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Pentosidein, C-Reactive Protein and Vitamin D in Diabetic Kidney Disease in Babylon Province .

A Thesis

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

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بسم الله الرحمن الرحيم

﴿وفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ﴾

صدق الله العلي العظيم

(76 - يوسف)

Supervisor Certification

We certify that this thesis entitled ((Pentosidein, C-Reactive Protein and Vitamin D in Diabetic Kidney Disease in Babylon Province)) was prepared by Maryam hyder under our supervision at the Department of Biochemistry, College of Medicine, University of Babylon, as a partial fulfillment of the requirements for the degree of master in Clinical Biochemistry.

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Dedication

To my ………………… mother
To my ……….…………….father
To my ………………….. husband
To my brothers and sisters
To everyone gave me support and help, especially
Prof. Dr. Wesam AL-Taher , and Bara Kalid
To my dear friends
To whom help me
To everyone I love.

Maryam Hyder Kadum

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جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

فرع الكيمياء والكيمياء الحياتية

البنتوسدين ,CRP و فيتامين دال لدى مرضى اعتلال الكلية السكري في محافظه بابل

رسالة

مقدمة إلى عمادة كلية الطب في جامعة بابل - وهي جزء من متطلبات نيل درجة الماجستير في العلوم \ الكيمياء الحياتية السريرية

من قبل

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اشراف

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List of Abbreviation

Abbreviation	Details
ATP	adenosine triphosphate (ATP
APC	Antigen-presenting cells
AUC	Area under the curve
BMI	Body Mass Index
BG	blood glucose
CNS	central nervous system
CRP	C-reactive protein
CV	cut off value
DN	diabetic nephropathy
DKD	diabetic kidney disease
ESRD	end-stage renal disease

DM	Diabetes mellitus
DR	Diabetic retinopathy (DR)
GDM	Gestational diabetes mellitus
DPN	Diabetic peripheral neuropathy
e GFR	Estimated glomerular filtration rate eGFR
FPG	Fasting plasma glucose
ROS	reactive oxygen species
IL	Interleuken
T2DM	Type 2 diabetes
PCr	phosphocreatine
PPV	positive predictive value.
Kg	Kilograms
LC	Langerhans cells
M GFR	measured glomerular rate

MHC	Major histocompatibility complex
OGTT	Oral glucose tolerance test (OGTT)
NPV	negative predictive value

Summery

Diabetic nephropathy (DN) is one of the most feared diabetic chronic microvascular complications and the major cause of end-stage renal disease (ESRD). Chronic hyperglycemia and high blood pressure are the main risk factors for the development of DN . The traditional presentation of DN is defined by hyperfiltration and albuminuria in the early stages, followed by a gradual loss in renal function.

Pentosidine is a biologically important advanced glycation endproduct, compose of pentose attached to tow amino acid lysine and arginine. It is a fluorescent protein cross-linked from human extracellular matrix, it was discovered at the end of the last century that involves lysyl and arginyl residues in an imidazo (4, 5b) pyridinium ring.

C-reactive protein (CRP) is an annular (ring-shaped) protein found in blood plasma . CRP 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. C-reactive protein (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.

Vitamin D is a fat-soluble vitamin Vitamin D has a key role in stimulating calcium absorption from the gut and promoting skeletal health, as well as many other important physiological functions. Its most important biological role is promoting enterocyte differentiation and intestinal calcium absorption, facilitating calcium absorption

Diabetic nephropathy (DN) is a progressive kidney disease caused by alterations in kidney architecture and function, and constitutes one of the leading causes of end-stage renal disease (ESRD). The aims of this study to summarize the state of the DN-biomarker field with a focus on the new strategies that enhance the sensitivity of biomarkers pentosidein and vitamin D3 to predict patients who will develop DN or are at risk of progressing to ESRD.

This study was designed as a case-sectional study . 120 individuals were involved , divided into three groups a control group diabetic without nephropathy , early stage and end stage of nephropathy , 40 patients was taken for each group identical in gender (20 male and 20 female) .

All the diabetic patients included in the current study were diagnosed by nephrologist conducted in nephrology Clinic at al-Emma al Sadeq Hospital in Hilla city and Department of Biochemistry in Collage of Medicine at University of Babylon from October 2022 to March 2023.

All participants in this study were informed before to collecting samples and verbal agreement was obtained from each of them.

Pentosidein was determined using the Enzyme-linked immunosorbent assay (ELISA) technique while C-reactive protein was determine by immunodetection Method and vitamin D 3 by chemiluminescence immunoassay, Blood glucose blood urea and serum creatinine were measured using a colorimetric methods.

The results of current study revealed a significant increase in serum pentosidine, CRP, blood glucose, blood urea and serum creatinine among diabetic nephropathy patients when compared to control and significant decrease of serum vitamin D 3, body mass index (BMI) and glomerular filtration rat in diabetic nephropathy patients than control.

1. Diabetes Mellitus

1.1 Definition

Diabetes mellitus (DM) is one of the most common chronic diseases and is the leading cause of end-stage renal disease (ESRD) . It is a metabolic disorder with insulin deficiency ,with insulin resistant or both , It is one of the most common non-communicable diseases worldwide and is in a growing concern [1].

The classic symptoms of diabetic mellitus are polyuria, polydipsia, weight loss, occasionally polyphagia and blurred vision . In most cases the disease develops progressively and the classic symptoms may remain unnoticed by the patient in the early stages of the disease . A serious long-term complication such as major microvascular complications of DM , diabetic kidney disease (DKD) or diabetic nephropathy (DN) are observed in approximately 20 – 40% of diabetic patients [2]. Ketoacidosis or a non-ketotic hyperosmolar conditions are the most serious clinical symptoms which can lead to dehydration , unconsciousness and death in the absence of adequate treatment . The prevalence of diabetes steadily increased worldwide especially in middle-income countries . By the year of 2014 there was approximately a 4-fold increase in the number of patients with diabetic since the year of 1980 , the estimation of diabetes patients has reached 425 million globally and by the year of 2045 may cross the mark of 625 million [3] . In the year of 2000 , India had topped in the number of diabetes cases followed by China and the USA , the epidemic of diabetes is estimated to be increase many more folds per year due to lack of awareness, unhealthy lifestyle and genetics [4] .

1.2. Diabetes Mellitus Classification

There are four major categories of diabetes: type 1, type 2 , gestational , secondary diabetes .

Type 1 Diabetes

Type 1 diabetes, or T1DM, is characterized by insufficient insulin secretion , it usually results from autoimmune destruction of the beta cells in the pancreas . People with type 1 diabetes need exogenous insulin they cannot make sufficient insulin to survive , they represent only 5% to 10% of all people with diabetes [5] . In the past , type 1 diabetes was called “insulin-dependent diabetes.” The American diabetes association changed the nomenclature as more patients with type 2 became dependent on insulin for glucose regulation , which was confusing to both patients and healthcare providers , so the name reverted back to “ type 1.” Type 1 diabetes has also been called juvenile diabetes because it typically appears in children and young adults . Type 1 diabetes can present as an acute illness , however , the destruction of the beta cells may have been occurring for weeks or even months prior to the acute symptoms [6] . Type 1 diabetes is rare during the first 9 months of life and it is at its highest incidence at the age of 12 years , when the patient is lacking insulin , glucose cannot enter body cells that require insulin-mediated uptake of glucose [7] . This can cause extremely high levels of blood glucose and diabetic ketoacidosis which may be life-threatening . This information suggests that a more vigorous autoimmune response may occur with type 1 diabetes in young children [8] . Type 1 diabetes is an autoimmune disease, in which cytotoxic CD8-T lymphocytes attack and destroy the pancreatic beta islands [9] . Although the precise causes of T1DM remain unknown , it is clear that both genetic and environmental factors play a role [10] . 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 action are mostly unknown [11] .

Type 2 Diabetes

Type 2 diabetes or T2DM is the most common form of diabetes and is characterized by insulin resistance or sluggish response of insulin after food consumption , Type 2 diabetes represents 90% of all people with diabetes , Insulin resistance is the reduced response of body cells to take up insulin , Type 2 diabetes is characterized by two main defects the first one is insulin resistance, in which many cells in the body become less responsive to insulin and the second one is the deterioration of beta cells in pancreas , which leads to sluggish production of insulin by it [6] . Even before the disease shows clinical signs and symptoms , mildly elevated blood glucose (BG) levels can be detected in tests at this stage , the disease is called prediabetes . The progression of type 2 diabetes is gradual , over the years , the prediabetes individuals worsens , especially if the they are over weights and inactive . Type 2 diabetes was once called “adult-onset diabetes” because the disease develops slowly and typically appears in older adults , 90% to 95% of all present cases of diabetes are type 2 , however, the age at which the condition is being diagnosed continues to lower, even including obese children , in the United States, type 2 diabetes is found in less than 2.5% of people aged 20 to 39 years , 10.5 % of people aged 40 to 59 years   and 23% of people 60 years of age or older [12]. Type 2 diabetes mellitus is a progressive disease that inevitably worsens over time, even with appropriate management and maintenance of the therapeutic regimen , 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 [13] . People with type 2 diabetes also have a progressively reduced secretion of insulin . Initially, many people with type 2 diabetes can live without additional insulin , however , the disease worsens , and many people eventually need insulin because of the duration of the disease and pancreatic fatigue . Given the rapid increase in the number of people with this condition and the increasingly younger age when the disease is diagnosed, healthcare providers need to be skilled in detection , management , education and prevention strategies in order to decrease the overall burden on health and finances to patients and their families [14].

Gestational Diabetes

Gestational diabetes mellitus (GDM) is diabetes that develops for the first time during pregnancy and is seen as persistent hyperglycemia , due to the overall stress of the pregnancy and with additional risk factors similar to those of type 2 diabetes such as obesity, sedentary lifestyle, high-fat diet, age, ethnicity and genetic predispositions , almost 21% of all pregnancies may develop hyperglycemia [15] .

Other Types of Diabetes

Types of diabetes that fall into the "other" class of diabetes mellitus include [16]

1) **MODY** which is a genetic mutation in an autosomal dominant gene that affects insulin production . Individuals with this diagnosis are generally children with a family history of diabetes for generations. These children still produce some insulin and are clinically closer to a type 2 .

2) **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 .

3) **Endocrinopathies** may include polycystic ovarian syndrome , pancreatic cancer or tumors and other hormonal disruptions in insulin production .

1. 3. Diabetes Diagnostic Criteria

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)
- 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 2 hours after the patient has ingested 75g of oral glucose . Diabetes is characterized by an OGTT > 200 mg/dl at the 2 hour time mark (11.1 mmol/l) .

Glycosylated hemoglobin (A1c) measures the saturation of hemoglobin molecules over the life of a red blood cell which is 3 months . The normal range is 4--6 mg/dL. In 2010 the American Diabetic Association adopted standards recommending the use of the A1c test to diagnose diabetes with a threshold set at 6.5% . The A1c test reflects the average glucose saturation over three months' time and is strongly predictive of diabetic complications at higher levels [17] .

1.4. Complication of Diabetes

Diabetes mellitus (DM) especially if uncontrolled over a prolonged period , is linked to the development of both macro and microvascular complications , Microvascular complications can be primarily categorized into diabetic nephropathy, diabetic retinopathy, and diabetic peripheral neuropathy , Diabetes is also accompanied by a substantial increase in atherosclerotic disease of large vessels , including cardiac , cerebral and peripheral vascular disease [18] , 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 a complication of its treatment , control of hypertension and dyslipidemia is an important step in minimizing the risk of complications [19] . Screening for kidney disease should be performed using estimated glomerular filtration rate and urine albumin measurement , The pathophysiology of diabetes complications involves several biological changes occurring within the vascular wall among those mechanisms, the accumulation of advanced glycation end-products (AGEs) due to non-enzymatic glycation seems to play a key role by promoting inflammation and endothelial dysfunction [20] .

1.4.1 Diabetic retinopathy (DR)

DR is one of the most common micro vascular complications of diabetes , it diagnosed in one-third of diabetic patients , it is the leading cause of blindness and it is an important cause of visual impairment in patients with diabetes . [28] , 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 [29] .

1.4.2 Diabetic peripheral neuropathy

Diabetic peripheral neuropathy (DPN) is a well-documented complication of DM, DPN is the most common cause of neuropathy worldwide affecting up to 50% of patients , during the clinical course patients with DPN are 10 to 20 times more likely to undergo a limb amputation than patients without DPN [22] .

Standard therapy depends on blood glucose and blood pressure control targeting A1c < 7%, and < 130/80 mmHg. Regression of albuminuria remains an important therapeutic goal , additional therapy is needed to prevent or ameliorate the condition . In addition to conventional therapies, vitamin D receptor activators, incretin-related drugs and therapies that target inflammation may also be promising for the prevention of DN progression [23] .

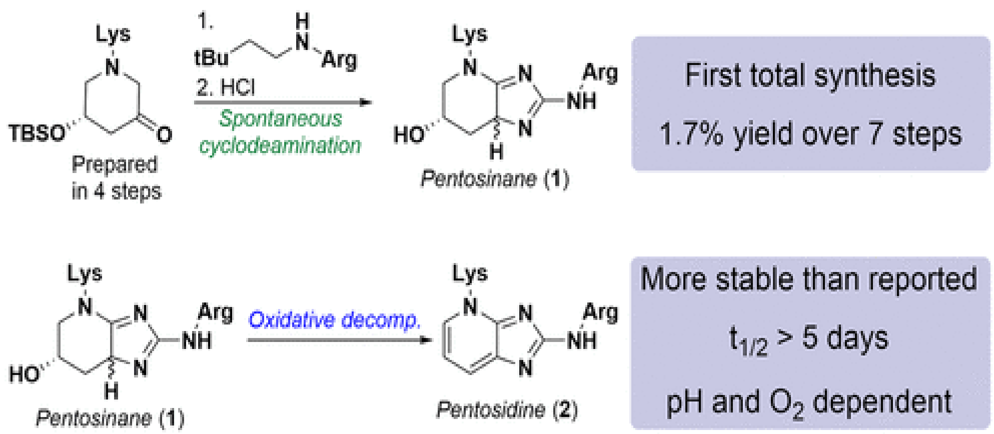
1.5. Kidney disease in patients with type 2 diabetes mellitus

Kidney disease in patients with diabetes can be a result of microvascular complications from diabetes , a concomitant kidney disease of other origin or a combination of the two , in type 1 diabetes patients, microvascular 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 the literature , while there is a whole spectrum of chronic kidney disease etiologies can cause kidney disease in type 2 diabetes patients [24] . 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 diabetes , one study from the United States which examined kidney biopsies in patients with type 2 diabetes and kidney disease found that typical diabetic microvascular 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 [25] . Interestingly, one study has found different insulin resistance phenotypes in diabetes to be associated with different risks for chronic kidney disease [26] . Regardless of kidney disease etiology, strict blood glucose control is on a group level the single-most important intervention 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: reduced hyperfiltration on the nephron level , reduced generation of toxic intermediates such as reactive oxygen species (ROS) and reduced activity in pathogenetic signaling pathways including the polyol, hexosamine , protein kinase C and advanced glycation end-product pathways , still as the chronic kidney disease progresses, GFR is reduced through nephron loss and hyperfiltration in remaining nephrons drives the process further[27]

1.6. Biochemical parameters of diabetic nephropathy of patients used in the study

1.6.1 pentosidine .

Pentosidine is a biologically important advanced glycation end product, compose of pentose attached to tow amino acid lysine and arginine [28] .



Figer (1-1) pentosidine equation

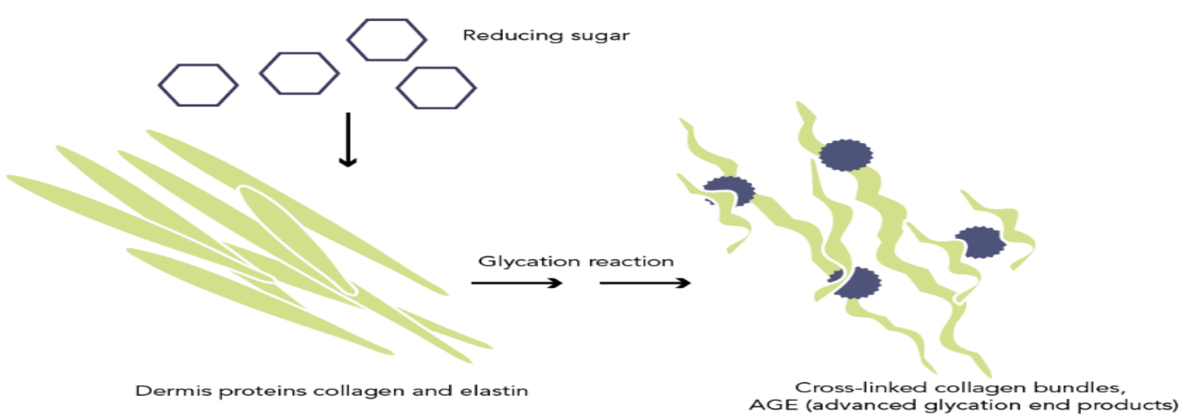


Figure (1-2) the pentosidine synthesis .

It is a fluorescent protein cross-linked from human extracellular matrix , it was discovered at the end of the last century that involves lysyl and arginyl residues in an imidazo (4, 5b) pyridinium ring . Pentosidine could be synthesized in vitro by the reaction of ribose , lysine and arginine . The potential biological significance of the molecule prompt to investigate its mechanism of formation from D-ribose and key intermediates, as well as from other potential precursor sugars [29] . This advanced glycosylation end-product and protein cross-link results from the reaction of pentose with proteins and was named pentosidine . Further work indicated that long-term glycosylation of proteins with hexoses also leads to pentosidine formation through sugar fragmentation [30] . Advanced glycation end products (AGEs) are heterogeneous compounds consisting of free amino groups of proteins, nucleic acids or lipids combined with reducing sugars . (AGEs) which are diverse compounds generated via a non-enzymatic reaction between reducing sugars and amine residues . One of the most frequently chemically characterized (AGEs) is pentosidine . AGEs are generated through normal metabolism and formation increases with aging hyperglycemia, dyslipidemia and oxidative stress [31].

1.6.1.2 Clinical role of pentosidine as an advanced glycation end product (AGE)

pentosidine is an advanced glycation end product (AGE) that marks oxidative stress caused by tissue exposure to hyperglycemia [32] . Accumulation of advanced glycation end products and inflammation also contribute to the process of diabetic nephropathies , AGEs which are accumulated in multiple tissues during aging provide information of a non-enzymatic reaction of proteins and carbohydrates that can be detected with an increased level in each related organ of patients with DM [33] . AGEs as a result of pathologically increased glycation due to chronic hyperglycemia , have a variable pathological expression in DM , kidney failure and tissue aging . Hence, AGEs can be regarded as a biomarker of aging and may lead to renal lesions in DM related to kidney aging [27] . In hyperglycemic circumstances the accumulated AGEs induce the accelerated aging of kidney dysfunction by inducing podocyte damage and apoptosis of mesangial cells and the expression of transforming growth factor-β (TGF-β) , the latter of which plays a pivotal role in fibrogenesis [34]. AGEs stimulate the activation of the receptor for AGEs (RAGE) which induces oxidative stress and cellular dysfunction . In the kidney, the RAGE-AGE activation also contributes to the induction of oxidative stress, endoplasmic reticulum stress inflammatory and fibrotic responses by activating different intracellular signaling pathways [35] .

1.6.2 Vitamin D3

Vitamin D is a fat-soluble vitamin . Cholecalciferol (vitamin D3) is mainly gain from animal sources while ergocalciferol (vitamin D2) is from plants. Cholesterol-like precursor (7-dehydrocholesterol) in skin epidermal cells can be converted after UV radiation into pre-vitamin D, which also isomerizes to vitamin D3, Both vitamin D3 and D2 are biologically inactive and need further enzymatic conversion to its active forms , they undergoes 25-hydroxylation in liver to form 25(OH)D (calcidiol), then converted in kidneys through 1-alpha-hydroxylation to their most active form , 1,25(OH)2D (calcitriol) [36]. This process is driven by parathyroid hormone (PTH) and other mediators, including hypophosphatemia and growth hormone vitamin D receptor (VDR) that is expressed in nucleated cells suggesting possible autocrine-paracrine role of that vitamin [37].

Vitamin D3 produced in the epidermis must be further metabolized to be active. The first step of 25-hydroxylation takes place primarily in the liver . 25OHD is the major circulating form of vitamin D. However, in order for vitamin D metabolites to achieve maximum biologic activity it must be further hydroxylated in the 1α position by the enzyme 1-alpha-hydroxylase . 1,25(OH)2D is the most potent metabolite of vitamin D and accounts for most of its biologic actions. The 1α hydroxylation occurs primarily in the kidney [38]. Vitamin D and its metabolites, 25OHD and 1,25(OH)2D, can also be hydroxylated in the 24 position. This may serve to activate the metabolite or analog as 1,25(OH)2D and 1,24(OH)2D have similar biologic potency and 1,24,25(OH)3D has activity approximately 1/10 than that of 1,25(OH)2D as illustrated in Fig (1-3) [39] .

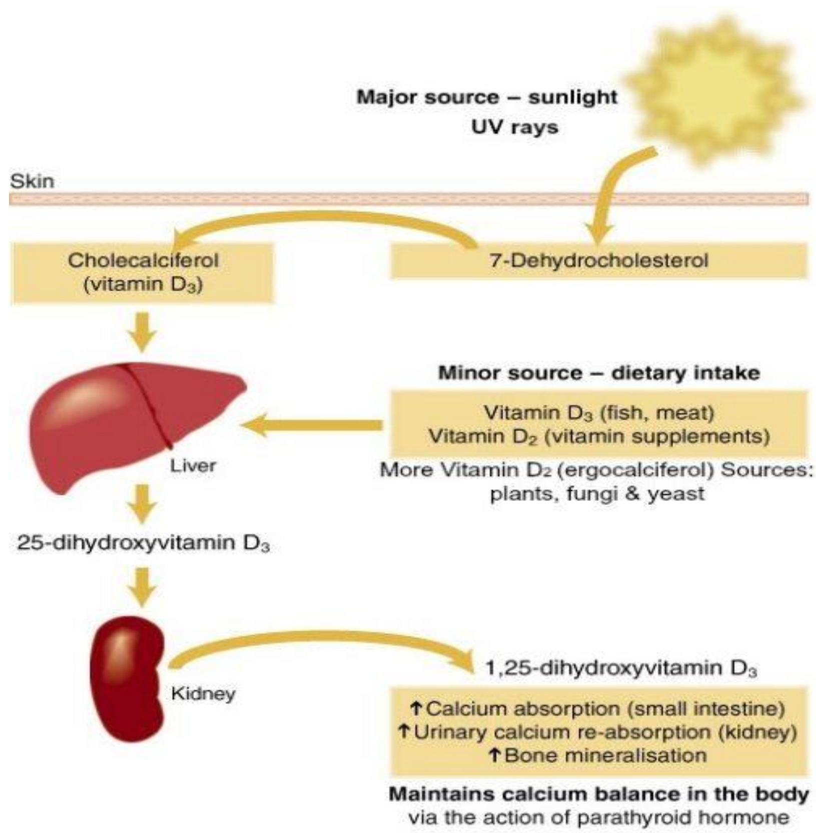


Figure (1-3) Vitamin D3 synthesis pathways in the body

1.6.2.2 Vitamin D3 interaction with receptor

The hormonal form of vitamin D (1,25(OH)2D) is the ligand for a transcription factor, the vitamin D receptor (VDR) . Most if not all effects of 1,25(OH)2D are mediated by VDR acting primarily by regulating the expression of genes whose promoters contain specific DNA sequences known as vitamin D response elements (VDREs) , there are thousands of VDREs throughout the gene, often thousands of base pairs away from the coding portion of the gene regulated [40] . However, some actions of 1,25(OH)2D are more immediate and may be mediated by a membrane bound vitamin D receptor that has been less well characterized than the nuclear VDR or by the VDR acting outside of the nucleus on the other hand , some actions of VDR do not require its ligand 1,25(OH)2D [41] .

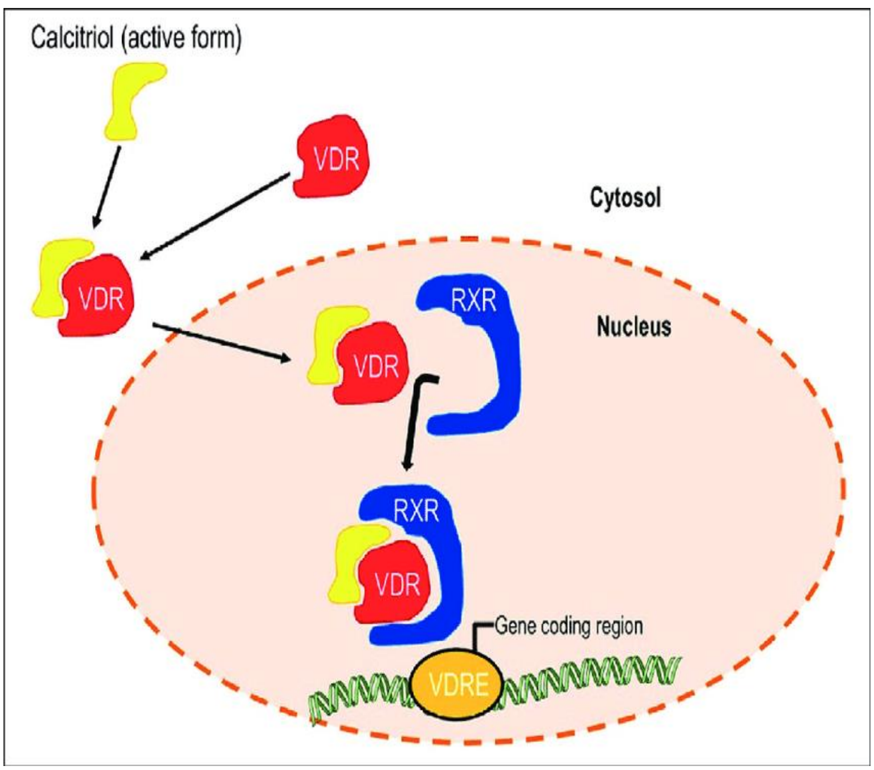


Figure **(1-4)** mechanisms of vitamin D3 actions

1.6.2.3 Biological role of vitamin D

Vitamin D has a key role in stimulating calcium absorption from the gut and promoting skeletal health , as well as many other important physiological functions, its most important biological role is promoting enterocyte differentiation and intestinal calcium absorption, facilitating calcium homeostasis [42]. Vitamin D regulates and modulates the physiology and function of multiple human systems including the skeletal muscle , the effect of vitamin D on the muscles has been widely investigated suggesting that this hormone can stimulate the proliferation and differentiation of skeletal muscle fibers, maintaining , improving muscle strength and physical performance with the capacity to modulate innate and adaptive immune function , cardiovascular function , proliferation and differentiation of both normal and malignant keratinocytes [43] . 1,25(OH)2D the biologically active form of vitamin D exerts most of its functions through the almost universally distributed nuclear vitamin D receptor (VDR) [44].

1.6.2.4 Role of vitamin D in diabetic nephropathy

Vitamin D has been suggested to harbor multiple biological activities, among them the potential of vitamin D in the protection of diabetic nephropathy (DN) has attracted special attention [45] . Both animal studies and clinical trials have documented an inverse correlation between low vitamin D levels and DN risk and on supplementation with vitamin D or its active derivatives has been demonstrated to improve endothelial cell injury, reduce proteinuria, attenuate renal fibrosis and resultantly retard DN progression [46] . Vitamin D exerts its pharmacological effects primarily via vitamin D receptor [47] . Vitamin D may reduce diabetic nephropathy not only by improving blood glucose and insulin levels, but also by modulating hexosamine pathways in kidney , calcitriol & vitamin D receptor

(VDR) signaling attenuated diabetic nephropathy and podocytes injury by restoring podocytes autophagy [48] .

1.6.3. C reactive protein

CRP 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 [49] . A phosphocholine (PCh)-binding site is located on the same face of each subunit in the homopentamer . The amino acids Phe 66 , Thr 76 and Glu 81 in CRP are critical for the formation of the PCh-binding site [50] . Once CRP is complexed with a substance with exposed PCh group, the complex activates the complement system through the classical pathway [50].

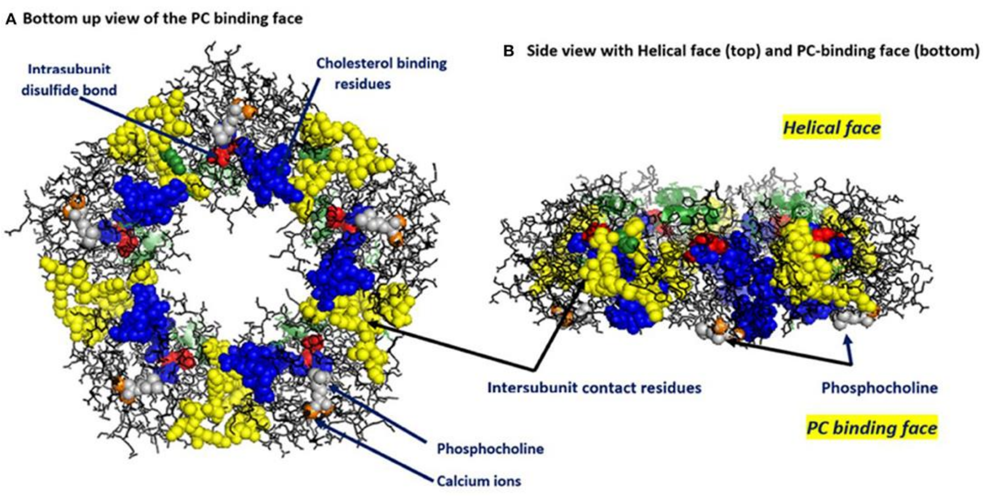


Figure (1-5) Different views of CRP with its active binding sites

1.6.3.2. Synthesis and biological role of CRP

C-reactive protein (CRP) is an annular (ring-shaped) protein 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 [51] . Human C-reactive protein (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[52] .

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 [53] . The pathogenesis of Diabetic Kidney Disease (DKD) is multifactorial where chronic inflammation and immune response are considered the major factors [54] . High-sensitivity C-reactive protein (hs-CRP) is a systemic inflammation marker that has been revealed to be correlated with DKD development . CRP is a reactant and sensitive marker in acute-phase of inflammation, and its 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 [55] .

1.7.Classical renal function tests.

The classical presentation of DN is characterized by hyperfiltration and albuminuria in the early phases which is then followed by a progressive renal function decline , The presentation of diabetic kidney disease (DKD) can vary where concomitant presence of other glomerular/tubular pathologies and severe peripheral vascular disease can become important confounders, All-cause mortality in individuals with DKD is approximately 30 times higher than that in diabetic patients without nephropathy and a great majority of patients with DKD will die from cardiovascular disease before they reach ESRD [56] .

The management of metabolic and hemodynamic perturbations for prevention and delay of progression of DKD is very important [57] .There is a progressive increase in the blood urea and serum creatinine with renal disease in diabetes , even higher blood urea > 50 mg/dL has been linked to increased incidence of diabetes mellitus [58] .The skeletal muscle has a fixed mass per unit of creatine and the breakdown rate of creatine to creatinine is also consistent which is the sole reason of the constant concentration of plasma creatinine throughout and is also a direct indicative of the skeletal muscle mass [59] .

Blood urea being a small non protein bound molecule is generally freely filtered at the glomerulus of the nephron , but because of its reabsorption in the tubules of the nephrons it is not a sensitive marker of GFR [60] . Rise in blood urea usually relates to the symptoms of the uremia as compared to creatinine whose concentration remains mostly constant and undergoes tubular secretion too , hence serum creatinine is considered a sensitive marker of the renal involvement in diabetes [61] .

1.7.1. Blood urea

Urea is the principal nitrogenous waste product of metabolism and is generated from protein breakdown , It is eliminated from the body almost exclusively by the kidneys in urine and measurement of its concentration first in urine and later in blood had clinical application in the assessment of renal function for over 150 years [62] . Urea is a small organic molecule (MW 60) comprising two amino (NH2) groups a linked with carbamyl (C=O) group . Proteins are first degraded to constituent amino acids which are in turn degraded (deaminated) with production of ammonia (NH3) which is toxic . In a series of five enzymatically controlled reactions known collectively as the “urea cycle” toxic ammonia resulting from protein breakdown is converted into non-toxic urea [63] .

The urea cycle begins in the mitochondria with the transfer of ammonia from either glutamate or glutamine to a phosphorylated molecule of bicarbonate by the enzyme carbamoyl phosphate synthetase 1 creating carbamoyl phosphate [64]. Carbamoyl phosphate then reacts with ornithine to form citrulline via the action of ornithine transcarbamylase and also in the mitochondria [65] . Citrulline is transported out of the mitochondria and into the cytoplasm via the ornithine-citrulline transporter where it reacts with aspartate to form argininosuccinate via the enzyme argininosuccinate synthetase , in turn argininosuccinate is broken down to arginine and fumarate via the action of argininosuccinate lyase , fumarate is then free to join the citric acid cycle while arginine is degraded to urea and ornithine via the arginase enzyme , ornithine is then transported back into the mitochondria via the ornithine-citrulline transporter, where the cycle repeated again [66].

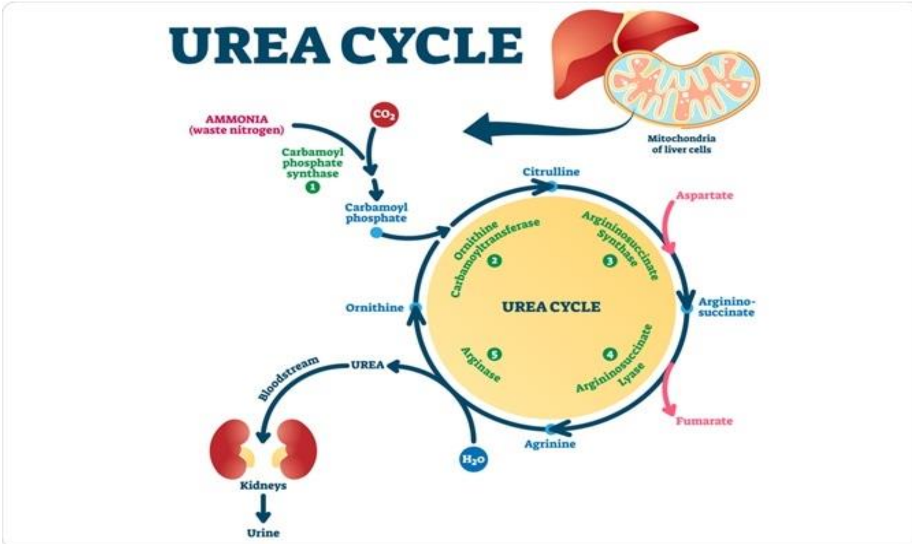


Figure (1-6) illustrating urea cycle in the body

1.7.2.Creatinine

Creatinine is the end product of creatine and creatine phosphate metabolism, it is a nitrogenous organic acid that is generated predominantly in kidney and liver and to some extent in pancreas using three amino acids glycine, arginine and methionine . This process consumes up to 10% of daily intake of glycine, 22% of arginine and 42% of methionine , serum creatinine is most widely used as a measurement of kidney function [67] . It has a complex metabolic pathway and due to multiple physiologic and technological issues related to its measurement the interpretation of serum creatinine as a marker of kidney function warrants some caution particularly in acute settings or among patients with liver disease or low muscle mass [68] .

Formation of creatinine begins with an aminotransferase reaction between l-arginine and glycine with the generation of Creatine phosphate or phosphocreatine (PCr) formed by phosphorylation of creatine in peripheral tissues in an enzymatic reaction using creatine kinase , Its basic role is to provide energy for cell activity and it acts together with creatine and creatine kinase as a cellular energy buffer [69]. It is considered that the PCr/creatine system is crucial for the synthesis of adenosine triphosphate (ATP) which is particularly important for tissues with high metabolic demands such as brain, skeletal muscles and heart .

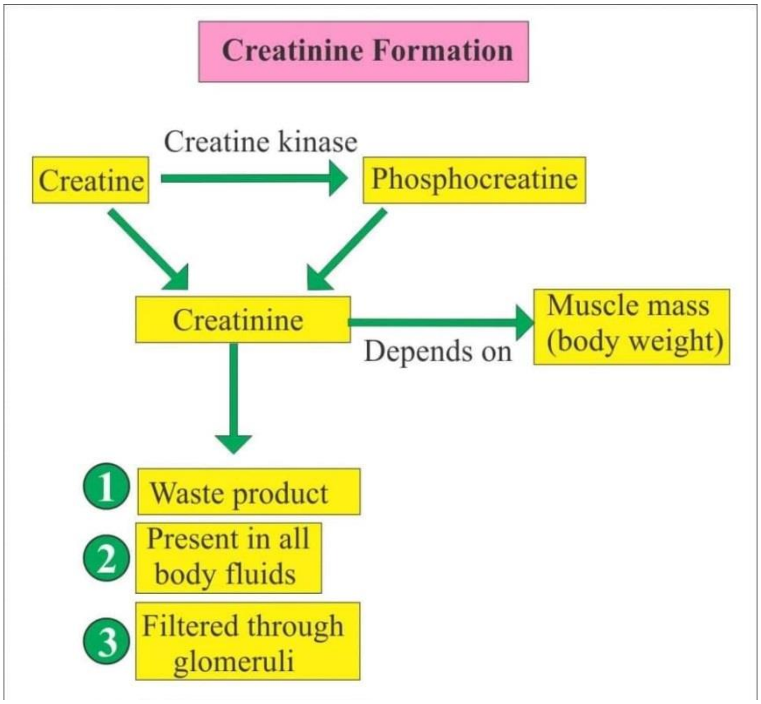


Figure (1-7)illustrating creatinine formation and excretion cycle in the body

1.7.3. Estimated glomerular filtration rate eGFR

Since 1957 over 70 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 [63] . 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 [70] . 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 [71] .

Evaluation of glomerular filtration rate (GFR) 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 (eGFR) were seen , both eGFR and mGFR are associated with some error compared with the true GFR [57] . 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 [72].

Aims of study

The current study aimed to evaluate a new Diabetic nephropathy biomarkers pentosidein , CRP and vitamin D3 to predict the patients with Diabetic nephropathy . To achieve this aim it should do the flowing :

1- Study the role of pentosidine in patients with diabetic nephropathy .
2- Estimate the level of vitamin D in patients sera with diabetic nephropathy and to determine the correlation between them .
3- Study the sensitivity and specificity of pentosidine vs CRP and eGFR in patients sera with diabetic nephropathy .
4- Study the severity of the disease on body weight through calculating the BMI for diabetic patients without complication , early stage diabetic nephropathy and end stage diabetic nephropathy .
5- Diabetic nephropathy (DN) is a progressive kidney disease caused by alterations in kidney architecture and function, and constitutes one of the leading causes of end-stage renal disease (ESRD) revealing biochemical changes in many critical parameters in the body . The study aim to investigate the differences of some of these important biochemical changes in the two groups (diabetic early and end stages of nephropathy) in comparison to normal control and to summarize the state of the studied DN-biomarker in this field to predict patients who will develop DN or are at risk of progressing to ESRD.

2. Materials and Methods

2.1 Materials

2.1.1. Chemicals and Kits

The chemicals and kits that used in this study were used just as the same as they arrived from the suppliers , without any further purification or modification .

Table 2-1 kits and compounds used in this study.

NO.	CHEMICALS	COMPANY / Manufactured Country
1	Creatinine	Biolabo \France
2	CRP	Boditech Med Incorporated \ Korea
3	Glucose	Ccrom\France
4	Pentosidin	Bioassay Technology Laboratory / china
5	Urea	Biolabo \France
6	Vit.D3	Shenzhen new industries biomedical engineering\ china

2.1.2. Instruments and Equipment's

The instruments and Equipment's used in this study are listed below .

Table 2 – 2 ; instruments and Equipment's used and their manufacturing companies

NO.	Instruments & Equipment	Company \ Country
1	AFIAS-6	Boditech Med Incorporated \ Korea
2	Centrifuge	Kokusan \ Japan
3	Deep Freezer	Samsung \Korea
4	Disposable cuvette	China
5	Disposable syringe (5 ml)	China
6	Elisa washer, reader and printer	Biotek \USA
7	Eppindorf tube (0.5 ml)	China
8	Kan tube	China
9	Micropipettes (5-50µl),(100-1000µl)	Slamed \ Germany
10	Maglumi 800	Shenzhen new industries biomedical engineering\ china
11	pipette tips with different sizes	China
12	Refrigerator	Agur \Turkish
13	Test tube with Separating gel	AFCO \ Jordan
14	Water Bath	HH-2 Chain

2.2 Study settings

2.2.1. Study population and designs

This study was approved on patients be present at Imam Sadiq Hospital in Babylon province in Hilla city. All samples were collected from the 1st of September 2022 till the 1st of January 2023. The practical part of the study was performed at the laboratory of biochemistry department in the faculty of Medicine / University of Babylon and in authorized private laboratories . Patients were diagnosed by the aforementioned nephrologist clinics depending on measuring renal diagnostic parameters such as blood urea, serum creatinine, proteinuria , and e GFR.

120 patients were selected and divided into three groups:

1) A control group of 40 diabetic mellitus patients (20 male and 20 female) with age range (30-65) years who were receiving insulin treatment with normal renal functions and without any complications (group 1) .
2) An 40 early stage nephropathy diabetic mellitus patients (20 male and 20 female) with age range (30-65) years who were receiving insulin treatment along with a renal drug regarded as group (2).
3) An 40 end stage nephropathy diabetic mellitus patients (20 male and 20 female) with age range (30 – 65) years who were receiving insulin prescribed for dialysis prior to the first kidney wash regarding them as group (3)

The study was designed as a case-sectional study . The sample size was determined according to Fisher's exact test for sample size . Subjects with, pregnancy, congestive heart failure, systemic lupus erythematous, and polycystic kidney disease were excluded from the study .

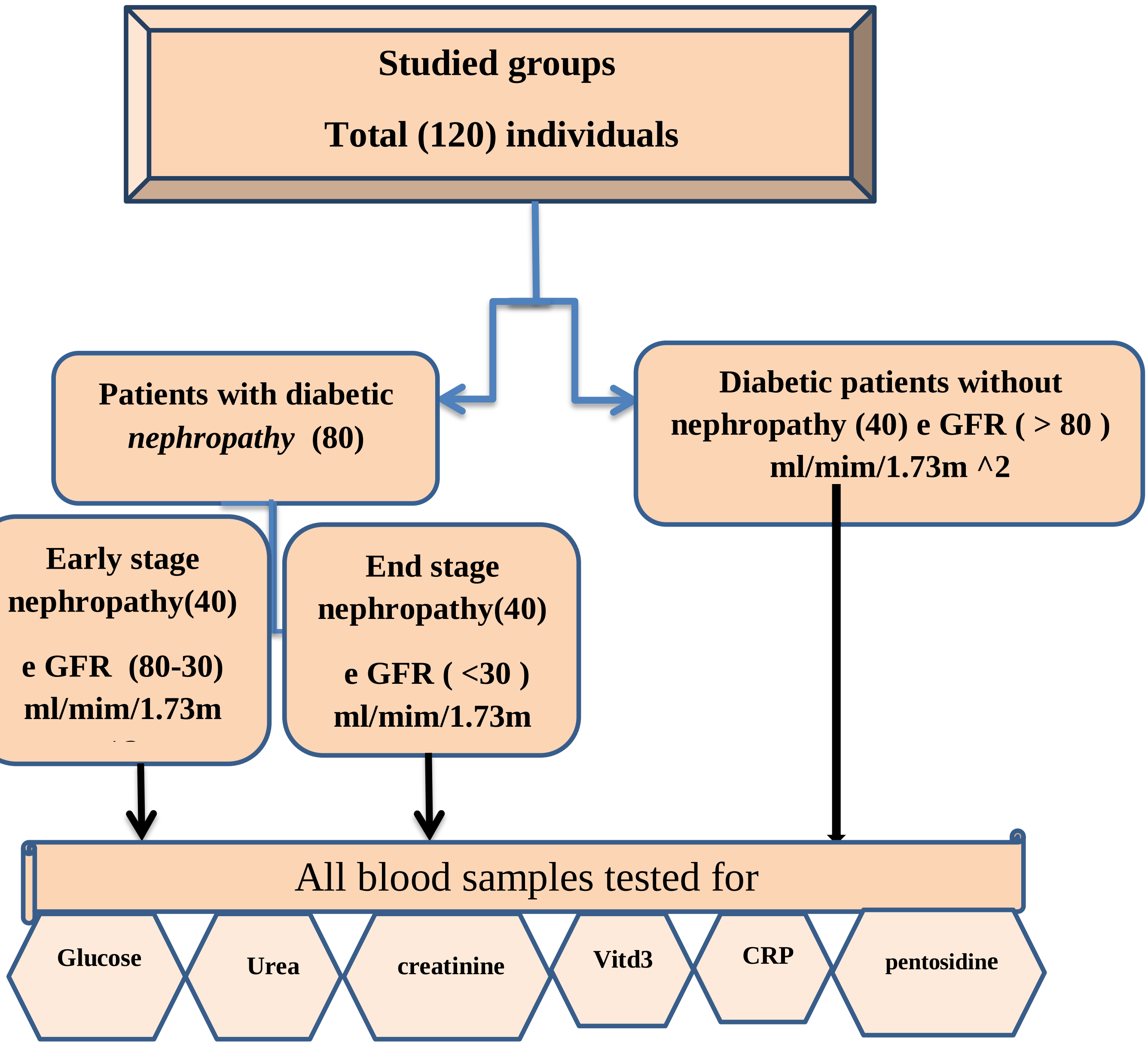


Figure (2-1)study flow chart

2.3. Methods

2.3.1 determination of serum pentosidine (PTSD) level

In this assay we used the quantitative sandwich enzyme linked immunosorbent assay technique (ELIZA). Antibody specific for Pentosidine has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Pentosidine present is bound by the immobilized antibody. After removing any unbounded substances, a biotin-conjugated antibody specific for Pentosidine is added , after washing , avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbounded avidin-enzyme reagent, a substrate solution is added and color develops in proportion to the amount of Pentosidine bound in the initial step. The color development is stopped and the intensity of color is measured using MR 96 Mindray microplate reader at 450 nm.

Preparation for pentosidine determination .

1) The standard solution ;

The standard vial was Centrifuged at 6000-10000rpm for 30s, reconstituted with 1.0 ml of Sample Diluent. This reconstitution produces a stock solution of 2000 pmol/ml , the standard mixed well and allowed to settle for a minimum of 15 minutes with gentle agitation prior to making dilutions . 250 µl of Sample Diluent was Pipetted into each tube (S0-S6) , the stock solution be Used to produce a 2-fold dilution series (below) , the undiluted Standard serves as the high standard (2000 pmol/ml). Sample Diluent serves as the zero standard (0 pmol/ml).

Tube	S7	S6	S5	S4	S3	S2	S1	S0
pmol/ml	2000	1000	500	250	125	62.5	31.250	0

1. 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 .

2. Volume of 100µl of standard and sample were added to wells and incubated for 2 hour at 37ºc

3. standard and sample were removed without washing .

4. volume of 100µl biotin-anti-pentosidine

Antibody was added to each well and Incubated at 37c for 60 minutes .
5.the wells content was aspirated and washing the plate 3 times .

6. 100µl of avidin-HRP was added to sample and standared wells. Mixed well . the plate covered with sealer and Incubated at 37c for 60 mintes .
7. the wells content was aspirated and washing the plate 5 times .

8. A 90µl of TMB substrate solution was added to each well and the plate incubated 30 minutes at 37 c in the dark .

9. A 50µl stop solution was added to each well and the blue color will change to

yellow .

10.within 10 minutes after adding stop solution ,the optical density of the wells was done using a micro plate reader set to 450 nm .

.

Figer (2-2) MR-96 A Mindray Microplate and Reader used for pentosidine determination in patients sera .

2.3.2 Determination of serum 25-OH Vitamin D

Principle of the test

The quantitative determination of total 25-OH vitamin D in human serum is a competitive chemiluminescence immunoassay and is of a two-incubation steps ,
ln the first incubation, the 25-OH vitamin D dissociate from its binding protein by displacing reagent and binds to the 25-OH vitamin D antibody on the magnetic microbeads forming an antibody-antigen complex Following a second incubation, the 25OH Vitamin D labeled ABEI are added. The rest unbounded material is removed during a wash cycle , subsequently, the starter 1+2 are added to initiate a flash chemiluminescent reaction. The resulting chemiluminescent reaction is measured as a relative light unit (RLUs), which inversely proportional to the concentration of 25-OH Vitamin D present in the sample (or calibrator/control, if applicable).

Components plates used enough for 100 tests

Magnetic Microbeads	Magnetic microbeads coated with 25-OH Vitamin
monoclonal antibody,	containing BSA, NaNe(<0.1%).2.5 mL 2.0 mL
Calibrator Low	Containing BSA and 25OH Vrtamin D antigen, NaN3(<0.1%). 3.0 mL 2.0 mL
Calibrator High	Containing BSA and 25OH Vitamin D antigen, NaN3(<0.1%). 3.0 mL 2.0 mL
Displacing Reagent	Acidic buffer 6.5 mL 4.0 ml
ABEI Label	25-OH Vitamin D antigen labeled with ABEI 12.5 mL 7.0 mL
Internal Quality Control	Containing BSA and 25OH Vitamin D antigen,NaN3(<0.1%). 2.0 mL 2.0 mL

Vitamin D status

Normal: 25-OH Vitamin D concentration 30-100 ng/ml (75-250 nmol/L)

Calculation of Results

The analyzer automatically calculates the 25-OH Vitamin D concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml

Figer(2-3) Maglumi 800 used for vitamin D3 determination in patients sera .

2.3.3. C-Reactive protein assessment

Principle of the test

The test uses a sandwich immune-detection method; 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 , an AFIAS instrument was used to determine CRP concentration in samples.

Test procedure

General Method (with pipette tip) ; 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 terms of mg/L.

1. The cut-off (reference value) of the kit that was used is : 10 mg/L

2. The working range of the AFIAS CRP is 0.5-200 mg/L

Steps of the test ;

1) 100 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) a tip be Inserted into the tip hole of the cartridge.

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

5) Tap the 'START' icon on the screen.

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

Figr (2-4) AFIAS - 6 this instrument used for CRP titer determination in patients sera

2.3.4.Determination of random blood glucose

Principle of the test ; Glucose oxidase catalyzed the oxidation of glucose to gluconic acid and hydrogen peroxidase . The formed hydrogen peroxide is detected by a chromogenic oxygen acceptor, a phenol and 4-aminophenazone (4-AP) in the presence of peroxidase to form a colored product. The intensity of the color formed is proportional to the original glucose concentration in the sample.

Glucose $+O_2 + H_2O$ $GDp\rightarrow$ H_2O + glucose

$2H_2O$ + phenol + 4- AP

$POD \rightarrow 4H_2O$ + Qoinonimine

GOD: Glucose Oxidase Enzyme , POD: Peroxidase Enzyme

2.3.5. Determination of Urea

Principle of the test ; Enzymatic and colorimetric method based on the specific action of urease which hydrolyses urea in to ammonium ions and carbon dioxide ammonium ions then form with chloride and salicylate a blue-green Complex , the absorbance of this complex was read at 600 nm against blank.

Assay procedure:

	Blank	Standard	Sample
Working Reagent (R1+R2)	1ml	1ml	1ml
Standard	-	5µl	-
Sample	-	-	5µl
Demineralized water	5µl	-	-
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C			
Base (vial R3) diluted ¼	1ml	1ml	1ml
Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm against blank.			

Calculations

$$\text{Urea (mg/dl)} = \frac{A\ Sample}{A\ Standard} * 40 \text{ (con. of stander)} \quad , \quad \text{(A is absorbance)}$$

The reference values of serum urea concentration according to this procedure was N.V. = 13 - 43 mg/dL (2.1 - 7.1 mmol/L) .

2.3.6. Determination of Creatinine

Principle of the test

Creatinine in a basic picrate solution forms a colored complex. The extinction at predetermined times during conversion is proportional to the concentration of creatinine in the sample

Assay procedure

	Blank	Standard	Sample
Standard		100 μl	
Sample			100 μl
Working Reagent (R1+R2)	1ml	1ml	1ml
Mix well. After 30 seconds, record absorbance A1 at 492 nm. Exactly 1 min after the first reading, record absorbance A2.			

Calculations

Creatinine (mg/dl) = $\frac{(A2-A1)\ Assay}{(A2-A1)\ Standard} * 2$ (con. of stander)

The reference values of serum Creatinine concentration according to this procedure was 0. 7-1.4 mg/dl (61.8– 132.6)mmol/L

2.3.7. Estimation of GFR

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

eGFR (mL/min/1.73 m2) = 175× (Scr)-1.154 × (Age)-0.203 × (0.742 if female) [73].

Statistical analysis

The data was analyzed by IBM SPSS for Windows, Version 22.0. One-way analysis of variance (ANOVA) was used for determining whether the mean variations among the three different analyzed groups are statistically significant. ROC curve analysis was used also in this study to estimate the strength of each marker to be useful in diagnosis of the disease .

3. Results and Discussion.

3.1. Demographic and Clinical Characteristics of The Study Group

3.1.1 Distribution of The Study Groups According to Gender

The early stage and the end stage of diabetic nephropathy patients included in the current study were classified according to gender into 20 (50%) males and 20 (50%) females for each group with a total number of patients of 80, also a Diabetic individuals without nephropathy regarded as controls were chosen at the same ratio of 20 (50%) normal renal function males and females for each as shown in Figure (3-1) .

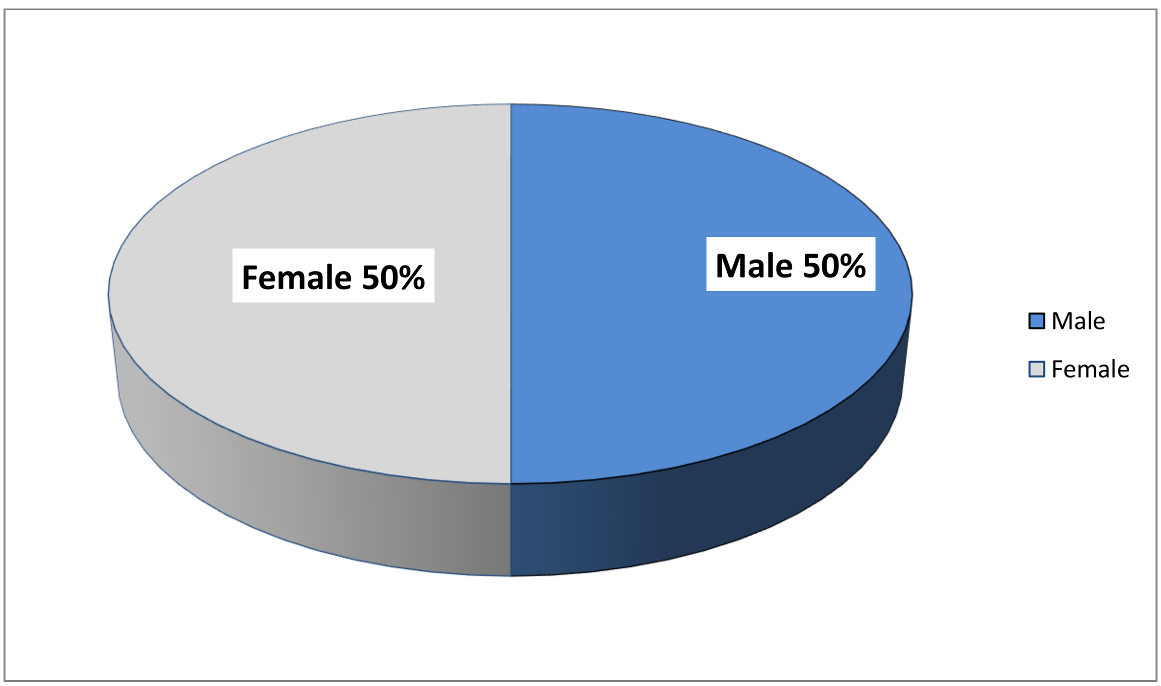


Figure (3-1) Gender distribution for all studied groups .

3.1.2. Body Mass Index (BMI) of The Participants.

The results of the present study revealed a Mean ± SD for BMI of early stage of diabetic nephropathy patients (24.08 ±0.42) Kg/M2 and a Mean ± SD BMI of end stage diabetic nephropathy (21.75 ±0.24) Kg/M2 , while the Mean ± SD was (24.01 ±0.64) in diabetic people of control group . There were a significant higher level (p value ≤ 0.05) in the BMI among end stage diabetic nephropathy patients group when compared to both early stage DN patients and control groups while the value was nonsignificant when comparing early DN with diabetic control group , as illustrated in Table (3 - 1) .

Table (3-1) showing mean and P values of the three tested groups

Parameter	Group	No	Mean ± SD	(P-value)
BMI (kg/m2)	Early stage	40	24.08 ±0.42	0.879 NS
	Control	40	24.01 ±0.64	
	End stage	40	21.75 ±0.24	0.0001 **
	control	40	24.01 ±0.64	
	Early stage	40	24.08 ±0.42	0.0001 **
	End stage	40	21.75 ±0.24	
	** (P≤0.01), NS: Non-Significant.			

Diabetics Patients in the early and end stages of nephropathy exhibited lower BMIs than diabetics with adequate renal function in the control group. As seen in Table (3-1) .

Insulin resistance, inflammation, oxidative stress and advanced glycation end-products (AGEs) are possible contributing factors of accelerates decrease in muscle mass and strength due to insulin resistance, AGEs, inflammation and diabetic complications [63] People with diabetic nephropathy may have damaged nephrons and develop proteinuria , loss of protein in urine has a negative effects on body weight. According to O'Sullivan, Lawson *et. al.*, patients with a relatively mild degree of chronic renal insufficiency are characterized by decreased lean body mass, bone mineral content, and basal energy expenditure[74] , 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 [75] . this study agree with Carrero *et al*. in that while muscular mass and muscle function are negatively affected by a variety of conditions inherent to chronic kidney disease (CKD) and to dialysis treatment .

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3.1.3. Age in The Patients and Control Groups .

Age in the patients and control groups .

The studied cases were separated into three groups: diabetic patients with early stage nephropathy (e GFR 80-30 ml/mim/1.73m ^2) ranging in age from 30 to 60 years with a Mean ± SE (52.36 ± 1.08) . DM patients with end stage nephropathy (e GFR less than 30 ml/mim/1.73m ^2) with age from 30 to 60 years with a Mean ± SD (59.40 ± 0.94) and DM patients with normal renal functions (e GFR more than 80 ml/mim/1.73m ^2) with a Mean of ± SD (47.94 ± 1.01) , as shown in Figure (3-2).

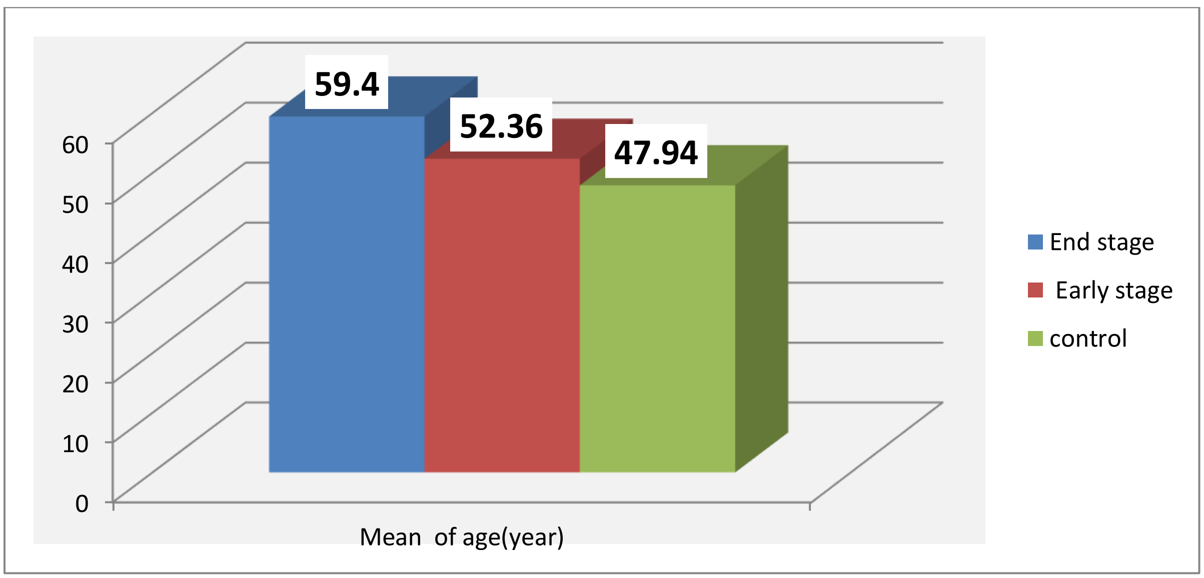


Figure (3 - 2) Mean of age distribution among different groups

the diabetics complication progress when disease duration increases . That supports the assertion made by Baek *et al*. that Renal function diminishes with disease duration in persons with DM diagnosed as a child or adolescent , DM diagnosed at an early age is a risk factor for DN [76] .

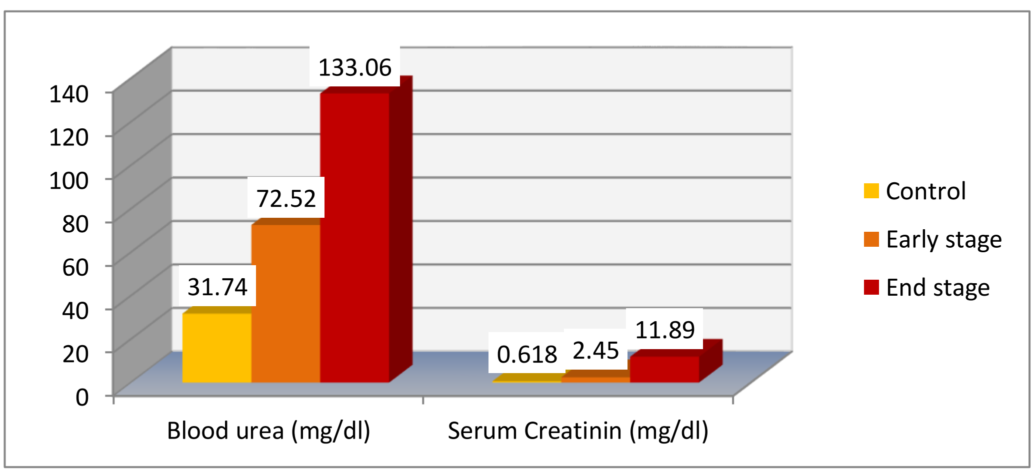
3.2. Biochemical Parameters

3.2.1. Blood Urea and Serum Creatinine Among Diabetic Nephropathies Groups and Control Group

The current results showed a significant rise in urea and creatinine levels, P-value ≤ 0.001 were reported in the diabetic nephropathies groups for both end and early stages when compared to diabetic non nephropathy individuals with normal renal function as a control groups Figure (3-3), Table (3-2). The purpose of this step in our study is to check the pattern of blood urea , serum creatinine and e GFR

Diabetic nephropathy and diabetic without nephropathy to relate both markers with various gradations of Diabetes for identification and prognosis of Diabetic Nephropathy. Serum creatinine and blood urea are two essential parameters that are used in the diagnosis and treatment of kidney diseases, adjustment of drug dosages, and decision-making regarding when to initiate renal replacement therapy

[77]



Fifer (3-3) Blood urea and creatinine among different groups

Table (3-2) Comparison between difference groups in Blood urea and Creatinine

Group		Mean ± SD	
	no	Blood urea (mg/dl)	Serum Creatinin (mg/dl)
End	40	133.06 ±10.02	11.89 ±0.42
Early	40	72.52 ±2.16	2.45 ±0.11
T-test (P-value)		**20.352 ** (0.0001)**	**0.862 ** (0.0001)**
End	40	133.06 ±10.02	11.89 ±0.42
Control	40	31.74 ±0.63	0.618 ±0.02
T-test (P-value)		**19.936 ** (0.0001)**	**0.834 ** (0.0001)**
Early	40	72.52 ±2.16	2.45 ±0.11
Control	40	31.74 ±0.63	0.618 ±0.02
T-test (P-value)		**4.473 ** 0.0001**	**0.228 ** 0.0001**
	** (P≤0.01).		

The principle of the effects of hyperglycemia on the renal cells in DN 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 [78] . Biochemical alterations of glomerular BM consist of an increased non-enzymatic glycosylation of collagen leading to un physiological crosslinking , this , in turn, may result in alteration of the size selective properties of the glomerular filtration unit [79] .

These findings revel that there is 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 Bauza who found that hyperglycemia is one of the major causes of progressive renal damage [80]. 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 Anjaneyulu *et al*. had found that increased urea and serum creatinine in diabetic rats indicates progressive renal damage[81] .

3.2.2 Fasting Blood Sugar and Estimated GFR Among Diabetic Nephropathies Groups and Control Group

Results revealed that there was a significant higher level ($P < 0.05$) in serum concentration of fasting blood sugar (FBS) among the diabetic nephropathy patient groups when compared with diabetic patient as control group. The results also revealed a significant higher serum concentration of FBS among end stage diabetic nephropathy patients when compared with early stage diabetic nephropathy group . Diabetes affects the kidney in stages or intervals , at the onset of diabetes the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed , most recent basic and clinical research had pointed toward sclerosis and kidney failure [82] . When DN early and end stages patients be compared to non-nephropathy diabetic individuals control group we noticed that their eGFR values declined significantly as seen in Figure (3 - 4) .

the increase glucose levels are known to cause hyper filtration (GFR above normal) of the kidney , and as glucose levels were lowered , the kidney's function of filtering materials goes back to the normal rate [60] .

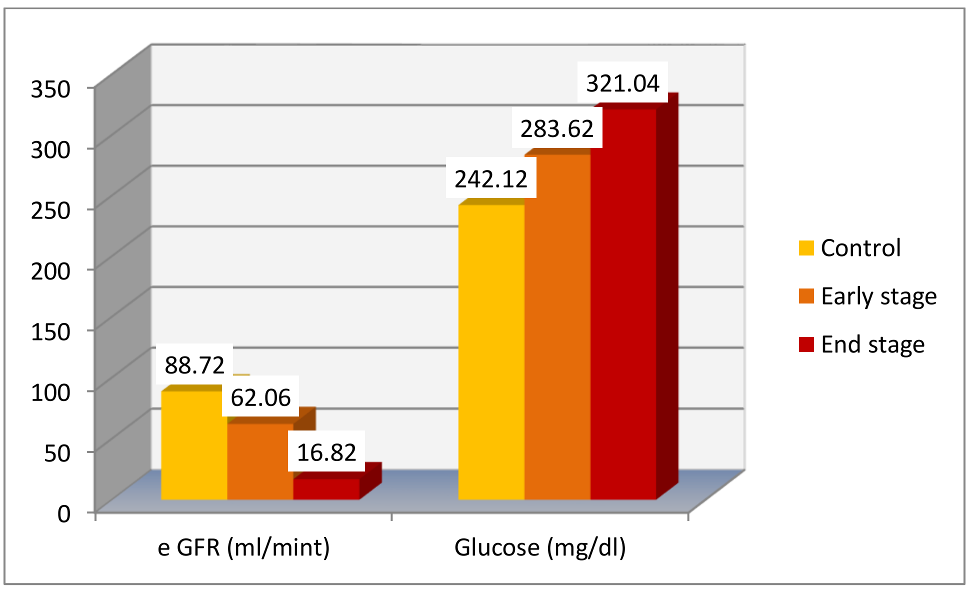


Figure (3-4) FBS and e GFR among early and end stages of diabetic nephropathy patients with control groups .

Table (3-3): Comparison between difference groups in e GFR and Glucose

Group		Mean ± SD	
	No	e GFR (ml/mint)	Glucose (mg/dl)
End	40	16.82 ±1.02	321.04 ±9.95
Early	40	62.06 ±1.69	283.62 ±7.81
T-test (P-value)		**3.917 ** (0.0001)**	**25.112 ** (0.0039)**
End	40	16.82 ±1.02	321.04 ±9.95
Control	40	88.72 ±0.64	242.12 ±6.13
T-test (P-value)		**2.387 ** (0.0001)**	**23.195 ** (0.0001)**
Early	40	62.06 ±1.69	283.62 ±7.81
Control	40	88.72 ±0.64	242.12 ±6.13
T-test (P-value)		**3.586 ** 0.0001**	**19.714 ** 0.0001**
	** (P≤0.01).		

The pathogenesis of DN is complex and involves a number of mechanisms , Hyperglycemia is the corner stone in the pathogenesis of DN via different pathways , Hyperglycemia promotes the shunt of glucose metabolism toward non-glycolytic mechanisms such as the polyol pathway , The polyol pathway involves two enzymatic reactions: the first is the reduction of glucose to sorbitol by the action of aldose reductase and the second oxidation of sorbitol to fructose by the action of sorbitol dehydrogenase, the second product of polyolpathway is increased several fold in tissues with an activated polyol pathway and can contribute to non enzymic fructosylation of proteins ,that produce the precursor to advanced glycation end products. After formation of polyol pathway prodect , which leads to increase production of reactive oxygen species (ROS) the related oxidative stress increases local and systemic inflammation , moreover , oxidative stress is responsible for both direct and indirect damage of kidney cells (podocytes , mesangial , and endothelial cells) these cells are crucial for the modulation of glomerular and filtration glomerular capillary structure [78] .

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 end stage renal disease and that's what we got in our study in somehow .

3.2.3 Serum Pentosidine levels Among Studied Groups

Results of the present study revealed that Pentosidine level is significantly increased in patients groups as compared to control group , there was a significant higher level ($P < 0.05$) in serum concentrations of pentosidine among the early stage diabetic nephropathy patients when compared to DM as control group the mean and SD are as follows (192.58 ± 6.21, 128.21 ± 2.70) . The results also revealed a significant higher serum concentration of pentosidine among end stage DN patients when compared to DM patients as a control group , the mean and SD are (303.41 ± 6.76 , 128.21 ± 2.70) respectively as shown in Table (3-4) . Accordingly it may play a significant role that leads to diabetic nephropathy progression and it may affect renal function in general , Thus, it may be employed as a good marker for the prediction of this disease.

Table (3-4) Serum pentosidine mean and P values among studied groups .

Parameters	Group	N0	Mean ± SD	(P-value)
Pentosidine (ng/ml)	Early stage	40	192.58 ± 6.21	0.0001 **
	Control	40	128.21 ± 2.70	
	End stage	40	303.41 ± 6.76	0.0001 **
	Control	40	128.21 ± 2.70	
	Early stage	40	192.58 ± 6.21	0.0001 **
	End stage	40	303.41 ± 6.76	
	** ($P \leq 0.01$)			

Patients with diabetic nephropathy experience a progressive and usually inexorable decline in renal function . Pentosidine forms through the Maillard reaction and some studies have shown that the kidney plays a key role in removing circulating AGEs, both clearing AGE peptides and metabolizing AGE proteins by the endolysosomal apparatus of the proximal tubule [23]. . Bansal et al thought that kidney has high levels of the enzyme 3- deoxyglucosone reductase, and they beleved that is to prevent protein glycation in the presence of the Amadori-derived reactive intermediate by 3-deoxyglucosone redacteas [24]. Thus, loss of renal mass might result in loss of expression of this enyme leading to accelerated rates of formation of AGEs .[83] . In addition, AGEs themselves can catalyze further AGE formation and this may explain the increase of pentosidine level in serum patients the increased cellular oxidant stress induced in cells by the uptake of AGEs, might lead to a vicious cycle in which accumulation of AGEs is accelerated and this was agree with Gostomska-Pampuch et [30] .

3.2.4. Serum C - Reactive Protein Among Diabetic Nephropathy Patients and Diabetic Control Group

The current study's C- reactive protein results revealed highly significant differences ($P \leq 0.01$) in early stage DN and end stage DN when compared to DM control group ,Where the mean and SD are (4.34 ± 0.17, 6.40 ± 0.20 , 2.27 ± 0.12) for the early , end stages diabetic nephropathy patients and DM control respectively . Also a significant differences between the two patients groups (early and end stage) ($P \leq 0.01$) were seen as illustrated in Table (3 – 5)

Table (3 - 5) Serum C.R.P. mean and P values among studied groups.

Parameters	Group	N0	Mean ± SD	(P-value)
C.R.P. (mg/dl)	Early stage	40	4.34 ± 0.17	0.0001 **
	Control	40	2.27 ± 0.12	
	End stage	40	6.40 ± 0.20	0.0001 **
	Control	40	2.27 ± 0.12	
	Early stage	40	4.34 ± 0.17	0.0001 **
	End stage	40	6.40 ± 0.20	
	** (P ≤ 0.01)			

Acute phase proteins are a class of proteins & their plasma concentrations increases or decreases in response to inflammations . 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 [54] . Among several markers of inflammations , C reactive protein (CRP) is found to be significant in people with diabetes [84]. 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 hs–CRP is found to be significant in people with diabetes [55] . CRP which is a pentameric protein produced by the liver has emerged as the ‘golden marker for inflammation , so diabetic nephropathy may be associated with abnormally high levels of CRP [85] . In some studies, they had found that there was a relationship between pentosidine and CRP levels in diabetic patients [86] , a similar result was found in our study too but there was a negative association between them and vitamin D concentration in our study , many studies found that [87]low grade inflammation as indicated by high CRP levels was an important predictor of diabetic nephropathy [88] . Increasing evidences shows 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 [89] . The current study provided experimental evidence that hs-CRP level was causally related to DN . These findings suggest that the elevated hs-CRP may be a causal risk factor for DN in patients with diabetes [90] .

3.2.5. Serum Vitamin D3 levels Among Different Studied Groups.

This study showed that the Mean and SD of serum vitamin D3 levels of patient for both groups early stage DN and end stage DN were (22.44 ± 1.06 , 11.58 ± 0.65) respectively and that for DM control group was (32.63 ± 0.78) as shown in Table (3-6).

Table (3 – 6)Serum Vitamin D3 mean and P values among studied groups .

Parameters	Group	No	Mean ± SD	(P-value)
Vit. D3 (ng/ml)	Early stage	40	22.44 ±1.06	0.0001 **
	Control	40	32.63 ± 0.78	
	End stage	40	11.58 ± 0.65	0.0001 **
	Control	40	32.63 ± 0.78	
	Early stage	40	22.44 ± 1.06	0.0001 **
	End stage	40	11.58 ± 0.65	
	** ($P \leq 0.01$)			

Vitamin D is a fat-soluble vitamin. dermal synthesis after ultraviolet-B (UVB) radiation remains the major route to obtain vitamin D, accounting for 90% of vitamin D replenishment. Cholecalciferol (vitamin D3) is from animal sources and ergocalciferol (vitamin D2) is from plants. it undergoes 25-hydroxylation in liver to 25(OH)D (calcidiol), the major circulating form of vitamin D Then it is converted in kidneys through 1-alpha-hydroxylation to its most active form, 1,25(OH)2D (calcitriol) this process is driven by parathyroid hormone (PTH) and hypophosphatemia [92]. The production of $1,25(OH)_2D_3$ in the kidneys is regulated by the plasma parathyroid hormone and serum calcium and phosphor concentrations . Vitamin D is transported through the human body in the bloodstream by binding to vitamin D binding protein (VDBP) , VDBP is a low-molecular-weight protein of 58 kDa, which predicts the bioavailability of $25(OH)D_3$ in the bloodstream to form the complex formation of VDBP/$25(OH)D_3$, filtration and reabsorption of this complex in the proximal renal tubular cells are critical for the retrieval and activation of vitamin D. people with renal damage, like diabetic patients with DKD, have increased urinary VDBP concentrations [48] . The effects of vitamin D are mediated through binding to its receptor (VDR) , which is present in a variety of tissues in human body, including kidneys and more specifically in the proximal and distal tubular epithelial cells , in glomerular parietal epithelium, in collecting duct cells, in mesangial cells and in podocytes as well as in the juxtaglomerular apparatus [38] . This indicates that kidneys play a crucial role in vitamin D metabolism by regulating the synthesis of the active form of vitamin D [93] . Complementary to these results, different studies proved that the prevalence of DN was high in T1DM and T2DM patients with low $25(OH)D_3$ concentrations [94]. Diabetic nephropathy (DN) is a severe complication of diabetes mellitus (DM) and represents one of the major complications associated with morbidity and mortality of patients.

Our study found that vitamin D3 levels decreased with progression of DN this result had agreement with previous studies that stated Vitamin D deficiency was related to DM and its complications [39]. As vitamin D levels are significantly lowered in DKD patients compared with diabetic patients without DKD and CKD patients without DKD, there seems to be a close association between vitamin D deficiency and DN , [95] . Patients with CKD produce less $1,25(OH)_2D_3$ due to impaired 1α-hydroxylase activity . In patients with T1DM or T2DM, vitamin D deficiency increases the risk of developing DKD , possibly due to the direct cellular effects, leading to podocyte loss and glomerulosclerosis [96] , also our study agree with [97] finding that assume Vitamin D deficiency was common among Korean T2DM patients; it was independently associated with microalbuminuria and HDL level, and positively related to diabetic nephropathy.

3.3 Correlation Between Different Parameters

3.3.1 Correlations Between Pentosidine and (Urea, Creatinine , Estimated Glomerular Filtration Rate and Glucose) .

The result of current study showed statistically significant positive correlation between pentosidein with urea R (0.58) Figure (3-5).

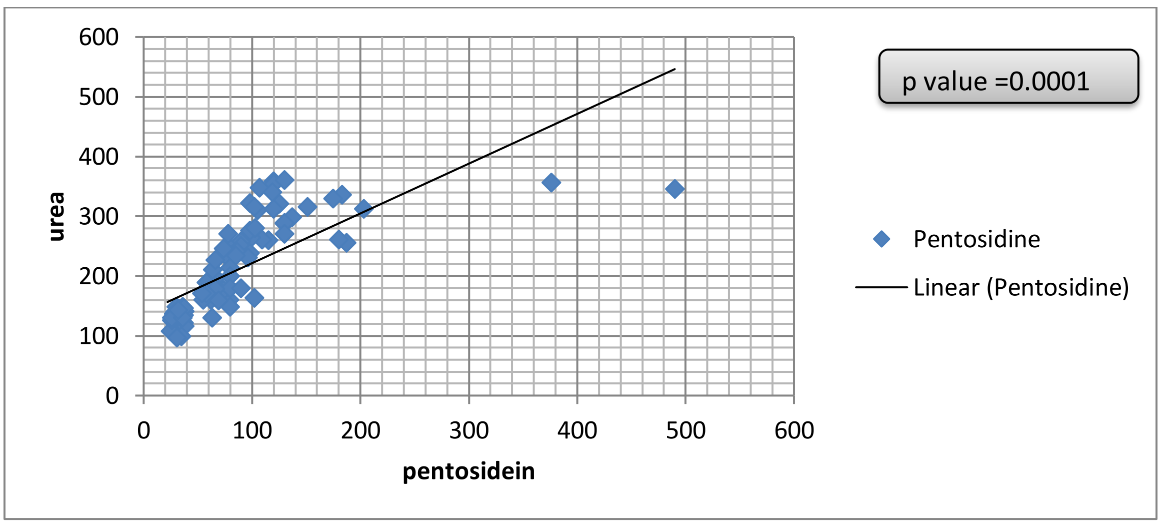


Figure (3-5) correlation between pentosidein and urea in patients with diabetic nephropathy .

The result of current study showed statistically significant positive correlation between pentosidine with creatinine R (0.89) Figure (3-6).

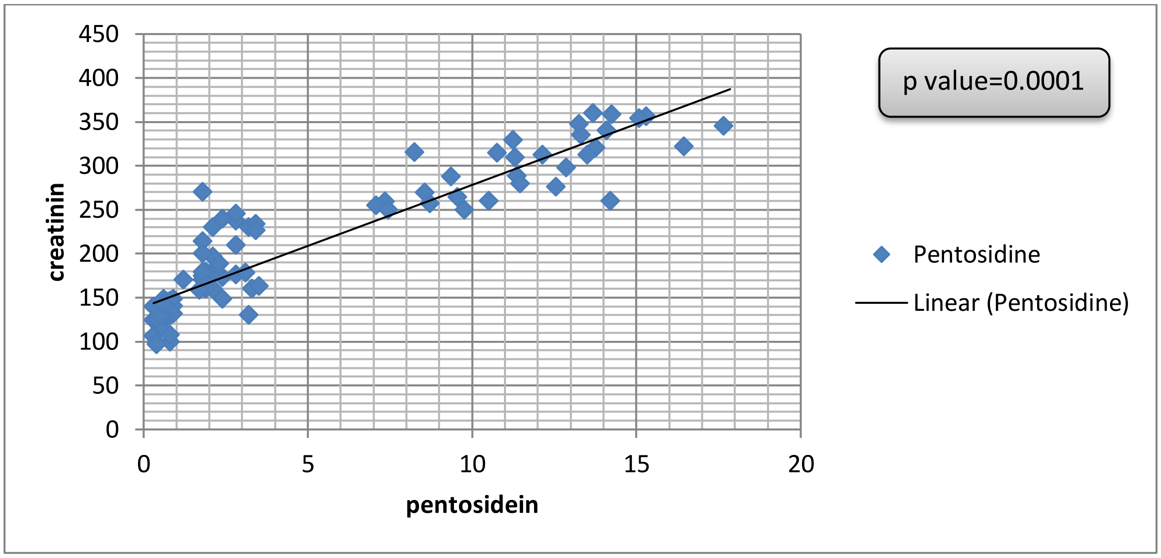


Figure (3-6) correlation between pentosidine and creatinine in patients with diabetic nephropathy .

The result of current study showed statistically significant negative correlation between pentosidein with e GFR R (-0.79) Figure (3-7) .

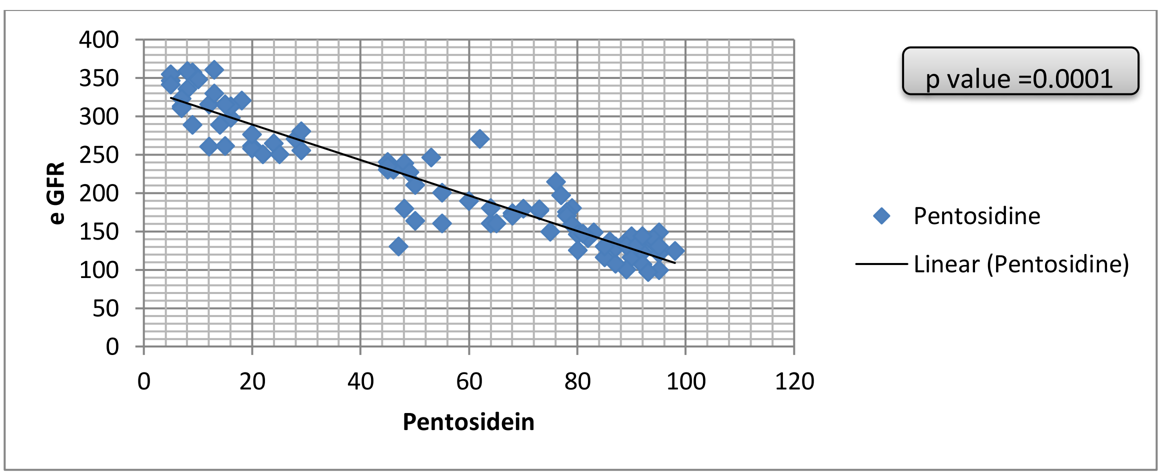


Figure (3-7) correlation between pentosidein and e GFR in patients with diabetic nephropathy

The result of current study showed statistically significant positive correlation between pentosidein with glucose R (0.38) Figure (3-8) .

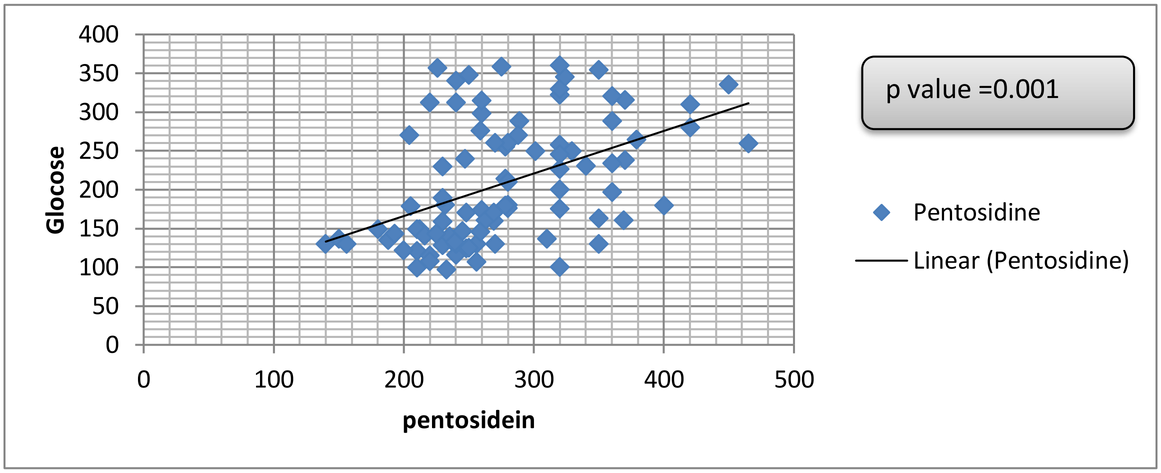


Figure (3-8) correlation between pentosidein and glucose in patients with diabetic nephropathy .

As illustrated in figure (3-8) pentosidine has a significant positive correlation with glucose this results agree with the fact that pentosidine is advance glycated end product formed by the non-enzymatic enter action between glucose and protein this study agree with[77]. As showed in Figure (3-5),(3-6)and (3-7) pentosidine has significant positive correlation with urea and creatinine , it has a significant negative correlation with e GFR these results improving the idea pentosidine has a significant role in DNP and high level of pentosidine may contribute to ESRD and this agree with [28] .

3.3.2 Correlations Between C Reactive Protein and (Pentosidein , Glucose , Urea, Creatinine and Estimated Glomerular Filtration Rate)

The result of the current study showed statistically significant positive correlation between CRP with pentosidine R (0.74) Figure (3-9) .

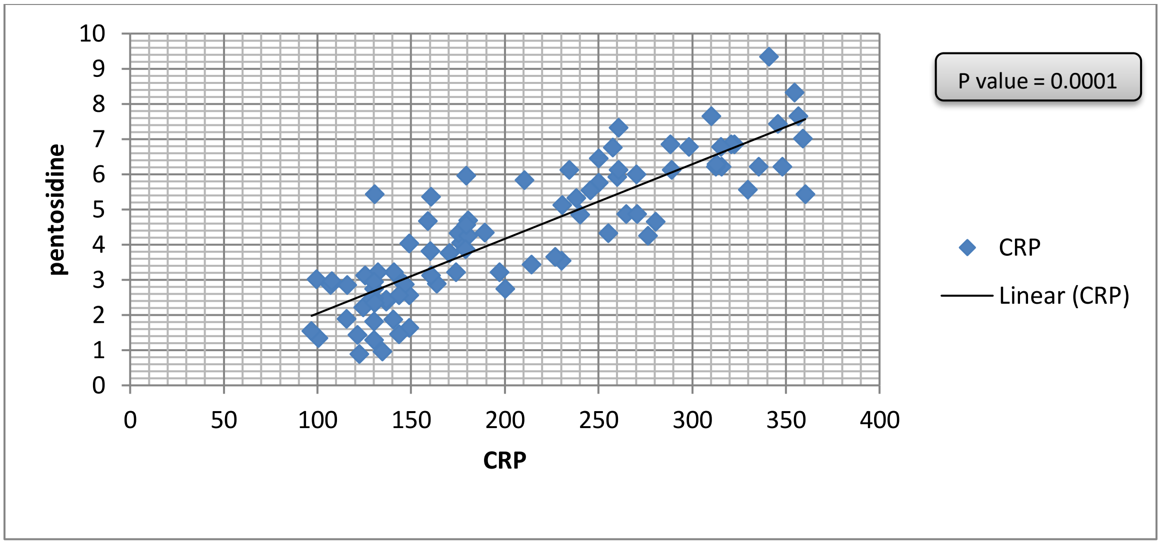


Figure (3-9) correlation between CRP and pentosidein in patients with diabetic nephropathy .

The result of the current study showed statistically significant positive correlation between CRP with glucose R (0.38) Figure (3-10) .

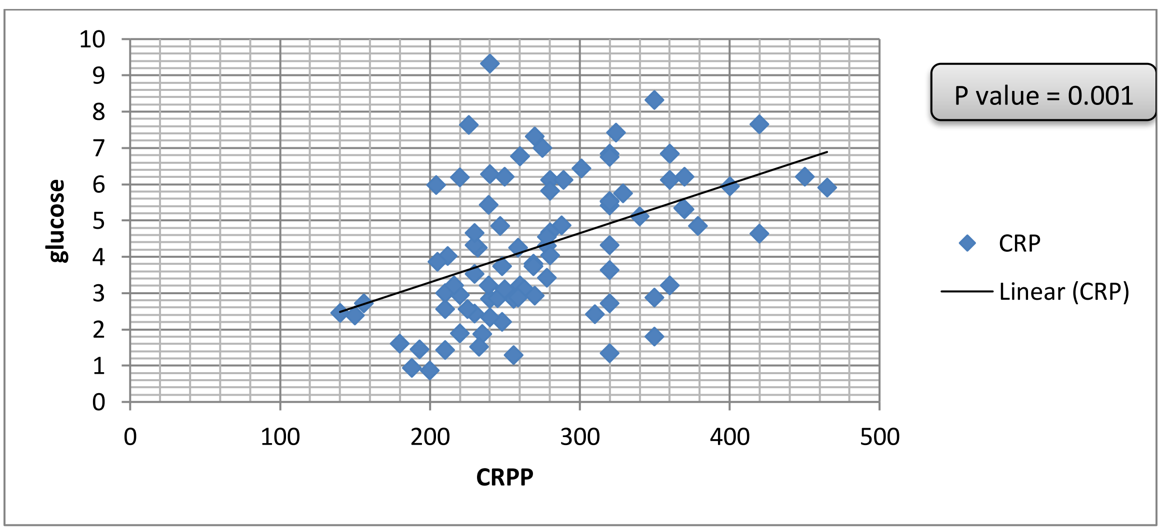


Figure (3-10) correlation between CRP and glucose in patients with diabetic nephropathy .

The result of the current study showed statistically significant positive correlation between CRP with urea R (0.48) Figure (3-11) .

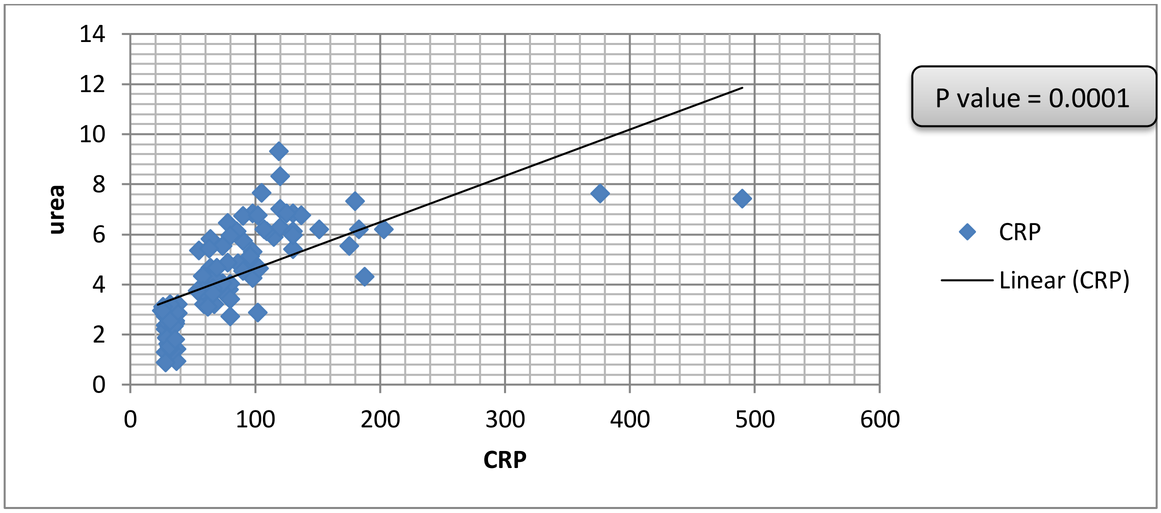


Figure (3-11) correlation between CRP and urea in patients with diabetic nephropathy .

The result of the current study showed statistically significant positive correlation between CRP with creatinine R (0.76) Figure (3-12) .

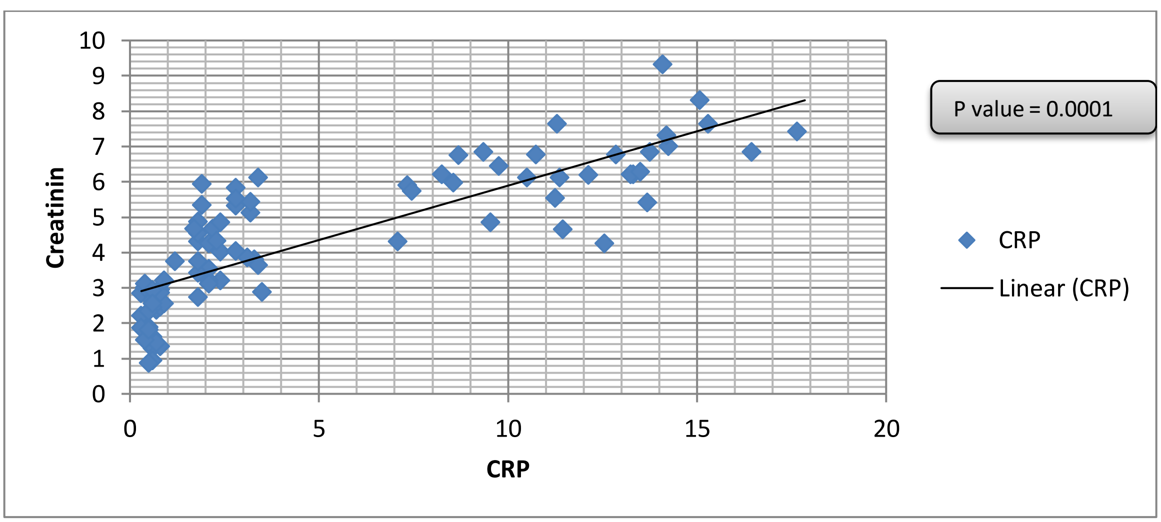


Figure (3-12) correlation between CRP and creatinine in patients with diabetic nephropathy .

The result of the current study showed statistically significant negative correlation between CRP with e GFR R (-0.79) Figure (3-13).

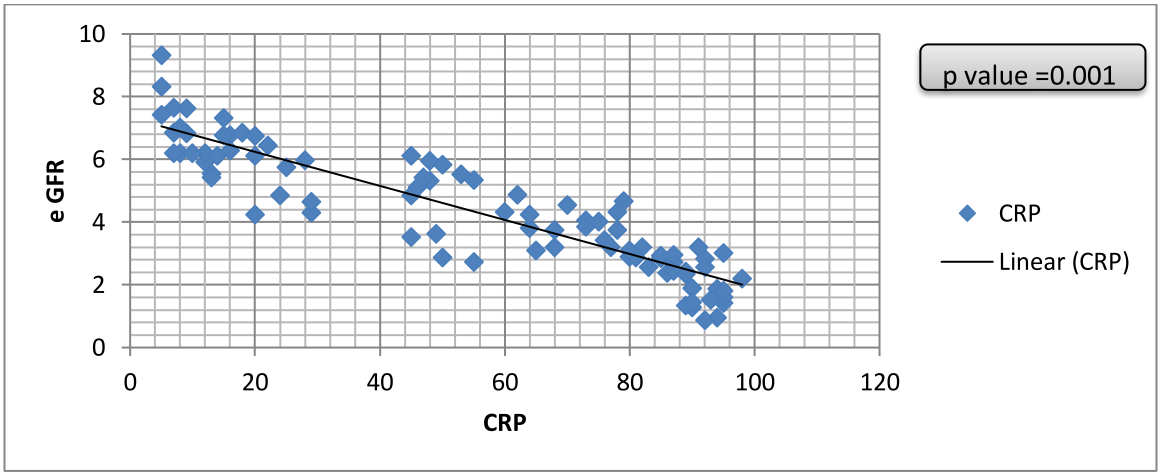


Figure (3-13) correlation between CRP and e GFR in patients with diabetic nephropathy .

As illstreted in Figures (3-9),(3-10) there is a significant posstive correlation

Between CRP with pentosidine and glucose and this result agree with [98] that pentosidune and glucose have stmulatary role in niflamation .this inflamentory mechanism accelerate progretion of DNP . As mentiond in the Figuers (3-11) ,(3-12), (3-13) ther is asgnificant postive correlation between CRP and renal falure and this agree with [51].

3.3.3 Correlations between vitamin D3 and (pentosidine, CRP , glucose ,urea, creatinine and estimated glomerular filtration rate)

The result of current study showed statistically significant negative correlation between vitamin D3 with pentosidine R (-0.64) Figure (3-14).

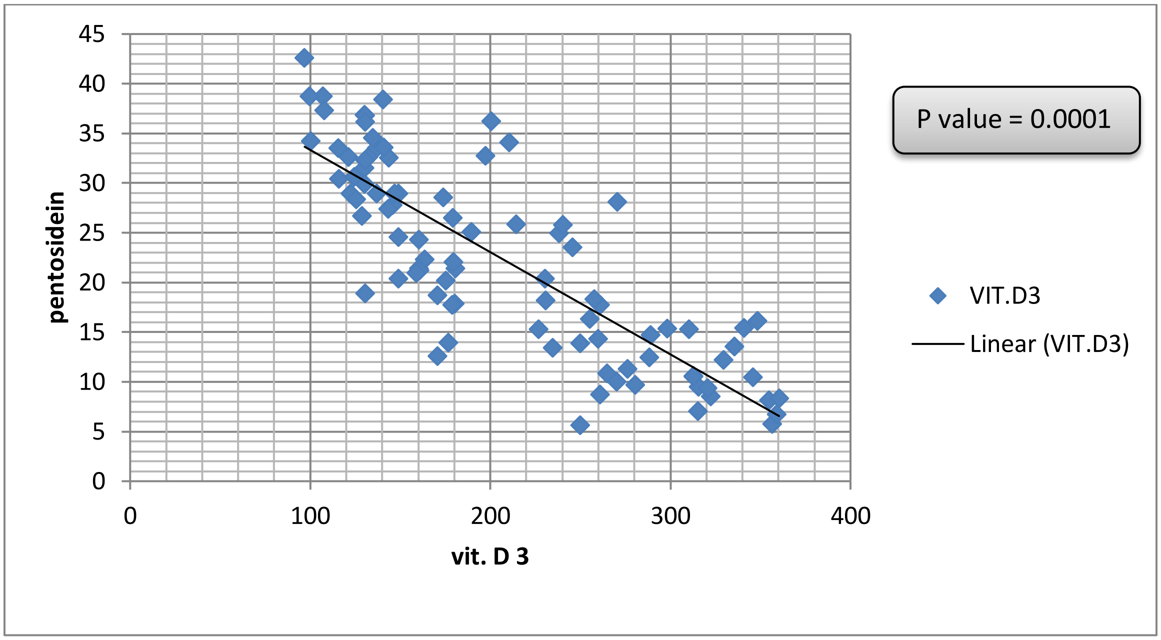


Figure (3-14) correlation between vit D 3 and pentosidein in patients with diabetic nephropathy .

The result of current study showed statistically significant negative correlation between vitamin D3 with CRP R (-0.58) Figure (3-15) .

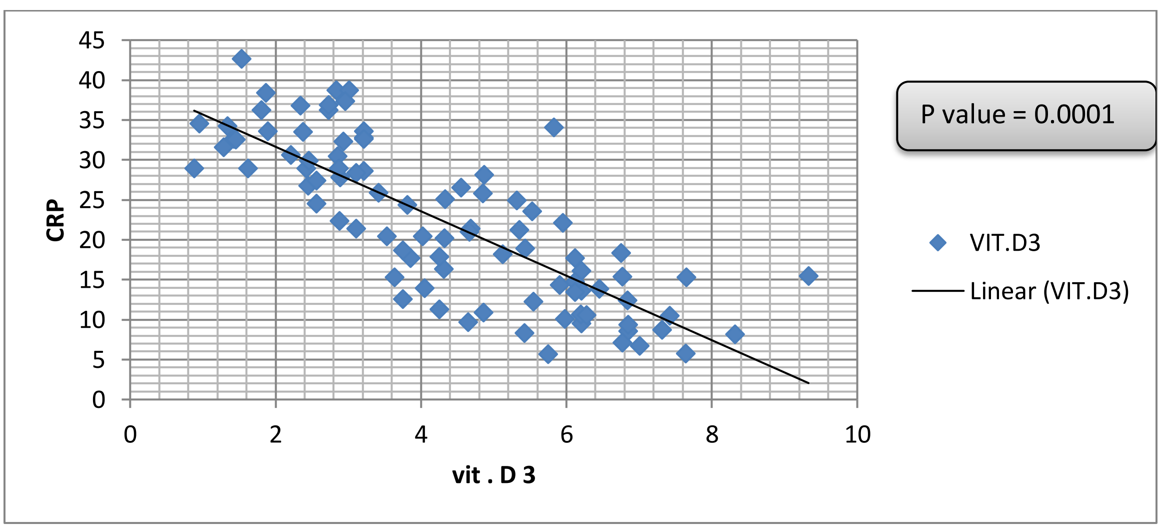


Figure (3-15) correlation between vit D 3 and CRP in patients with diabetic nephropathy .

The result of current study showed statistically significant negative correlation between vitamin D3 with glucose R (-0.35) Figure (3-16) .

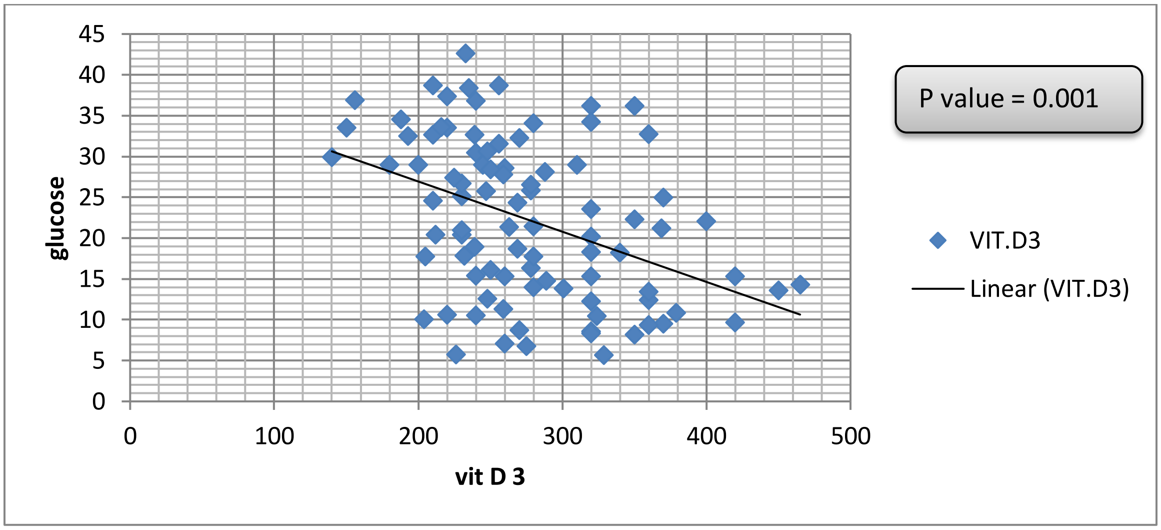


Figure (3-16) correlation between vit D 3 and glucose in patients with diabetic nephropathy .

The result of current study showed statistically significant negative correlation between vitamin D3 with urea R (-0.44) Figure (3-17) .

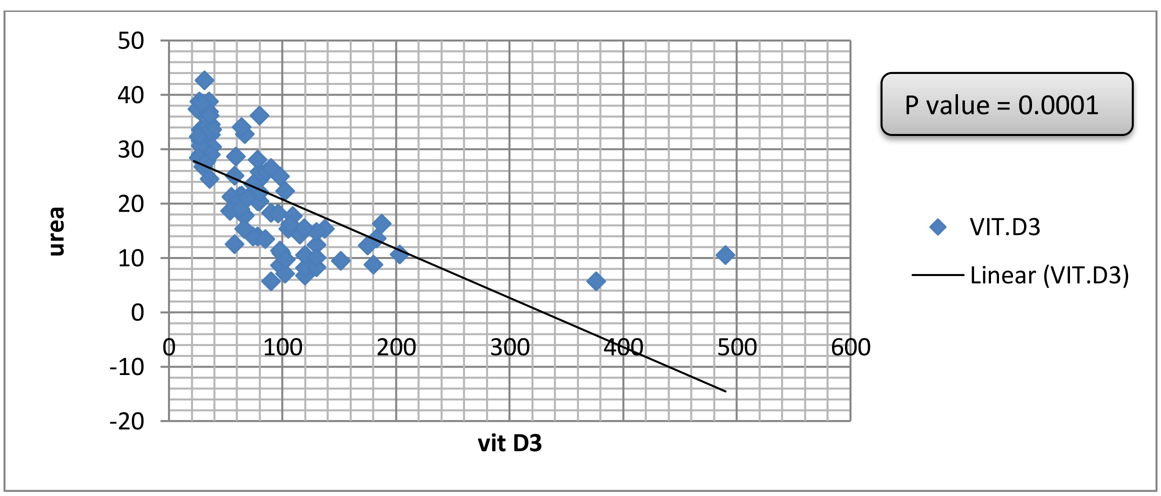


Figure (3-17) correlation between vit D 3 and urea in patients with diabetic nephropathy .

The result of current study showed statistically significant negative correlation between vitamin D3 with creatinin R (-0.74) Figure (3-18) .

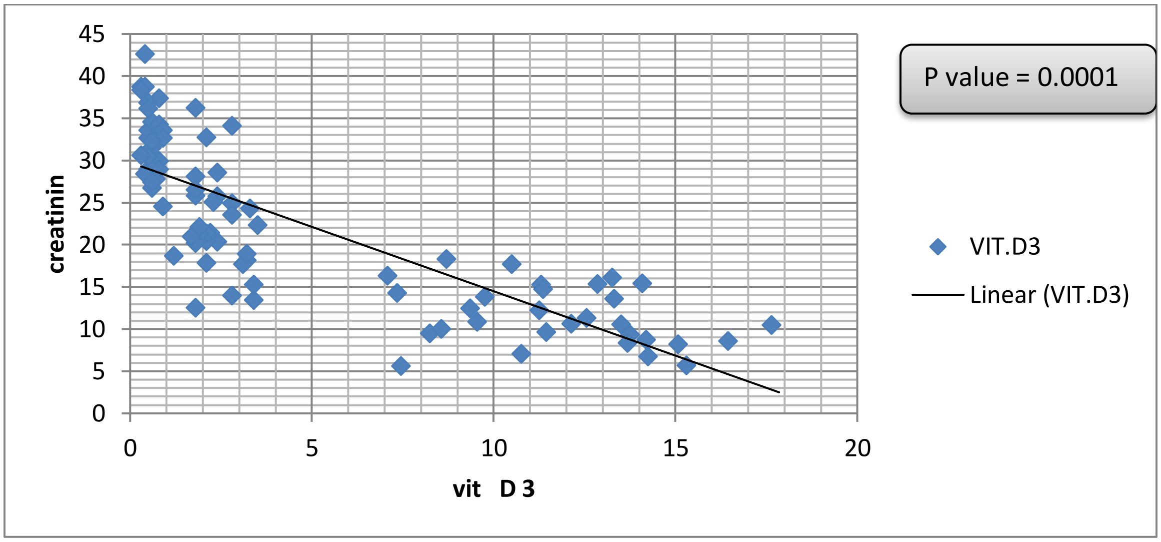


Figure (3-18) correlation between vit D 3 and creatinin in patients with diabetic nephropathy .

The result of current study showed statistically significant positive correlation between vitamin D3 with eGFR R (0.69) Figure (3-19) .

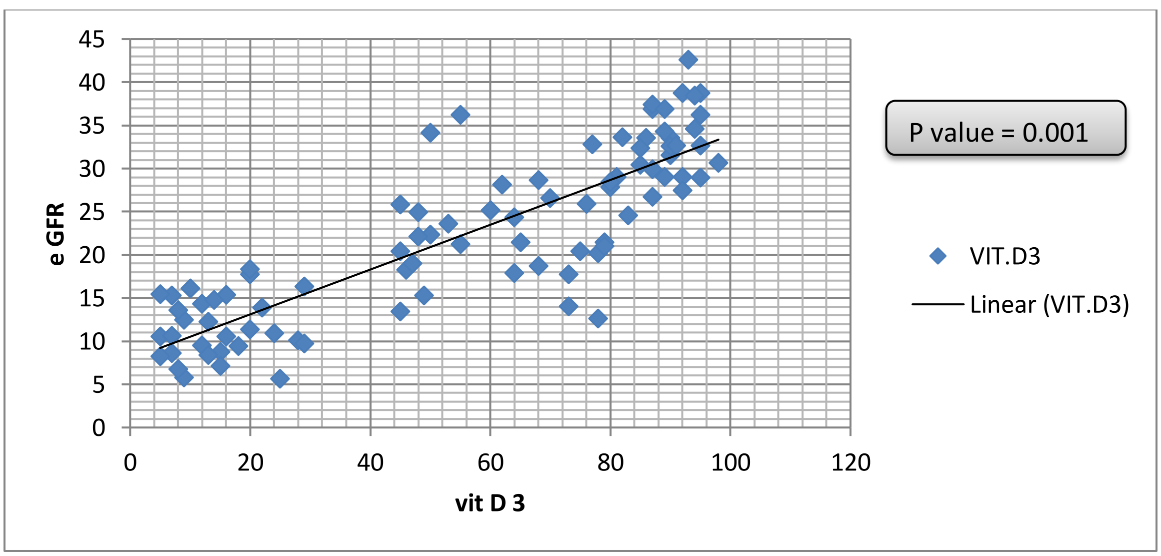


Figure (3-19) correlation between vit D 3 and e GFR

this study showed an association between vitamin D deficiency with diabetic nephropathy , this result agree with Previous studies that suggest patients with chronic kidney disease exhibit reduced 1α,25-(OH)2D3 levels and frank rickets or osteomalacia due to a deficiency of 1α,25-(OH)2D3 caused by lack of renal-1a-hydroxylase and this agree with [38].

3.4. ROC curve of biochemical parameters

3.4.1 Receiver Operator Characteristics (ROC) Analysis of pentosidein

The ROC analysis data demonstrate that pentosidine possesses an excellent ability to predict nephropathy in the diabetic group , which included patients with early and end stage of diabetes in comparison to DM without nephropathy as a control . 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 (3-3) .

Table(3-7) ROC analysis data of Pentosidine level in early and end stages diabetic nephropathy patients related to control groups

Pentosidine	AUC	P value	CV ng/dl	Spfcificity	Sensitivity	PPV	NPV
End stage VS control	0.86	0.00	256	90%	88%	96%	92%
Early stage VS control	0.85	0.00	150	99%	97%	90%	98%

AUC: area under the curve, CV: cut off value, , NPV: negative predictive value, PPV: positive predictive value.

Pentosidine showed an excellent capability (AUC= 0.86) to identify and predict end stage diabetic nephropathy patients from those without any disease . In term of prior probability, the P value was found to be 0.00 with very high sensitivity and specificity values 88% and 90% respectively . In terms of posterior probability, the PPV value was 96.0% and the NPV value was high too (92.0%) , which indicates that this marker has equal roles as for confirming and excluding disease . The best cut- off point (> 256 ng/ml) derived from the ROC curve . Accordingly, a test value above 256 ng/ml is considered abnormal (end stage diabetes with nephropathy) . A value of pentosidine > 150 ng/ml indicates that the patients in probability have nephropathy compared to the normal persons , as shown in Table (3-7) . Also Pentosidine showed a good ability (AUC = 0.85 and P value = 0.00) to distinguish diabetic patients with early stage from normal control .

Pentosidine level can be considered as a strong parameter to diagnose early stage DM patients since the AUC value was found to be 0.85 . The best cut-off point derived from the ROC curve, with a sensitivity of 97% and specificity of 99% was found to be 150 ng/ml. Accordingly, values above 150 ng/ml are considered abnormal (diabetes with early stage nephropathy). In term of posterior probability of the PPV is 90% and NPV is very high 98.0% , this indicate that this marker have equal roles as for confirming and excluding disease . The significance level is obtained at (P = 0.00). Other parameters including C.R.P. and Vit. D. may have the same role and explanations but at a different strength .

Pentosidine , which act as markers for the formation and accumulation of AGEs . The formation of pentosidine can occurs as a result to hyperglycemia , the elevated plasma level of pentosidine in diabetes may also be caused by increased oxidative stress , plasma pentosidine is strongly associated with low GFR , high oxidative stress and inflammatory conditions in diabetic kidney disorder [100] [101] . DN is a causative disorder characterized by association of hemodynamic and metabolic factors that include elevated blood sugar and advanced glycation end products (AGEs) [102] . Our finding in this study showed elevated pentosidine levels with progresses of DN and this result agree with others [98]

. Pentosidine was well described as a biomarker for the production and accumulation of AGEs that are to play an important role in diabetes and vascular disorders , Checking diabetic patients with the use of pentosidine would provide a strong long-term glycemic management tool that may have a significant impact on the levels of glycated hemoglobin [104] .

Also the findings of this study had agreed with Kuzan et al [35] who stated that Pentosidine has a pivotal role in diabetic complications, probably as a consequence of the diverse properties of this compound which alters the structure and function of molecules in biological systems [33] . Grady *et al* they found elevated pentosidine concentrations were reported to be a beneficial biomarker for clinical outcome surveillance in end-stage renal disease [105] .

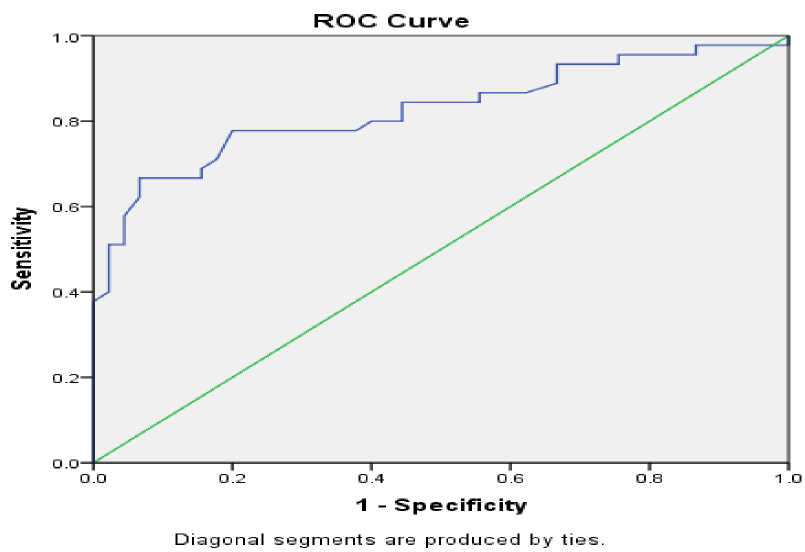


Figure (3-20) ROC Curve of pentosidein at end stage diabetic nephropathy

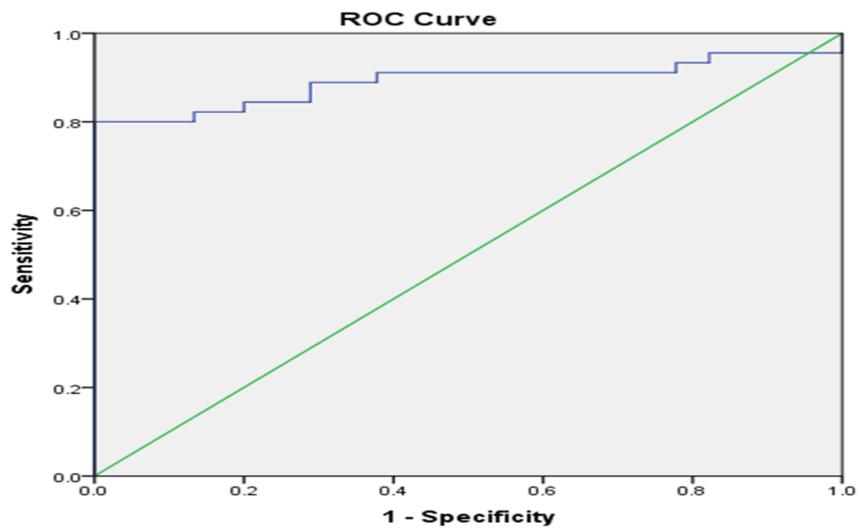


Figure (3-21) ROC Curve of pentosidine at early stage diabetic nephropathy

3.4.2.Receiver Operator Characteristics (ROC) Analysis of CRP

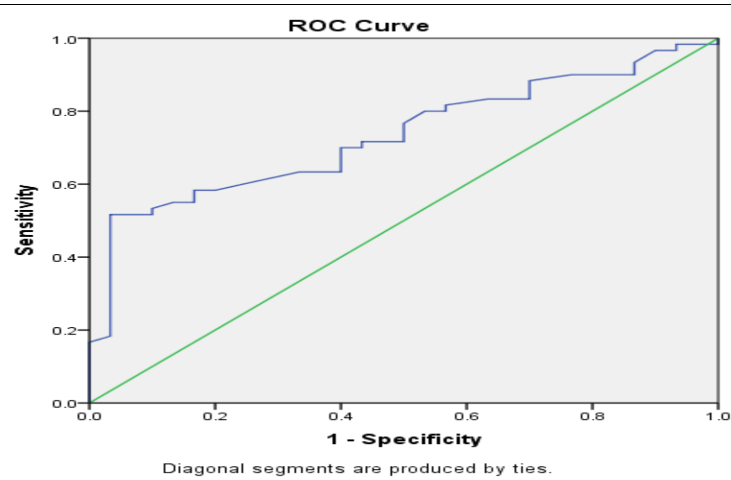
The ROC analysis data demonstrated that C reactive protein possesses an excellent ability to predict nephropathy in the diabetic group , which included patients with early and end stage of diabetes in comparison to DM without nephropathy as a control . This result was achieved based on investigations that included the sensitivity and specificity of CRP (mg/dl) for diagnosis of DNP , Cut-off points for end and early stage were (3.25 , 2.15) md/dl , AUC (0.8, 0.86) , P value ≤ 0.001 for both end and early , the sensitivity and the specificity were 80% , 76% for end stage and 85% , 88% for early stage , positive predictive values (PPV) were 79% at end stage and 80% at early stage , negative predictive values(NPV) were 74% at end stage and 76% at early stage as shown in Table (3-8).

For C reactive protein, our result state that good diagnostic value in the diagnosis of diabetic nephropathy in both end and early stage according to the area under the curve(AUC) in ROC curve.

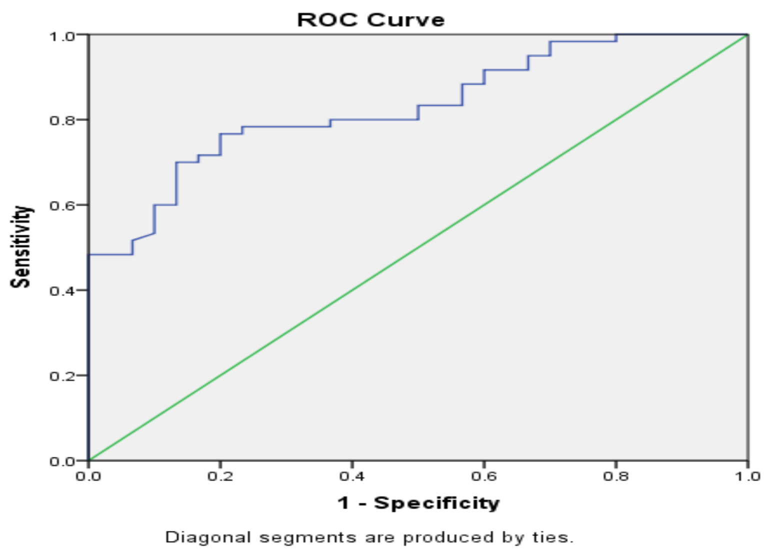
Table(3-8) ROC analysis data of CRP level in early and end stages diabetic nephropathy patients related to control groups .

CRP	AUC	P value	CV mg/dl	Specificity	Sensitivity	PPV	NPV
End stage VS control	0.81	0.00	3.25	76%	80%	79%	80%
Early stage VS control	0.86	0.00	2.15	88%	85%	74%	76%

AUC: area under the curve, CV: cut off value, , NPV: negative predictive value, PPV: positive predictive value.



Fig(3-22): Roc curve of CRP at end stage diabetic nephropathy .



Fig(3-23): Roc curve of CRP at early stage diabetic nephropathy

3.4.3. Receiver Operator Characteristics (ROC) Analysis for vitamin D3

The ROC analysis data demonstrate that vitamin D 3 possesses an excellent ability to predict nephropathy in the diabetic group , which included patients with early and end stage of diabetes in comparison to DM without nephropathy as a control . This result was achieved based on investigations that included the sensitivity and specificity of vitamin D 3 (ng/ml) for diagnosis of DNP , Cut-off points for end and early stage were (30.8 , 10.36) mg/ml , AUC (0.90, 0.88) , P value ≤ 0.001 for both end and early , the sensitivity and the specificity were 80% , 77% for end stage and 75% , 76% for early stage , respectively, positive predictive values (PPV) were 82% end 86% early , negative predictive values(NPV) were 76% end and 80% early as shown in Table (3-9).

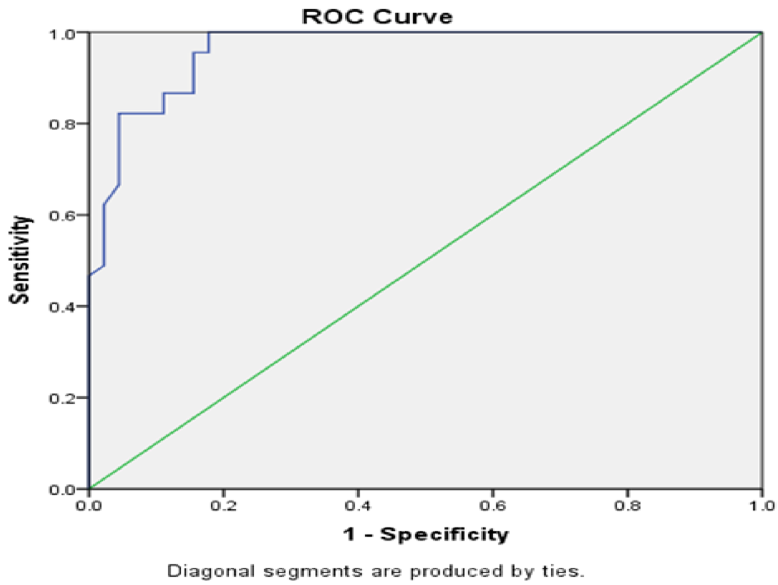
For vitamin D 3, our result state that good diagnostic value in the diagnosis of diabetic nephropathy in both end and early stage according to the area under the curve(AUC) in ROC curve.

On the basis of area under the curve(AUC) that highest in vitamin D 3 (0.90 in end stage and 0.88 in early stage) among the CRP(0.81 in end stage and 0.86 in early stage) and the pentosidein (0.86 in end stage and 0.85 in early stage) the vitamin D 3 biomarker was less sensitive and specific among CRP and pentosidine in the diagnosis of diabetic nephropathy patients

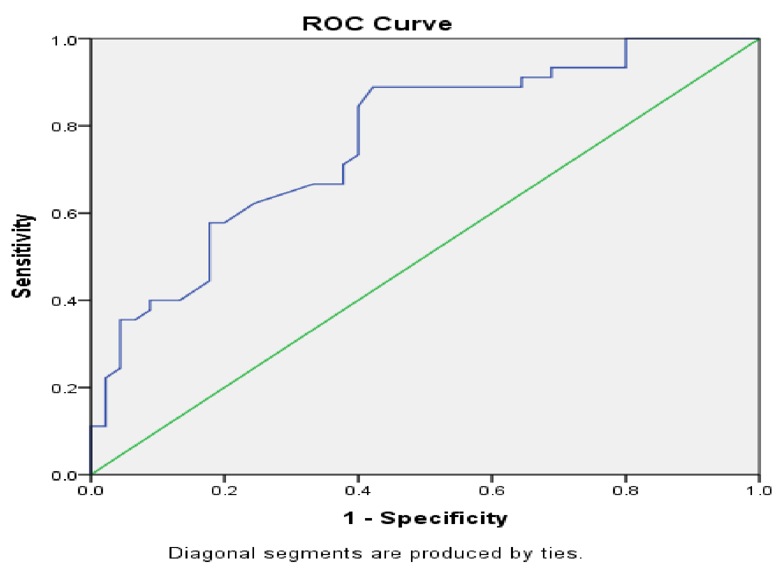
Table(3-9) ROC analysis data of vitamin D3 level in the early and end stages diabetic nephropathy patients related to control groups .

Vitamin D3	AUC	P value	CV ng/dl	Spfcificity	Sensitivity	PPV	NPV
End stage VS control	0.90	0.00	30.8	77%	80%	82%	76%
Early stage VS control	0.88	0.00	10.36	76%	75%	86%	80%

AUC: area under the curve, CV: cut off value, , NPV: negative predictive value, PPV: positive predictive value.



Fig(3-24): Roc curve of vitamin D3 at end stage diabetic nephropathy



Fig(3-25): Roc curve of vitamin D3 at end stage diabetic nephropathy

Conclusions

- Diabetes mellitus and long duration hyperglycemia may be one of the important causes of diabetic nephropathy through its direct role in pentosidein formation and accumulation of inflammatory factors in the kidney .

- The biochemical parameters included in this study such as pentosidein and CRP are significantly higher in DN patients with complications than DM patients without complication regarded as a control group and they are influenced by the severity and duration of DN, but vitamin D3 levels showed significantly lower value in DN patients with complications control .

- CRP could be considered as a biomarker of inflammation in DN and is more sensitive and specific than vitamin D3 so that it’s a good marker for diagnosis of DN patients with early or end stage of nephropathy .

- Pentosidine can be used as a new marker for evaluation of disease severity and progression in DN nephropathy .

Recommendations

- A future study is to be done on larger sample size to give results that are more reliable .

- Other early markers of diabetic nephropathy such as**,** TNF , IL6 , parathyroid hormones (PTH) , iron , calcium, phosphorus , micro and macro albuminuria may took in consideration .

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الخلاصه

اعتلال الكليه السكري هو واحد من اشهر مضاعفات داء السكر . ويعد السبب الرئيسي للمراحل المتقدمه من اعتلال الكله ويعتبر ارتفاع السكر المزمن و ضغط الدم المرتفع من العوامل المسببه لاعتلال الكليه السكري حيث يتم تشخيصه عن طريق ارتفاع مستوئ البروتين في الادرار و ارتفاع في وضاءف الكليه وانخفاض مستوئ الترشيخ الكبيبي .

Pentosidine هو احد معقدات البروتين المتكون من تفاعل سكر خماسي pentose مع الاحماض الامينيه lysine. arginine . ويعتبر ال pentosidine بروتين مشع يتكون خارج الخلايا بتفاعل لا انزيمي وتم اكتشافه في اواخر القرن الماضي .

CRP هو بروتين حلقي موجود في الدم . CRP هو بروتين يفرز في الدم من الكبد عند وجود التهابات حاده او عدوئ . يتم تصنيع CRP في الكبد نتيجه تخفيزه ببروتينات المناعه IL6 التي تفرز من الخلايا الدهنيه والخلايا البلعمي . حيث يرتع تركيزه بسرعه في الدم .

فيتامين D هو فيتامين ذائب في الدهون . له دور رئيسي في امتصاص الكالسيوم من الامعاء و تعزيز صحه الهيكل العظمي بالإضافة الئ الدور الفسيولوجي له دو في تمايز الخلايا في الامعاء وبذلك يزيد من امتصاص الكالسيوم .

تم تصميم هذه الدراسة كدراسة حالة وضبط. شارك 120 فردًا ، مقسمون إلى ثلاث مجموعات ، مجموعة ضابطة مصابة بمرض السكر بدون اعتلال الكلية ، ومرحلة مبكرة و مرحله متقدمه من اعتلال الكلية ، وتم أخذ 40 مريضًا لكل مجموعة و تتكون المجموعه من (20 ذكرًا و 20 أنثى).

تم تشخيص جميع مرضى السكري المشمولين في الدراسة الحالية من قبل أخصائي أمراض الكلى الموجود في عيادة أمراض الكلى بمستشفى الإمام الصادق في مدينة الحلة وقسم الكيمياء الحيوية في كلية الطب بجامعة بابل من أكتوبر 2022 إلى مارس 2023

تم تحديد Pentosidein باستخدام تقنية (ELISA) بينما تم تحديد CRP عن طريق طريقة الكشف المناعي و D 3 عن طريق المقايسة المناعية الإشعاعية الكيميائية.

كشفت نتائج الدراسة الحالية عن زيادة معنوية في pentosidine في الدم و CRP وجلوكوز الدم

References

واليوريا في الدم وكرياتينين في الدم بين مرضى اعتلال الكلية السكري مقارنة بالسيطرة وانخفاض معنوي في فيتامين D3 في الدم ومؤشر كتلة الجسم (BMI), e GFR . في مرضى اعتلال الكلية السكري مقارنه بمجموعه السيطره .

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