml/min/1.73 m2, moderately to severely decreased
- Stage 4 GFR of 15 to 29 ml/min/1.73 m2, severely decreased
- Stage 5-GFR less than 15 ml/min/1.73 m2 (end-stage renal disease) or kidney failure .

Normal eGFR is equal to or greater than 90ml/min/1.73 m2.

Chapter Three

material and method

3.1 Study Population and Designs

This study was carried out for DM patients at Marjan Teacheing Hospital and AlHilla General Teaching Hospital in Hilla city. All the diabetic patients that included in the current study were diagnosed by endocrinologist depending on measuring RBS,HbA1C diagnostic parameters such as urea, serum creatinine , and e GFR also the healthy group subjected to same tests during the period from October 2023 to March 2024.

All participants were included in this study divided into four group with age range 32-64 years as fallowing figure(2-1)

➢Group 1 : Control group consist from 30 appearantly healthy persons

➢Group 2: Included 30 diabetic mellitus patients without any complication

➢Group 3:Included 30 Diabetic foot patients with eGFR more than sixty

➢Group 4:Included 30 Diabetic foot patients with chronic kidney disease

The study was designed as case control study and sample size was determined according to G power program for sample size determination .

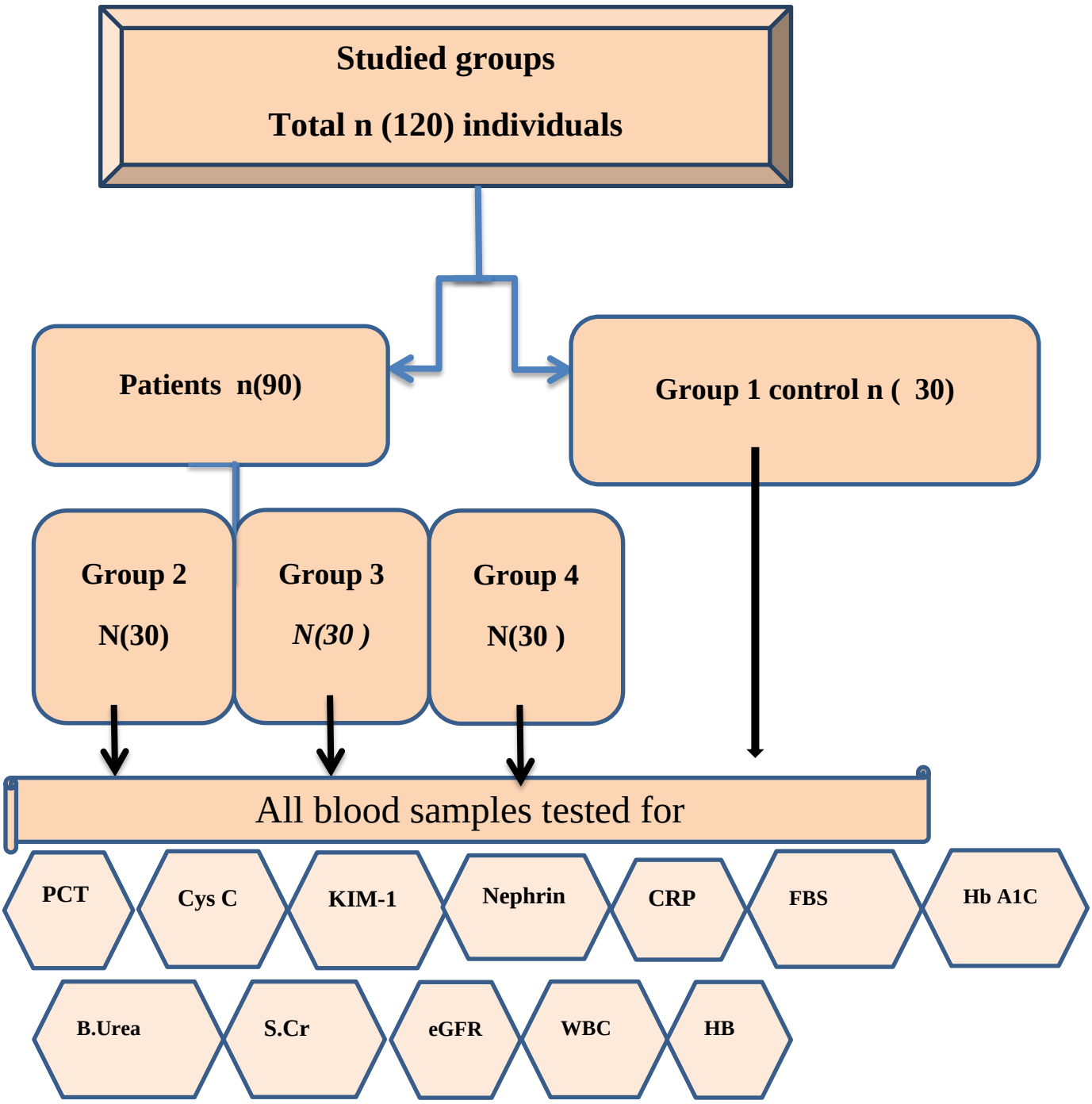
Inclusion criteria:

All patient with diabetic foot (both types 1,2) , whose age ranged from 32 to 64 years, and who have been examined clinically by endocrinologist doctor and the control group was selected as apparently healthy individuals.

Exclusion criteria:

Patients with Tumors, Patients under 18 years old, pregnancy, congestive heart failure, systemic lupus erythematous, thyroid disease and incomplete data were excluded from the study.

Figure (3-1) Study designs



N= Number

3.1.2 -Blood Sampling

Ten milliliters (ml) of venous blood were withdrawn from all participant using disposable syringes in the sitting position. The blood was discharged slowly and divided into two part the first one discharged into anticoagulant tubes in which used in hematological test CBC and the second one was discharged into plain disposable test tubes in which the blood allowed to clot at 37˚C for 10-15 minutes, and then centrifuged at 1000 xg for approximately 10-15 minutes in order to obtained the serum in which the serum divided into two parts the first part used immediately for routine test that included serum FBS, CRP, B.Urea and S.Cr and the second part was stored at -80˚C until complete sample collection for analysis of PCT, Cys C, KIM-1 and Nephrin concentrations **.**

3.2 Materials

3.2.1. Instruments and Equipment's

The instruments and Equipments used in this study are listed below in (Table 3-1).

Table (3–1); instruments and Equipments used and their manufacturing companies

NO.	Instruments & Equipment	Company	Country
1	AFIAS-6	Boditech Med Incorporated	Korea
2	Auto chemistry analyzer CS-T180	DIRIU	China
3	Centrifuge	Kokusan	Japan

4	Deep Freezer	Samsung	Korea
5	Disposable cuvette	Cybow	China
6	Disposable syringe (5 ml)	Sakaria	Malizia
7	Elisa washer, reader and printer	Biotek	USA
8	Eppindorf tube (0.5 ml)	Cybow	China
9	Kan tube	Cybow	China
10	Micropipettes (5-50µl),(100-1000µl)	Dragon-lab	China
11	pipette tips with different sizes	Afma .despo	Jordan
11	Refrigerator	Agur	Turkish
12	Test tube with Separating gel	AFCO	Jordan
13	Water Bath	Memmert	Germany
14	Dymind	Biotech	China

3.2.2 Diagnostic Kits

The kits that used in this study were used just as they arrived from the suppliers, without any further purification or modification listed in (Table 3-1).

Table (3-2) kits used in this study with their suppliers.

NO.	CHEMICALS	COMPANY	Manufactured Country
1	Creatinine	DIRIU	china
2	CRP	Boditech Med Incorporated	Korea
3	FBS	DIRIU	china
4	Procalcitonin	Bioassay Technology Laboratory	china
5	Nephrin	Bioassay Technology Laboratory	china
6	Kidney injury molecule -1	Bioassay Technology Laboratory	china
7	Cystatin c	Bioassay Technology Laboratory	china
8	Urea	DIRIU	china
9	CBC	Dymind	France
10	HbA1C	Boditech Med Incorporated	Korea

3.3. Methods

3.3.1 Determination of Serum Procalcitonin Levels

In this assay used the quantitative sandwich ELISA. Antibody specific for PCT has been pre-coated onto a microplate. Standards and samples were pipetted

into the wells and any PCT present was bound by the immobilized antibody. After removing any unbounded substances, a biotin-conjugated antibody specific for PCT was added , after washing , avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a washed to remove any unbounded avidin-enzyme reagent, a substrate solution was added and color develops in proportion to the amount of PCT bound in the initial step. The color development was stopped and the intensity of color was measured using MR 96 microplate reader at 450 nm.

Preparation for Procalcitonin Determination.

All reagents were brought to room temperature before use

The standard vial was centrifuged at 6000-10000 rpm for 30S in order to prepare standard solution, reconstituted with 1.0 ml of Sample Diluent. This reconstitution produced a stock solution of 2.4 ng/ml , the standard mixed well and allowed to settle for a minimum of 15 minutes with gentle agitation prior to making dilutions . Prepare duplicate standard points by serially diluting the standard stock solution (1.2 ng/ml) 1:2 with standard diluent to produce 0.6 ng/ml, 0.3 ng/ml, 0.15 ng/ml and 0.075 ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml).Any remaining solution frozen at -20°C and used within one month. Dilution of standard solutions suggested were given in following table (Table 3.3) serial dilution of standard solution of PCT

Tube	S6	S5	S4	S3	S2	S1	S0
ng/ml	2.4	1.2	0.6	0.3	0.15	0.075	0

- All reagents and standard solutions were prepared as instructed by the provider companies. The assay on patients samples performed at room temperature and before starting all reagents were brought to room temperature too.

- One hundred microliter of standard and sample were added to wells and incubated for tow hour at 37ºC

- Standard and sample were removed without washing.

- One hundred microliter biotin-anti-PCT Antibody were added to each well and Incubated at 37c for 60 min.

-The wells content was aspirated and the plate washed three times.

- One hundred microliter of avidin-HRP were added to sample and standared wells and Mixed well. The plate covered with sealer and incubated at 37C for 60 min.

- The wells content was aspirated and washing the plate 5 times.

- Ninety microliter of TMB substrate solution was added to each well and the plate incubated 30 min at 37 c in the dark.

- Fifty microliter of stop solution was added to each well and the blue color will change to yellow.

-Stop solution was added and read the optical density after 10 min by using a micro plate reader set to 450 nm.

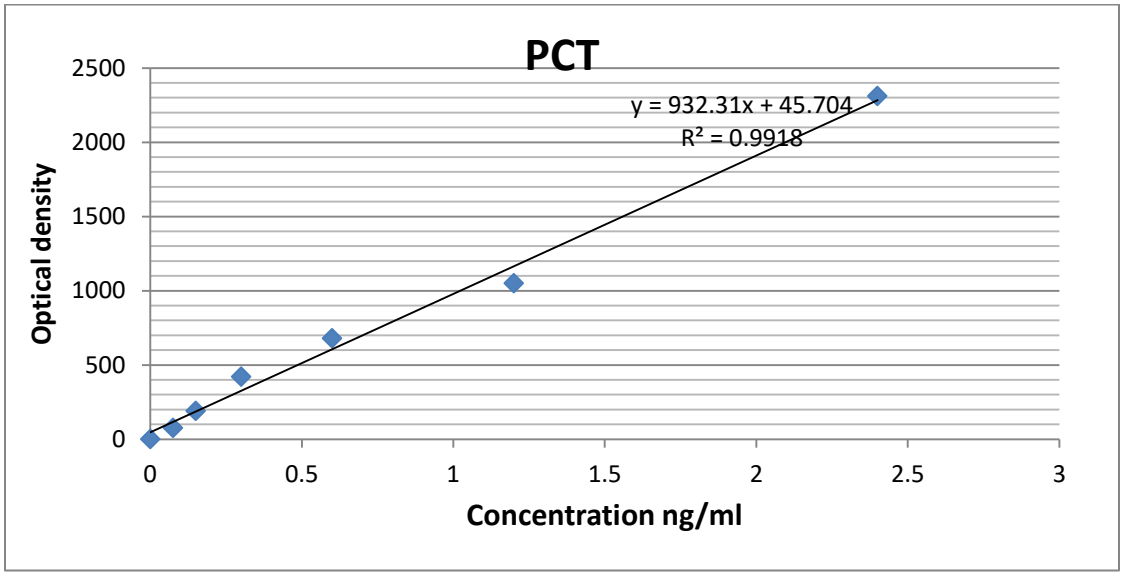


Figure (3-2) Calibration curve of PCT

3.3.2 Determination of Serum Cystatin C (cys-c) Levels

In this assay ,we used the quantitative sandwich ELISA. The plate has been pre-coated with Human Cys-C antibody. Cys-C present in the sample was added and binds to antibodies coated on the wells, biotinylated Human Cys-C Antibody is added and binds to Cys-C in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated Cys-C antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human Cys-C. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

All reagents were brought to room temperature before use

The standard vial was Centrifuged at 6000-10000 rpm for 30 S in order to prepare standard solution , Standard Reconstitute the 120 µl of the standard (4.8 mg/dl) with 120µl of standard diluent to generate a 2.4mg/dl standard stock solution. The standard allowed sitting for 15 mins with gentle agitation prior to

making dilutions. Prepare duplicate standard points prepared by serially diluting the standard stock solution (2.4mg/dl) 1:2 with standard diluent to produce 1.2mg/dl, 0.6 mg/dl, 0.3 mg/dl and 0.15 mg/dl solutions. Standard diluent serves as the zero standards (0 mg/dl). Any remaining solution was frozen at -20°C . Dilution of standard solutions suggested were as following table

(Table 3.4) serial dilution of standard solution of Cystatin C

Tube	S6	S5	S4	S3	S2	S1	S0
mg/dl	4.8	2.4	1.2	0.6	0.3	0.15	0

Wash Buffer Twenty milliliter were diluted of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved

Assay Procedure

1. All reagents prepared standard solutions and samples as instructed.All reagents bringed to room temperature before use. The assay was performed at room temperature.

2. The number of strips that required for the assay determined the strips in the frames for use inserted and the unused strips stored at 2-8 ° C.

3. Fifty microliter of standard were added to standard well.

4. Forty microliter of sample were added to sample wells and then Ten microliter of anti-Cys-C antibody were added to sample wells, then add 50 µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. The plate with a sealer was covered and incubated 60 min at 37°C and mixed well.

5.The sealer removed and washed the plate fife times with wash buffer .Wells soaked with 300ul wash buffer for 30s to 1 min for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. The plate blotted onto paper towels or other absorbent material.

6. Fifty microliter of substrate solution A were added to each well and then 50µl of substrate solution B were added for each well.The plate covered with a new sealer incubated for 10 minutes at 37°C in the dark.

7. Fifty microliter of stop solution were added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well immediately determined by using a microplate reader setted to 450 nm within 10 minutes after adding the stop solution.

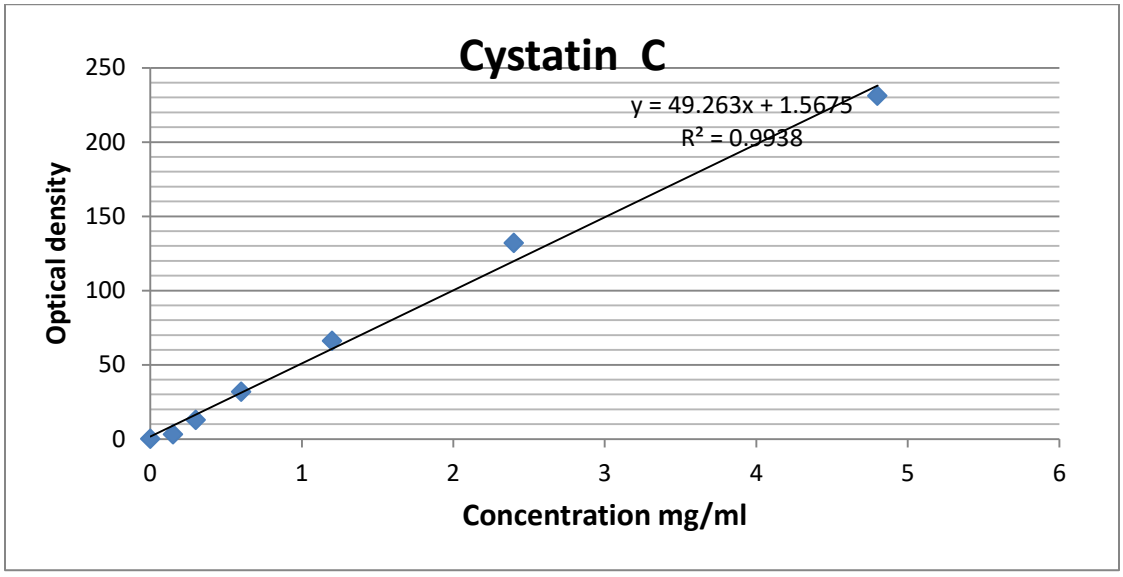


Figure (3-3) Calibration curve of Cys c

3.3.3 Determination of Serum Nephrin (Nphs1) Levels

In this assay we used the quantitative sandwich ELISA. The plate has been pre-coated with Human Nphs1 antibody. Nphs1 present in the sample was added and binds to antibodies coated on the wells, biotinylated Human Nphs1 Antibody was added and binds to Nphs1 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated Nphs1 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human Nphs1. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm

Preparation for Nphs1 determination.

All reagents brought to room temperature before use

The standard solution:

Standard Reconstitute 120µl of standard diluent to generate 24ng/ml standard stock solution. The standard was allowed to sit for 15 min with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (24 ng/ml) 1:2 with standard diluent to produce 12 ng/ml, 6 ng/ml, 3 ng/ml and 1.5 ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml).Any remaining solution frozen at -20°C and used within one month. Dilution of standard solutions suggested were as following table

(Table 3.5) serial dilution of standard solution of nephrin

Tube	S6	S5	S4	S3	S2	S1	S0
ng/ml	48	24	12	6	3	1.5	0

Wash Buffer twenty milliliter of Wash Buffer Concentrate 25x were diluted with deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, gently mixed until mixer the crystals have completely dissolved

Assay Procedure

1. All reagents prepared, standard solutions and samples as instructed. All reagents ringed to room temperature before use. The assay is performed at room temperature.

2. The number of strips required for the assay were determined .The strips inserted in the frames for use. The unused strips stored at 2-8°C.

3. Fifty microliter of standard were added to standard well.

4. Forty microliter of sample were added to sample wells and then 10 µl of anti-Nphs1 antibody added to sample wells, then 50 µl streptavidin-HRP added to sample wells and standard wells (Not blank control well), then well mixed. The plate covered with a sealer and incubated for 60 min at 37°C.

5. The sealer removed and the plate 5 times washed with wash buffer was wished and soaks wells with 300 µl ash buffer for 30 s to 1 minute for each wash. For automated washing each well aspirated or decanted and fife times washed with wash buffer. The plate was blotted onto paper towels or other absorbent material.

6. Fifty microliter of substrate solution A were added to each well and then 50µl substrate solution B were added into each well. The plate covered with a new sealer for 10 min incubated at 37°C in the dark.

7. Fifty microliter of Stop Solution were added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) was determined for each well immediately using a microplate reader setted to 450 nm within 10 min after adding the stop solution.

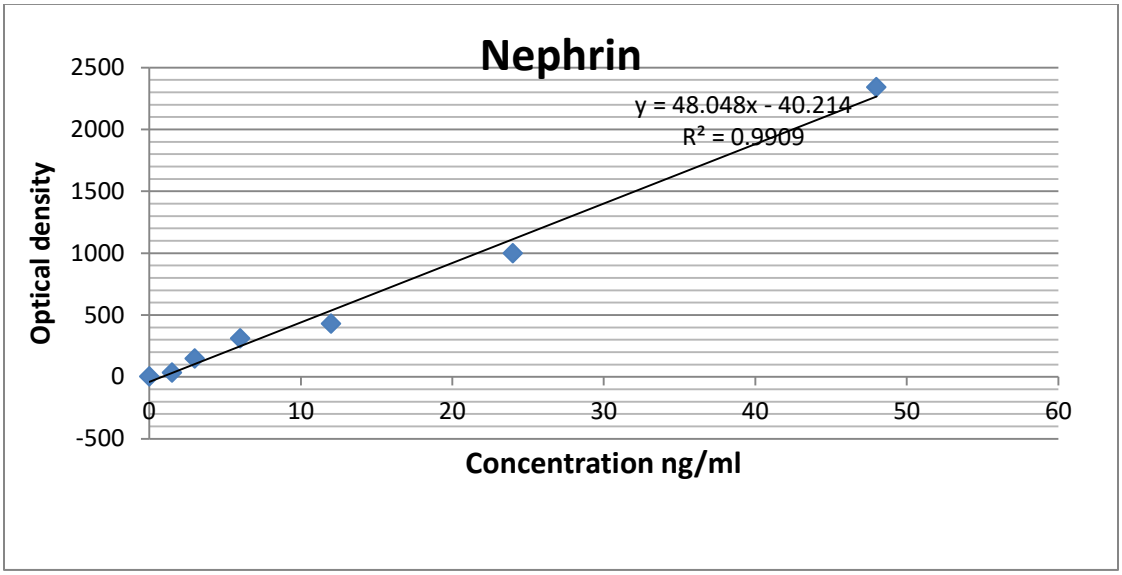


Figure (3-4) Calibration curve of Nephrin

3.3.4 Determination of Human Serum Kidney Injury Molecule -1(KIM-1) Concentration:

Principle of the test: In this assay quantitative sandwich ELISA used. The plate has been pre-coated with Human Kim-1 antibody. Kim-1 present in the sample was added and binds to antibodies coated on the wells, then biotinylated Human Kim-1 Antibody was added and binds to Kim-1 in the sample. Then Streptavidin-HRP was added and binds to the Biotinylated Kim-1 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of Human Kim-1. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm

Preparation for KIM-1 determination

All reagents were brought to room temperature before use

The standard solution

One handered twenty microliter of the standard (12.8 ng/ml) reconstituted with 120 µl of standard diluent to generate a 6.4ng/ml standard stock solution. The standard allowed sitting for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points prepared by serially diluting the standard stock solution (6.4ng/ml) 1:2 with standard diluent to produce 3.2ng/ml, 1.6ng/ml, 0.8ng/ml and 0.4ng/ml solutions. Standard diluent serves as the zero standard (0ng/ml). Any remaining solution frozen at -20°C

(Table 3.6): serial dilution of standard solution of KIM-1

Tube	S6	S5	S4	S3	S2	S1	S0
ng/ml	12.8	6.4	3.2	1.6	0.8	0.4	0

Wash Buffer: Twenty milliliter of Wash Buffer Concentrate 25x diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer

- All reagents, standard solutions and samples prepared as instructed and Bringed to room temperature before use. The assay is performed at room temperature.

- The number of strips required for the assay were determined and the strips in the frames for use inserted. The unused strips stored at 2-8°C.

-Fifty microliter of standard were added to standard well. Note: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

- Forty microliter of sample were added to sample wells and then 10µl of anti-Kim-1 antibody were added to sample wells, then 50µl streptavidin-HRP were added to sample wells and standard wells (Not blank control well). Which well mixed . The plate with a sealer covered and incubated 60 minutes at 37 °C.

-The sealer removed and the plate 5 times washed with wash buffer. Wells soaked with 300 µl ash buffer for 30 s to 1 minute for each wash. For automated washing, each well aspirated or decanted and 5 times washed with wash buffer. The plate blotted onto paper towels or other absorbent material.

-Fifty microliter of substrate solution A were added to each well and then 50µl substrate solution B were added to each well. The plate covered with a new sealer for 10 min incubated at 37°C in the dark.

- Fifty microliter of Stop Solution were added to each well, the blue color will change into yellow immediately.

- The optical density (OD value) of each well determined immediately using a micro plate reader setted to 450 nm within 10 minutes after adding the stop solution.

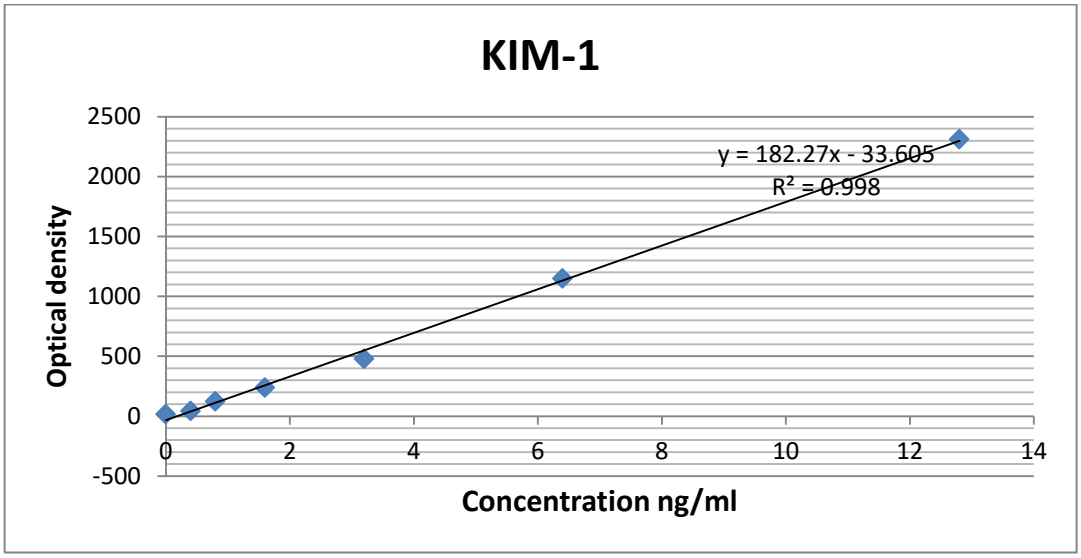


Figure (3-5) Calibration curve of KIM-1

3.3.5 C-Reactive Protein Assessment and HbA1c

Principle

The sandwich immune-detection method was used ; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complex and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on the test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector

Test procedure

In this study we used AFIAS instrument to calculate CRP concentration in the sera of the patients and control group, the test is semi-automated and the results displayed automatically on the instrument screen showing CRP concentrations in mg/L.

Cut-off (reference value) of the kit that was used is : 10 mg/L

Working range of the AFIAS CRP is 0.5-200 mg/L

Steps of the test:

1) One hindered µL of serum was taken with a micro pipette and dispensed into the sample well on the cartridge.

2) The cartridge then inserted into the cartridge holder.

3) Tip was inserted into the tip hole of the cartridge.

4) The "General Mode" in the instrument of AFIAS tests was Selected.

5) The test result will be displayed on the screen after 3 minutes.

3.3.6 Routine Biochemical Tests

Chemical analyzes such as RBS, B.Urea and S.Creatinine were measured by the Dirui device, this full automated device, where a tube containing serum was placed in the place designated for it, and then pressed on the Start button, as it took 15 minutes to obtain the results

3.3.7 Hematological Tests

Tow milliliter of sample of venous blood in an EDTA tube was used to calculate the CBC

3.3.7.1 – Complete Blood Count:

The blood components were measured by the CBC device, where the EDTA tube was placed in the place designated for it, and then the start button was pressed.

3.3.8- Determination and Categorization of Body Mass Index (BMI):

Body mass index (BMI) was calculated according to the following equation:

BMI = (weight in kilogram) / (height in meter)2

According to World Health Organization, the BMI was separated into seven categories (Hales *et el* ., 2018) as shown in table (2-8):

Table (3-7): BMI classification (Hales *et et.*, 2018)

Weight status	BMI range (kg/m2)
Severely Underweight	< 16.5
Underweight	< 18.5
Normal weight	18.5 to 24.9
Overweight	25 to 29.9
Obese class I	30 to 34.9
Obese class II	35 to 39.9 O
Obese class III	> 40

3.3.9 Estimation of GFR

The eGFR was calculated using serum creatinine among other variables such as age and sex. The most frequently used assessment equation is the abbreviated Modified Diet Renal Disease (MDRD) equation.

eGFR (mL/min/1.73 m2) = 175× (Scr)-1.154 × (Age)-0.203 × (0.742 if female) (Hoefield *et el*.,2021)

3.4- Statistical Analysis

By using Statistical Package was used for Social Science (SPSS 24 IBM, Armonk, USA). The results were expressed as mean ± standard deviation (SD), the differences in means of the variables between control and patient groups were analyzed by analysis of variance (one way ANOVA) test. The correlations between all of the studied variables were evaluated using Pearson's correlation coefficient ® and linear regression analyses were used for the evaluation of data. P-value of < 0.05 was considered to be statistically significant. The receiver operating characteristic (ROC) curve was used to demonstrate the cut-off values, sensitivity, specificity and area under the curve of the studied research variables.

Chapter four

Result and Dissection

4.1. Demographic and Clinical Characteristics of The Study Groups

4.1.1 Distribution of the Study Groups According to Sex

The results of sex distribution among the study groups were in Control group 16(53.3%) male and 14(46.7%) female ,DM group 17 (56.7%)male and 13 (43.3)female ,DFU group 22(73.3%) male and 8 (26.7%)female , DFU&CKD group 24(80%) male and 6 (20%) female .The distribution of study groups into males and females was shown in table (4-1) .

Table (4.1) Frequencies of Sex for control and patient groups.

The frequencies of group	Male	Female
Control N=30	(16) 53.3%	(14) 46.7%
DM N=30	(17) 56.7 %	(13) 43.3%
DFU N=30	(22) 73.3%	(8) 26.7 %
DFU&CKD N=30	(24) 80 %	(6) 20 %

N= number, DM=diabetic mellitus group, DFU=diabetic foot ulcer group, DFU&CKD= diabetic foot ulcer group and chronic kidney disease.

The results showed increasing in frequency of male than female in(DM,DFU, and DFU&CKD) groups , Which agreed with Fan *et al* that found males were more commonly affected than female after age of puberty .Diabetic foot was more prevalent in males than in females (Fan *et al., 2021*). Male sex has been identified

as a risk factor for the development of DFU. These differences were most pronounced in the older age categories. Men is more workable than women due to lifestyle needs and outside activity which may lead to more feet exposure to different risks and more plantar pressure , these results agreement with (Vanherwegen A-S *et al* ,2023).

4.1.2 Frequencies of Smoker and Nonsmoker Among Study Groups

Table (4.2) showed the frequency of Cigarette smoking and non-smoking in each group in which the control group were (26.7 %, 73.3%), DM group were (33.3%, 66.7%), DFU group were (56.7%, 43.3) and DFU&CKD were (63.3, 36.7) these results indicated that there is increase in frequencies in diabetic foot patient group and diabetic foot with chronic kidney disease group than diabetes group and control group.

This results were agreed with (Lu *et al.*, 2021), that found smoking risk factor increase diabetic foot amputation, so were agreed (Alam *et al.*, 2021), that found Cigarette smoking effect in vascular system and neuropathy and act as risk factor for diabetic foot ulcer and delayed wound healing.

Table (4.2)- Frequencies of smoking for Study groups

The frequencies of groups	Smoking	Non-smoking
Control N=30	26.7 %	73.3 %
DM N=30	33.3 %	66.7%
DFU N=30	56.7 %	43.3%
DFU with CKD N=30	63.3 %	36.7 %

N= number, DM=diabetic mellitus group, DFU=diabetic foot ulcer group, DFU&CKD= diabetic foot ulcer group and chronic kidney disease.

As a source of free radicals and oxidants, cigarette smoke could induce cellular oxidative stress in many organs, including the nervous system and blood vessels, leading to cellular damage and even apoptosis. In vitro and in vivo evidence shows that cigarette smoke contains glycotoxins. These glycotoxins are highly reactive glycation products that can rapidly induce advanced glycation end-products (AGE) formation outside the cells. The increased modified proteins and lipids in the circulation of smokers bind to the receptor for AGE, which activates nicotinamide adenine dinucleotide phosphate oxidase and expression of pro-inflammatory cytokines and chemokines, inducing oxidative stress (Xia *et el*., 2019). The excessive ROS caused by cigarette smoking results in the production of nitric oxide synthase and an overload of glutamate in the synapses, and the consequent influx of Ca2+ leads to mitochondrial dysfunction, deoxyribonucleic acid damage, inflammation and even apoptosis. Therefore the smoking act as risk factor that increase cause of diabetic foot

4.1.2 Body Mass Index (BMI) and Age of the Participants among Groups.

The results of mean BMI and age among the study groups for control group and patients groups (diabetic mellitus, diabetic foot ulcer and diabetic foot ulcer with chronic kidney disease) were shown in figures (4.1 ,4.2) .The mean ± SD of age and BMI for control group were a 48.93 ±7.02 years, 27.05 ±2.22 Kg/m^2 ,DM group were 53.6 ±6.1 years, 25.1 ±2.59 Kg/m^2 , DFU group were 57.3 ±3.4 years ,24.1 ±1.4 Kg/m^2, and (DFU&CKD)were 59.3 ±4.03 years, 22.3±1.2 Kg/m^2 respectively. There were significant deference ($P<0.05$) in the mean of age and BMI in patients groups (DM,DFU,DFU&CKD) when compared with control group, also there were significant difference ($P<0.05$) between Diabetic mellitus group and (DFU , DFU&CKD) groups, while there were non-significant difference ($P>0.05$) in mean of age between diabetic foot group with diabetic foot with chronic kidney disease group and significant decrease ($P<0.05$) in mean of BMI in diabetic foot with chronic kidney disease group when compared with diabetic foot ulcer group .

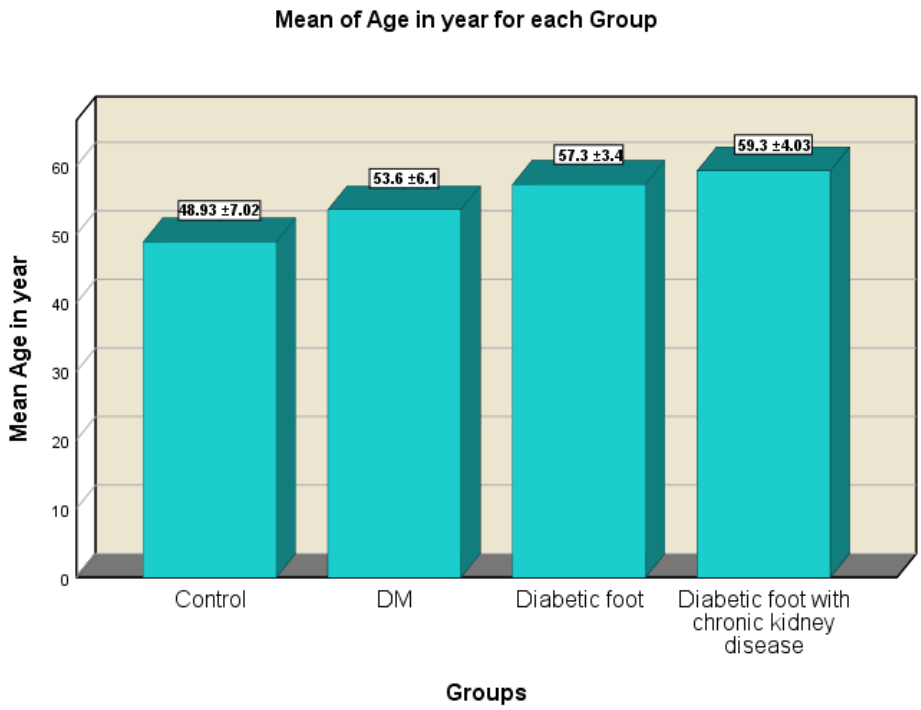


Figure (4.1): Histogram Showing Mean ± SD of Age in Study Groups

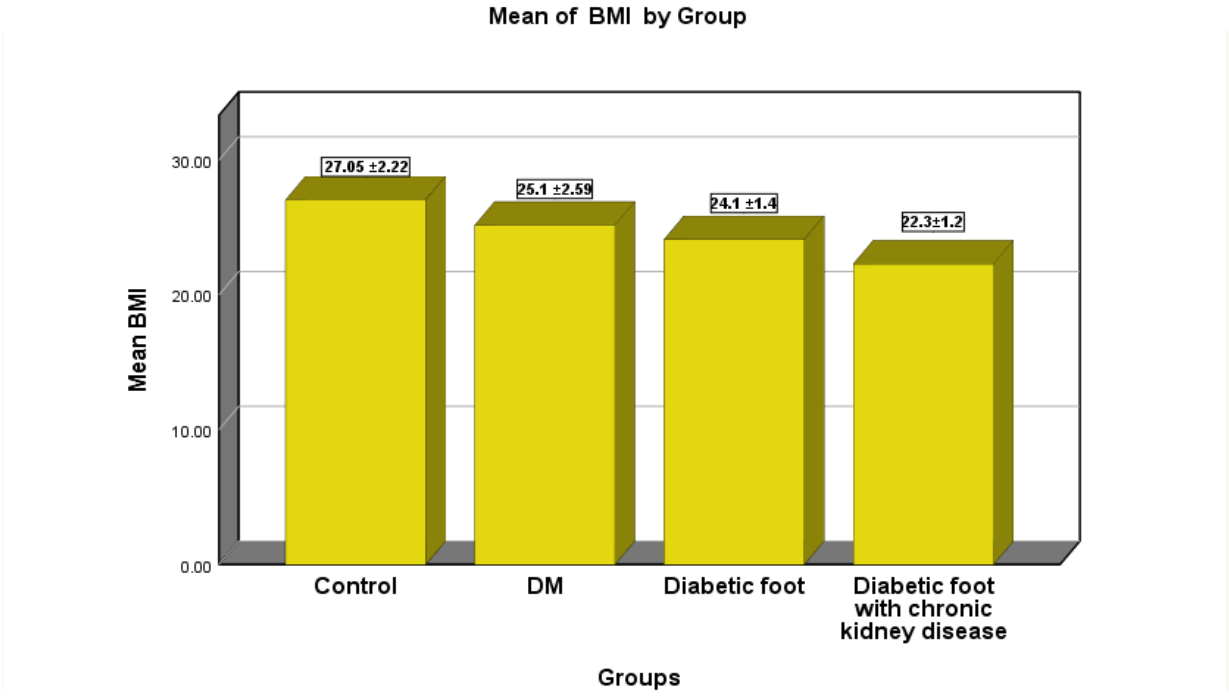


Figure (4.2): Histogram Showing Mean ± SD of BMI in Study Groups

Body mass index in (DFU&CKD, DFU ,DM) groups were serially lower than control group, this study agree with (Suk *et al.*, 2019). In that while muscular mass and muscle function are negatively affected by a variety of conditions as diabetic and chronic kidney disease (CKD).

The patients with diabetic foot ulcer were older, had a lower body mass index, longer diabetic duration, and had more hypertension and smoking history than patients without diabetic foot ulceration (Lauwers *et al.*, 2022) . The obesity paradox theory in which for every 5 kg/m2 increase in body mass index (BMI), a reduced risk of DFU was found,(Pham *et al*, 2022) , reported a low risk of DFU among overweight and obesity patients with a BMI of 25–34.9. (Biasucci *et al.,2024*), reported wound healing in obese patients was better because there was an increase endothelial progenitor cell levels that function as a protective vascular factor against atherosclerosis. People with diabetic foot ulcer and chronic kidney disease may have damaged in nephrons and develop proteinuria, loss of protein in urine has a negative effects on body weight. According to (Troutman *et el*.,2024). patients with a relatively mild degree of chronic renal insufficiency are characterized by decreased lean body mass, bone mineral content, and basal energy expenditure (Barutta *et el* ., 2022) ,reduced appetite and dietary limitations lead to insufficient food intake (real under nutrition) , other highly prevalent characteristics are necessary for the entire syndrome to occur ,Uremia-induced changes include increased energy expenditure, prolonged inflammation, acidosis, and a variety of endocrine abnormalities that cause hyper metabolism and excess catabolism of muscle and fat (Oliveira *et el*., 2021) .

4.2 Hematological and Biochemical Results

4.2.1 Blood Urea, Serum Creatinine and eGFR Among Study groups

The results of mean±SD values of B.urea in Control group was 28.7 ±5.6, compared with DM, DFU , DFU&CKD were 34.4 ±2.2 , 38.03 ±4.5 , 100.9 ±18.6 respectively .The results of mean ±SD of S.creatinine in Control group was 0.76 ±0.13 compared with DM , DFU ,DFU&CKD were 0.84±0.10 , 0.9 ±0.12 , 2.9±0.38 respectively.The results of mean ± SD e GFR in Control group was 109.6± 10.2 compared with DM, DFU,DFU&CKD 95.7±10.4, 82.6±13.5 , 21.4±4.2 all this data listed in Table (4-3).

Table (4.3) The mean ±SD of serum levels of B.urea, S.cr and eGFR for both control group and patients groups (DM, DFU and DFU&CKD).

Mean ±SD	Control N=30 Mean ±SD	DM N=30 Mean ±SD	DFU N=30 Mean ±SD	DFU & CKD N=30 Mean ±SD
B.urea (mg/dl)	28.7 ±5.6	34.4 ±2.2 $^{a▲*}$	38.03 ±4.5 $^{a▲**}$ **b**=ns	100.9 ±18.6 $^{a▲***}$ **b▲*** c▲***
S.CR(mg/dl)	0.76 ±0.13	0.84±0.10 **a**=ns	0.9 ±0.12 $^{a▲**}$ **b**=ns	2.9±0.38 $^{a▲***}$ **b▲***c▲***
eGFR (1.73 ml /min /m2)	109.6± 10.2	95.7±10.4 $^{a▼***}$	82.6±13.5 $^{a▼***}$ $^{b▼***}$	21.4±4.2 $^{a▼***}$ $^{b▼***}$ $^{c▼***}$

N: Number, SD: Standard deviation

a= ANOVA test between control, DM, DFU and DFU &CKD groups: ▲*= significant increase (P <0.05) , ▲** =high significant increase(P <0.01), $^{a▼***}$ very higher significant decrease(P <0.001)

▲***=very higher significant increased (P <0.001), NS= Non-Significantb= ANOVA test between DM and DFU, DFU & CKD groups: ns =non- significant . $^{b▲***}$ very high significant, $^{b▼***}$ very higher significant decrease (P <0.001,c= ANOVA test between DFU and DFU & CKD groups: C=▲***=very higher significant increased (P <0.001), $^{c▼***}$ very higher significant decrease (P <0.001)

These results significant increase ($P < 0.05$) in mean serum levels of B.urea in DM group when compared with control group , also there were high significant increases ($P < 0.01$) in mean of B.urea in diabetic foot ulcer group when compared with control group, there were very high increase significantly ($P < 0.001$) in mean of B.urea in diabetic foot &chronic kidney disease group when compared with control group.

There were no statistical differences ($P > 0.05$) in mean serum levels of s.cr between control groups and DM group ,in contrast significant increase ($P < 0.01$) in DFU group when compared with control group , while there were very high significant increases ($P < 0.001$) in mean of s.cr in diabetic foot ulcer with CKD group when compared with control group .

The level of eGFR in DM, DFU and (DFU&CKD) groups high significant decreases ($P < 0.001$) compared with control group .

Serum creatinine and urea are two essential parameters that are used in the diagnosis and monitor treatment of kidney diseases, adjustment of drug dosages, and decision-making regarding when to initiate renal replacement therapy (Ohishi *et al.*, 2022).the principle of the effects of hyperglycemia on the renal cells in Diabetic kidney disease can significantly improve our understanding of glomerular and tubular pathological processes at the cellular level , influence of diabetes on basement membrane (BM) components has been studied (Andrésdóttir *et al.*, 2019) . Biochemical alterations of glomerular BM consist of an increased non-enzymatic glycation of collagen leading to un physiological crosslinking , this in turn may result in alteration of the size selective properties of the glomerular filtration unit (Dong and Xu, 2022) . These findings revealed that there is a strong relationship of blood sugar level with urea and creatinine levels, as there is increase in blood sugar level an increase in urea and creatinine levels has been detected. This corroborates with the findings of Sharchil who found that

hyperglycemia is one of the major causes of progressive renal damage (Sharchil *et al.*, 2022). An increase in urea and creatinine levels is seen when there is damage to the kidney or the kidney is not functioning properly. Increment of blood urea and creatinine levels with the increment of blood sugar level clearly indicates that the increase of blood sugar level causes damage to the kidney. Research conducted by(Collins *et al.*, 2022) ,had found that increased urea and creatinine in DM indicates progressive renal damage.

Diabetes affects the kidney in stages or intervals , at the onset of diabetes the kidney become large and the glomerular filtration rate (GFR) becomes disturbed , most recent basic and clinical research had pointed toward sclerosis and kidney failure (Ciardullo and Perseghin, 2022) . When patient groups be compared to the control group it was noticed that their eGFR values declined significantly as seen in table(4-3).

The increase glucose levels were known to cause hyper filtration (GFR above normal) of the kidney , and as glucose levels were lowered the function of kidney filtering materials goes back to the normal rate (Levey *et el* ., 2022) .

According to kidney Disease Improving Global Outcomes Work Group (KDIGO) guidelines, CKD is defined as a reduction of estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m2 and/or presence of albuminuria this classification includes patients who have increased albuminuria but normal eGFR (stage I and II) and those who have low eGFR with or without albuminuria (stage III, IV, and V). The classical description of diabetic nephropathy is a slow and progressive increase in albuminuria, followed later in the disease by a decrease in estimated glomerular filtration rate (eGFR) below 60 mL/min, which can eventually lead to chronic kidney disease (Selen *et el*.,2022) .

4.2.2 Blood Glucose and Glycated hemoglobin Diabetic Patient Groups and Control Group

The mean ± SD of blood glucose and HBA1C for study groups (control ,DM, DFU and (DFU&CKD) were listed in table (4.4), the mean of glucose were 102.3 ±9.8 mg/dl, 256.7 ±29.6 mg/dl, 357.4 ±17.9 mg/dl**,** 363.7± 28.3 mg/dl and the mean of the HBA1C 4.7 ±0.44 %,10.3 ± 1.15 %, 12.9±0.81 %, 13. 2±0.69 % respectively .These results increased very high significantly ($P < 0.001$) in the mean of serum glucose and HBA1C found in patients groups (DM and DFU , DFU &CKD) compared with the control group, also there were increased high significant ($P < 0.01$) in mean serum glucose and HBA1C found in DFU ,DFU&CKD group when compared with DM groups , also there were very high significant increases ($P < 0.001$) in the mean of serum glucose found in DFU&CKD group when compared with DFU groups . There were non-significant increases ($P > 0.05$) in mean HBA1C found in DFU group when compared with DFU &CKD group, listed in table (4-4)

Table (4.4): Mean ±SD of Serum levels of Glucose, and glycated hemoglobin for both control group and patients groups (DM, DFU and DFU&CKD) groups

Mean ±SD	**Control No=30** Mean ±SD	**DM No=30** Mean ±SD	**DFU No=30** Mean ±SD	**DFU & CKD No=30** Mean ±SD
FBS (mg/dl)	102.3 ±9.8	256.7 ±29.6 **a▲*****	357.4 ±17.9 **a▲*** b▲*****	363.7 ±28.3 **a▲*** b▲*** c▲****
HBA1C %	4.7 ±0.44	10.3±1.15 **a▲*****	12.9 ±0.81 **a▲*** b▲*****	13.2±0.69 **a▲*** b▲***c=** ns

N: Number, SD: Standard deviation

a= ANOVA test between control, DM, DFU and DFU &CKD groups:) , ▲*** = very high significant increase(P <0.001), b= ANOVA test between DM and DFU, DFU & CKD groups:. **b▲***** very high significant increase , , c= ANOVA test between DFU and DFU & CKD groups groups: C=ns = non-significant **C▲***** very high significant increase(P <0.001),

The investigators found a significant association between hyperglycemia and the risk of vascular complications in the lower limb. Another study by Mariadoss et al, showed that ulcer healing was faster in patients with low glycosylated hemoglobin levels; however there was no improvement of neuropathy or vasculopathy after 12 week of treatment (Mariadoss *et al.*, 2022) .

A target of glycated hemoglobin less than 7% is acceptable in all diabetic patients and the same is applicable for diabetic foot ulcer patients as well. This study indicated that the progression to the complication of diabetic foot ulcers was strongly correlated to the level of HbAlC as found in a similar study by (Surowiec *et al.*, 2022) .The beneficial effect of considering measurement of HbA1C as a routine test for diabetic patients and maintaining an optimum level of less than 7%. Hb1Ac was the best predictor of outcome and optimization of diabetic control will

lead to improved outcome, with fewer incidences of diabetic ulcer and amputation. High levels of HbA1c is an important risk factor for diabetic ulcer in patients with diabetes and supports the strategy for lowering glucose levels to reduce diabetic foot in patients with diabetes. Similarly, effective control of hyperglycemia will result in lesser period of hospital stay (Ozougwu *et al.*, 2021).

4.2.3 Procalcitonin, C - reactive protein and WBC Levels among Study Groups

The results of Procalcitonin ,C- reactive protein and WBC for control group and patients groups (DM, DFU and DFU&CKD) were listed in table(4.5),the mean of Procalcitonin were (0.17 ±0.04,0.28±0.07,1.44±0.16, 1.48±0.22) in Control ,DM, DFU and DFU&CKD respectively, the mean of C- reactive protein were(4.4 ± 1.19,4.66±0.82, 53.5± 16.6 ,77.08±11.2) in Control ,DM, DFU and DFU&CKD respectively ,and the mean of WBC (6.0±1.2, 5.6 ± 1.15 , 13.9± 1.6 , 13.8 ± 1.3 in Control ,DM, DFU and DFU&CKD respectively.

There were non significant difference ($P > 0.05$) in mean serum PCT found in patients groups (DM) compared with the control group, while there were very high significant increases ($P < 0.001$) in mean of serum PCT found in (DFU , DFU&CKD) groups when compared with control group , also there were very high significant increases ($P < 0.001$) in mean of serum PCT found between DM group and (DFU , DFU&CKD) groups and between DFU group with DFU &CKD .There were non-significant difference ($P > 0.05$) in mean serum CRP,WBC found in patients groups (DM) compared with the control group, while there were very high significant increases ($P < 0.001$) in mean serum CRP and WBC found in (DFU ,DFU&CKD) groups when compared with control group ,and between DM group with DFU, (DFU&CKD) groups and DFU groups with DFU &CKD

Table 4.5: The mean ±SD of Serum levels of Procalcitonin, C reactive protein and WBC for both control group and patients groups (DM, DFU and DFU&CKD)

Mean ±SD	Control N=30	DM N=30	DFU N=30	DFU & CKD N=30
Procalcitonin (ng/ml)	0.17 ±0.04	0.28±0.07 **a**=NS	1.44±0.16 **a▲*** b▲***	1.48±0.22 **a▲*** b▲*** c▲**
C-reactive protein mg/dl	4.4 ±1.19	4.66±0.82 **a**= ns	53.5±16.6 **a▲*** b▲***	77.0±11.2 **a▲*** b▲*** c▲***
WBC	6.0± 1.2	5.6±1.15 a= NS	13.9±1.6 **a▲*** b▲***	13.8±1.3 **a▲*** b▲***C**=NS

N: Number, SD: Standard deviation

a= ANOVA test between control, DM, DFU and DFU &CKD groups: ▲*= significant increase (P <0.05) **a▲*** very higher significant increase(P <0.001) , NS= non-significant ,b= ANOVA test between DM and DFU, DFU & CKD groups: **b▲*** very high significant increase (P <0.001)' c= ANOVA test between DFU and DFU & CKD groups: C= **c▲*** = very high significant increase (P <0.001) **c▲**** = high significant increase (P <0.01, NS= non-significant

The correlation of the PCT values in (DFU&CKD) group were positive significant correlation with CRP (r= 0.882, p < 0.05) as well showed positive significant correlation with Cystatin C (r = 0.771, p < 0.05), PCT showed positive significant correlation with WBC (r = 0.690, p < 0.01).

The correlation of the PCT values in DFU group were positive significant correlation PCT with CRP (r= 0.480, p < 0.05) as well showed positive significant correlation with Cystatin C (r = 0.862, p < 0.05), PCT showed positive significant correlation with WBC (r = 0.761, p < 0.01) and weak positive correlation with HBA1C(r=0.32 p <0.05).

The correlation of the PCT values in diabetic mellitus group were positive significant correlation with CRP (R=672 p < 0.05) as well showed positive

correlation with Cystatin C (R= 0.865, $p < 0.05$), PCT showed positive significant correlation with WBC (R = 0.934, $p < 0.01$), as shown in the table (4.6), figure (4.3), (4.4),(4.5)

Table (4-6): The correlation analysis using Pearson correlation of PCT in DFU&CKD, DFU and DM groups with CRP, Cystatin C, WBC, parameters

PCT Correlation		CRP	Cystatin C	WBC
Control group	Person correlation (R)	0.431*	0.719***	0.432*
	Sig. (2-tailed)	0.017	0.0001	0.017
DM group	Pearson Correlation (R)	0.762***	0.865***	0.934***
	Sig. (2-tailed)	0.0001	0.0001	0.0001
DFU group	Pearson Correlation (R)	0.480**	0.862*	0.761***
	Sig. (2-tailed)	0.007	0.02	0.0001
DFU&CKD group	Pearson Correlation (R)	0.882***	0.771***	0.690***
	Sig. (2-tailed)	0.0001	0.0001	0.0001

*** Correlation significant at $p < 0.001$ level (2-tailed). a:, b: c
** Correlation significant at $p < 0.01$, level (2-tailed).
* Correlation significant at $p < 0.05$, level (2-tailed).

These results agree with(Massara *et al* ,2017) who indicated that the relationship between these markers and the onset of diabetic foot ulcer showed that PCT and CRP markers had the highest diagnostic values for predicting the incidence of diabetic foot ulcers .(*Umapathy et al*, 2018) study showed that PCT which can be used as a good marker for realizing infection in Indian patients with

DFU and it was greater than for other traditional markers. PCT level were significantly increased in diabetic foot ulcer and diabetic foot with chronic kidney disease patients and *(Uzun et al* ,2017*)*,study showed increased sensitivity when PCT is combined with CRP

Recently, PCT has been suggested as an important marker of inflammation, which increases in inflammatory processes, especially bacterial infections (localized or bacteremia). The PCT values have a progressive increasing pattern in bacterial infections, while the elevation is only mild in other inflammatory conditions(Korkmaz *et al.*, 2018) .PCT is produced in direct response to bacterial endotoxins and indirectly to mediators such as interleukin (IL-1), tumor necrosis factor-α β, and IL-6, it is strongly correlated with severity of infection. When compared with CRP, Procalcitonin is detectable in serum 3 hours after bacterial infection and peaks 6–12 hours later, whereas CRP peaks after 36–50 hours (Hadavand *et el* , 2019). Furthermore, PCT has better sensitivity and specificity at diagnosing infections including infected DFU. A group of diabetic patients without foot complication was enrolled, to exclude the inflammatory process accompanying diabetes that may cause an increase of PCT concentration. The results here showed no significant difference in PCT concentration in patients with DM than that in control group, confirm that a higher level of serum PCT is present of infected DFUs.

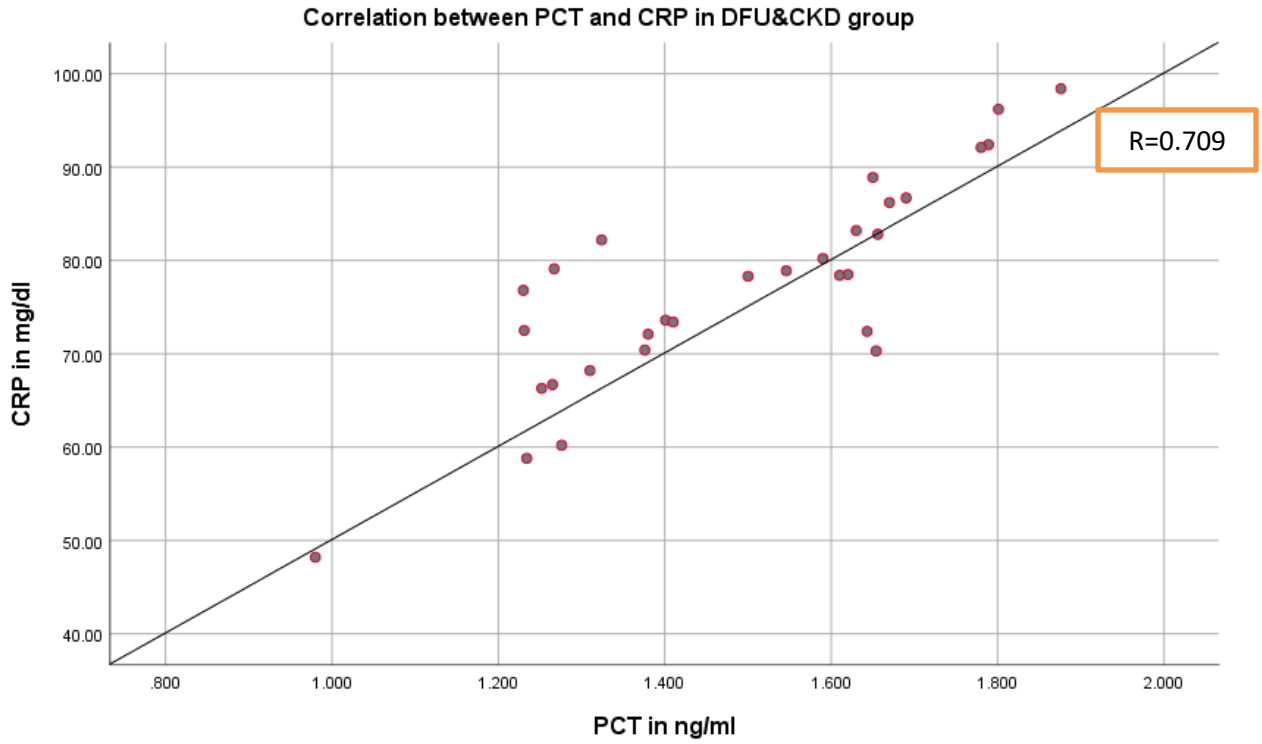


Figure (4.3) The correlation between serum levels of PCT (ng/ml) with CRP (mg/dl) in the Diabetic foot ulcer with chronic kidney disease group

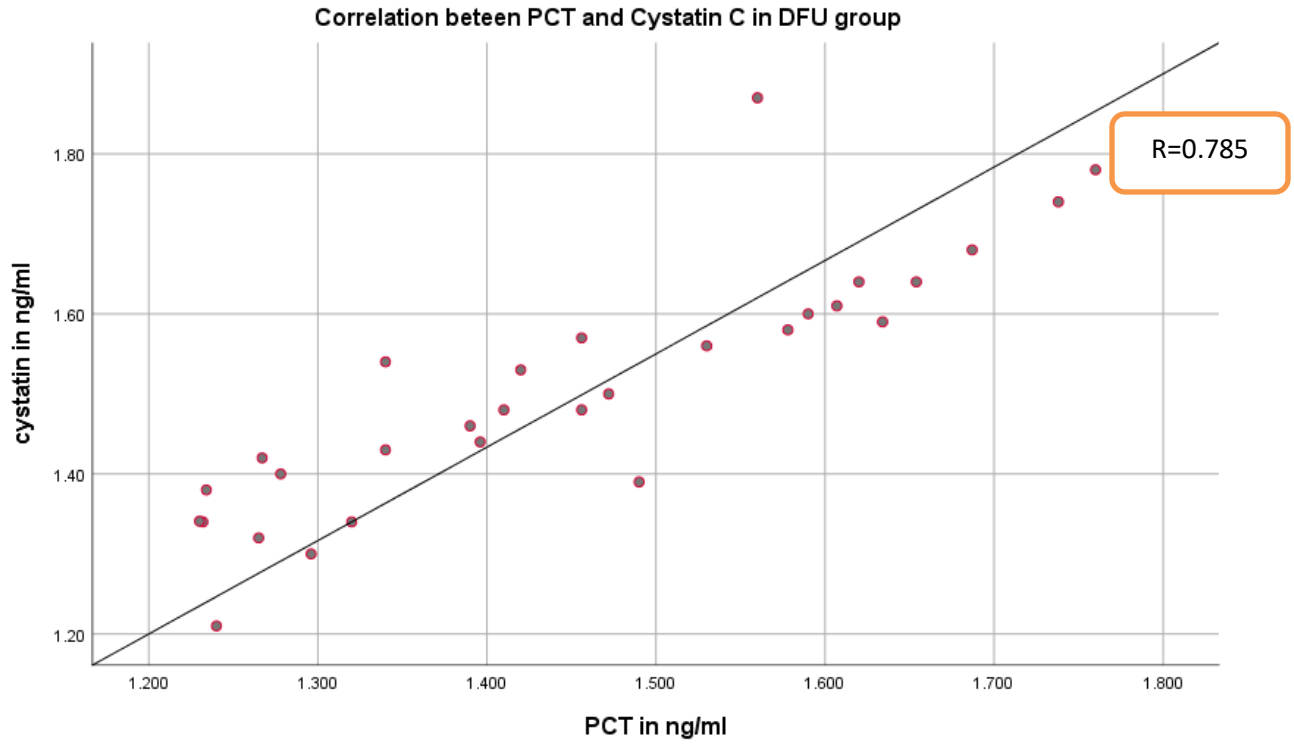


Figure (4.4) The correlation between serum levels of PCT (ng/ml) with Cystatin C (ng/ml) in the Diabetic foot ulcer group

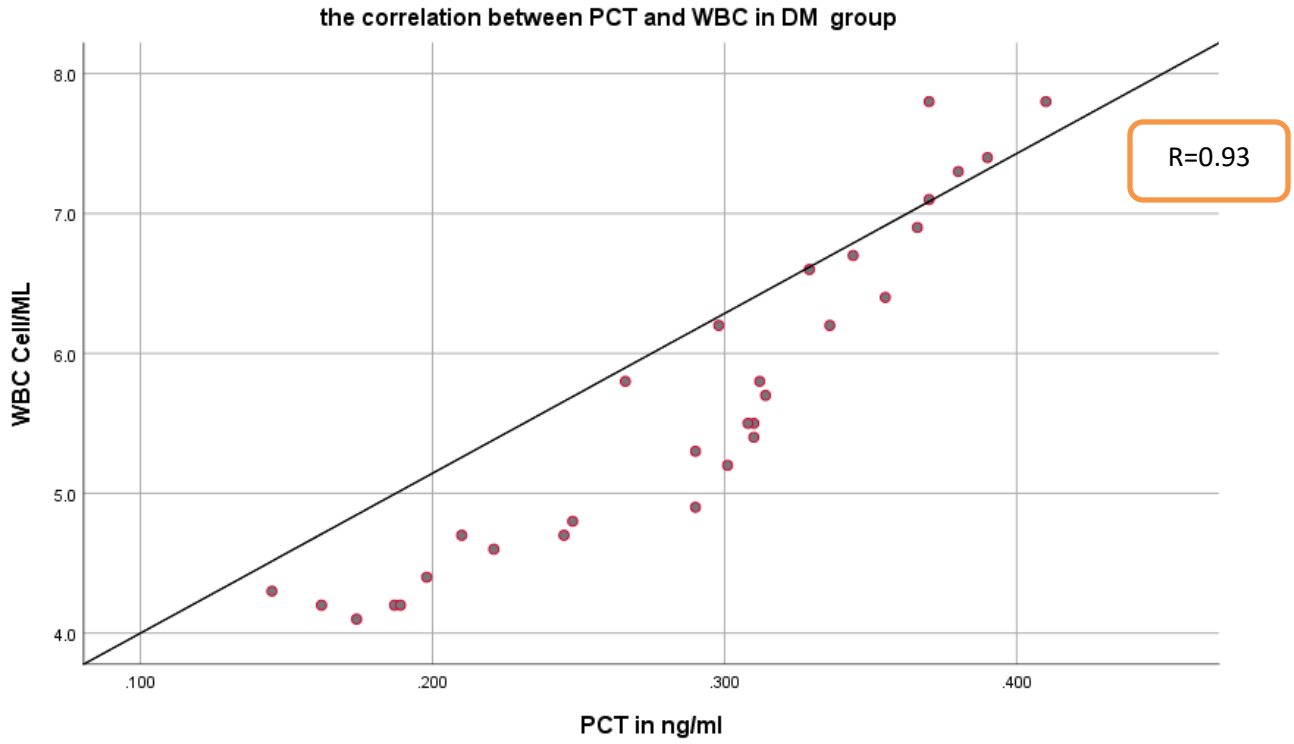


Figure (4.5) The correlation between serum levels of PCT (ng/ml) with WBC (cell/ml) in the DM group

Acute phase proteins are a class of proteins and their plasma concentrations increases or decreases in response to inflammation. Previous studies had demonstrated that inflammation played an important role in the pathogenesis of DM , an inflammatory basis for Diabetes and its complications has attracted interest (Dmitriyeva *et al.*, 2022) . Among several markers of inflammations , C reactive protein (CRP) is found to be significant in patient with diabetic foot (Sushith *et al.*, 2020). Diabetic nephropathy may be associated with abnormally high levels of CRP it can binds to many biological materials and subsequently activates complement , an inflammatory basis for Diabetes and its complications has attracted interest and among several markers of inflammation , CRP is found to

be significant in people with diabetic (Stanimirovic *et al.*, 2022) . CRP which is a pentameric protein produced by the liver has emerged as the ‘golden marker for inflammation , so diabetic foot associated with abnormally high levels of CRP (Nguyen *et al.*, 2022) .Some studies, they had found that there was a relationship between PCT and CRP levels in diabetic foot patients , a similar result were indicated in this study , many studies were agreed with current study (Selen *et el*., 2022)low grade inflammation as indicated by high CRP levels was an important predictor of diabetic foot and diabetic nephropathy (Gao *et al.*, 2020) .

Increasing evidences showed that C-reactive protein (CRP) is not only an inflammatory biomarker but also an important risk factor associated with ageing-related diseases including cardiovascular disease, hypertension, diabetes mellitus and kidney disease . It might be the possibility that chronic inflammation being a background process of diabetic nephropathy which in turn increased with advancement of age was leading to rise in CRP (Lin *et al.*, 2023) . The current study provided that CRP level were good indicator causally related to DN. These findings suggested that the elevated CRP may be a causal risk factor for DN in patients with diabetic foot (Santos *et al.*, 2022)

Table (4-5) showed there was a highly significant CRP concentrations differences in diabetic foot group than other groups. This were agreement with (Park *et al*, 2017) who showed PCT and CRP measurement correlated positively with the grades of infection of DFUs, CRP was useful for distinguishing localized diabetic foot infection grades.

WBC is inflammatory markers for diabetic foot ulcers and other diseases and the level of WBC can reflect the severity of DFU infection. This study showed that higher baseline WBC levels predicted a higher probability of major amputation.

(Lauri *et al* .,2019), study results also showed that increased WBC was associated with increased risks from DFU to major amputation in China .WBC count levels were also markers of infection as confirmed in our study, in which WBC count levels was significantly higher in diabetic foot group in comparison with other groups.

4.2.4 Cystatin C among Study Groups

The mean ± SD of Cystatin C for control group (0.53 ±0.11) ,DM group (0.75±0.16), DFU group (1.6±0.59) and DFU&CKD group (4.6±0.61) respectively. There were non-significant increases (P> 0.05) in mean of serum Cystatin C found in patients groups (DM) compared with the control group, also there was a very significant increase (P < 0.01) in mean serum Cystatin C found in(DFU) and very high significant increase (P < 0.001) (DFU&CKD) groups when compared with control group .

Table (4.7) the mean ±SD of Serum levels of Cystatin C for both control group and patients groups (DM, DFU and DFU&CKD)

Mean ±SD	Control N=30	DM N=30	DFU N=30	DFU & CKD N=30
Cystatin C(ng/ml)	0.53 ±0.113	0.75±0.16 **a**=ns	1.60±0.59 **a▲**** **b▲*****	4.6±0.61 **a▲***** **b▲*** c▲****

N: Number, SD: Standard deviation

a= ANOVA test between control, DM, DFU and DFU &CKD groups: NS= non-significant , a▲** = significant increase(P <0.01), **a▲***** very higher significant increase(P <0.001) b= ANOVA test between DM and DFU, DFU & CKD groups: ▲**=high significant increased (P <0.05). **b▲***** very high significant **increase (p value** <0.05) c= ANOVA test between DFU and DFU & CKD groups groups: C= **c▲**** = high significant increase (P <0.01)

The correlation of the Cystatin C values of DFU group were positive significant correlation Cystatin C with CRP (r= 0.509, $p < 0.05$) as well showed weak positive significant correlation with HBA1C (r = 0.352, $p < 0.05$), Cystatin C showed positive significant correlation with WBC (r = 0.742, $p < 0.01$) and positive correlation with PCT(r=0.871 $p < 0.05$), showed in figures (4-6, 4-7).

The mean of Serum CysC levels in DFU group were higher significantly than non-DFU group, the serum Cystatin indicator for diabetic foot ulcer, and therefore, serum CysC considered being a probable marker for DFU in DM populations. This study was agreed (Al-Nori *et al*, 2019*)* that showed renal disease had significant higher rates of diabetic lower extremity amputation compared with diabetic patients without renal disease, the kidney disease is predictor to DFU , the prevalence of DFU is higher in DM patients with age, duration of DM and bad control of blood glucose ,therefor Cystatin c is believed to be a sensitive biomarker for screening out PAD in the diabetic populations. Serum CysC has slowly gained more widespread acceptance as an alternative endogenous filtration marker.

Serum CysC has been proved as a strong relationship with cardiovascular disease (CVD) and peripheral vascular disease atherosclerosis, hardening and narrowing of vessel walls leading to arterial occlusion. (Collins *et al,* 2022). Showed that elevated CysC was associated with increased long-term rates of community-acquired sepsis, independent of abnormal estimated glomerular filtration rate, albumin creatinine ratio, and high sensitivity C reactive protein (Barutta *et al.*, 2022). Agree with our results. CysC is significantly associated with inflammation markers, such as white blood cell and C-reactive protein, and these results were indicated that increased CysC in DFU group and DFU&CKD group and also may be as indicator for renal dysfunction in diabetic patients with overt kidney disease .

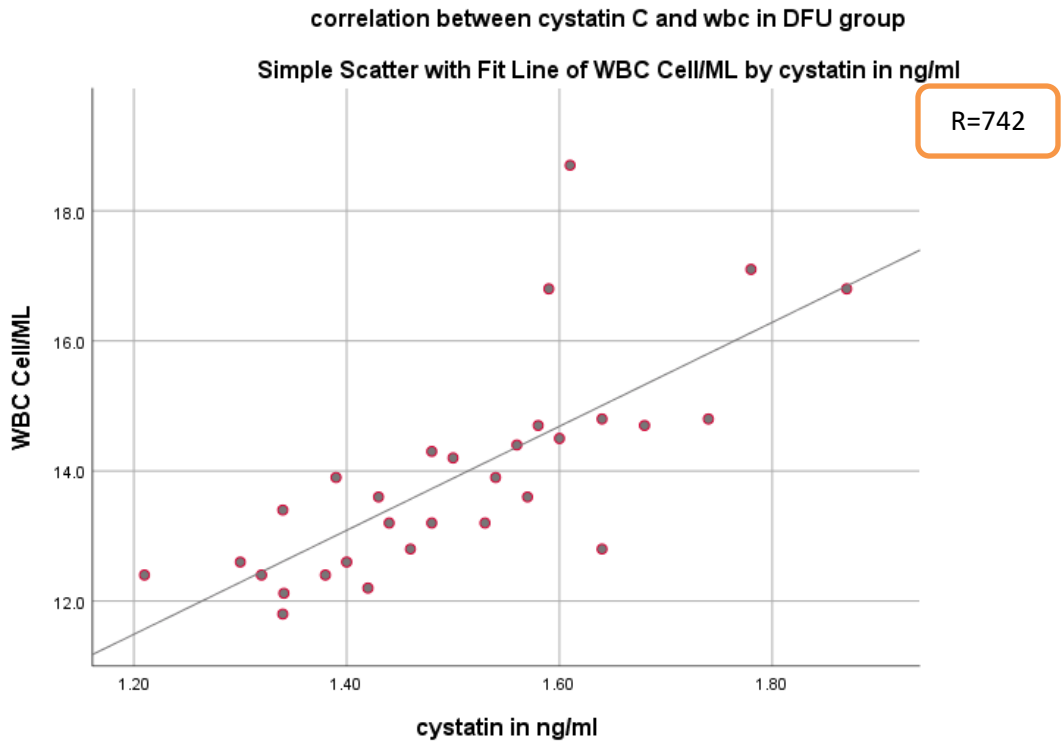


Figure (4.6) The correlation between serum levels of Cystatin C (ng/ml) with WBC (cell/ml) in the DFU group

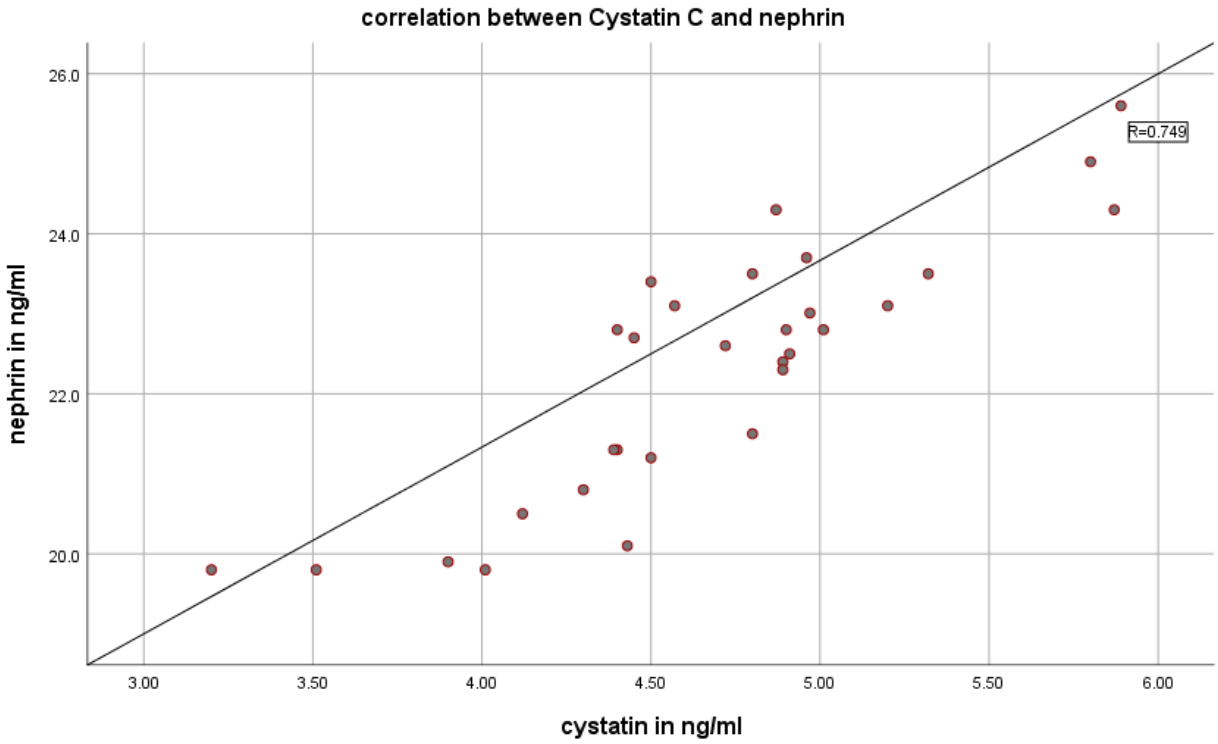


Figure (4.7) The correlation between serum levels of Cystatin C (ng/ml) with Nephrin (ng/ml)in the DFU&CKD group

4.2.4 Nephrin among Control Group and Diabetic Patient Groups

ANOVA test for the mean ± SD of Nephrin for control group and patients groups (DM, DFU and DFU&CKD) were 5.03 ±0.66, 8.2±1.2,9.8±0.63, 25.7±2.4 respectively. There were significant increase ($P < 0.05$) in mean serum nephrin found in patients groups (DM) compared with the control group, also there were high significant increases ($P < 0.01$) in mean serum nephrin found in(DFU) group and very high significant increase ($P < 0.001$) in (DU&CKD) group when compared with control group as shown in table (4-8) .our results were agreed with (Oliveira *et el.*, 2021) that showed significant increase of serum Nephrin in diabetic nephropathy patients.

Table 4.8 The mean ±SD of Serum levels of Nephrin for both control group and patients groups (DM, DFU and DFU&CKD)

Mean ±SD	Control No=30	DM No=30	DFU No=30	DFU & CKD No=30
Nephrin(ng/ml)	5.03 ±0.66	8.2±1.2 $^{a▲*}$	9.8±0.63 $^{a▲**}$ $^{b▲***}$	25.7± 2.4$^{a▲***}$ $^{b▲***}$ $^{c▲**}$

N: Number, SD: Standard deviation

a= ANOVA test between control, DM, DFU and DFU &CKD groups: a▲* = significant increase (P <0.05), , a▲** = significant increase (P <0.01), ^{a}▲*** very higher significant increase (P <0.001)

b= ANOVA test between DM and DFU, DFU & CKD groups: b▲***=very higher significant increased (P <0.001), NS= Non-Significant . c= ANOVA test between DFU and DFU & CKD groups: C= $^{c▲**}$ = high significant increase (P <0.01)

The results of nephrin in our study were in consistence with (Aljorani *et el*., 2023) revealed significant increase in serum and urine Nephrin in patient with early diabetic nephropathy. Long-term high blood sugar often leads to cause kidney disorders. It was indicated that the renal dysfunction can occur during long duration of the most patients with diabetes mellitus. Diabetic nephropathy is considered as a silent disease during a long period without any symptoms. Subsequently, chronic hyperglycemia affects different types of kidney cells which finally leads to progressive glomerular and tubular damage resulting in kidney failure (Chen *et el*., 2018), these changes cause increase nephrin production and release so increase its level in serum and urine. These indicators typically capture a single mechanism of the disease process, such as glomerular or tubular damage, inflammation or oxidative stress, these findings suggest that podocyte destruction may occur in DM patients prior to the development of microalbuminuria , Several

studies showed that nephrinuria was associated with higher urine albumin concentrations and diabetes status (Kostovska *et al.*, 2020), thus, given that hyperglycemia is likely to cause further damage to renal vasculature and glomerular filtration barrier over time .

Nephrin may provide an early indicator of renal damage, even if not all diabetic patients with nephrinuria progress to kidney disease. The current study was consistent with a previous study that indicated nephrin loss significantly and redistribution in the glomeruli of diabetic patients with microalbuminuria ; this observation suggested that nephrin loss and redistribution may precede the development of glomerular lesions and be an early event in the progression of diabetic nephropathy. It also revealed that patients with diabetes and nephropathy have structural changes to the glomerular filtration unit, such as increased width of podocyte foot processes and filtration slits (Veluri *et el* ,2022) . As a result, one can infer that nephrin can be found in the systemic circulation or that nephrin secreted by podocytes while passing through the nephron can be reabsorbed in the renal tubular system and discovered in the serum.

The presence of nephrin and other podocyte-specific proteins in urine indicate only damage of podocytes, independently of the other two components of the glomerular filtration barrier (Sharchil *et el* .,2022).Thus, it is thought that podocyte damage is present before the appearance of microalbuminuria and proteinuria, hence, podocyte proteins such as nephrin are considered as earlier and more specific markers for diagnosis of DN compared to microalbuminuria and presence of micro - albumin in urine suggests damage of all three components of the glomerular filtration barrier (endothelium, glomerular basement membrane and podocytes), and its diagnostic accuracy is limited by the fact that structural damage might precede microalbumin excretion(Kondapi *et al.*, 2021).

The correlation of the Nephrin values of DFU&CKD group were positive significant correlation Cystatin C with CRP (r= 0.866, $p < 0.05$) as well showed positive significant correlation with WBC (r = 0.584, $p < 0.05$), Nephrin showed positive significant correlation with KIM-1 (r = 0.955, $p < 0.01$) and positive correlation with PCT(r=0.629 p <0.05).

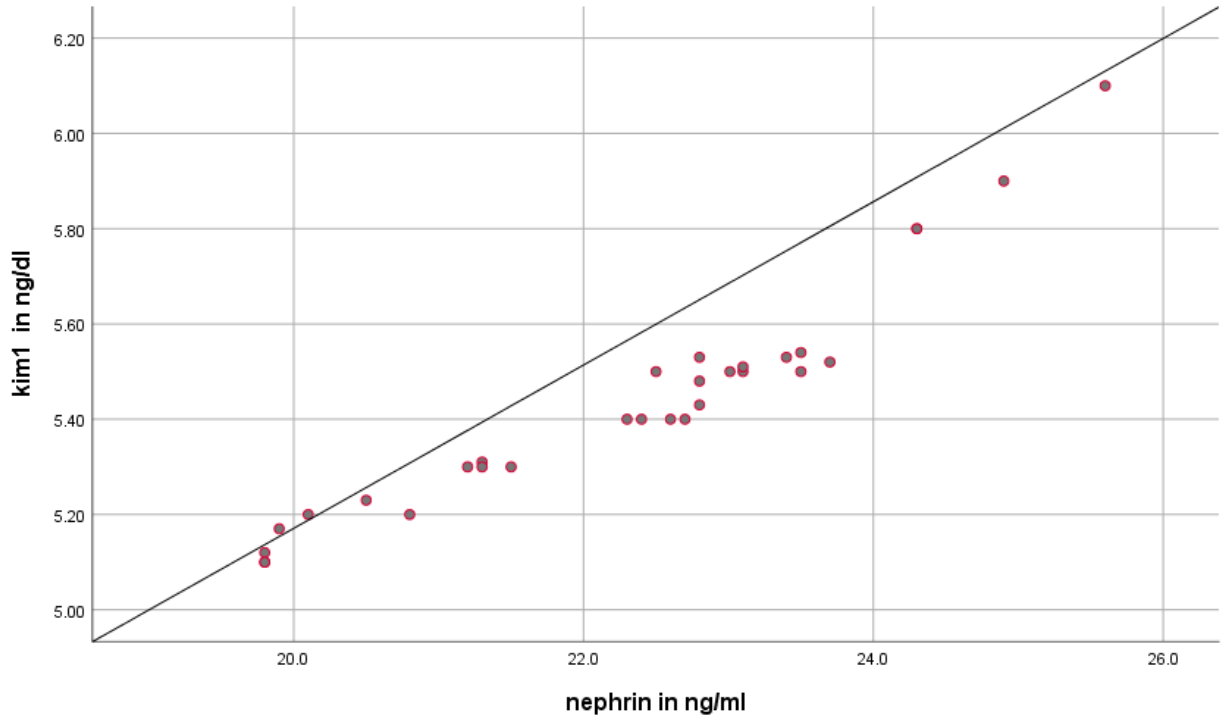


Figure (4.8) The Correlation between serum levels of Nephrin (ng/ml) with KIM-1 (ng/ml)in the DFU&CKD group

4.2.4 Kidney Molecule injury-1(KIM-1) Among Control Group and Diabetic Patient Groups

The mean ± SD of KIM-1 for control group and patients groups (DM, DFU and DFU&CKD) were 0.54 ±0.04, 0.71±0.11, 1.28±0.1, 5.4±0.4 respectively. There were non-significant deference ($P > 0.05$) in mean of serum KIM-1 in patients groups (DM) compared with the control group . There were increased high significantly ($P < 0.001$) in mean of serum KIM-1 in(DFU ,DFU&CKD) groups when compared with control group, also there were increased high significantly ($P < 0.001$) in mean serum KIM-1 found in(DFU ,DFU&CKD) groups when compared with DM group and high significant increase ($P < 0.001$) between DFU group with DFU &CKD as shown in table (4-9) .

Table 4.9 The mean ±SD of serum levels of KIM-1 for both control and patients groups (DM, DFU and DFU&CKD)

Mean ±SD	Control N=30	DM N=30	DFU N=30	DFU & CKD N=30
KIM-1(ng/ml)	0.54 ±0.04	0.71±0.11 a =ns	1.28±0.1 a▲** b▲***	5.4± 0.4 a▲*** b▲*** c▲**

N: Number, SD: Standard deviation a= ANOVA test between control , DM, DFU and DFU &CKD groups: , a▲** = significant increase(P <0.01), a▲*** very higher significant decrease(P <0.001) , b= ANOVA test between DM and DFU, DFU & CKD groups: b▲***=very higher significant increased (P <0.001), c= ANOVA test between DFU and DFU & CKD groups: C= ▲** = high significant increase (P <0.01)

The correlation of the KIM-1 values of DFU&CKD group was positive significant correlation with CRP ($r= 0.475$, $p < 0.05$) and positive correlation with PCT($r=0.626$ $p <0.05$) in figure (4-9), as well showed positive significant correlation with WBC ($r = 0.453$, $p < 0.05$), KIM-1 showed positive significant correlation with Nephrin ($r = 0.867$, $p < 0.01$) and showed in figure (4-8)

According to results obtained in the current study, in addition to hyperglycemia, DM causes weight loss and increased the serum concentration of urea, and creatinine. In addition, this results showed increase in mean of KIM-1 in diabetic foot group and diabetic foot with chronic kidney disease that because DM induced oxidative stress in the kidney tissue and significantly increases the serum levels and renal expression of KIM-1, KIM-1 as a phosphatidylserine receptor that recognizes apoptotic cells and directs them to lysosomes, that transforms kidney proximal epithelial cells into semi-professional phagocytes. (Al-Bataineh *et al.*, 2021) showed significantly increase in serum KIM-1 in chronic kidney disease patients .

As a consequence of its role in enhancing the clearance of dead cells by the surviving tubular cells. KIM-1 protects the kidney early after injury via processes facilitated by apoptotic cell uptake.

The expression of KIM-1 in chronic kidney disease because inflammation of proximal cells in diabetic nephropathy. Hypoxia is a powerful stimulus of KIM-1 expression increase in proximal tubular cells which, in its turn, may result in the induction of chronic interstitial inflammation.Membrane-bound as well as free KIM-1 was considered to be involved in signaling interactions between the cells of the damaged renal proximal tubules and macrophages acting as autocrine-paracrine factor in relation to the epithelial and stromal cells because that increase KIM-1 in DFU, DFU&CKD groups.

In parallel with these finding (Dong *et al*, 2022), revealed that kim-1 was elevated significantly of KIM-1 in insulin resistance at elderly individuals. Impaired insulin sensitivity and compensatory hyperinsulinemia have been suggested to contribute to development of renal injury by promotion of mutagenic

and fibrotic processes via different pathophysiologic pathways such as activation of insulin-like growth factor1, transforming growth factor-b, endothelin-1, and the renin–angiotensin–aldosterone system. Insulin resistance is closely associated with oxidative stress, pro-inflammatory cytokines and adipo-kines, which also could promote renal injury. But the opposite chain of events is also possible; an increased inflammatory activity due to ongoing kidney damage could also impair insulin sensitivity. Tubulointer-stitial injury is present in all forms of chronic kidney disease and is thought to be a better predictor of disease progression and long-term prognosis than is the severity of damage to glomeruli, by measuring KIM-1, this "tubular phase" of renal damage could be detected before the development of albuminuria, the currently used marker of early diabetic nephropathy (Yin *et el* .,2016).

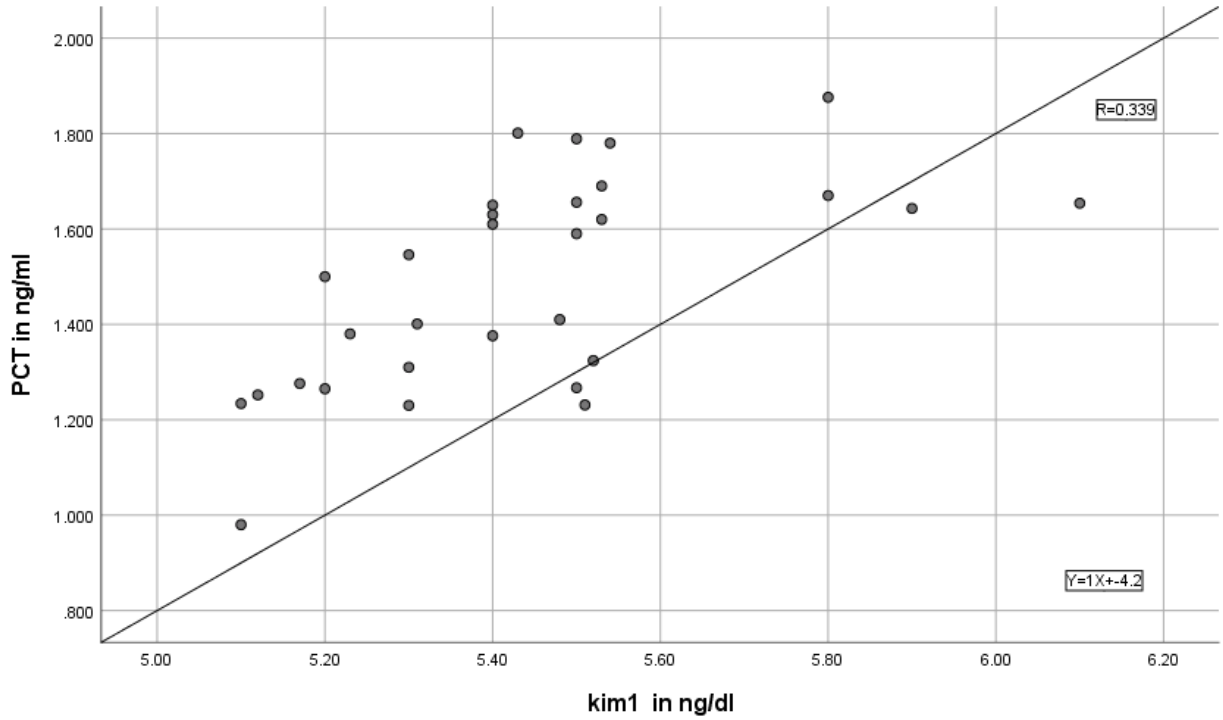


Figure (4.9) The correlation between serum levels of KIM-1 and PCT (ng/ml)in the DFU&CKD group

4.2.5 The Level of Hemoglobin among Control Group and Diabetic Patient Groups

The mean ± SD of hemoglobin for control and patients groups (DM, DFU and DFU&CKD) were (14.2±1.7), (13.6±1.4),(12.8±1.04),(12.4±0.8)respectively. There were no significant deference (P> 0.05) in mean of hemoglobin found in patients groups compared with the DM group, also there were significant decrease in DFU and DFU&CKD groups (P< 0.05) when compared with control group as shown in figure (4-10) .

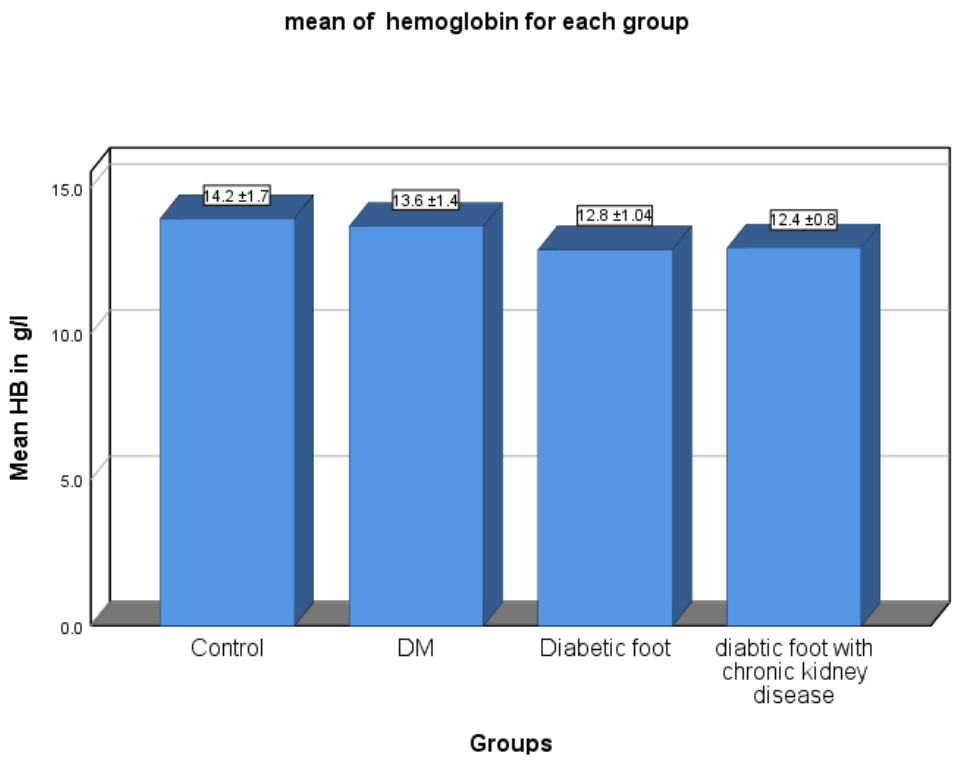


Figure (4.10) Histogram Showing Hemoglobin Mean ± SD for each Study Groups

Complications of diabetes (nephropathy, retinopathy, and neuropathy) are associated with anemia that may lead to impaired wound healing, and macrovascular disease ,it was suggested that low blood oxygen level accompanying low hemoglobin levels may consequently worsen ischemia of the lower limb. Anemia has been severally reported as a complication of diabetes mellitus, (Li *et el*.,2023) According to previous studies, the proteins of RBC membrane undergo oxidation through non-enzymatic glycosylation due to increased oxidative stress in diabetes and reduce PCV, Hb, RBC levels that may lead to hemolysis and consequently to anemia .This study agreed with (Xie *et al.*, 2023)had similar findings whereby the prevalence of anemia was higher in diabetic patients in spite of having safeguarded renal function. The measured deformable RBC in diabetic patients with and without DFU (JA *et el*, 2020), They found significant increase in the deformable RBCs percent in DFU patients in comparison with that in patients without DFU. Chronic inflammation is considered to be a common cause of anemia in diabetic patients, especially with DFU. These due to systemic inflammation, repeated superficial, deep tissue infection, osteomyelitis, and antibiotic use which may delay healing of foot ulcer (Li et el .,2023).

4.3 Receiver operating characteristics(ROC) for diagnostic markers

Receiver operator characteristic curve was used for the analysis of significant differences of indices of diabetic patient groups. Determination of the diagnostic performance is based on the area under the curve (AUC) as follows: AUC = 0.9–1.0, excellent; AUC = 0.8–0.9, good; AUC = 0.7–0.8, fair; AUC= 0.6–0.7, poor; and AUC < 0.6, not useful (Anjum *et al*., 2020) ,(Rahman *et al.*, 2021)

4.3.1 Results of Receiver Operator Characteristics Analysis of Procalcitonin, Cystatin C ,Kidney injury molecule -1 and Nephrin

The ROC analysis data demonstrated that PCT possesses an excellent ability to predict DFU in the diabetic group, in the included groups DM, DFU, (DFU&CKD) by comparison to control group. This result were achieved based on investigations that included the outcome of sensitivity and specificity for test, as well as area under the curve and some other relevant characteristics, as shown in Table (4-10)

PCT showed an excellent capability (AUC= 0.96) to identify and predict DFU patients from those without any disease. In term of prior probability, the P value was 0.001 with very high sensitivity and specificity values 99% and 93% respectively .Which indicated that this marker has equal roles as for confirming and excluding disease. The best cut- off point (0.231> ng/ml) derived from the ROC curve. Accordingly, a test value above 0.231 ng/ml is considered abnormal. A value of PCT> 0.231 ng/ml indicated that the patients in probability have DFU compared to the normal persons, as shown in figure (4 -11).

Table (4-10): The ROC results of PCT, Nephrin ,KIM-1 andCystatin C biomarkers

Test Variable positive actual critical state.	AUC	P value	Cut-off points	Specificity	Sensitivity
PCT	0.964	0.001	0.231 ng/ml	99 %	91.1%
Cystatin C	0.98	0.001	0.71 mg/dl	99.7%	96.7%
KIM-1	0.939	0.001	0.66 ng/ml	99.3%	93.3%
Nephrin	0.94	0.001	9.4 ng/ml	83.3 %	99.6%

PCT level can be considered as a strong parameter to diagnose DFU group since the AUC value was 0.96. PCT, which act as good markers for the infection and inflammation. The elevated plasma level of PCT in diabetes can occurs as a result to inflammation of foot ulcer (Mansoor *et al.*, 2022) . This study showed elevated PCT levels with progresses of diabetic disease and this result agree with others (Kerkeni *et al.*, 2019) , Other studies were support the diagnostic accuracy of PCT in diabetic foot infections.(Uzun *et al* 2017*)*.Studied the usefulness of inflammatory markers, including PCT, in detecting bacterial infection in patients with diabetic foot ulcers.

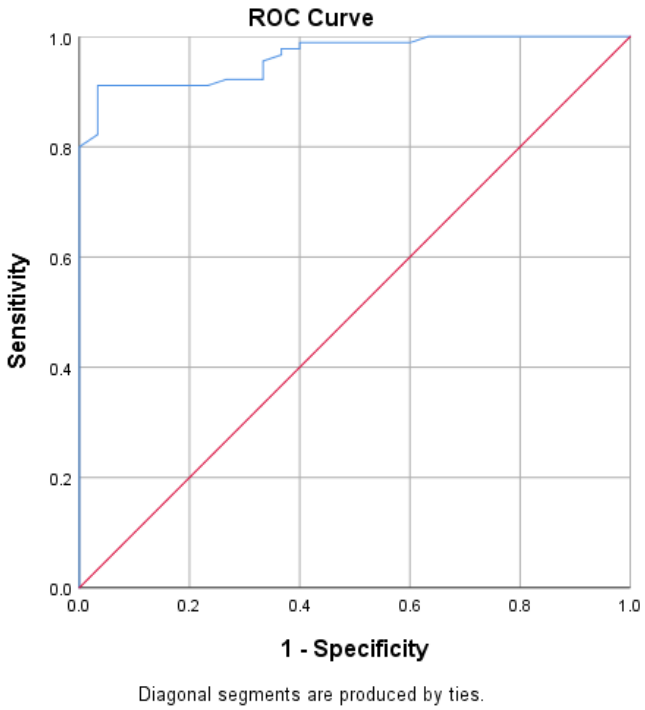


Figure 4-11: Receiver operating characteristic curve for PCT showing sensitivity and specificity

The ROC analysis data demonstrated that Nephrin possesses an excellent ability to predict DFU&CKD in the diabetic group, in the included groups, DFU, (DFU&CKD) in comparison to DM group. This results were achieved based on investigations that included the parameters of sensitivity and specificity of the test, as well as area under the curve and some other relevant characteristics, as listed in Table (4-10)

Nephrin showed an excellent capability (AUC= 0.94) to identify and predict DFU&CKD patients from those without kidney disease. In term of prior probability, the P value was found to be 0.001 with very high sensitivity and specificity values 99.6% and 83.3% respectively .Which indicates that this marker has equal roles as for confirming and excluding disease. The best cut- off point (9.4> ng/ml) derived from the ROC curve. Accordingly, a test value above 9.4 ng/ml is considered abnormal. A value of Nephrin > 9.4 ng/ml indicates that the patients in probability have CKD compared to the DM patients , as shown in figure (4 -12).

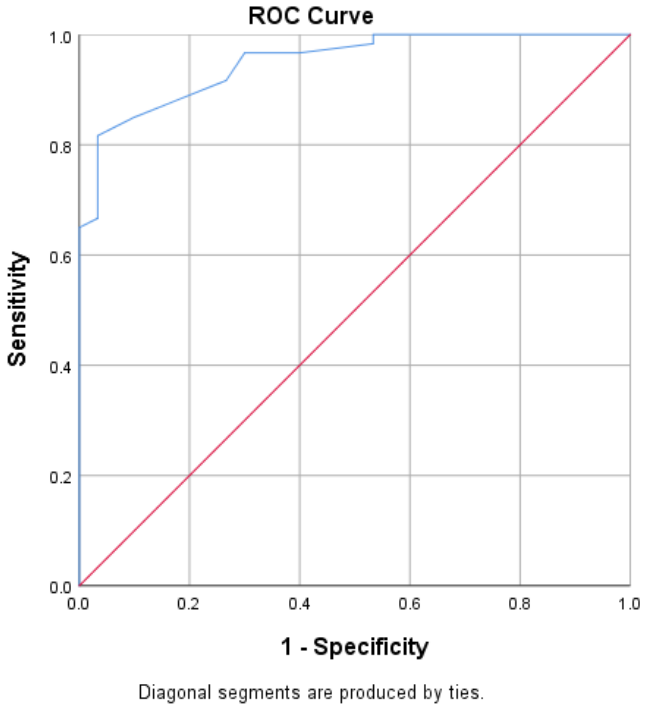


Figure 4-12: Receiver operating characteristic curve for Nephrin showing sensitivity and specificity

The ROC analysis data demonstrated that KIM-1 possesses an excellent ability to predict DFU&CKD in the diabetic group, which included groups DM, DFU,(DFU&CKD) in comparison to Control group . This result was achieved based on investigations that included the parameters of sensitivity and specificity of the test, as well as area under the curve and some other relevant characteristics, as shown in Table (4-10)

KIM-1 showed an excellent capability (AUC= 0.93) to identify and predict DFU&CKD patients from those without any disease. In term of prior probability, the P value was found to be 0.001 with very high sensitivity and specificity values 99.3 % and 93.3% respectively .Which indicates that this marker has equal roles as for confirming and excluding disease. The best cut- off point (0.66> ng/ml) derived from the ROC curve. A value of KIM-1 > 0.66 ng/ml indicates that the patients in

probability have DFU or CKD compared to the normal persons, as shown in Figure (4 -13).

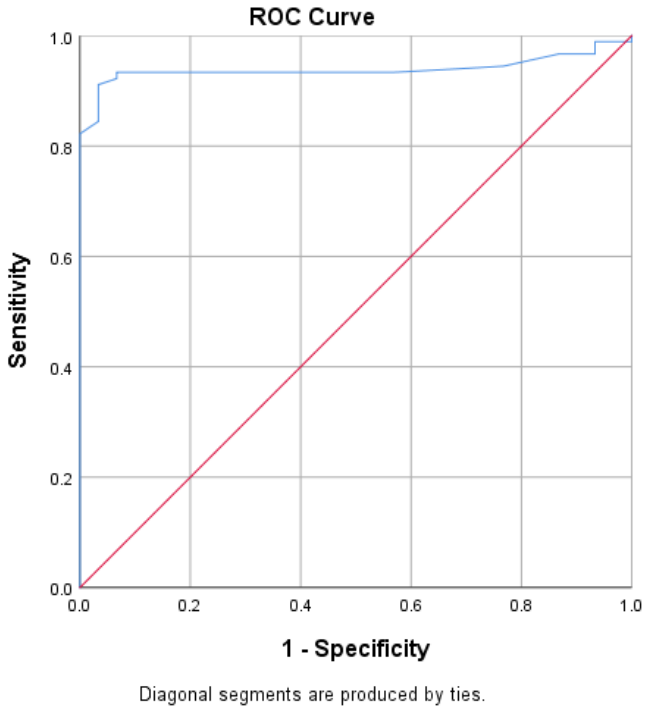


Figure 4-13: Receiver operating characteristic curve for KIM-1 showing sensitivity and specificity

The ROC analysis data demonstrated that Cystatin C possesses an excellent ability to predict DFU&CKD in the diabetic group, which included groups DM, DFU, (DFU&CKD) in comparison to Control group. This result was achieved based on investigations that included the parameters of sensitivity and specificity of the test, as well as area under the curve and some other relevant characteristics, as shown in Table (4-10)

Cystatin C showed an excellent capability (AUC= 0.98) to identify and predict DFU&CKD patients from those without any disease. In term of prior probability,

the P value was be 0.001 with very high sensitivity and specificity values 96.7 % and 99.7% respectively .Which indicates that this marker has equal roles as for confirming and excluding disease. The best cut- off point (0.71> mg/ml) derived from the ROC curve. Accordingly, a test value above 0.71 mg/ml is considered abnormal figure (4.14)

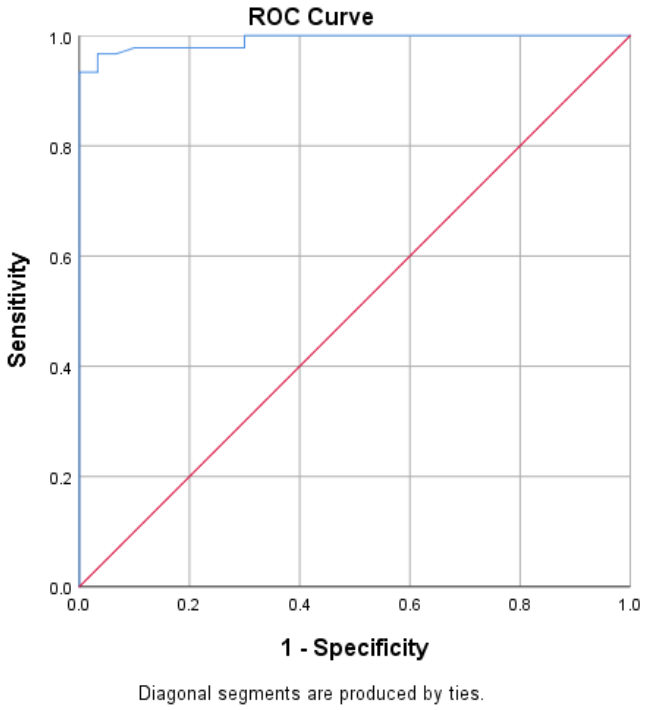


Figure 4-14: Receiver operating characteristic curve for Cystatin C showing sensitivity and specificity

Conclusion and Recommendation

1- PCT inflammatory biomarkers may be considered as a predictor of early infection without clear clinical signs in the diabetic foot patient and increase in moderate-to-severe infection. PCT may be useful for early diagnosis of systemic inflammatory response including nonseptic patients

2- Cystatin C as biomarker of kidney function and complications of diabetes .This study suggested that serum CysC level associated with the patients with diabetic foot inflammation. The measurement of CysC level was value importance for screening out the DM patients with more risk of foot disease. Therefore, serum CysC may be a useful early biomarker for DFU in DM patient

3- KIM-1 was pro-inflammatory marker that significantly increased in diabetic foot and chronic kidney disease and predictive to diabetic kidney disease

4- Positive correlation between PCT and Cystatin C ,CRP and WBC and the correlation between KIM-1 and Nephrin were positive

5- The ROC analysis show high specificity and sensitivity for PCT , Cystatin C, KIM-1, and Nephrin for predicting chronic kidney disease in patients with diabetic foot ulcer.

Recommendation

1- Study the role of PCT in in other complication of diabetes mellitus

2- Other early markers of diabetic foot such as**,** TNF , IL6 ,pentarixin 3

3- Study gene expression of KIM-1

4- Increase sample size to study sub groups (type 1 and type 2 DM)

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الخلاصة

داء السكري هو مرض ايضي يتميز بارتفاع مستوى السكر في الدم بسبب نقص الانسولين او مقاومته ومن أكثر المضاعفات التي يخشى مرضى السكري حدوثها هو التهاب القدم السكري وممكن ان تؤدي إلى بتر القدم اذا لم يتم علاجها . ارتفاع السكر في الدم المزمن وأمراض الشرايين المحيطية ، الاقفار والاضطرابات هي عوامل خطر تؤدي الى القدم السكري .عند حدوث ضرر في الجلد أو كسر في منطقة القدم تؤدي الى حوالى 34% من مرضى السكري النوع الاول والثاني الى قرحة القدم تصل الى مدى الحياة .ربما تؤدي الى مضاعفات عديدة مثل الكنكرين وبتر الاطراف فيما لو لم يتم التعرف عليه في وقت مبكر.

هدفت الدراسة الى دراسة مستويات البروكالسيتونين (Procalcitonin (PCT ،سيستاسين سي Cystatin C،نفرين Nephrin وجزيئة الضرر الكلوي kidney injury molecule-1 في مرضى القدم السكري، وكذلك دراسة خصوصيتها وحساسيتها واقل قيمه طبيعية لهذه العوامل وارتباطها مع مختلف العوامل الاخرى والمعلمات السريرية .

صممت الدراسة وفق دراسة الحالة المرضية - السيطرة. تضمنت 120 شخص ،قسمت الى أربعة مجاميع وهي :مجموعة السيطرة، مجموعة السكري بدون مضاعفات ،مجموعة المصابين بالسكر مع قرحة القدم السكري ومجموعة القدم السكري المقترن مع مرض الكلى المزمن.

تم تشخيص جميع مرضى السكري من قبل طبيب غدد الصم في مركز السكري في مستشفى مرجان التعليمي و مستشفى الحلة التعليمي في مدينة الحلة للفترة من تشرين الاول 2023 لغاية آذار 2024.

تم إبلاغ جميع المرضى في الدراسة الحالية وحصول موافقاتهم قبل عملية جمع العينات.

تم تحديد مستويات البروكالسيتونين ،سيستاسين سي ،نفرين وجزيئة الضرر الكلوي -1 باستخدام تقنية الامتصاص المناعي المرتبط بالأنزيم (الركيزة) بينما تم تحديد مستويات البروتين المتفاعل -سي باستخدام طريقة الكشف المناعي وتم تحديد سكر الدم ،يوريا الدم وكرياتنين المصل باستخدام طريقة الكشف اللوني.

أشارت نتائج الدراسة الحالية ازدياد معنوي في مستويات البروكالسيتونين ،سيستاسين سي ،نفرين ، جزيئة الضرر الكلوي ، البروتين المتفاعل-سي ،يوريا الدم وكرياتنين المصل في مرضى القدم السكري مقارنة مع عامل السيطرة ،بينما انخفض معنويا مؤشر كتلة الجسم ، معدل الترشح الكبيبي في مرضى القدم

السكري مقارنة مع عامل السيطرة.أظهرت هذه الدراسة إن البروكالسيتونين ربما يعد أداة تشخيصية للقدم السكري .بينت النتائج الحالية إن مستويات منحى المقطعي ،الحساسية ،النوعية ،نقطة القطع هي (0.96, 0.91, 0.99, 0.231) على التوالي عند المعنوية $p < 0.001$. كانت ارتباط البروكالسيتونين مع كل من السيستاتين ،البروتين المتفاعل- سي وكريات الد م البيض إيجابي

في الختام أشارت الدراسة الحالية إن مستويات البروكالسيتونين وسستاتين سي تزاداد في مرضى القدم السكري وممكن استخدامها لتشخيص الاصابة وكذلك جزيئ الضرر الكلوي -1 ،النفرين ازدادت معنويا عند مرضى القدم السكري ومرضى الكلى المزمن وكان هناك ارتباط معنوي بين القدم السكري ومرض الكلى المزمن.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

تقييم مستويات البروكالسيتونين ,سستاتين سي, جزيئي اصابة الكلى والنفرين في مرضى القدم السكري وعلاقتها ب اعتلال الكلى المزمن

الرسالة مقدمة

إلى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل

شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

(احمد مالك حسن)

بكالوريوس تقنيات تحليلات مرضية / كلية التقنيات الصحية والطبية

الجامعة التقنية الوسطى

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