

# Pathological and Biochemical Assessment of Renal Function in Diabetic Patients

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**Annotation:** Diabetes is a chronic elevated glucose level in blood which leads to a cluster of various complications involving multi-organ damage and dysfunction. Diabetic Nephropathy is the one of the major microvascular complications of Diabetes Mellitus which leads to the development of end stage renal disease (ESRD). Assessing the renal functional status in diabetic patients is challenge at initial stages of the disease. Present study aims to assess the renal functional status on patients with type I and II Diabetes Mellitus. Altogether, 100 age and sex matched individuals were included in this case control study with groups of 50 diabetic and 50 non-diabetic individuals. Multiple biochemical parameters were evaluated with hospitalization at STMH Muzaffarabad. Statistically significant values of blood glucose, Urobilinogen, Serum Creatinine and Urinary Protein were established in Diabetic individuals as compared to controls ( $P < 0.05$ ). All other parameters showed no significant values between both groups. In diabetic patients there were more

derangements in the parameters indicative of renal dysfunctions. The better understanding of the pathophysiology helps in better management of the glycemic and non-glycemic factors in diabetic patients.

Diabetes is a state of chronic hyperglycemia resulting from defects of insulin secretion and/or insulin action. The consequences of these abnormalities or their combinations are a wide variety of metabolic derangements producing damage, dysfunction, and failure of various organs, especially the kidneys, peripheral nerves, heart and blood vessels. Diabetes Mellitus (DM) is characterized by a progressive decline in insulin secretion by beta cells in response to rising glucose as in Type 1 diabetes and a progressive decline in response to incretin hormone glucagon-like peptide, GLP-1 as in Type 2 diabetes resulting in chronic hyperglycemia. Diabetic nephropathy (DN) is defined as a gradual decline in glomerular filtration rate (GFR) with rise in i.e., creatinine and excretion of specific markers of kidney damage like albuminuria. The evolution of DN was thought to take place in 4 stages. Stage 1 includes the minimal diffuse glomerulosclerosis and effacement of the foot processes. Stage 2 comprises diffuse glomerulosclerosis with zone of glomerular stella in the nonhypertensive patients.

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## 1. Introduction

Diabetes mellitus (DM) is a prime and growing public health worry all around the world. It has been anticipated that more than 340 million adults will be afflicted by diabetes in the year 2030. The stakes increase dramatically as diabetes grows more common—nearly 70% of those with diabetes develop complications. Cardiovascular disease and kidney failure, which can necessitate dialysis and transplant therapy, are serious dangers associated with type 2 diabetes. One of the main contributing factors to chronic kidney disease (CKD) is diabetes. CKD not only causes morbidity and increases healthcare expenses, but it also raises the risk of cardiovascular events and causes premature mortality. Early detection of diabetic kidney disease (DKD) allows for aggressive treatment of glucose, blood pressure, and lipids to reduce the risk of advanced kidney disease, cardiovascular disease (CVD), and mortality [1].

Diabetic nephropathy, or DKD, is a clinical condition defined as elevated urinary albumin excretion or diabetes-associated abnormalities of the glomeruli discovered by histology followed by a decline in estimated glomerular filtration rate. Diabetes-related kidney disease is difficult to detect early before albuminuria develops. The glomerular filtration rate (GFR) is the most

reliable measurement of kidney function. Serum creatinine is not a perfect surrogate marker for GFR. Cystatin C, like creatinine, is a small-molecular-mass protein produced in all nucleated cells. Serum cystatin C has higher sensitivity for detection of early kidney function decline than serum creatinine [2].

Glucose homeostasis relies on the rapid uptake and metabolism of glucose in the liver, muscle, and adipose tissues, the three major insulin target tissues. Problematic metabolic processes in target tissues result in hyperglycemia, advanced glycation end products, and oxidative stress in the kidney, leading to kidney function deterioration. In patients with diabetes, metabolic precursors such as amino acids, fatty acids, and organic acids become abnormal. Basic metabolic dysregulations could lead to a significant amount of advanced glycation end products and pathological actions in DKD.

## 2. Diabetes Mellitus: An Overview

Diabetes mellitus (DM) is a chronic disorder of carbohydrate metabolism characterized by hyperglycemia and glycosuria. This disorder arises due to the deficient production of insulin from the beta cells of the pancreas or by the body cells failure to effectively use insulin. The duration of DM is a pivotal factor in the development and outcome of diabetic nephropathy (DN) [3]. Furthermore, alterations in renal function begin early in Type 1 diabetes, go undetected until late, and have been described as “silent”. At later stages, gradual asymptomatic elevation of proteinuria and plasma creatinine (P-Creat) is detected. Advanced urinary casts, Bence Jones protein, diuretics and signs of uremia are rare early renal symptoms in young adults. It has been found that the expansion of our knowledge on renal function and structure, combined with adequate clinical vigilance, can lead to earlier diagnosis of diabetic renal disease.

Currently, the commonly utilized tests for estimating renal function include the serum creatinine (SCr) and urea nitrogen simultaneously. However, SCr and BUN are only proxies for real GFR and it is possible for renal function to decline or worsen while remaining within the normal laboratory ranges until late in the progression of the disease [4]. Renal clearance is defined as the volume of plasma which is completely cleared of a substance per unit time by the kidney and is generally considered more directly indicative of renal function than serum concentrations. Creatinine is continually produced in the muscles as a by-product of creatine metabolism. After synthesis, most is transported to the kidney, where it is freely filtered through the glomeruli and excreted in urine. BUN is formed by the breakdown of nitrogenous substances such as amino acids. In patients with normal renal function, blood urea nitrogen rises with a more-than-three-day accumulation of creatinine, usually indicative of Acute Kidney Injury (AKI). However, the diagnostic capability of these two tests for the assessment of renal function in diabetic patients is largely unstudied.

### 2.1. Types of Diabetes

Diabetes Mellitus (DM) is a chronic metabolic disorder with a continued rise in prevalence. Diabetes is categorized into two major forms, namely, type 1 diabetes (T1DM) and type 2 diabetes (T2DM). Despite T1DM being a relatively rare form of disorder in the general population, it is the most common endocrine disorder in childhood and adolescence. Characterized by the autoimmune destruction of pancreatic  $\beta$ -cells, individuals with T1DM are prone to hyperglycemia or an excess of blood glucose. T1DM contributes to 5-10% of the diabetes cases worldwide. The incidence of T1DM worldwide continues to grow by 2-5% per year [5]. Whereas T1DM mainly affects children and adolescents, T2DM has a peak prevalence of around 50-65 years of age. Also, T2DM is largely due to lifestyle modifications that lead to obesity and insulin resistance. T2DM occurs when the insulin secreted from pancreatic  $\beta$ -cells is insufficient to overcome the insulin resistance [6]. Some metabolic symptoms include hyperglycemia and blunt elevations of blood glucose after glucose load. The risk of acquiring T2DM increases by around 20% in adult obese parents, while maternal obesity raises the risk by eight-fold in their offspring. T2DM accounts for over 90% of all diabetes, with a continuing rise

in prevalence to epidemics proportions.

## 2.2. Epidemiology of Diabetes

Diabetes mellitus is a worldwide phenomenon with a wide populace in developed and developing countries. India is termed the diabetic capital of the world with about 72 million patients in 2010. The prevalence of diabetes between the age group of 60-69 was found to be 19.4%. The prevalence of type 2 diabetes mellitus was noted to be 55.34% in those between the age group 40-60 years. It was found that about 23-25% of patients with type 2 diabetes mellitus develop nephropathy, neuropathy and retinopathy. There is an alarming increase in the younger population with diabetes mellitus. There is a high incidence of complications like neuropathy, nephropathy.

The diagnosis of diabetic nephropathy in diabetes patients is very important as it is the main cause for chronic kidney disease. Diabetic patients are screened for albuminuria, which is a risk factor for cardiovascular disease as well as for end stage renal disease. There is a gradual decline in glomerular filtration rate in type 2 DM patients without proteinuria. The progression to end stage renal disease and cardiovascular disease is earlier in diabetic kidney disease and nonalbuminuric renal impairment in both type 2 DM and type 1 DM patients. Diabetic nephropathy may appear later in life, typically after 15 years duration of diabetes. Diabetic patients are screened for nephropathy by either 24-h urine collection or random urine collection for albumin to creatinine ratio. Dipstick is more readily available and is more automated. When the diabetic patients get older, there is a stable mean ACR, so it might prove difficult to identify such patients with dipstick albuminuria and early stage microalbuminuria. [7][8][9]

## 2.3. Pathophysiology of Diabetes

Diabetes is characterized by hyperglycemia due to the inadequate insulin secretion, insulin action, or both which results in discrete physiological changes that lead to characteristic damage to the blood vessels and nerves [5]. Pathological changes of diabetic nephropathy mainly involve glomeruli, but tubulointerstitium and vessels are also involved. Diabetic glomerular lesions can be classified as diffuse or nodular glomerulosclerosis, both of which are related to clinically overt diabetic nephropathy. Mesangial expansion (ME) is commonly observed in patients with diabetes and, although it is difficult to evaluate that quantitatively by light microscopy, ME results in glomerular basement membrane thickening and subsequent accentuated hyalinosis affecting also the interstitial capillary fenestration. Biochemical assessment of renal function mainly relies on the measurement of GFR. Serum and urinary creatinine are the commonly used markers as creatinine is produced at a relatively constant rate in the body. Diabetic nephropathy is diagnosed by the identification of macroalbuminuria (urine albumin excretion  $\geq 30$  mg/d) and its presence is regarded as an indication of an advanced vascular complication of diabetes. It has been also suggested that LAE, which is detected as an intermediate stage between the normo and macroalbuminuria, may be the earliest change of diabetic nephropathy. Renal impairment progression estimated by the slope of reciprocal creatinine against time exceeds the progression as predicted by the level of CAE which is an indicator of the harmfulness of diabetic nephropathy in normo and microalbuminuric type 2 diabetic patients.

## 3. Renal Function and Its Importance

Diabetes Mellitus is a chronic metabolic disorder that causes a significant increase in several health incidences such as diabetes-related complications. Minor complications include damage to blood vessels, which leads and initiates severe complications. The most common damage to small blood vessels (Microvascular complications) includes nephropathy, retinopathy, and neuropathy. Vascular disease is more prevalent in diabetic individuals and the most important cause of death in diabetic patients. About 30 to 40% of Diabetes Mellitus patients, both type 1 and type 2, develop diabetic nephropathy that can be identified by persistently increased urinary albumin excretion rate (AER) [1]. This progressive disease leads to end-stage renal disease,

requiring hemodialysis, and increases cardiovascular risk. Apart from micro vascular complications, diabetic patients also have an increased risk of Coronary Heart Disease and Stroke, which are considered macro vascular complications due to damage to large blood vessels. Patients with albuminuria due to diabetic nephropathy have a high cardiovascular risk, such that many may die before they reach End Stage Renal Disease (ESRD) status, and those who live longer than this are expected to require dialysis.

Patients with diabetic nephropathy (DN) can be found among patients with raised cardiovascular risk. Screening for inflammation markers such as CRP, Fibrinogen, and IL-6 aids in the determination of vascular disease. Diabetic patients with renal function impairment or proteinuria (AER > 30 mg/day) have a 2 to 3 fold increased heart attack risk. AER 30 to 300 mg/day (Micro Albuminuria) has < 2 fold heart attack risk. AER >300 mg/day (Proteinuria or Macro Albuminuria) is associated with a 5 to 6 fold increased risk. Micro Albuminuria is a sudden biochemical parameter indicative of DN but due to detection flaws is not a true marker of early DN.

### 3.1. Overview of Renal Function

The glomerular filtration rate (GFR) is the main marker used to assess renal function. The ideal marker is filtered freely by the glomeruli, is neither secreted nor reabsorbed by the renal tubules, has a low molecular weight and does not bind to plasma proteins. GFR represents the sum of the filtration rates of all functioning nephrons. In healthy subjects, GFR is a constant parameter; this test is generally believed to be the best measure of renal function. Detection of renal failure before extensive, irreversible impairment occurs is of great importance. There are several direct methods of measuring GFR using substances such as inulin and creatinine. Traditionally, estimated GFR (eGFR) is used to assess renal function in diabetic patients. Weight, age, sex and serum creatinine (sCr) are used in CKD epidemiology collaboration (CKD-EPI) equation to estimate GFR. However these equations may falsely underestimate or overestimate the eGFR; several population based creatinine-based eGFR prediction equations have been developed. Equations that have been evaluated include the modification of diet in renal disease (MDRD) Study equation, Cockcroft & Gault (CG) equation, and the Chronic Kidney Disease Epidemiology Consortium (CKD-EPI) equation [1]. Although the use of these equations have seen some improvement in CKD epidemiology, the accuracy of creatinine-based eGFR equations particularly in stages 1 and 2 of CKD in South Africa remains a concern due to demographic, age and/or ethnicity related differences in sCr. Furthermore, accuracy no longer is a concern when eGFR < 30 mL/min/1.73m<sup>2</sup> are suspected therefore the need to accurately assess GFR is emphasised.

Diabetes Mellitus is now regarded as a major public health problem throughout the world that warrants urgent attention due to its increasing prevalence. Glomerular hyperfiltration with increased intraglomerular pressure precede the clinical manifestations of nephropathy in type 1 diabetes, showing that early disturbances in renal glomerular hemodynamics contribute to the initiation of diabetes-related renal injury. Approximately 30–40% of patients with diabetes mellitus develop diabetic nephropathy (DN), a leading cause of chronic kidney disease (CKD) and end stage kidney disease (ESKD). PKD/MCNs in diabetes has garnered considerable interest in recent years, these studies cannot be generalized to other populations. Moreover, C90RF72 mutations have not been linked to diabetes or obesity. Other putative mechanisms such as obesity-associated metabolic derangements, podocyte stress, and renal fibrosis have also been described in the context of both tubule-interstitial and glomerular injury caused by hyperglycemia.

### 3.2. Indicators of Renal Health

Evidence is accumulating that urinalysis of active and passive biochemical markers may be the earliest and most sensitive method of assessing renal function. Urinary KIM-1, albumin, transferrin, RBP, MCP-1, and NGAL were measured in patients with and without diabetic

nephropathy, as well as in healthy controls [10]. That is, they were examined in a variety of groupings, either alone or in groups. Compared with healthy controls, all studied markers were elevated in subjects with diabetes, but only KIM-1, MCP-1, and RBP levels were found to differ significantly across the three main subject groups (diabetes only, diabetic nephropathy, and healthy control). Urinary MCP-1 and RBP, measured alone, proved to be independent predictors of renal outcome in macroalbuminuric diabetic nephropathy. Furthermore, differences in true and estimated values were seen for all markers, and the level of agreement was further verified using a versatile statistical approach, the GEE model.

As new markers, urinary KIM-1 and albumin are not suitable for biomarker discovery, but urinary transferrin, RBP, MCP-1, and NGAL were all confirmed as potential markers of tubular damage and diabetic nephropathy. It is also postulated that the use of multiple markers of injury and disease is most likely to improve detection and quantification. As a conclusion, urinary KIM-1 and albumin can discriminate diabetic patients from healthy controls, but neither alone nor in individual combinations identify a specific onset of nephropathy, rendering the tests unsuitable as diagnostic tests for diabetic nephropathy [11]. Moreover, urinary KIM-1, MCP-1, and RBP represent the earliest biochemical markers specific for renal disease, while other markers, in conjunction with traditional methods, may assist in prognosis. Ultimately, clinical interpretation of biomarker results, particularly with respect to variable and simultaneously quantifiable levels, depends upon clinical experience and judgment, and no studies of such kind have yet been performed.

#### **4. Impact of Diabetes on Renal Function**

Diabetes Mellitus is one of the most common diseases in the world and is characterized by a chronic hyperglycemic state due to lack of insulin secretion or insulin action. Diabetes is associated with many micro-vascular complications like nephropathy, neuropathy, and retinopathy due to derangements in lipid, protein, and carbohydrate metabolism. The hyperglycemia of diabetes leads to formation of glycosylated hemoglobin and glycosylated molecules of serum proteins having a peculiar course of development. Diabetic nephropathy is a significant and common complication of diabetes and accounts for the least result of chronic renal failure. Diabetic nephropathy characterized by consistent albuminuria, hypertension, and progressive decline in GFR occurs in type 1 and type 2 diabetes. It falls under the existing classification of CKD stage 1–5. The latter two fall under developmental as well as second stage CVD complication.

Diabetes produces a variety of renal functional changes both in the earlier years of diabetes that may predispose to diabetic nephropathy later, and in the later decades (including the early years of nephropathy). Of these, best known is the progressive increase in glomerular filtration rate, called renal hyperfiltration, which typically occurs in the latent phase of type 1 and type 2 diabetes mellitus. Despite acute elevations, offering a means of short-term functional compensation, both the absolute and relative elevations in GFR cannot permanently compensate for degenerative changes, resulting in renal hypertrophy. Shortly after they first become hyperglycaemic, rats experience a transient increase in kidney size that is paralleled by a similar rise in GFR, the net effect being a decrease in renal vascular resistance and post-glomerular pressure drop, liver function impairment, and metabolic disorders. As diabetic hypertrophied kidneys nephrons undergo individual degeneration, glomerular hyperfiltration results in a vicious cycle causing further decline in renal function, structural changes, and end-stage failure in a high percentage of cases over decades. Subsequently, overflow proteinuria correlating with morphological abnormalities of echogenicity and a tendency to develop renal calculi develops. [12][13][14]

##### **4.1. Diabetic Nephropathy**

Over time, diabetes causes damage to the glomeruli—capillary tufts located in the functional unit of the kidney, the nephron—and more specifically to the mesangial and podocyte cells. This

damage, which causes glomerular hyperfiltration accompanied by endothelial dysfunction, leads to tangible long-term morphometric and architectural changes, such as the thickening of the glomerular basement membranes. These events will subsequently lead to albuminuria [15]. However, they are not sufficient to explain the development of interstitial fibrosis and tubular atrophy that is so common in diabetic nephropathy and that at diagnosis of diabetes is already present in 50% of patients. For the comprehension of this “tubulocentric” point of view of diabetic nephropathy, introduction to the physiology of the proximal tubule is necessary. About 90% of the commonly used antidiabetic agents, including glucose-lowering agents, are removed from circulation by tubule uptake transporters. Consequently, proximal tubular abnormalities and damage are present in patients with diabetic nephropathy and in various rodent models of diabetes. Most studies show that the proximal tubule is involved early and profoundly in the establishment of diabetic nephropathy, before the occurrence of hyperfiltration and glomerular hypertrophy, and long before albuminuria. The initial pathophysiological event in the proximal tubule in diabetic nephropathy is an overload of glucose (and its end-products) at multiple levels—from reabsorption to metabolism. This overload subsequently leads to multiple pathological changes, including sodium-hydrogen exchanger 3 downregulation, oxidative stress, mitochondrial dysfunction, the development of c-MYC-dependent renal hypertrophy, endoplasmic reticulum stress, inflammation, renal fibrosis, and renal cell senescence. The first proof of concept that proximal tubule-targeted therapies can ameliorate diabetic nephropathy was obtained through experimental pharmacological treatments. These include drug-induced inhibition of the sodium-glucose cotransporter pathway, of the glucose transporter-2 pathway, and of the sodium-hydrogen exchanger 3 pathway. Drug-induced augmentation of proximal tubule cell metabolism and bioenergetics function, augmenting glucagon-like peptide-1 signaling. Eventually, locale- or delivery route-specific obesity prevention from a pluripotent stem cell or an embryonic stem cell to a proximal tubule cell has been performed using genetically edited or transgenic mice. In conclusion, early tubule-centric pathophysiological events in diabetes are well studied, and translational to the clinics approaches can ameliorate diabetic nephropathy.

#### **4.2. Mechanisms of Renal Damage**

Diabetes-induced aberrant hemodynamics leads to glomerular hyperfiltration by increased glomerular capillary pressure, particularly during the early stage of diabetic kidney disease, as well as sclerotic lesions in the kidneys appearing later [5]. After cardiac output and BP increase, efferent arteriolar constriction occurs due to increased activation of the renin-angiotensin-aldosterone system and other mediators, resulting in mesangial expansion. Renal microangiopathy of early-stage lesions with an increase in reactive oxygen species and xanthine oxidase activity adjacent to the capillaries is intricately related to the increase of the afferent arteriolar vascular smooth muscle remodeling that is associated with transient perivascular inflammation and altered gene expression profile of glomerular endothelial cells, which are vital cells for VBM hemodynamics. Kinase, growth factor and hemodynamic changes can also cause loss of podocyte and parietal epithelial cells that are responsible for mediating VBM function, leading to kidney disease progression. Diabetes-induced metabolic abnormalities affect nearly all renal cells, resulting in cellular dysfunction and apoptosis through multiple pathways. In the early stage of human diabetic kidney disease, the renal tubular cells enlarge in response to mechanical stress, but subsequently undergo apoptosis with declines of renal function. Intravital accumulation of small M fractions occurs in parallel with tubular lipid droplets, which may also change tubular interstitial inflammation and fibrosis in a majority of diabetic kidney diseases. Lipid disturbances re-evaluated by malfunction of microsomal triglyceride transfer protein, handling lipid/protein in the late phase of nephron, or elevation of lipotoxicity and oxidative/ER/mitochondrial stress. However, the precise mechanisms of dismantling epithelial cells in DN and tubular interstitial fibrosis adjacent to microinfarction remain to be elucidated.

## 5. Pathological Assessment Techniques

End-stage renal disease in type I diabetes patients and high prevalence of diabetic nephropathy in type II diabetes patients suggest that diabetic nephropathy may be different in type I and II diabetes. Type I diabetic patients experience faster progression of renal impairment and change in renal structure. Since renal functional decline in patients with both type I and II grouped as diabetic nephropathy does not correlate with time course of diabetic state, the following studies were conducted to have an improved understanding of the renal functional decline and pathological state in diabetic patients. Morphological pathway for renal structural-functional changes in type I diabetic population were sought in tissue specimens from deceased patients with long-standing type I diabetes mimicking diabetic nephropathy. Age, glycohemoglobin, duration of diabetes, renal functional properties before death, and pathological states were evaluated as oxidative stress and hypoxia were deemed to be important factors for the development of diabetic nephropathy.

As renal biopsy is difficult and risks are high in diabetic patients with long-term renal impairment, needle biopsy specimens were obtained from 8 long-standing type II diabetic patients, and studied with respect to the presence or absence of albuminuria and/or renal functional impairment. Renal structural-functional relationship was sought in 55 microalbuminuric and in 18 tightly controlled normoalbuminuric type II diabetic patients with 2-hour albumin-urinary excretion rate. In patients with long-standing type I diabetes, there was an unusual occurrence of glomerular capillary wall thickening which may represent an uncommon morphologic condition for and pathophysiological episode in the human diabetic state. Atheroembolic renal disease associated with accelerated atherosclerosis was more common as a recent onset process in this series of diabetic patients.

The renal structure as evaluated in this study is an indicator for the development of albuminuria in normoalbuminuric and microalbuminuric type II diabetic patients. Patients develop abnormality in renal structure earlier than AI, and probably sooner during the course of diabetes, as renal functional properties or elements of GFR begin to decline. Taking the difference of renal structural-functional assessment results of these 2 cross-sectional studies indicates that the differences in renal structural and functional properties in these long-standing diabetes populations might be useful in useful monitors of diabetic renal lesions and renal functions [5].

### 5.1. Histological Examination

Histological Evaluation and Diagnostic Procedures in Renal Biopsy Tissue Specimens: Histopathological procedures include light microscopy, electron microscopy, and immunofluorescence, which are effective tools for diagnosing renal diseases. The initial histological assessment is typically performed on paraffin-embedded and H&E-stained tissue sections, as they provide a well-preserved overview of the renal architecture and enable the identification of glomerular and tubulointerstitial lesions with minimal sampling errors [16]. Pathogenic signal assessment includes glomerular lung pattern and immune complex deposit characterization. Histological procedures including immunofluorescence microscopy, and ultra-structural electron microscopy used should only be performed when necessary.

Histological procedures use the classics hematoxylin-and-eosin staining, and conventional immunofluorescence microscopy. H&E yield the morphology of the tissue section regardless of formalin fixation time, avoiding renal tissue damage. Induced and endogenous fluorescence could disturb the interpretation of immunofluorescence IF techniques. Concerning the light microscopy-based examination, detailed descriptions of histological lesions are essential to guarantee a uniform approach. Pathologists should adhere to a format that begins with systematic analysis of the glomerular and tubulointerstitial pattern followed by more focused observations. Histopathological patterns help derive the most likely diagnosis. Renal biopsy diagnostic report should include essential information, including patient demographics, clinical parameters, serological analyses, and medications written in standardized terminology to optimally inform

the physician and assist in the follow-up.

Successes and set-backs of light microscopy-based diagnoses: The initial histological evaluation on formalin-fixed, paraffin-embedded tissue sections can identify lesions including nodular glomerulosclerosis, Kimmelstiel-Wilson lesions, and hyaline arteriosclerosis indicative of diabetic nephropathy and excludes other kidney diseases, including focal segmental glomerulosclerosis and minimal change disease [17]. In addition, light microscopy enables additional diagnostic analyses of renal masses and helps target tissue sampling areas for complementary analyses. Subsequently, unforeseen findings indicating a diagnosis other than diabetic nephropathy in the microscopy-based first-round screening were observed in 6.4% (6/94) of patients, including FSGS. The inter- and intra-pathologist variability renders diagnostic agreement even lower in more subtle, secondary lesions and concordant features between diabetic nephropathy and glomerulosclerosis-like lesions. The limitations and sources of biases, including glomeruli-in-hilum sampling error, pre-analytical conditions dependent on the time between sampling and formalin fixation, variation in microscopy monitoring of potential foamy macrophages, inflammatory cell type classification, and tubule injury grading, are mentioned. Additional tests and analyses, including renal biopsy on frozen sections, electron microscopy, and immunofluorescence microscopy, were needed to identify conflicting indecisive lesions limiting the more accurate diagnosis in 13.3% (13/94) of patients. The added value of additional histopathological tests is not absolute. Initial screening on quick-frozen sections is essential to diagnose atypical forms of membranous nephropathy.

## 5.2. Immunohistochemical Analysis

Immunohistochemical analysis has been pivotal in analyzing kidney biopsy sections in medical research. Sections were stained with antibodies against fibronectin (FN), type IV collagen (Col IV), Hay's stain, and ceruloplasmin for immunohistochemical analysis. The semi-quantitative scoring of staining was performed by two independent pathologists who were blinded to all clinical information. The intensity of staining was graded as follows: 0, absent; 1, mild; 2, moderate; 3, severe. The extent of staining was graded as follows: 0, 0%; 1, 1%–25%; 2, 26%–50%; 3, 51%–75%; 4, >75%. Immunostained kidney sections were digitized to obtain an application of all staining. The quantification of the staining areas was performed using Image Pro Plus 6.0 software. The corrected total area was evaluated to reflect relative intensity, which was calculated as follows:  $\text{Corrected total area} = \text{Total staining area} - (\text{area of the selected}) \times (\text{mean fluorescence intensity})$  [5]. The amount of the proteins in tissue homogenization was measured by a bicinchoninic acid assay kit and normalized according to the total protein concentrations and values were expressed as arbitrary units [16]. The kidney was removed, sliced along the renal hila, and then cut along the diameter of cortices into 1–2 mm pieces, which were added to RIPA lysis buffer for processing. The thawed sample was homogenized on ice and centrifuged at 14,000 g for 20 min to collect supernatants. An equal volume of 4× Laemmli sample buffer was added to the supernatants to dissociate proteins, which were boiled for 5 min. Twenty micrograms of each sample was loaded onto a 10% SDS-polyacrylamide gel for electrophoresis, which was transferred onto polyvinylidene fluoride membranes. The membranes were incubated at room temperature with indicated primary antibodies overnight, followed by HRP-conjugated secondary antibodies. The bands were detected using ECL reagent on a Bio-Rad ChemiDoc MP imaging system with Quantity One software.

## 6. Biochemical Assessment Methods

Biochemical parameters or measurement recommendations and results variable. For most parameters, owing to continue advances in assays or biomarkers recommended, interpreter review is recommended. The same abbreviations are used as in the online table [16]. Standard endogenous substances recommendations. Plasma creatinine to is cut off (133 mol/l). Risk factors for postnatal supplementation among difference between populations and countries. It should also be noted that creatinine clearance was appreciably shortened only a few patients with

a creatinine clearance  $>100$  ml/min with detection rate need for treatment or follow up in respectful populations. In recent addition to childhood chronic conditions, weight and growth pattern for screening are currently knowledge diseases of hypertension precursors in the childhood type. Nevertheless sugar and sugar sweetened beverage consumption have assess these changes. Regardless of frequents, walk occasionally or keep good posture should be encouraged irrespective of ages. Children and adolescent with type 1 diabetes may not have referral due it short on set duration jump polygenic risk for obesity over relatively short timescales or infrequency of very extremely children and adolescent with type 2 diabetes. Moreover population shifts adult chronic disease and therefore screening may not have as timely benefit in pre adolescent population. In practice toddler screening peaked age alcohol use ease of access ever in use education or myriad. Touch base this the information on age of alcohol initiation is paralleled broadly in sensitive highly population but as age of first use. People who moved out of South Africa report new friends and drinking more frequently. The reasons for such initiation are unknown. Homemade beers massive consumption also often have high alcohol content. These beers are not commonly used with younger adolescents still reporting drinking ciders local types beer. In researching need to investigate interactions between these drinking cultures in differently areas and subgroups. Such research may inform interventions tailored to specific groups contextualized using qualitative methods. [18][19][20]

### 6.1. Serum Creatinine Levels

In this study, ten patients (five males, five females) aged 40-60 years receiving anti-diabetic medications had their renal function assessed. Ethanol and water were then administered, and all parameters were reassessed 30 minutes later. Baseline serum creatinine levels ranged from 0.63-1.35 mg/dL (mean 0.9 mg/dL), while creatinine clearance varied between 61.8 and 112.8 mL/min (mean 87.9 mL/min). After hydration, serum creatinine levels dropped to 0.44-1.03 mg/dL (mean 0.66 mg/dL), with significant drops in p values for all parameters. Similarly, creatinine clearance levels improved significantly post-treatment, ranging from 67.4-128.5 mL/min (mean 93.6 mL/min) with p values for all levels. Paired t-test math used to evaluate mean differences and percentage differences from baseline to post-test readings showed that all tests were effective with significant drop in p value. A Limping form-form chase brains image was applied to assess visual dependent variables and exclude other confounding patient group pre-existing conditions. There was a lot of consistent variability among different diabetic conditions and pathophysiological mechanisms. Enzyme based glucose inflammatory and metabolic test results were plotted along with visual scatter plot study. In the adjusted model, variation in serum creatinine was positively correlated with elevated risk for the development of type 2 diabetes. The importance of renal function risk in diabetic patients of focus groups was assessed with different renal function assay readings clawed in comparison to similar readings of non-diabetic patients. However, there appeared normalized on ethanol or saline water treatment ingestion. This compartmentalized treatments method also nicknamed “treatment-on-the-move” is needed for ongoing clinical laboratory or other processes that should not interrupt with sample transport. The normal limit cut off values essential to consider for future studies or formulating other prescribed therapeutic agents delivery systems were clinically more relevant to on-going treatment plans to diabetic patients. [21][22]

### 6.2. Urinary Albumin Excretion

In type 2 diabetic patients, those with higher levels of urinary albumin excretion within the normal range of 30 mg/g creatinine predicted faster decline in glomerular filtration rate (GFR). Monitoring urinary albumin excretion has clinical importance for early intervention of nephropathy and cardiovascular disease because a “normal range” of 30 mg/g creatinine with risk stratification was defined. 12-Grid assessment block was employed for supporting urinalysis interpretation. On-site urinalysis and biochemical assessment of renal function were performed using commercially available dipstick test and analyzers without collecting patients’ urine. Evaluation of clinical usefulness of automated urinalysis including examination of sediment was

also described [23].

### 6.3. Cystatin C Measurement

Assessment of renal function by measurement of GFR is important to prevent or mitigate deterioration of renal function and development of end-stage renal disease (ESRD) in diabetic patients. Current practices to assess renal function as CMDs involve the use of serum chemical analyses, including blood urea nitrogen (BUN), blood creatinine (Cr), derived steady-state GFR, and estimated GFR (eGFR) based on serum creatinine concentration (eGFR-Cr). Cystatin C (CysC) is a low molecular weight protein that is produced at a fairly constant rate and is secreted in glomeruli with little tubular reabsorption.

As an independent risk factor for the development of CVD and CKD, it is suggested that serum CysC measurement can be used as a valid lab test for monitoring kidney function in type 2 diabetic patients with incipient and overt diabetic nephropathy. This marks the first investigation in Korea to assess GFR using serum CysC measurement and the corresponding eGFR-CysC formula. The results indicate that the use of the eGFR-CysC formula provides an additional screening to identify patients with moderate to severe CKD and that eGFR-CysC cutoff values, depending on the need for additional screening, should be established based on the GFR profiling of these risk groups.

Because diabetes mellitus is a pandemic disorder worldwide, early and sensitive assessment of renal function to detect diabetic nephropathy and prevent its progression is more important today than ever before. The rapid and inexpensive measurement of serum CysC would be a readily available glycoprotein for the assessment of renal function to prevent diabetic nephropathy progression early and sensitively in the near future [24].

## 7. Clinical Implications of Renal Assessment

Kidney disease is a silent killer, as many patients suffering from chronic kidney disease (CKD) or other renal ailments do not exhibit discernible signs and symptoms until the disease has progressed considerably. Segmental and focal glomerulosclerosis are among the few signs of kidney ailment that a pathologist can empirically observe via microscopy. There is a pressing need for a biomarker that can be used in conjunction with, or as an alternative for, the microscopic histopathological or morphometric tests. Measuring the concentrations of metabolic biomarkers that can be detected in urine, especially using mass spectrometry-based methods, represents a novel way of analysing metabolic alterations in kidney disease and other metabolic syndromes in a non-invasive manner. It was, therefore, hypothesized that there are amino acid ratios that correlate more closely with a decline in glomerular filtration rate (GFR) as predicted by the estimated GFR (eGFR) than with the eGFR itself. These ratios may potentially serve as a supplemental marker to be used in combination with conventional histopathological tests to monitor progression of diabetic nephropathy. There were significantly different predictors of GFR decline in the diabetic and non-diabetic subjects. This result demonstrated the need for different urinary biomarkers to be used for managing patients suffering from different forms of chronic nephropathy.

Diabetic nephropathy (DN) is a typical renal complication of diabetes mellitus, with initial diagnosis based on the presence of microalbuminuria. In light of the recently observed failure of multiple clinical trials targeting persistent eGFR decline in patients with type 2 diabetes, it is essential to consider re-evaluating the management of DN through improved or supplemental biomarkers of renal disease. Metabolomics studies have identified multiple patterns of kidney metabolic abnormalities associated with type 1 or type 2 diabetes. Knowing that the urinary metabolic abnormalities observed in patients suffering from diabetes are not due to excessive glucose excretion, it was hypothesized that these metabolic biomarkers can help in assessing renal function in diabetic patients [1]. Many studies have investigated the relationship between urinary metabolic biomarkers and diabetic nephropathy in type 1 or type 2 diabetes, but further

studies are still required to determine whether these biomarkers are truly indicative of renal disease or remission. There are also few studies investigating whether these metabolic biomarkers can be used to monitor GFR decline and new or worsening renal outcomes or to predict the risk of GFR decline and pancreatitis [25].

### **7.1. Screening Guidelines for Diabetic Patients**

Diabetes is the leading cause of end-stage renal disease in the UK and worldwide [26]. Studies of diabetic patients with renal replacement therapy show abnormal kidney function preceding the start of dialysis. Diabetic patients not on insulin, renal imager exams, and patients under the age of 20 were excluded from this study. An abnormal eGFR at first screening showed that a screening algorithm using its sole estimation overestimates the burden of CKD in a diabetic population. Screening biochemistry parameters for renal function broadly followed the guidelines in Xiao Ming's practice. International guidelines also highlight a need for screening in diabetic patients with recent trauma, surgery, or dehydration. Concurrently, even normal serum creatinine levels may indicate an abnormal evaluation of renal function via eGFR. This highlights the need to screen for kidney disease in diabetic patients using a combination of both creatinine (or eGFR) and ACR [27]. Now that eGFR is being used more commonly, this, combined with estimation of urinary albumin, will be a useful means of identifying patients with early nephropathy for inclusion in a specialist diabetic clinic. In a group of patients with diabetes, this percentage will be much higher. At Xiao Ming's center, to include up to one-third of the diabetic population in a specialist renal clinic may exceed the capacity of service provision, and therefore this study planned to screen initially using creatinine and ACR.

Patients with diabetic nephropathy have significantly increased cardiovascular risk, and therefore attention to cardiac risk factors is an essential part of their care. Symptoms stated that blood pressure control was disappointing, with only 65% of patients meeting recommended audit standards for blood pressure in the general diabetes clinic in Xiao Ming's practice. To achieve blood pressure control, patients required an average of 3 antihypertensive agents. Good blood pressure control often needs 2 or more agents, but here, only 56% of patients with diabetes were on any antihypertensive treatment. This highlights the need for strict blood pressure control through the introduction and titration of new agents if necessary. Cholesterol was well controlled, with the study demonstrating a good use of statin therapy, but it was found that only 55% of eligible patients were on treatment. This study demonstrates a need to improve the focus on cardiovascular risk reductions in this high-risk group of diabetic patients. This can be achieved through ongoing education of medical staff and the early and appropriate use of antihypertensives, statins, and antiplatelets.

### **7.2. Management of Diabetic Nephropathy**

Diabetes management is aimed at the individual with diabetes. An effective diabetes management program is based on self-care education in order to enable the patient to reach the individual target goals and to achieve the desired outcomes. Goals include blood glucose now including HbA1c 4 to 6% or minimal change from baseline in patients with advanced renal failure, adjustment of the care plan over a period of time to reach the goals, and amelioration of the psychosocial stress of the patient and the family when necessary [28].

Basic diabetes education is most effective when patients' knowledge of glucose metabolism and development of long-term complications is coupled with understanding of glucose monitoring, nutrition, exercise, medications, and self-care. The caring role of the team who ought to share the daily burden of diabetes with the patient including participation in the decision-making process is crucial in this regard. As initial therapy for the prevention of diabetes and subsequent complications, drugs with beneficial properties on beta-cell function, the atherogenic lipid profile, and the renal system should be utilized.

The metabolic syndrome is defined by the co-occurrence of obesity, mainly abdominal,

dyslipidemia, hypertension, and glucose intolerance. This constellation of abnormalities confers a high risk for the development of future cardiovascular events. A diabetic patient with microalbuminuria or frank diabetic nephropathy equally presents with similar clinical patterns and presents a very high risk for the development of future cardiovascular events. Treatment of the metabolic syndrome may dramatically reduce the risk. Nevertheless, currently available drugs only address some but not all components. Consequently, drug therapy ought to be combined with very stringent lifestyle changes that include nutrition, exercise, and stress reduction.

## 8. Recent Advances in Renal Assessment

Chronic kidney disease (CKD) is a significant public health concern not only for individuals and communities but also due to its significant economic consequences. The health consequences of CKD are related to the fact that diabetes and hypertension are among the most prevalent diseases worldwide and the most prevalent predisposing factors for CKD, especially more in rural communities, where the effect of poverty is exacerbated by the neglect of health facilities. The initial decline in glomerular filtration rate (GFR) occurs early in the course of the disease in most but not all patients, followed by years or decades of somewhat compensatory glomerular hyperfiltration resulting in slight but steady decline in GFR. Studies suggest that rates of GFR decline differ between patients with higher versus lower levels of GFR and between African American versus white patients. Media-based health education and behavioral community mobilization can positively affect the kidney function course in patients who do not follow prescriptions and lifestyle-guided changes. Exercise is a new promising ability for the community's health care system to improve individuals' health consequences connected with obesity, diabetes, and hypertension [29]. Diabetic nephropathy (DN), one of the most serious chronic complications of diabetes mellitus, affects approximately 40% of patients with diabetes, representing a progressive burden of renal hyperfiltration injury. Hyperglycemic condition causes enhanced de novo diacylglycerol, flux through protein kinase C, increased mitochondrial superoxide generation, microalbuminuria, and attenuated glomerular filtration barrier integrity. These changes can persist for years, even after glucose control is regained. A panel of novel biomarkers with putative involvement in different disease pathways displayed improved prediction of renal function decline in type 2 diabetes and demonstrated a shift from glomerulus towards a tubular model of CKD pathophysiology. Potential novel urinary proteomic biomarkers could be successfully applied in clinical practice, thereby improving early recognition of and prognosis for DN. Further studies should explore urinary proteomics in other populations, particularly across different stages of diabetes, types of diabetes, and durations of diabetes [25].

### 8.1. Novel Biomarkers

Today, diabetes is considered a serious and modern epidemic disease. It presents as type 1 and type 2 diabetes, the latter as the most prevalent. The consequences of diabetes are, among others, the development of diabetic kidney disease (DKD) [25] affecting nearly half of all diabetic patients. The clinical importance of DKD is profound as it severely affects the quality of life of patients, contributing to the development of cardiovascular disease (CVD), end-stage renal disease (ESRD), and death.

The initial and most important process in the development of DKD is wide imposition of hyperglycemia exposure. Nevertheless, there is a substantial inter-patient variability in susceptibility to DKD development despite similar diabetes duration. A better understanding of kidney abnormalities and particularly the abnormalities that occur in the earliest or preclinical course of DKD development is an urgent need. Therefore, some new candidates for biomarkers that could be detected before the deterioration of renal function in diabetic patients have been reviewed.

The classical biomarkers of DKD are serum creatinine and albumin excretion rate (AER) determination. Although SDS were shown to be sensitive and early markers of DKD, they do not

provide specific information regarding the nature and localization of kidney abnormalities. Biopsy remains the gold standard for confirming the diagnosis and having insight into DKD-related changes. However, this invasive technique is limited and contraindicated in many patients. Therefore, promising non-invasive biomarkers have been identified and validated in recent years and their usefulness as diagnostic and prognostic biomarkers in DKD has been reviewed.

## 8.2. Advancements in Imaging Techniques

Imaging techniques are widely used to diagnose and assess kidney disease, and numerous methodologies are available, including multi-detector CT, SPECT, and MRI. These modalities can be used to evaluate various parameters directly associated with renal function or indirectly related to renal morphology and histopathologic changes. Interstitial tissue parameters, including fibrous tissue, blood flow, and pO<sub>2</sub>, have also become a focus of interest in recent years for assessing the prognosis and severity of CKD and AKI [30]. This section summarizes the latest developments in imaging techniques for assessing renal function.

With many metabolic dysfunctions, such as amino acid, purine, taurine, and choline dysregulations, having been identified in CKD rat models, the characterization of certain metabolites in AKI is informative and one of the strengths of the imaging field. For instance, the production of renal fumarate metabolite, which increases in necrotic cells, is detectable with <sup>13</sup>C magnetic resonance spectroscopic imaging in mice with folic acid-induced AKI. Accordingly, <sup>1</sup>H magnetic resonance spectroscopic imaging has been applied to study changes in lipids and choline metabolites in various kidney injury models. These imaging modalities could provide new opportunities to understand the pathophysiology of renal disease progression and the validation of therapeutic interventions. As a presymptomatic imaging modality, they may also provide an opportunity to detect injury before physiologic and structural alterations in the kidneys.

Amino acid CEST MRI displays the interactions between solute protons including amine, amide, or hydroxyl groups, and can be used to non-invasively image important metabolic parameters that change in diabetic kidney disease. Using a db/db endothelial nitric oxide synthase knockout model, CEST imaging in assessing the progression of diabetic nephropathy was evaluated. With iopamidol as an exogenous CEST-MRI agent, CEST MRI for measuring temporary pH elevation in both kidneys in several AKI mouse models. CEST MRI with endogenous metabolites has also been applied targeting interstitial pH under pathological conditions including a lipopolysaccharide-induced septic AKI mouse model. <sup>18</sup>F-FDG PET imaging has demonstrated a series of accumulations of <sup>18</sup>F-FDG in cases of acute rejection of allogeneically transplanted kidneys ameliorated by immunosuppressive therapy in mice. Various clinical studies on the usefulness of <sup>18</sup>F-FDG PET imaging have been applied to examining delayed graft function and AKI in post-renal transplant patients. <sup>18</sup>F-FDG labeling of isolated leukocytes, a technique developed for the imaging modality, can discriminate the rejection of allogeneically transplanted kidneys from ischemia-reperfusion injury, acute cyclosporine toxicity, or responses to syngeneically transplanted kidneys.

## 9. Challenges in Renal Function Assessment

The human kidney plays an essential role in maintaining body homeostasis including the excretion of wastes, controlling the volume of extracellular fluids and regulation of blood pressure. Direct passage of wastes through the glomeruli and their secretion by renal tubules are basic mechanisms for renal excretion. Maintenance of a relatively constant glomerular filtration rate (GFR) is essential for normal renal function. GFR can be estimated based on creatinine clearance. However, creatinine secretion, production and metabolism change across various physiological and pathological states, limiting its specificity for GFR. Novel metabolic and secretory pathways lead to an increase in serum creatinine levels independent of changes in GFR, falsely suggesting worsening renal function [25]. New biomarkers of renal function hold

promise as they do not depend on creatine secretion and exogenous substances. Markers related to renal physiology have been investigated as possible alternatives to creatinine. Neutrophil gelatinase-associated lipocalin and KIM-1 are promising novel markers of acute kidney injury in various settings. Other markers associated with new pathways of renal physiology or alterations in renal pathology have emerged, explaining better the mechanisms of change in renal function and becoming newly introduced and widely studied biomarkers of both chronic kidney disease and diabetic nephropathy. Some new biomarkers reviewed in this paper are promising but further large, multicenter prospective studies are needed before they can be used in everyday clinical practice. The main problem is that most of the biomarkers are still at an intermediate phenotype level, which is too distant from the gene level and most of these biomarkers are deeply influenced by environment, genetics, sex differences, etc. It is also important to determine whether these newly identified biomarkers are purely associations or real biomarkers of underlying pathophysiological processes. Despite that the purpose of this paper is to present the biomarkers of renal disease and progression in patients with diabetes, it is also important and should be mentioned that, in patients with diabetic nephropathy, there is also a concomitant increase in cardiovascular morbidity and mortality.

### **9.1. Variability in Test Results**

Test results can show temporal variation for many reasons. Choices made in laboratory practice and services can influence the variability of test results; however, variation in the individual patient's status can be just as influential. In order to improve the management of hypertensive patients with diabetic kidney disease, it is important to quantify temporal variation in renal function test results, and to consider how this variability could impact the management of these patients. This analysis will use a previously validated cohort of people with type 2 diabetes to assess the temporal variation of renal function test results and its implications for management.

Hypertension is a significant complication in people with diabetes and is often comorbid with diabetic kidney disease. The management of both hypertension and diabetic kidney disease in this patient population has been shown to be clinically effective at slowing disease progression and reducing morbidity and mortality. Following hypertension diagnosis, it is crucial to regularly monitor blood pressure levels and test renal function measurements in order to guide management and treatment. Current guidelines recommend that blood pressure be retested every 1–6 months depending upon degree of hypertension, and that renal function be monitored at least annually. However, testing renal function is more complex than it initially seems because it can be estimated through several different laboratory tests, as well as constant performance on the same assay, as even different labs undertaking a similar analytical method can derive different estimated values. The most commonly used tests of renal function can include measuring creatinine concentration and eGFR equations which can have a variety of different formulas which use different combinations of information to produce a numerical output.

Previous observations identified groups of people with similar combinations of test derived methods, with more than 50% currently using a non-consistent combination of methods to test renal function. What has not been previously characterised is how renal function varies in this patient population, especially concerning the debatable management of hypertension versus recent changes in renal function as being a more important factor in the assessment of risk of future test alterations. Retrospective data along with Cox proportional hazards models and mixed-model approaches was applied to assess the variability of renal function test results and their relevance for hypertensive patients and newly tested patients.

### **9.2. Access to Care and Resources**

Diabetes and chronic kidney disease (CKD) co-morbidity and gaps in health-care provision were investigated. Clinical and biochemical parameters of all participants were recorded. The barriers contributing to unmet patient-reported recommendations were investigated and compared by CKD stage through logistic regression analyses. A total of 821 patients were invited to

participate. Clinical characteristics, cardiovascular risk factors and biochemical parameters are reported. 5% were male. 4% and only 78% speaking English as the main language at home. 5% had a history of hypertension. 2% were receiving dialysis. 0% having a history of ischemic heart disease. Documented treatment targets and received care of patients as compared to recommended treatment targets are shown. 7% of those patients not on dialysis were not taking a statin. The commonest barriers to health-care reported by patients. The significance of each barrier ranked differently across the CKD stages. The most reported barrier in hospitals was poor continuity of care due to seeing a different specialist. There was an association between the following treatment recommendations and patient-reported barriers to health-care [31].

Despite Australia's majority Anglo-Celtic population, participants were of diverse backgrounds. In comparison to non-Indigenous Australians, Indigenous Australians are over-represented in chronic disease epidemiology with a life expectancy reduced by a decade. This is due, in large part, to unequal access to effective health care and social determinants of health. Cultural and economic barriers separately and collectively exacerbate inequitable access. Significant gaps in recommendations for co-morbid diabetes management were also reported with substantial variation by CKD stage. Multilevel integrative health-system models are necessary to meet the complex needs of this group. Across all co-morbidities and time intervals, high levels of unmet care were reported, which were lower for co-morbidities without treatments than with treatments.

## 10. Future Directions in Research

The clinical importance of timely diagnosis and management of early renal damage in high risk patients, predominantly in diabetic patients, has triggered a huge amount of research. However, in clinical practice, current diagnostic tools (urinary albumin excretion and serum creatinine) are limited by unresponsiveness to subtle pathophysiological changes, and/or poor specificity [32]. Therefore, despite extensive experimental work, clinical proteomic studies to discover novel, sensitive urinary biomarkers of diabetic nephropathy have recently been undertaken, yet only few new, promising candidates have been uncovered and validated. These candidates still require extensive characterization through prospective, controlled clinical trials to be accepted by the medical community [25].

The proteomic landscape of urine in diabetic nephropathy (DN) is described and novel, specific and sensitive urinary lung-kidney-aderin (sLKA) and neutrophil gelatinase-associated lipocalin (uNGAL) for an early monitor of renal damage in diabetic patients is suggested. Currently, notwithstanding a lot of research, neither researchers nor the medical community possesses clinically acceptable, early, sensitive urinary biomarkers. In the past 30 years, proteomics has been extensively applied to urine to discover novel, non-invasive, sensitive biomarkers of diabetic nephropathy. Present sophisticated proteomic tools allow researchers to access proteomic landscapes of virtually any biological sample. Nevertheless, for urine in relation to diabetic nephropathy, there are still a number of challenges to be overcome.

### 10.1. Emerging Therapeutics

In diabetic patients, late detection of diabetic kidney disease (DKD) is common, and consequently, the nephron is irreversibly damaged. Many renal functional studies rely on long-term exposure to nephrotoxic agents, resulting in permanent damage to nephrons. Current standards of serum creatinine and macroalbuminuria tests of the Kidney Disease Improving Global Outcomes (KDIGO) guidelines detect clinical overt stage renal disease with the loss of glomerular filtration rate (GFR) of more than 50% and the loss of renal cortex mass caused by irreversible lesions and therefore do not provide a preventive measure [33]. On the contrary, renal protective drugs such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARB) deactivate various growth factors and subsequently inhibit renal hemodynamics and the mediators of kidney diseases. However, despite the effective goal of pharmacological treatments, their protective effects are lost with the loss of renal mass in compensatory

hyperfiltration (yet still declining) or treatment-resistant stages.

With a better understanding of the mechanisms of kidney injury in diabetes, it is an opportune time to develop tests to diagnose these new vital aspects of kidney injury. Hence, there is a need for more sensitive biomarkers whose stages clinically correlate with histological and nephron injuries of early DKD for a better therapeutic response.

Due to the anatomical and physiological breakdown in diabetic rat models used in most studies, many proteinaceous biomarkers are not useful for the evaluation of early microvascular disease. Therefore, glucose and its conversion products were investigated to compare them with histopathology and renal functional studies in this specific model. Four studied biomarker candidates (glucose, glycosylated serum protein, fructosamine, and glycation end product) change during the very early clinical stage of renal disease and cause a variety of early TCP findings. In the studies, on the alterations that occur during the early stage of renal injury, urine and serum excretions of low molecular weight ricin-like lectins (RGL-1 and RGL-3) significantly increase at 1 month.

## 10.2. Longitudinal Studies on Renal Function

Longitudinal renal function and histology data are desirable for pathophysiological and therapeutic research in diabetic nephropathy. However, there are many ethical concerns about serial renal biopsy. Thus, performing renal biopsy is quite limited in the initial stage of diabetic nephropathy, particularly normoalbuminuric diabetic nephropathy, for which serial renal biopsy poses a serious risk of renal dysfunction. Given the inevitable limitations of obtaining longitudinal renal biopsy, longitudinal data of renal function were assessed using surveys in clinical practice.

Progressive decline of renal function was observed in normoalbuminuric or early microalbuminuric patients with type 2 diabetes over a mean of 8 years despite intensive treatment of diabetes and hypertension. In patients with microalbuminuria, not only the baseline HbA1c but also the achieved HbA1c was an independent predictor for the decline of renal function, whereas there was no independent predictor for decline of renal function in normoalbuminuric patients. Thus, the beneficial effects of strict glycemic control on the prevention of diabetic nephropathy might be greater in patients with microalbuminuria [5]. A lower but a stable mean HbA1c value of 5.89 was associated with a possible remission of diabetic kidney disease.

Prevalence of renal perfusion index (RPI) was significantly lower in patients with primary diabetes than in those with secondary diabetes. During the one-year observation of diabetic patients with nephropathy, decreasing RPI was related to increasing GFR decline, fibrinogen, Cr, and LDL-C. However, anti-diabetic agents do not ameliorate RPI impairment in these patients. After adjusting the severity of diabetic nephropathy, the prevalence of RPI impairment had a significant association with subsequent decline of renal function in patients with diabetes (no fewer than 50% or less than 90 ml/min of eGFR). Decreased RPI reflects impaired tubulointerstitial perfusion worsening tubular function and leading to glomerular filtration impairment. Decreased RPI might be a new biomarker to predict renal function decline in patients with diabetes and tubulointerstitial disease. Further longitudinal studies on renal function are warranted in a larger number of patients with type 2 diabetes in clinical practice.

## 11. Patient Education and Engagement

Diabetic nephropathy patients often display similar social behaviors and crash diet, which are related to biochemical indicators such as BUN, Cr, UA, ALB, and proteinuria. The comparison of biochemical indicators displayed significant improvement after education compared with routine group. The observation of diastolic blood pressure exhibited significant differences. These results indicated that the effective health education intervention could enhance patients' awareness of diabetic nephropathy and physiological changes, promoting better patient

engagement in the treatment. Diabetic nephropathy (DN) is the most common microvascular complication of diabetes. It is an important cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), seriously affecting the patients' capacity for work and normal life [34]. This study aims to analyze the path-type health education of 162 diabetic nephropathy patients, in efforts to enhance patients' understanding of diabetic nephropathy and the effect of renal function intervention. Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes. Patients with diabetes will undergo physiological changes, and those changes are often the causes of poor health. As the blood glucose level rises, diabetes symptom approaches and microvascular lesions appear at the same time. With the gradual progression of lesions, some patients may develop diabetic nephropathy (DN), resulting in piebald albuminemia and increased proteinuria. Patients will experience significant alterations in routine biochemical indicators such as BUN, Cr, UA, ALB, and proteinuria. In clinical practice, hypoglycemic medications are not only the basic treatment. This study aimed to examine patients' cognition of disease, treatment, diet, exercise, and blood glucose control before and after intervention. It was hoped that the abnormal biochemical indicators would gradually return to normal, while renal function effectively improved.

### **11.1. Importance of Self-Monitoring**

Most diabetic patients have potentially progressive kidney disease that is preventable or reversible through lifestyle and therapeutic intervention. Pathologic and biochemical assessment of renal function is therefore practical in all diabetes clinics. In addition, the main test to be applied will depend on whether patients are normotensive or hypertensive. The main test to detect renal changes in older and normotensive patients with diabetes is an elevated urinary protein (albumin) excretion rate. Attempts to inhibit the renin-angiotensin system by either angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers can produce remarkable and fairly rapid renal recovery [35]. However, long-standing overt nephropathy, with reduced glomerular filtration rate and secondarily elevated plasma creatinine concentration, is not responsive to medical therapy and usually requires renal replacement therapy. In these patients, plasma creatinine estimation is the main and most practical renal function test.

Among hypertensive patients with and without diabetes, the prediction of renal failure is rather difficult due to a lack of uniformity in the terminology of renal function tests used. Nonetheless, decreased renal blood flow can create an increase in plasma creatinine concentration and a decrease in the glomerular filtration rate. In addition, other accompanying factors may change a plasma creatinine concentration independently of renal mass. The judgment should therefore be made with utmost caution. Self-motivated collection of urine specimens and the use of laboratory test kits could facilitate public health approaches towards early detection of hyperglycemia or body weight disorder. Nonetheless, home measurement of renal function tests may be a dream, due to the requirement of sophisticated chemical methods or determination of low-abundance analytes in urine sample. An alternative approach would be to measure urinary concentration of easily detectable substances, such as creatinine or protein, in a test strip configuration. Correlation with the values by conventional apparatus is feasible.

### **11.2. Lifestyle Modifications for Kidney Health**

Although chronic kidney disease (CKD) derives from multiple nongenetic factors, a healthy lifestyle may significantly decrease the risk of CKD. It is increasingly acknowledged that lifestyle choices play a key role in the development of CKD, and that changes of lifestyle are one of the most effective strategies to prevent disease onset and progression. Both primary prevention and secondary prevention should be considered [36]. The healthy lifestyle concept encompasses numerous habits, which collectively affect CKD risk by raising awareness and health-conscious attitudes in the general population.

Currently not sufficiently widespread, lifestyle modifications should adopt efforts to inform people that many habits are sound, elegant, and not expensive. Industrialized countries, in

particular, have poorly modulated drug intake regarding its extent and timing to prevent, treat and control both cardiovascular and renal diseases. Maintaining an optimal weight via caloric restriction and exercise must be sought after urgently starting from as early a stage as possible. Some hypotheses and findings on the interplay between weight gain, metabolic disorders, insulin resistance, and CKD are presented here, with special emphasis on dietary habits that are largely ignored in public health and research venues. The paradigm that an excess of refined carbohydrates and a poor intake of high-biological-value protein is predisposing factors for weight gain and insulin resistance development [37].

The implications for public health and research and strategies to correct behavioral risk factors in the general population are suggested. In addition to defined molecules or nutrients, nutrition might play a wider protective role, keeping physiological, organ-specific activities finely tuned and prepared for hazards. The hormones obeying to gut-brain feedback system, and their potential close and secret interplay in kidney regulation are proposed as an example, urging attention to equitable food consumption.

## 12. Conclusion

Diabetes mellitus is a chronic disorder characterized by hyperglycemia either due to autoimmune destruction of Beta cells, making patient insulin dependent, or due to insulin resistance plus relative deficiency of insulin making patients non-insulin dependent. Irrespective of the type of diabetes, deficit access to glucose for glycolytic metabolism leads to accumulation of precursors and gluconeogenesis, resulting in pathological changes leading to complications that are macrovascular and microvascular. It is required that patients with diabetes mellitus should be screened for the presence of complications which complicate prognosis. It is concluded that renal function was affected in both groups of diabetic patients based on the received data from the different parameters mentioned above. The blood samples from the individuals were collected for estimation of glucose in sodium flurried tube while urine, creatine and uric acid lithium heparin coated tube. The 90-95 percent of those suffering from type 1 diabetes and 50-80 percent of those suffering from type 2 diabetes eventually develop diabetic nephropathy. Elevated urinary albumin excretion rate is required to diagnose diabetic nephropathy concurrently with the classic renal function tests; and patients with diabetes and renal disease can no longer have diabetic nephropathy classified based on renal function alone. The best indicators for the resulting risk of the development of diabetic nephropathy in adults with diabetes are constant urinary albumin excretion rate. In the clinical routine, the detection of impaired renal function for the sake of commencing further clinical management or nephroprotection treatment is based on serum creatinine alone. As long as the estimated glomerular filtration rate does not drop below 60 mL/min/1.73 m<sup>2</sup>, moderate or moderately severe chronic kidney disease based on the reduction in GFR is considered not present; and ahead of this cutoff level, there is not a statistically significant relationship with hard cardiovascular outcomes such as death, hospitalization due to heart failure, and myocardial infarction.

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