Association between SIRT1 Gene Polymorphisms and Susceptibility to Diabetic Kidney Disease

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Basma Gamal
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<td>ACACB</td>
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<td>AceCS2</td>
<td>Acetyl coenzyme A synthetase 2.</td>
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<td>Albumin Creatinine Ratio.</td>
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<td>ADA</td>
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<td>ADIPOQ</td>
<td>Adiponectin gene.</td>
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<td>ADP</td>
<td>Adenosine diphosphate.</td>
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<td>AGES</td>
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<td>AGTR1</td>
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<td>AngII</td>
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<td>Angiotensin II type 1 receptor.</td>
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<td>ATP</td>
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<td>Bcl2L11</td>
<td>Bcl-2-like protein 11.</td>
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<td>BMI</td>
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<td>Blood pressure.</td>
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<td>CCr/CrCl</td>
<td>Creatinine clearance rate.</td>
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<td>CKD-EPI</td>
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<td>CoA</td>
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<td>COUPTF</td>
<td>Chicken ovalbumin upstream promoter transcription factor.</td>
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<td>CR</td>
<td>Calorie restriction.</td>
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<td>DCCT</td>
<td>Diabetes Control and Complications Trial.</td>
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<td>DKD</td>
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<td>DM</td>
<td>Diabetes Mellitus.</td>
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<td>DN</td>
<td>Diabetic nephropathy.</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid.</td>
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<td>ECM</td>
<td>Extracellular matrix.</td>
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<td>EDIC</td>
<td>Epidemiology of Diabetes Interventions and Complications.</td>
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<td>EDTA</td>
<td>Ethylene diamine tetra-acetic salt.</td>
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<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate.</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay.</td>
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<td>ELMO1</td>
<td>Engulfment and cell motility protein.</td>
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<td>ENaC</td>
<td>Epithelial sodium channel</td>
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<td>EPO</td>
<td>Erythropoietin</td>
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<td>ER</td>
<td>Endoplasmic reticulum</td>
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<td>ESRD</td>
<td>End-stage renal disease</td>
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<td>FIA</td>
<td>Fluorescence Immunoassay</td>
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<td>FOXO</td>
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<td>GCKR</td>
<td>Glucokinase regulatory protein</td>
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<td>Glutamate dehydrogenase</td>
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<td>GENIE</td>
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<td>Genome-wide association studies</td>
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<td>HDAC</td>
<td>Histone deacetylase</td>
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<td>HDL</td>
<td>High density lipoproteins</td>
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<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
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<td>HES1</td>
<td>Hairy and enhancer of split 1</td>
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<td>HEY2</td>
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<td>HTN</td>
<td>Hypertension</td>
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<td>HU</td>
<td>Hyperuricemia</td>
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<td>IDF</td>
<td>International Diabetes Federation</td>
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<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
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<td>IR</td>
<td>Insulin resistance</td>
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<td>IRS</td>
<td>Insulin-receptor substrate</td>
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<td>KDIGO</td>
<td>Kidney Disease Improving Global Outcomes</td>
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<td>KDOQI</td>
<td>Kidney Disease Outcomes Quality Initiative</td>
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<td>LP-CKD</td>
<td>Low proteinuric chronic kidney disease</td>
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<td>MDRD</td>
<td>Modified Diet in Renal Disease</td>
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<td>Mets</td>
<td>Metabolic syndrome</td>
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<td>MTSs</td>
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<td>N A-DKD</td>
<td>Normo albuminuric DKD</td>
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<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
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<td>NDR</td>
<td>National Diabetes Register</td>
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<td>NKF</td>
<td>National Kidney Foundation</td>
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<td>NLSs</td>
<td>Nuclear localization sequences</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<td>PGC-1α</td>
<td>Peroxisome proliferator-activated receptor γ coactivator 1α</td>
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<tr>
<td>PPAR γ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
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<td>Ptpn</td>
<td>Protein tyrosin phosphatase non receptor</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>Q</td>
<td>Quencher</td>
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<td>R</td>
<td>Reporter</td>
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<td>RAS</td>
<td>Renin-angiotensin system</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<tr>
<td>RIACE</td>
<td>Renal Insufficiency and Cardiovascular Events</td>
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<td>RID</td>
<td>Radial immunodiffusion</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>RRT</td>
<td>Renal replacement therapy</td>
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<td>RT-PCR</td>
<td>Real time polymerase chain reaction technique</td>
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<tr>
<td>S</td>
<td>Significant</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>SGLT2</td>
<td>Sodium glucose co transporter 2</td>
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<tr>
<td>SIGN</td>
<td>Scottish Intercollegiate Guidelines Network</td>
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<tr>
<td>Sir2</td>
<td>Silent information regulator 2</td>
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<tr>
<td>Sir2p</td>
<td>Silent information regulator 2 protein</td>
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<td>SNPs</td>
<td>Single Nucleotide Polymorphisms</td>
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<td>TCF7L2</td>
<td>Transcription factor 7-like 2</td>
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<td>Triglycerides</td>
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<td>TGFβ1</td>
<td>Transforming growth factor-β1</td>
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<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
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<td>UA</td>
<td>Uric acid</td>
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<td>UACR</td>
<td>Urinary albumin creatinine ratio</td>
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<td>UAE</td>
<td>Urinary albumin excretion</td>
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<td>UCP2</td>
<td>Uncoupling protein 2</td>
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<td>UK Prospective Diabetes Study</td>
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<td>UMDO</td>
<td>Uromodulin gene</td>
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<td>USFs</td>
<td>Upstream stimulatory factors</td>
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<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<td>VEGFA</td>
<td>Vascular endothelial growth factor A</td>
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Diabetic kidney disease (DKD), or CKD attributed to diabetes, occurs in 20–40% of patients with diabetes and is the leading cause of end-stage renal disease (ESRD). Diabetic kidney disease typically develops after diabetes duration of 10 years, or at least 5 years in type 1 diabetes, but may be present at diagnosis of type 2 diabetes (Tuttle et al., 2014).

Chronic kidney disease (CKD) is diagnosed by the presence of elevated urinary albumin excretion (albuminuria), low estimated glomerular filtration rate (eGFR), or other manifestations of kidney damage (Kidney Disease Improving Global Outcomes (KDIGO, 2012)).

Albuminuria and glomerular filtration rate (GFR) are recommended for use as clinical markers of diabetic nephropathy, and albuminuria has been considered as the first clinical sign of diabetic nephropathy (Wada et al., 2012).

However, a growing evidence from interventional and observational studies, has demonstrated that reduction in GFR may precede the development of albuminuria in many diabetic patients and that there is a discrepancy between the progression/ regression of albuminuria and decline in GFR in patients with DKD (Dwyer et al., 2012).

Over the last decade, it has been noted that nonalbuminuric chronic kidney disease (NA-CKD) is common, ranging between 25% and 50% among people with diabetes and estimated glomerular filtration rate (eGFR) less than 60ml/min/1.73m2. Nonalbuminuric diabetic kidney disease has been found to be increasingly prevalent with advanced age and female gender (Wada et al., 2012).
The pathogenesis of diabetic NA-CKD remains to be elucidated. In normoalbuminuric patients with type 1 diabetes and reduced GFR, the presence of relatively advanced classical diabetic nephropathy lesions could explain the GFR reduction. However, this is less well studied in type 2 diabetes. Hypothesized etiologies of NA-CKD include renal hypertensive and vascular diseases, age related renal senescence and masking of albuminuria by RAS inhibitors (Macisaac and Jerums, 2011).

Numerous studies have shown that genetic and environmental factors play considerable role in the process of CKD occurrence and progression (Kato and Natarajan, 2014).

DKD has the feature of familial aggregation, and the genetic factor plays an important role in the pathogenesis of DKD. The regulation of histone modification has a certain influence on the DKD related key epigenetic mechanism (Hang et al., 2016). SIRT1, a kind of NAD+ histone deacetylase in mammals, can regulate many biological functions in the body (Fang et al., 2015).

The function of SIRT1 in the occurrence and development of renal disease has been of increasing concern in humans, SIRT1 makes a contribution towards innate cell apoptosis reduction in the kidney, kidney failure retardation caused by increase in age, inflammation reduction, renal interstitial fibrosis inhibition, blood pressure regulation and autophagy induction (Kume et al., 2007).

In addition, SIRT1 can regulate energy metabolism under the conditions of caloric restriction and fasting through deacetylation of histones, nuclear transcription factor and related enzymes (Li et al., 2010).
SIRT1 activation can affect the development of age and obesity-related diseases such as diabetes, angiocardioathy and neurodegenerative diseases (Kume et al., 2013).

The SIRT1 pathway is a new therapeutic target for DKD. Some research work has shown that SIRT1-related SNPs rs10823108, rs3818292 and rs4746720 and the level of urine protein are associated with ESRD (Maeda et al., 2011).
Aim Of The Work

This study aims to investigate the association between SIRT1 gene polymorphism and development of diabetic kidney disease (DKD) in patients with type 2 diabetes (T2DM) based on the presence or absence of albuminuria.
Diabetic kidney disease

Definition of diabetic kidney disease (DKD):

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics. Diabetes may be classified as type 1 (autoimmune β-cell destruction and absolute insulin deficiency), type 2 (relative insulin deficiency and resistance), and other types (e.g. pancreatic disease) (Afkarian et al., 2016).

Diabetic kidney disease is usually a clinical diagnosis based on the presence of albuminuria and/or reduced eGFR in the absence of signs or symptoms of other primary causes of kidney damage. The typical presentation of diabetic kidney disease includes a long-standing duration of diabetes, retinopathy, albuminuria without hematuria, and gradually progressive kidney disease. However, signs of CKD may be present at diagnosis or without retinopathy in type 2 diabetes, and reduced eGFR without albuminuria has been frequently reported in type 1 and type 2 diabetes and is becoming more common over time as the prevalence of diabetes increases in the U.S. (de Boer et al., 2011 and Molitch et al., 2010).

As such, this clinical diagnosis requires only basic clinical and laboratory evaluations. The normal range for albuminuria is <30 mg/g, and the abnormal range is ≥30 mg/g, but values within both these ranges may be associated with an elevated risk of renal and cardiovascular diseases (American Diabetes Association, 2017).
The presence of moderately elevated urine albumin excretion (microalbuminuria) (30–300 mg/g) is widely regarded as a precursor of diabetic nephropathy, both indicating early risk and providing a target for intervention. However, in some cases microalbuminuria can display remission, either spontaneously or owing to treatment, resulting in a lower renal risk compared with progression of albuminuria (Hovind et al., 2004).

The broader term “kidney disease in diabetes” is used for patients with CKD (impaired renal function: estimated glomerular filtration rate [eGFR] < 60 ml/min per 1.73 m2 or proteinuria) regardless of the background. Although impaired renal function with normal albuminuria (ACR < 30 mg/g) is prevalent, particularly in elderly individuals, it is much less likely to progress if albuminuria is not present (Thorn et al., 2015).

Epidemiology:

Diabetes has become the most common single cause of end-stage renal disease (ESRD) in the U.S. and Europe; this is due to the fact that diabetes, particularly type 2, is increasing in prevalence and diabetic patients now live longer. About 20–40% of patients with type 1 and type 2 diabetes develop evidence of nephropathy but a considerably smaller fraction of those with type 2 diabetes progresses to ESRD. However, because of the much greater prevalence of type 2 diabetes, such patients constitute over half of those diabetic patients starting on dialysis. In the U.S., diabetic nephropathy accounts for about 40% of new cases of ESRD (Molitch et al., 2010).

In Egypt, the prevalence of DKD as a cause of end-stage renal disease (ESRD) increased from 8.9% of patients in 1996 to 14.5% in
2002. The mean age of diabetic nephropathy patients was higher than that of patients with ESRD due to other causes, mortality among diabetic patients with ESRD in Egypt is higher than mortality for all other causes of ESRD which is probably related to the well-known cardiovascular complications of diabetes (Afifi et al., 2004).

**Stages of DKD:**

Albuminuria is one of the most characteristic clinical signs in DKD. In the past, especially from the observations in patients with type 1 DM, the clinical stages of DKD were considered to begin from early glomerular hyperfiltration, followed by the development of microalbuminuria, macroalbuminuria, and then declined GFR. In untreated type 1 diabetics, approximately 80% of patients with sustained microalbuminuria increase their albumin excretion by 10%–20% per year until overt nephropathy develops, which normally takes 10–15 years. With the development of overt nephropathy, the GFR declines at a rate of 2–20 mL/minute/year and ESRD develops in 50% within 10 years and in 75% by 20 years (Drummond et al., 2012).

However, in many studies of type 2 DM, many patients with DKD do not manifest above classic step-by-step changes. Therefore, the concept of natural history of DKD is changing and continuing to be evolved. Albuminuria is now considered an active and deteriorating condition rather than a sequent process in DKD (Retnakaran et al., 2006).

For CKD in general, including in patients with diabetes, it has been recommended to stage the severity of the condition using a combination of etiology (if known), level of urinary albumin excretion, and eGFR category (Figure 1).
The National Kidney Foundation; Kidney Disease Outcomes Quality Initiative (KDOQI) working group for diabetes and CKD suggested that absence of retinopathy, fast deterioration of GFR, rapidly increasing or nephrotic-range albuminuria (>2500 mg/g), active urinary sediments, refractory hypertension, or signs or symptoms of other systemic diseases should raise suspicion of nondiabetic causes of CKD (*Kidney Disease Improving Global Outcomes (KDIGO, 2012)*).

**Risk Factors:*

The two main risk factors for DKD are hyperglycemia and arterial hypertension. However, DKD develops in only about 40% of patients, even in the presence of hyperglycemia and elevated blood pressure (BP) for long periods of time. This observation raised the concept that DKD will develop only in a susceptible subset of patients (*Krolewski et al., 2001*).

Furthermore, family studies have confirmed a genetic contribution for the development of DKD in both type 1 and type 2 diabetes (DM).
Once DKD is present, progression factors may act, favoring evolution to more advanced stages. There is evidence that some factors involved in the development of proteinuria are also common to the loss of GFR, but others are unique to each one of them (*Placha et al., 2005*).

1- Hyperglycemia:

Hyperglycemia is well known risk factor for DKD and it recognized that intensive glucose control reduces the risk of DKD. Specifically, during the Diabetes Control and Complications Trial (DCCT), nearly normalization of blood sugar decreased the risks of incident microalbuminuria and macroalbuminuria by 39% (95% CI 21%- 52%) and 54% (95% CI 29%-74%), respectively, compared with conventional therapy (*Gheith et al., 2014*).

One of the main potentially modifiable risk factors for diabetic nephropathy initiation and progression in susceptible individuals is sustained hyperglycemia (*Stratton et al, 2011*).

Intensive diabetes management with the goal of achieving near normoglycemia has been shown in large studies to delay the onset of microalbuminuria and the progression to macroalbuminuria in patients with type 1 and type 2 diabetes (*Reichard et al., 2010*).

Improvement of glycemic control, reflected by glycosylated hemoglobin values of about 7.0%, is associated with lower urinary albumin excretion and a decrease in the incidence of diabetic nephropathy (*Parving et al., 2011*).
2- Arterial Hypertension:

Hypertension (blood pressure > 140/90 mmHg) is a common comorbidity of diabetes, affecting 20-60% of people with diabetes, depending on age, obesity, and ethnicity. Hypertension is also major risk factor for cardiovascular diseases (CVD) and microvascular complications such as retinopathy and nephropathy. In type 1 diabetes, hypertension is often the result of underlying nephropathy. In type 2 diabetes, hypertension is likely to be present as part of the metabolic syndrome (i.e., obesity, hyperglycemia, dyslipidemia) that is accompanied by high rates of cardiovascular diseases (CVD) (American Diabetes Association, 2011).

Arterial hypertension is a main risk factor for the development of DKD, and probably the best known relevant factor related to its progression. Analysis of UK Prospective Diabetes Study (UKPDS) showed that every 10 mmHg reduction in systolic BP is associated with a 13% reduction in the risk of microvascular complications, with the smallest risk among those patients with systolic BP <120 mm Hg (Adler et al., 2000).

High blood pressure accelerates the progressive increase in the level of albuminuria in patients with type 2 diabetes who have initially normal albumin levels and accelerates the loss of renal function in those with overt nephropathy. Both effects are prevented or limited by antihypertensive therapy (Bakris, 2001).

3-Genetic Factor:

The exact genetic model underlying DKD susceptibility is uncertain, but theoretically few genes with a major contribution and some with
minor interaction with the environment could cause DKD. Unfortunately, no gene with a major effect had been identified so far. The knowledge of which gene(s) predisposes to DKD will allow the identification of patients at high risk for this complication, and adoption of preventive measures (Krolewski et al., 2001).

The increase in risk cannot be explained only by the duration of diabetes or hypertension, or the degree of glycemic control. Environmental and genetic factors must, therefore, have roles in the pathogenesis of diabetic nephropathy (Adler, 2004).

4- Dyslipidemia:

Raised plasma triglycerides and low levels of high density lipoproteins (HDL) have been correlated with the development of diabetic nephropathy. Triglycerides and cholesterol reduction had not been found to alter the progression of renal disease (Mirsa et al., 2003).

Several studies indicate that diabetic dyslipidemia, i.e., high triglycerides (TG) and/or low high-density lipoprotein cholesterol (HDL-C) levels, is an independent residual risk for DKD in patients with atarget values of blood glucose and BP. Although the pathophysiological bases linking dyslipidemia to DKD are largely undefined, it has been suggested that either deficient and/or dysfunctional HDL particles, hampering reverse cholesterol transport from renal cells, may contribute to intra-renal lipid accumulation leading to glomerulosclerosis and tubule-interstitial damage (Vaziri, 2010).

5- Proteinuria:

Proteinuria may hasten nephropathy development. Exposure of tubular cells to high concentrations of filtered proteins and growth factors
has been shown to trigger a tubulointerstitial inflammatory response that induces progressive interstitial fibrosis (Rabkin, 2003). Proteinuria is generally regarded as a marker for the degree of glomerular damage: the level of proteinuria correlates well with the prognosis of renal function and interventions that retard the progression of diabetic renal disease also reduce proteinuria (Remuzzi and Bertani, 1990). Proteinuria > 2 g/day was associated with disease progression and adverse renal outcome, independent of the underlying renal lesion. The magnitude of proteinuria probably reflects the severity of underlying renal disease, and proteinuria presence is tubulotoxic. Thus, the prognostic value of proteinuria 2 g/day applies not only to diabetic patients in general but also to those with superimposed or isolated non diabetic renal lesions (Wong et al., 2002).

6- Glomerular hyperfiltration:

Elevated GFR values are present in about one third of type 2 DM patients and theoretically it could cause DKD due to glomerular damage. Studies led to controversial findings regarding its role as a risk factor for the development of DN (Ficociello et al., 2009).

Type 2 DM patients with a single-kidney more often present increased urine albumin excretion (UAE) levels (Silveiro et al., 1998). On the other hand, type 1 DM patients with only one kidney do not have a more aggressive disease. Glomerular hyperfiltration probably plays a small role, if any, in the development of DKD (Chang et al., 2008).

7- Smoking:

Patients with type 2 diabetes who smoke have a greater risk of microalbuminuria than patients who do not smoke, and their rate of
progression to end-stage renal disease is about twice as rapid (Orth et al., 2011).

8- Protein Intake:

Increased dietary protein intake seems to be associated with the presence of higher UAE values, at least in patients with type 1 DM. In patients with type 2 DM this association has not been documented. The source of proteins in the diet also seems to be related to the presence of DKD. A higher intake of fish protein is related to a lower risk of microalbuminuria in type 1 DM patients. The mechanisms involved in these findings are unknown but probably related to hemodynamic factors (Mollsten et al., 2001).

Dietary protein restriction has also been reported to retard progression of diabetic nephopathy in patients with type 1 diabetes (Zeller et al., 1991).

9-Overweight:

High body mass index (BMI) increases the risk of development of chronic kidney disease (CKD) in patients with DM. Furthermore, adequate diet and reduction in body weight decrease proteinuria and improve kidney function in those patients. The role of overweight as a risk factor for DN (independent of DM and glycemic control) has not been clearly confirmed (Saiki et al., 2005).

10- Uric acid and the metabolic syndrome:

Several cross-sectional observational studies have documented a strong association between increased serum uric acid levels and reduction of GFR or albuminuria in patients with T2D. In addition, the duration of
diabetes appeared to be a modulator of the link between serum uric acid and incidence of DKD, as the link was weaker in patients with short disease duration (Viazzi et al., 2017).

It is well known that hyperuricemia (HU) is strongly associated with metabolic syndrome (Mets) and its individual components, even after adjusting for obesity and insulin resistance (IR). Since both UA and Mets are predictors and they contribute to the onset of DKD independently of each other. In addition, the incidence of low eGFR was higher in patients with HU also when the coexistence or absence of Mets was taken into account, while albuminuria developed more frequently in those with HU and Mets compared to the group of reference (De Cosmo et al., 2015).

Pathology and pathophysiology

The histopathological lesions of DKD have been classified (Table 1). Renal pathological changes are present in patients with long-standing diabetes prior to the onset of microalbuminuria (Adler et al., 2002).

Development of DKD is associated with many alterations in the structure of multiple kidney compartments (Figure 2). The earliest consistent change is thickening of glomerular basement membrane, which is apparent within 1.5–2 years of DM diagnosis. It is paralleled by capillary and tubular basement membrane thickening (Caramori et al., 2013). In glomeruli, there is mesangial expansion, thickening of the basement membrane, and characteristically, nodular glomerulosclerosis (Kimmelstiel–Wilson nodules) (Weil et al, 2012).

Other glomerular changes include loss of endothelial fenestrations, mesangial matrix expansion, and loss of podocytes with effacement of
Chapter 1

Diabetic kidney disease

foot processes. Mesangial volume expansion is detectable within 5–7 years after DM diagnosis (Fioretto and Mauer, 2007).

Figure (2): Normal kidney morphology and structural changes in diabetes mellitus.

Diabetic kidney disease induces structural changes, including thickening of the glomerular basement membrane, fusion of foot processes, loss of podocytes with denuding of the glomerular basement membrane, and mesangial matrix expansion (Radica et al., 2017).

In early cases, tubular hypertrophy is present but eventually interstitial fibrosis with tubular atrophy develops, along with arteriolar hyalinosis in advanced cases, there is an infiltrate of macrophages and T-lymphocytes. Ultra structurally, there is podocyte loss and reduced endothelial cell fenestration (Weil et al., 2012)

In patients with type 1 DM, GFR, albuminuria, and hypertension are strongly correlated with mesangial expansion and somewhat less strongly associated with glomerular basement membrane width. Renal structure changes in patients with type 2DM are similar to those seen in type 1DM, but they are more heterogeneous and less predictably associated with clinical presentations (Fioretto and Mauer, 2007).
Table (1): Pathological classification of glomerular lesions in diabetic kidney disease:

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Inclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Mild or non-specific light microscopy changes and GBM thickening proved by electron microscopy</td>
<td>Near normal light microscopy Biopsy does not meet any of the criteria for class II, III or IV GBM &gt;395 nm in female and &gt;430 nm in male subjects 9 years of age or older</td>
</tr>
<tr>
<td>IIa</td>
<td>Mild mesangial expansion</td>
<td>Mild mesangial expansion &lt; 25% of the observed mesangium. Biopsy does not meet criteria for class III or IV</td>
</tr>
<tr>
<td>IIb</td>
<td>Severe mesangial expansion</td>
<td>Severe mesangial expansion &gt; 25% of the observed mesangium Biopsy does not meet criteria for class III or IV</td>
</tr>
<tr>
<td>III</td>
<td>Nodular sclerosis (Kimmelstiel–Wilson lesion)</td>
<td>At least one convincing Kimmelstiel–Wilson lesion. Biopsy does not meet criteria for class IV</td>
</tr>
<tr>
<td>IV</td>
<td>Advanced diabetic glomerulosclerosis</td>
<td>Global glomerular sclerosis in &gt;50% of glomeruli. Lesions from class I–III</td>
</tr>
</tbody>
</table>

Pathogenesis:

DKD is characterized by an accumulation of extracellular matrix (ECM) proteins such as collagen and fibronectin in renal compartments, resulting in tubular interstitial fibrosis, glomerular mesangial hypertrophy and expansion, thickening of the glomerular basement membrane, podocyte foot process effacement and inflammation due to the infiltration of monocytes and macrophages. All of these factors contribute to renal dysfunction and can ultimately lead to ESRD (Kanwar et al., 2011).

Diabetogenic stimuli, including high blood glucose levels; advanced glycation end products (AGEs); growth factors such as transforming growth factor-β1 (TGFβ1), angiotensin II (AngII) and...
platelet-derived growth factor; and inflammatory cytokines, have been implicated in the pathogenesis of DKD owing to their adverse effects on multiple renal cell types (Ruggenenti et al., 2010). Up regulation of the above-mentioned growth factors and cytokines activates signal transduction pathways, including protein kinase C and AKT kinase cascades, leading to the activation of key effector transcription factors such as SMADs, nuclear factor-κB (NF-κB) and upstream stimulatory factors (USFs) (Fig. 3). Together, these mechanisms promote the expression of genes associated with fibrosis, hypertrophy, apoptosis, inflammation, oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial dysfunction and autophagy (Kato et al., 2019).

In particular, TGFβ1, which is overexpressed in several renal cell types in diabetes mellitus, has been widely studied in DKD progression owing to its pro-fibrotic effects (Kanwar et al., 2011).

![Figure (3): Different pathways involved in the initiation and progression of diabetic kidney disease. (Radica et al., 2017).](image-url)
1- Hyperglycemia, Advanced Glycosylation End Products (AGEs) (Metabolic pathway):

Persistent hyperglycemia is a strong risk factor for DKD and causes the proliferation of mesangial cells and their matrix, as well as the thickening of the basement membrane. Hyperglycemia increases the expression of VEGF in podocytes causing increased vascular permeability (Yamagishi et al., 2007).

Hyperglycemia may contribute to alterations in endothelial and mesangial cell structure, synthetic capacity, function and podocyte injury. For example, in endothelial cells, hyperglycemia leads to changes in cell shape, generalized basement membrane thickening, vasoconstriction, and reduction in endothelial cell life span (Caglierro et al., 2011).

If hyperglycemia is sustained, AGEs may be formed. Hyperglycemia causes non enzymatic glycation of proteins resulting in products such as HbA1c (Caglierro et al., 2011).

AGE formation on collagens and matrix proteins tends to decrease endothelial cell adhesion and replication, favor mesangial cell vasoconstriction and growth (Gnudi et al., 2003).

2- Haemodynamic and hypetrophic pathways:

Glomerular hyperfiltration leads to the occurrence of DKD. Hyperglycemia causes afferent arteriolar dilatation by release of vasoactive mediators, such as insulin-like growth factor 1 (IGF-1), glucagon, nitric oxide (NO), vascular endothelial growth factor (VEGF) and prostaglandin. On the other hand, alterations in renal tubular function also occur in the early stage of DM, and are related to the degree of glycemic control. Due to high filtrated load of glucose,
reabsorption of both glucose and sodium chloride is increased because of up regulation of sodium glucose co transporter 2 (SGLT2) in the proximal tubules. Thus, the delivery of sodium chloride to the maculadensa of distal tubules is decreased, and then causes dilatation of afferent arteriole because of tubuloglomerular feedback. At the same time, constriction of efferent arteriole occurs due to high local level of angiotensin II, and then causes changes of autoregulation and glomerular hypertension (Tuttle, 2017).

In the kidney, activation of endothelin-receptor A is not only associated with vasoconstriction, but also podocyte injury, oxidative stress, inflammation, and fibrosis (de Zeeuw et al., 2014).

3- Growth Factors and Cytokines:

A series of circulating markers of inflammation such as C reactive protein, interleukins and TNF-α are increased in DKD and their levels correlate with albuminuria and progression to ESRD. In addition, hyperglycemia, TGF-β1 and angiotensin II stimulate the secretion of VEGF, causing the production of endothelial nitric oxide, vasodilation and glomerular hyper filtration (Shaker et al., 2014).

Hyperglycemia, possibly mediated by oxidative stress, also induces angiotensin II to the synthesis of TGF- β, type IV collagen and fibronectin, contributing to progressive glomerulosclerosis (Pezzolesi et al., 2009).

Inflammatory factors are also involved in the development of tubulointerstitial lesion, and appear to lead to accumulation of macrophages in the tubular interstice in animal models designed to study DKD. Macrophages also produce free radicals, inflammatory cytokines
and proteases that induce tubular damage. Furthermore, glomerular and renal cells also produce a series of inflammatory factors when they are exposed to glomerular hyper filtration and increased UAE, intensifying this process \((Brosius \textit{et al.}, 2008)\).

4- Genetics:

Role of inheritance in the pathogenesis of diabetic nephropathy is demonstrated by the high concordance rate for diabetic nephropathy in families -and the fact that different rates of nephropathy exist in different racial groups, however a simple Mendelian inheritance model does not occur in diabetic nephropathy, making the approach to genetic studies very difficult \((Borch \textit{et al.}, 2010)\).

The ethnic background plays an important role because some races are more susceptible to diabetic nephropathy than others. In fact, the rate of developing ESRD is five times higher in relatives of black patients with type 2 diabetes in renal replacement therapy (RRT) \((Friedman \textit{et al.}, 2007)\).

**Pathogenesis of normoalbuminuric renal insufficiency in diabetes:**

There are several possible pathogenic mechanisms that may account for the development of normoalbuminuric renal insufficiency in diabetes. One possibility is that renal ischemia due to intrarenal arteriosclerosis may be related to the development of normoalbuminuric renal insufficiency. A negative correlation between GFR and the intrarenal arterial resistance index was found in type 2 diabetes, regardless of albuminuria stage \((MacIsaac \textit{et al.}, 2006)\).

In addition, carotid intimal medial thickness, carotid stiffness, and silent cerebral infarction were also reported to be associated with
impaired kidney function in type 2 diabetes, independent of microalbuminuria (Uzu et al., 2010).

The other possibility is that genetic susceptibility may contribute to the development of normoalbuminuric renal insufficiency. Polymorphisms of the protein kinase C-b gene were reported to be associated with accelerated decline of estimated GFR (eGFR) in type 2 diabetes without overt proteinuria (Araki et al., 2006).

Although the increasing use of renin-angiotensin system (RAS) blockade may be related to the increasing prevalence of normoalbuminuric renal insufficiency, the RIACE study showed that the use of RAS blockade was more common in patients with albuminuric renal insufficiency than in those with normoalbuminuric renal insufficiency (Penno et al., 2011).

**Screening for Diabetic Kidney Disease:**

Screening begins at diagnosis of type 2 diabetes and usually 5 years after onset of type 1 diabetes. Timed urine collections can also be utilized and will average out diurnal variations in albumin excretion (normal <20 μg/minute) (American Diabetes Association, 2014).

Most guidelines recommend screening with a spot urine albumin/creatinine ratio (ACR; normal <30 mg/g creatinine), from either first morning (preferred) or random specimens. An abnormal result is repeated once or twice over a few months for consistency. This is coupled with an assessment of renal function, using the Modification of Diet in Renal Disease or Chronic Kidney Disease Epidemiology Collaboration formulas for estimated GFR (eGFR) in order to stage chronic kidney disease (CKD) (Johnson et al., 2012).
Screening for kidney damage can be most easily performed by urinary albumin creatinine ratio (UACR) in a random spot urine collection. Timed or 24 hours urine collections are more burdensome and add little to prediction or accuracy. Measurement of a spot urine sample for albumin alone (by immunoassay or by using a sensitive dipstick test specific for albuminuria) without simultaneously measuring urine creatinine (Cr) is less costly but liable to false-negative and false positive determinations as a result of variation in urine concentration due to hydration. Normal UACR is defined as 30 mg/g Cr, and increased urinary albumin excretion is defined as ≥30 mg/g Cr. Because of variability in urinary albumin excretion, two of three specimens of UACR collected within a 3 to 6 month period should be abnormal before considering a patient to have albuminuria. Exercise within 24 h, infection, fever, congestive heart failure, marked hyperglycemia, menstruation, and marked hypertension may elevate UACR independently of kidney damage (American diabetes association, 2016).

1- Screening for albuminuria:

Screening for microalbuminuria should be performed on diagnosis of type 2 diabetes. Microalbuminuria rarely occurs with short disease duration in type 1 diabetes; therefore, screening is recommended after 5 years of disease duration. Because microalbuminuria is also a marker for cardiovascular morbidity and mortality in diabetic patients, its presence is an indication to screen for possible vascular disease and to aggressively control cardiovascular risk factors (American Diabetes Association, 2012).

Microalbuminuria is urinary albumin excretion of 30-300mg/24 hrs (equivalent to 20μg/min on a timed specimen or 30 mg/g creatinine on a
random sample). Microalbuminuria is an early marker of diabetic nephropathy and is also a valuable marker of cardiovascular risk in type 2 diabetes (Goud et al., 2011).

Estimation of microalbuminuria is done by semiquantitative methods as microalbumin urine test strip or quantitative methods as radial immune assay (RIA), enzyme-linked immunosorbent assay (ELISA), radial immune diffusion (RID), and immune turbidimetry (Goud et al., 2011).

Albumin specific measurements are required; as measurements of urinary total protein are insufficiently sensitive, timed overnight collections for the albumin excretion rate are the gold standard but are difficult to carry out in large population. The ratio of albumin to creatinine is simple, requiring patients to bring a spot urine sample (which preferably should be passed on rising in the morning) with them to the clinic. The albumin creatinine ratio (ACR) measured on such samples relates well to the timed albumin excretion rate (American Diabetes Association, 2012).

In addition to its being an early manifestation of nephropathy, microalbuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either type 1 or type 2 diabetes. Thus, the finding of microalbuminuria is an indication for screening for possible vascular disease and aggressive intervention to reduce cardiovascular risk factors (Molitch et al., 2010).

Isolated microalbuminuria or macroalbuminuria usually indicates the presence of diabetic nephropathy, but the presence of other abnormalities on urine analysis suggests another renal disease. Screenings for background or proliferative retinopathy are especially important in all patients with urinary abnormalities. If retinopathy is present, albuminuria
can be attributed with confidence to diabetic nephropathy; if there is no evidence of retinopathy; one should look for other causes of albuminuria (Yun et al., 2013).

All abnormal test must be confirmed in two out of three samples collected over a 3 to 6 month period due to the known day to day variability in urinary albumin excretion (UAE) (Eknoyan, 2009).

Screening should not be performed in the presence of conditions that increase UAE, such as urinary tract infection, hematuria, acute febrile illness, vigorous exercise, short-term pronounced hyperglycemia, uncontrolled hypertension, and heart failure (Mogensen and Kher, 2011).

Table (2): Definitions of abnormalities in albumin excretion:

<table>
<thead>
<tr>
<th>Category</th>
<th>Spot collection (mg/g creatinine)</th>
<th>24h collection (mg/24hours)</th>
<th>Timed collection (μg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30-300</td>
<td>30-300</td>
<td>20-200</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

(National Kidney Foundation, 2007).

2-Monitoring glomerular filtration rate (GFR):

Because up to 36 % of persons with type 2 diabetes have renal insufficiency without microalbuminuria or macroalbuminuria, annual screening for diabetic nephropathy should also include measurement of serum creatinine and estimation of GFR (Roett et al., 2012).

Estimated Glomerular Filtration Rate: Describes the flow rate of filtered fluid through the kidney, while Creatinine clearance rate (CCr or CrCl): is the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the
GFR. Creatinine clearance exceeds GFR due to creatinine secretion, which can be blocked by cimetidine. In alternative fashion, overestimation by older serum creatinine methods resulted in an underestimation of creatinine clearance, which provided a less biased estimate of GFR. (*Stevens and Levin, 2013*).

Both GFR and CCr may be accurately calculated by comparative measurements of substances in the blood and urine, or estimated by formulas using just a blood test result (eGFR and eCCr). The results of these tests are used to assess the excretory function of the kidneys (*Stevens and Levin, 2013*).

In clinical practice, GFR can be estimated by prediction equations that take into account serum creatinine concentration and some or all of the following variables: age, sex, race, and body size. The recommended equation by the National Kidney Foundation is that of the MDRD (Modified Diet in Renal Disease): $GFR = 186 \times [serum\ creatinine\ (mg/dl) – 1.154 \times age\ (years) – 0.203 \times (0.742\ if\ female) \times (1.210\ if\ African\ American)]$ (*Levey et al., 2003*).

The Cockroft-Gault equation: $creatinine\ clearance\ (ml/min) = \left(\frac{140\ age\ (years)\times weight\ (kg)}{72 \times serum\ creatinine(mg/dl) \times (0.85\ if\ female)}\right)$. The reference range of GFR values in young individuals is from 80 to 130 ml/min/1.73 m2, declining at 10ml/ min/ decade after 50 years of age (*Granerus and Aurell, 1981*).
Diabetic kidney disease

Table (3): stages of nephropathy:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or increased eGFR*</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mildly decreased eGFR</td>
<td>60 - 89</td>
</tr>
<tr>
<td>3</td>
<td>Moderately decreased eGFR</td>
<td>30 - 59</td>
</tr>
<tr>
<td>4</td>
<td>Severely decreased eGFR</td>
<td>15 – 29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure, 15 or dialysis</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

(American Diabetes Association, 2016).

Diagnosis of Diabetic Kidney:

Kidney biopsy is the definitive method to establish the diagnosis of DKD, although in most cases, careful evaluation of patients can identify subjects most likely to have DKD without the need for kidney biopsy. Albuminuria and GFR are recommended for use as clinical markers of DKD, and microalbuminuria has been considered as the first sign of the disease. However, studies demonstrated that diagnostic accuracy of microalbuminuria is limited by the fact that structural damage might precede albumin excretion (Currie et al., 2014).

Renal damage may underlie early stage of DKD, which, in most cases, is asymptomatic. Proteinuria is present in patients with advanced stage. Early detection and intervention in diabetic patients with DKD will possibly reverse, or even eliminate, underlying renal damage. Therefore the early diagnosis and treatment of DKD are crucial (Menne and Haller, 2011).

The criteria for early diagnosis of DKD vary among different guidelines (Ahmed et al., 2016).
NKF guidelines published in 2007, do not consider albuminuria (microalbuminuria or macroalbuminuria) for the diagnosis of DKD in T2D unless accompanied by retinopathy (National Kidney Foundation, 2007). In most people with diabetes, CKD should be considered in the presence of (i) macroalbuminuria or microalbuminuria plus retinopathy, and (ii) in people with type 1 diabetes (T1D), in the presence of microalbuminuria plus duration of diabetes longer than 10 years. Based on studies showing positive relationship between the duration of diabetes and DKD, they expressed that the presence of elevated albuminuria in T1D of short duration should raise possibilities about non-DKD. In 2012, NKF has expressed concern that the presence of macroalbuminuria without retinopathy, present within 10 years of onset in patients with T1D, suggests a need for investigations to rule out non-DKD; as there is spontaneous remission of microalbuminuria in up to 40% of patients with T1D. About 30–40% remains with microalbuminuria and do not progress to macroalbuminuria over 5–10 years of follow-up (National Kidney Foundation, 2012).

American Diabetes Association (ADA, 2019), in their standards of medical care in diabetes 2019, has stated DKD to be a clinical diagnosis that is based on the presence of albuminuria and/or reduced eGFR in the absence of signs or symptoms of other primary causes of kidney damage. However, signs of CKD may be present at diagnosis or without retinopathy in T2D, and reduced eGFR without albuminuria has been frequently reported in type 1 and T2D and is becoming more common over time as the prevalence of diabetes increases in the US (American Diabetes Association, 2019).

Intercollegiate Guidelines Network (SIGN) 2010 (updated November 2017), classifies DKD, on the basis of the extent of urine
albumin excretion, as either microalbuminuria or diabetic nephropathy. They consider microalbuminuria as the earliest, clinically detectable manifestation of classic DKD. Remission of microalbuminuria may occur and so the presence of microalbuminuria does not imply an inexorable progression to diabetic nephropathy. Diabetic nephropathy is defined by a raised urinary albumin excretion of >300 mg/day. This represents a more severe and established form of renal disease and is more predictive of total mortality, CV events and ESKD than microalbuminuria. The SIGN do not consider DKD in the absence of albuminuria even if there is a sustained low GFR (Scottish Intercollegiate Guidelines Network, 2010).

According to National Institute for Health and Care Excellency (NICE) guidelines of United Kingdom 2014 (modified on March 2015), CKD has been defined as abnormalities of kidney function or structure present for more than 3 months, with implications for health. In people with diabetes microalbuminuria should be considered clinically significant (NICE guidelines, 2014).

International Diabetes Federation (IDF) guideline, diagnose DKD on the basis of a raised urine albumin (microalbuminuria or ACR >30 mg/gm) or a reduced eGFR (<60 mL/min/1.73m2) in a patient with diabetes (International Diabetes Federation, 2017).

According to Malaysian guidelines 2015, the diagnosis of DKD is made clinically by the presence of persistent proteinuria (either microalbuminuria or macroalbuminuria). Microalbuminuria has been considered as the earliest sign of diabetic nephropathy as it predicts increased CV mortality and morbidity and ESKD (Ministry of Health Malaysia, 2015).
Prognosis of Diabetic Kidney:

The classification of diabetic kidney disease based on albuminuria and eGFR level is simple, provides prognostic information, and is helpful to guide therapeutic decisions; but it is not perfect. Not all patients with abnormal albuminuria progress to ESRD or cardiovascular disease, and the same is true of many patients with impaired renal function (eGFR < 60 ml/min per 1.73 m²). Therefore an intensive search for new biomarkers in blood or urine that could improve diagnostic and prognostic precision in early or later stages of diabetic kidney disease has been ongoing during the past decades. The underlying hypothesis is that the development from uncomplicated diabetes to renal damage, impaired renal function, and finally ESRD, cardiovascular events, or death takes years, and that an increased risk of progression or early changes in structure or function are reflected by changes in such biomarker (Levey et al., 2011).

The overall prevalence of microalbuminuria and macroalbuminuria in both types of diabetes is approximately 30-35%. Microalbuminuria independently predicts cardiovascular morbidity, and microalbuminuria and macroalbuminuria increase mortality from any cause in diabetes mellitus. Microalbuminuria is also associated with increased risk of coronary and peripheral vascular disease and death from cardiovascular disease in the general non diabetic population (Anderson et al, 2011).

Patients in whom proteinuria did not develop have a low and stable relative mortality rate, whereas patients with proteinuria have a 40-fold higher relative mortality rate (Parving et al., 2011).
Sirtuins

Sirtuins or Sir2 (silent information regulator 2)-related enzymes defined as a family of nicotinamide adenine dinucleotide-dependent enzymes that deacetylate lysine residue on various proteins. Certain sirtuins have in addition an ADP-ribosyltransferase activity. The sirtuins are remarkably conserved throughout evolution from archaebacteria to eukaryotes. The mammalian sirtuins SIRT1–SIRT7 are implicated in a variety of cellular functions ranging from gene silencing, over the control of the cell cycle and apoptosis, to energy homeostasis (Yamamoto et al., 2007).

The founding member of the sirtuin protein family was the silent information regulator 2 protein (Sir2p) of Saccharomyces cervisiae, a nicotinamide adenine dinucleotide (NAD$^+$)-dependent histone deacetylase (HDAC) that regulates chromatin silencing (Denu, 2003).

Yeast strains with abnormal levels of Sir2p show defects in many cellular functions, including transcriptional and recombinational silencing, senescence, and DNA repair. In S. cervisiae, there are four sirtuins (NAD$^+$-dependent histone deacetylases Hst1–Hst4) in addition to Sir2p, whereas in mammals seven homologs, i.e. SIRT1–SIRT7, have been identified (Frye, 1999).

The remarkable conservation of members of the sirtuin gene family from yeast to humans indicates that these proteins play vital physiological roles (Frye, 2000).
Table (4): Localization, substrates, functions and enzyme activities of different sirtuins:

<table>
<thead>
<tr>
<th>Sirtuin</th>
<th>Activity</th>
<th>Substrates</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIRT1</td>
<td>Deacetylase</td>
<td>p53, Foxo1, Foxo3, Bax, Hif-1α, Hif-2α, HSF1, Ku70, b-catenin, E2F1, Myc, STAT3, PGC-1α, NF-κB, TORC2, LXR, FXR, SREBP, PER2, CLOCK</td>
<td>Energy metabolism, stress response</td>
</tr>
<tr>
<td>SIRT2</td>
<td>Deacetylase</td>
<td>Tubulin, H4, Foxo3a</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>SIRT3</td>
<td>Deacetylase</td>
<td>Oxidative phosphorylation complex I, AceCS2, LCAD, HMG-CoA synthase 2, IDH2, MnSOD, SOD2</td>
<td>ATP production, anti-oxidative stress, thermogenesis</td>
</tr>
<tr>
<td>SIRT4</td>
<td>ADP-ribosyltransferase</td>
<td>GDH</td>
<td>Insulin secretion, fatty acid oxidation</td>
</tr>
<tr>
<td>SIRT5</td>
<td>Deacetylase</td>
<td>CPS1</td>
<td>Urea cycle</td>
</tr>
<tr>
<td>SIRT6</td>
<td>Deacetylase/ADP-ribosyl-transferase</td>
<td>H3K9, H3K56, CtIP, SIRT6</td>
<td>DNA repair, metabolism, inflammation</td>
</tr>
<tr>
<td>SIRT7</td>
<td>Deacetylase?</td>
<td>p53?</td>
<td>rDNA transcription</td>
</tr>
</tbody>
</table>

Among the large HDAC protein family, sirtuins were originally categorized as class III HDACs. Whereas classes I and II HDACs use zinc as a cofactor and are inhibited by trichostatin A (Lamming et al., 2005), sirtuins are not inhibited by trichostatin A and convert acetylated protein substrates in a reaction that uses NAD⁺ into a deacetylated protein, nicotinamide, and the acetyl ester metabolites 2′-O- and 3′-O-acetyl-ADP ribose (AADPR), which are formed by the transfer of the acetyl group to the ADP-ribose portion of NAD⁺ (Smith and Denu, 2006) (Fig. 4).

The deacetylase activity of the sirtuins is controlled by the cellular [NAD⁺]/[NADH] ratio, i.e. NAD⁺ works as an activator, whereas nicotinamide and reduced nicotinamide adenine dinucleotide (NADH) inhibit their activity (Revollo et al., 2004).
Because sirtuins are class III HDACs, it was logical that their function initially became associated with transcriptional repression. Acetylated histones H1, H3, and H4 are known to be physiological substrates for the sirtuins, and lysine 16 in histone H4 appears to be the most critical residue for sirtuin-mediated transcriptional silencing (Liou et al., 2005). Afterwards, it has been recognized that a growing number of nonhistone proteins are also deacetylated by the sirtuins, largely expanding their biological roles. These nonhistone sirtuin substrates include several transcriptional regulators, such as the nuclear factor-κB (NFκB), forkhead box type O transcription factors (FOXO), and the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α), but also enzymes, such as acetyl coenzyme A (CoA) synthetase 2 (AceCS2), and structural proteins, such as α-tubulin (D’Mello, 2009).

Figure (4): The histone deacetylase (HDAC) family (D’Mello, 2009).
The mammalian genome encodes seven different sirtuins (SIRT1-7), with varying subcellular localizations and chemical activities \((\textit{Du J et al., 2011})\).

A common approach to divide them into different classes is by subcellular localization (Fig 5), four sirtuins, SIRT1, SIRT3, SIRT6, and SIRT7, are nuclear proteins, but their subnuclear localizations are distinct. SIRT1 is detected in the nuclei but is excluded from the nucleoli, whereas SIRT6 and SIRT7, are associated with heterochromatic regions and nucleoli, respectively \((\textit{Mostoslavsky et al., 2006})\), SIRT2 is generally localized in the cytoplasm, but, during the G2/M phase, it binds chromatin in the nucleus \((\textit{Vaquero et al., 2006})\), SIRT3, SIRT4, and SIRT5 are present in the mitochondria. Although initially described as a mitochondrial protein, recent studies suggest that SIRT3 can also be a nuclear protein that transfers to the mitochondria during cellular stress \((\textit{Scher et al., 2007})\), the exact localization of the SIRT3–SIRT5 in the mitochondria has, however, not yet been defined experimentally.

However, these locations will change conditionally. For example, some cells showed nuclear expression of SIRT1, while others expressed SIRT1 separately in cytoplasm \((\textit{Tanno et al., 2007})\). SIRT2 proactively shuttled between the nucleus and cytoplasm. Multiple cell localizations were also found in SIRT3 and SIRT7 \((\textit{North et al., 2007})\). Moreover, some studies have shown that SIRT3 is mainly located in the nucleus under normal conditions \((\textit{Osborne et al., 2014})\).
Structural Domains of the Mammalian Sirtuins:

The seven mammalian sirtuins show significant sequence homology and contain conserved catalytic and NAD$^+$ binding domains. Although based on sequence similarities, eukaryotic sirtuins have been divided into four broad phylogenetic groups, with SIRT1, SIRT2, and SIRT3 composing class I, SIRT4 constituting class II, SIRT5 forming class III, and SIRT6 and SIRT7 forming class IV; there is no obvious correlation between this classification and the specific biological functions of the sirtuins (Frye, 2000).

SIRTs differ in sequence and length in both their N- and C-terminal domains, partially explaining their different localization and functions. SIRTs can catalyze both deacetylation and ADP-ribosylation (Carafa et al., 2012).

Their best-characterized activity is NAD$^+$-dependent lysine deacetylation, but recent studies demonstrated that some SIRTs also remove other acyl groups such as succinyl, malonyl, glutaryl, and long-chain fatty acyl groups (Tan et al., 2014).
Sirtuins

Figure (6): Primary structure of sirtuins. The seven mammalian sirtuins (SIRT1–7) are aligned with yeast Sir2p (yeast has four other SIR2 paralogs, HST1–4). The conserved, catalytic domain that all sirtuins have in common is in yellow. Nuclear localization sequences (NLSs) and mitochondrial targeting sequences (MTSs) are also indicated. Numbers refer to amino acid residues in the proteins (Imai et al., 2000).

It exists as a long chain of 257 amino acid molecules (44 KDa), and the N-terminal of the molecules contains a mitochondrial targeting sequence. When under stress, the targeting sequence of the long chain is cut off by substrate processing enzyme (MPP) to create the active short chain which has the antistress activity and then will be sent into the mitochondria to play a role (Osborne et al., 2014).

SIRT2 was the first subtype reported. They first uncovered the Sirtuin protein structure and provided useful information about the Sirtuin catalytic core (Finnin et al., 2001) (Fig. 7).

The catalytic core consists of two main parts, as shown in Fig.7: a large and a small domain. The large domain contains an inverted Rossmann fold, which is a typical NAD+(H) binding site, consisting of 6 beta strands (β1-3 and β7-9) and 6 alpha helices (α1, α7, α8 and α10 and α12), and which is parallel to the β-sheet (residues 53–89, 146–186 and 241–356) (Bellamacina, 1996).
The small domain contains a helical module (residues 187–240) and a zinc finger module (residues 90–145). The spiral module consists of four $\alpha$-helices ($\alpha_3$-$\alpha_6$), which consist of structural zinc ions and three anti-parallel $\beta$-sheets ($\beta_4$-$\beta_6$) and one $\alpha$-helix ($\alpha_9$). It is coordinated by four Cys residues \textit{(Finnin et al., 2001)}.

The two domains are joined by a small-domain polypeptide chain and three large-domain polypeptide chains, all of which form a large groove. The junctional groove includes the NAD$^+$ binding site, and the residues therein are conserved among the Sirtuin family. Mutation of these sites will directly lead to the loss of deacetylation catalytic activity, indicating that this huge junction groove is the catalytic core \textit{(Finnin et al., 2001)}.

The catalytic core substrate binding pocket is characterized by three subpockets (subpocket A\B\C). The ADP-ribose moiety will bind to subpocket A and nicotinamide-ribose interacts with the B subpocket. However, during the catalysis, nicotinamide will bind to the C subpocket, allowing the acetylated group to transfer from the lys residue to the ribose portion of the substrate \textit{(Finnin et al., 2001)}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sirtuin_catalytic_core.png}
\caption{The Sirtuin catalytic core structure (Wang et al., 2019).}
\end{figure}
Mechanism of action:

Sirtuins share a common NAD\(^+\)-binding catalytic domain, and the deacetylation is the most important and common activity. Sirtuins catalyze the deacetylation of proteins by breaking the bonds between NAD\(^+\) and niacinamide ribosomes, transferring the acetylated groups from proteins to ADP-ribose, then releasing the deacetylated products. Therefore, it is more accurate to treat Sirtuins as transacetylases rather than deacetylases, which makes niacinamide rightly considered an endogenous inhibitor of Sirtuins (Lee et al., 2008).

![Figure (8): The mechanism of deacetylation by Sirtuin (Wang et al., 2019).](image)

In addition to deacetylation, there are other enzymatic activities for Sirtuin. SIRT4 is mainly used to transfer ADP-ribose to a specific protein-glutamate dehydrogenase (GDH) and considered to have very weak deacetylation activity (Rauh et al., 2013). Research has shown that SIRT5 has weak deacetylation activity, yet it has strong desuccinylation and depropanediylation activity. Its catalytic activity is \(\sim 1000\) times stronger than deacetylation (Roessler et al., 2015).

Compared with the relatively weak deacetylation activity of the Sirtuin members mentioned above, SIRT2 and SIRT6 share the long adipose chain diacylation and stronger deacetylation activity. Other studies have shown that SIRT6 can catalyze ADP-ribose transfer similar to SIRT4 (Jiang et al., 2013).
Various enzyme activities direct various functions of this conservative family. The related effects of Sirtuin have been found to point to lifespan-related diseases such as diabetes, metabolic syndrome, cancer, inflammation and neurodegenerative diseases (Rogina and Helfand, 2004).
Sirtuin1 (SIRT1)

Definition:

Sirtuin 1 (SIRT1) is the largest of the seven members of the sirtuin family of class III nicotinamide adenine dinucleotide (NAD$^+$)-dependent protein deacetylases, whose activation is beneficial for metabolic, neurodegenerative, inflammatory and neoplastic diseases (Finkel et al., 2009).

SIRT1 was the first SIRT family member to be discovered and is still the most studied. Its involvement in many neuronal processes (Li, 2013).

Physiological role:

SIRT1 can deacetylate a variety of substrates and is, therefore, involved in a broad range of physiological functions, including control of gene expression, metabolism and aging (Yamamoto et al., 2007).

SIRT1 catalyzes an enzymatic reaction that generates nicotinamide and the acetyl group of the substrate is transferred to cleaved NAD, generating a unique metabolite, O-acetyl-ADP ribose (Pillarisetti, 2008).

The list of SIRT1 substrates is continuously growing and includes several transcription factors: the tumor suppressor protein p53, members of the FoxO family (forkhead box factors regulated by insulin/Akt), HES1 *(hairy and enhancer of split 1), HEY2 (hairy/enhancer-of-split related with YRPW motif 2), PPARγ (peroxisome proliferator-activated receptor gamma), CTIP2 (chicken ovalbumin upstream promoter transcription factor (COUPTF)- interacting protein 2), p300, PGC-1α (PPARγ coactivator), and NF-κB (nuclear factor kappa B)( Michan and Sinclair, 2007).
SIRT1 is the closest to yeast Sir2 in terms of sequence and enzymatic activity, and is also the mammalian sirtuin most extensively studied to date. SIRT1 is a key regulator of metabolism, and its activity is regulated by nutritional status, being up regulated throughout the body during fasting and calorie restriction (*Tissenbaum and Guarente, 2001*). SIRT1 up regulates mitochondrial biogenesis in several tissues, stimulates fat and cholesterol catabolism in liver, skeletal muscle, and adipose tissue, induces the gluconeogenic genes and repress glycolytic genes and activate fatty acid oxidation systemically (*Silva and Wahleste, 2010*). SIRT1 controls the gluconeogenic/glycolytic pathways through the transcriptional co activator PGC 1α, which leads to an increase in the mitochondrial mass and function in animal and in vitro models (*Nemoto et al., 2005*).

![Figure 9: SIRT1 substrate](image)

**Figure (9):** SIRT 1 substrate. The diverse functions of SIRT1 in central nutrient sensing and peripheral energy metabolism. The activity of SIRT1 is regulated by the cellular metabolic status, small molecule activators, interacting proteins, as well as post-translational modifications. After activation, SIRT1 modulates a variety of metabolic activities systemically and locally through either direct protein deacetylation or indirect chromatin remodeling (*Li and Kazgan, 2011*).
SIRT1 and insulin resistance:

Insulin resistance is the critical pathological feature of type 2 diabetes mellitus, obesity, metabolic syndrome, and aging (Newsholme et al., 2007). Although the precise pathogenesis of insulin resistance remains ill-defined, several factors have been proposed to have a role in this process, such as adipokines, defects in the insulin signaling pathway, mitochondrial dysfunction and inflammation (Muoio and Newgard, 2008).

SIRT1 has a prominent role in metabolic tissues, such as the liver, skeletal muscle and adipose tissues, where it deacetylates a range of substrates, including PGC1α, UCP2, NFκB and FoxO1 proteins, which results in a pronounced effect on glucose homeostasis and insulin secretion (Rodgers et al., 2005). SIRT1 regulates the activity of the nuclear receptor PPARγ and thus influences adipogenesis as well as fat storage in white adipose tissue, glucose and lipid metabolism in the liver and differentiation of muscle cells (Fulco et al., 2003).

Moreover, increasing evidence suggests that decreased SIRT1 expression or activity might contribute to the pathogenesis of diseases related to insulin resistance. SIRT1 protein levels were reduced in mice fed a high-fat diet and in two mice models of aging, both of which conditions are associated with insulin resistance (Deng et al., 2007).

In addition, inhibition of SIRT1 induces insulin resistance in cultured insulin-sensitive cells and tissues (Sun et al., 2007). Thus, reduced SIRT1 levels might directly cause or at least substantially contribute to insulin resistance in vivo and in vitro. In support of this theory, activators of SIRT1 enhance insulin sensitivity in vitro in a
SIRT1-dependent manner and ameliorate insulin resistance in vivo (Feige et al., 2008).

Figure (10): Proposed roles and targets of SIRT1 in insulin resistance. By its deacetylase activity, sirT1 represses the inflammatory response, and regulates insulin secretion from β cells, hepatic metabolism of glucose and lipids, mitochondrial homeostasis and rOs levels, adipogenesis and adiponectin secretion, the insulin signaling pathway, and myogenesis. Abbreviation: rOs, reactive oxygen species. (Liang et al., 2009).

A number of studies suggest that SIRT1 has a role in the regulation of insulin secretion from pancreatic β cells. Overexpression of SIRT1 in β cells enhances ATP production by repressing UCP2, which mediates the uncoupling of ATP synthesis from glucose, and an elevated ATP level leads to cell membrane depolarization and Ca\(^{2+}\)-dependent exocytosis (Bordone et al., 2006).

SIRT1 is also involved—directly or indirectly—in the insulin signaling pathway. Firstly, it represses the transcription of Ptpn (Sun et al., 2007), which acts as a negative regulator of insulin signaling, mainly through dephosphorylation of the insulin receptor and insulin-receptor substrate (IRS) 1 (Goldstein et al., 2000), (Figure 13).

Moreover, SIRT1 also regulates insulin-induced tyrosine phosphorylation of IRS-2 through deacetylation of this substrate, which
affects a crucial step in the insulin signaling pathway. Inhibition of SIRT1 activity also directly interferes with insulin signaling at both the protein and mRNA level (Zhang, 2007) (Figure 13).

**SIRT1 in renal disease:**

**Sirtuins in Renal Physiology**

In the kidney, SIRT1 is widely expressed in tubular cells and podocytes. The abundance of SIRT1 expression has been taken to suggest that it is possibly involved in sodium and water handling. SIRT1 decreases epithelial sodium reabsorption by interacting with methyl transferase, the disruptor of telomeric silencing-1, ultimately repressing the transcription of the α-subunit of the epithelial sodium channel (ENaC) in cultured inner medullary collecting duct cells. The inhibitory effect of SIRT1 on the promoter of ENaC is independent of its deacetylase activity (Zhang et al., 2009).

The capacity of SIRT1 to regulate sodium and water handling in the kidney might ultimately affect BP. Data are also available to indicate a counterregulatory role of SIRT1 on renin-angiotensin system activation. Overexpression of SIRT1 downregulates angiotensin II type 1 receptor (AT1R) in vascular smooth muscle cells (Miyazaki et al., 2008). Whereas the reduced expression of SIRT1 is associated with the increased transcription of AT1R in podocytes (Chandel et al., 2017).

These findings, together with the evidence that SIRT1 upregulates endothelial nitric oxide synthase, points to SIRT1 as a potential player in BP control (Mattagajasingh et al., 2007).
Figure (11): Functional activity of SIRTs 1, 3, 6, and 7 and their specific targets in the different compartments of the kidney (Morigi et al., 2018).

Sirtuins in diabetic nephropathy:

The protective effects of SIRT1 agonists on some metabolic parameters, such as glucose tolerance, fasting blood glucose levels, and insulin resistance resulting in a prolongation of animal lifespan, have been described in several experimental models of diabetes (Pfluger et al., 2008). Beside its beneficial effect on the metabolism, SIRT1 has a protective role in limiting podocyte injury in DN. There are data that confirm that the conditional deletion of Sirt1 in the podocytes of diabetic db/db mice results in acetylation of the p65 subunit of NF-κB and STAT3, which likely translates into increased levels of urinary protein excretion and more severe renal damage compared with db/db mice without the genetic deletion (Liu et al., 2014).

Consistently, the downregulation of SIRT1 expression is functionally linked to FOXO4 hyperacetylation and the induction of the proapoptotic factor Bcl2L11 in both injured podocytes in culture and glomeruli of db/db mice with DN (Chuang et al., 2011).
It has been suggested that complex functional interplay between proximal tubules and glomeruli coordinated by SIRT1 primes DN. The targeted disruption of SIRT1 in proximal tubules of DN mice results in ectopic expression of the tight junction protein claudin-1 in podocytes, an event that leads to albuminuria and renal function impairment. In search of a potential explanation for such results, the authors provide in vitro data showing that proximal tubular cells exposed to high glucose concentration secrete less of the nicotinamide mononucleotide that lowers SIRT1 in podocytes and upregulates claudin-1 expression (Hasegawa et al., 2013). The evidence of reduced SIRT1 expression in both tubular cells and glomeruli from patients with DN provides additional clues regarding the potential involvement of SIRT1 in human DN (Zhao et al., 2017).
Genetics of DKD

Basic genetics

The human genome is the complete set of nucleic acid sequences of humans encoded as DNA (Deoxyribonucleic acid). DNA is a molecule that carries the genetic instructions used in the growth, development, functioning, and reproduction of all known living organisms. DNA is a ladder-like helical structure which is found in the cytoplasm chiefly in the nucleus, a small fraction is present in mitochondria. It is composed of two strands that twist together to form a helix. The monomeric unit of DNA is the nucleotide, to be specific, deoxyribonucleotides. The nucleotide itself is made of three components, a sugar (deoxyribose), phosphate and a base (there are four of them: two purines - adenine and guanine; and two pyrimidines-thymine and cytosine). These bases pairs, adenine pairs with thymine and guanine with cytosine (Alberts, 2014).

A gene is the basic physical and functional unit of heredity. Genes which are made up of DNA, act as instructions to make molecules called proteins. Human genomes include both protein-coding DNA genes and noncoding DNA genes (Abecasis et al., 2012).

Coding DNA is defined as those sequences that can be transcribed into mRNA and translated into proteins during the human life cycle, these sequences occupy only a small fraction of the genome (<2%). Noncoding DNA is made up of all of those sequences (98% of the genome) that are not used to encode proteins. Numerous classes of noncoding DNA have been identified, including genes for noncoding RNA (e.g. tRNA and rRNA), pseudogenes, introns, untranslated regions of mRNA, regulatory DNA sequences and repetitive DNA sequences (Morris, 2015).
A gene is said to be polymorphic if more than one allele occupies that gene’s locus within a population. In addition to having more than one allele at a specific locus, each allele must also occur in the population at a rate of at least 1% to generally be considered polymorphic. Gene polymorphisms can occur in the coding or non-coding region of the genome. The majority of polymorphisms are silent, meaning that they occur in the non-coding region and therefore do not alter the function or expression of a gene if a gene polymorphism is located in the coding region of the genome, it can alter the expression of the gene and create variation within a given population. A polymorphic variant of a gene can lead to the abnormal expression or to the production of an abnormal form of the protein; this abnormality may cause or be associated with disease (Karki et al., 2015).

Gene polymorphisms are caused by duplications, deletions, and a mutation of triplication of high quantity of DNA base pairs sequences. In addition, Polymorphisms may occur due to changes inside introns or changes in regions for one or multiple DNA bases that are between genes. There are four types of gene polymorphisms, single nucleotide polymorphisms, small-scale insertions and deletions, polymorphic repetitive elements and microsatellite variation (Mills et al., 2011).

**Single nucleotide polymorphisms (SNPs)** are a single nucleotide changes that happen in the genome in a particular location. The single nucleotide polymorphism is known to be the most common form of genetic variation, where two or occasionally three alternative nucleotides are common in the population. In most cases, an SNP has two alternative forms, termed alleles, for example, A or G at a certain position in the genome. SNPs may occur in both coding and non-coding regions of the
genome so it may cause a disease through the affection in a specific gene or regulatory region near this gene resulting in disturbance in the gene's function (Gomy et al., 2017).

There are variations between human populations regarding SNPs, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another (Varela and Amos, 2010).

Genetic risk Factors of DKD:

Both clinical and epidemiological studies have demonstrated that there is familial aggregation of DKD in different ethnic groups, indicating that genetic factors contribute to development of the disease. Furthermore, genetic risk factors in DKD interact with the environmental factors (for example, lifestyle, diet and medication (Kato and Natarajan, 2014).

Genetic and epigenetic factors may influence the development and progression of DKD. Although there is evidence for genetic susceptibility for the development of DKD, identification of causative genes has proved to be elusive. Familial aggregation of DKD is a well-recognized phenomenon. Diabetic siblings of patients with ESRD due to diabetes are known to be at 5-fold higher risk of ESRD compared with those without a family history (Thomas et al., 2012).

Despite this recognized association, many of these high-risk individuals are not being targeted for early and intensive risk-factor modification. One study has shown that diabetic siblings of patients with ESRD due to diabetes have a high frequency of albuminuria (46%), suboptimal BP control (65%), suboptimal glycemic control (HbA1c >
7.0%; 43%), smoking (26%), and failure to receive RAAS-modifying agents (42%) (Bleyer et al., 2008).

1-Genetic Predisposition:

Chronic hyperglycemia is necessary but not sufficient for the development of diabetic kidney disease because many patients with uncontrolled diabetes will never develop nephropathy, and conversely, individuals with excellent glycemic control may develop microvascular complications of diabetes. The evidence that genetic factors may contribute to diabetic kidney disease, like many other similar complex diseases, is based mainly on the observation of familial aggregation in epidemiologic studies. The notion that certain individuals with diabetes are at differential risk for developing nephropathy on account of familial aggregation of kidney disease was first reported in 1989, when Seaquist et al examined probands of type 1 diabetic patients with and without diabetic kidney disease and found that 83% of diabetic siblings of probands with nephropathy had evidence of nephropathy compared with only 17% of diabetic siblings of probands without nephropathy (Seaquist et al., 1989).

Genetic studies of DKD are mainly focused on association analyses between genomic DNA variation (for example, single nucleotide polymorphisms (SNPs), copy number variants (CNVs) and microsatellites) and clinical phenotypes of the disease (Florez, 2016).

Genome-wide association studies (GWAS) focus on the most common kind of genetic variation in the human genome, a single nucleotide polymorphism (SNP). SNPs are common substitutions of a single base with another, which occur with high frequency in the
human genome (1 every 300–500 base pairs) (*International HapMap, 2003*).

Genome-wide association studies (GWAS) identified 16 loci that were associated with renal function and CKD at genome-wide significance level \((P < 5 \times 10^{-8})\). Several of these loci had previously been linked to renal disease, as for example rare, mutations in the UMOD locus, which cause rare, autosomal-dominant renal diseases such as familial juvenile hyperuremic nephropathy and medullary cystic kidney disease type 2 (*Köttgen et al., 2010*).

UMOD encodes the most abundant protein excreted in urine, uromodulin, also known as Tamm–Horsfall protein; however, its physiological function is not fully understood (*Serafini et al., 2003*). Other variants were in genes related to nephrogenesis glomerular filtration barrier formation and podocyte function, angiogenesis, solute transport, metabolic functions of the kidney and the function of primary cilia (*Köttgen et al., 2010*).

SNPs are the most common form of genomic DNA variation. The updated dbSNP database of more than 500 million reference SNPs (rs) with allele frequency data has provided fundamental information for genetic studies of complex diseases including, DKD. The genetic studies in DKD have implicated previously unsuspected biological pathways and subsequently improved knowledge for understanding of the genetic basis of the disease (*Allis and Jenuwein, 2016*). Several other loci associated with DN and ESRD have been discovered. Variants in the engulfment and cell motility 1 (ELMO1) locus were associated with renal disease in individuals with type 1 diabetes in a
Caucasian population, and type 2 diabetes in a Japanese and African American populat (Pezzolesi et al., 2009), Overexpression of ELMO1 was shown to contribute to the progression of chronic glomerular injury (Shimazaki et al., 2006).

2-Epigenetics:

Because DNA is the basic blueprint for all cellular activity, DNA mutations have long been understood to play a role in diabetes and diabetic kidney disease. At the same time, much of a cell’s identity is set by modifications to its chromatin, which comprises DNA and histones, the proteins that bind and package it. Epigenetic information provides another layer of control over gene expression by controlling the interpretation of the underlying genetic sequence defining a cell’s identity (Berger et al., 2009).

Epigenetic studies of DKD examine potentially heritable changes in gene expression that occur without variation in the original DNA nucleotide sequence (Keating et al., 2018). Epigenetic regulation is a posttranslational modification of histones that allows for the conversion of inaccessible, tightly packaged, and inactive heterochromatin to the accessible euchromatin state that offers a more permissive environment for active transcription of genes (Bird, 2007).

The main epigenetic changes include DNA methylation (covalent attachment of methyl groups at CpG dinucleotide) and histone modifications, which mainly include acetylation, methylation, and phosphorylation (Dawson et al., 2012).
In DKD, the effects of DNA methylation have been studied in terms of trans generational inheritance of the disease to explore environmental and other non-genetic factors that may influence epigenetic modifications in the genes involved in DKD \((\text{Jones, 2012})\). Identification of differentially methylated CpG sites in promoters or other functional regions of genes and the analysis of the DNA methylation changes that are associated with DKD have become the most common approaches used in epigenetic studies of the disease. Furthermore, ncRNAs, particularly long ncRNAs are known to be involved in epigenetic processes. ncRNAs certainly play an important role in chromatin formation, histone modification, DNA methylation and consequently gene transcription silencing \((\text{Thomas, 2016})\).

Epigenetics is a potential mechanism that links genes and the environment with DKD \((\text{Tonna et al., 2010})\). Potential epigenetic markers for DKD progression have been identified by comparing DNA methylation in individuals with or without DKD. Different levels of DNA methylation in individuals with DKD have been linked with a predisposition to ESRD \((\text{Sapienza et al., 2011})\). Of possible relevance to DKD, methylation and demethylation of the proximal promoter region of the genes, which regulate ACE expression, have been shown to upregulate or silence ACE expression. However, prospective studies are required to show that epigenetic factors predispose patients to DKD \((\text{Riviere et al., 2011})\).

Although many studies have established the association of histone modifications with models of diabetic glomerulosclerosis, the role of histone methylation in diabetic kidney disease is unknown. Whether particular gene promoter histone methylation patterns are altered in
kidney cells under diabetic conditions is an intriguing possibility requiring further study (Sayyed et al., 2010).

Therefore, epigenetic studies of DKD may provide information to help understand how environmental factors modify the expression of genes that are involved in DKD progression. Combined genetic, epigenetic and phenotypic studies together may generate information to understand new pathogenic pathways and to search for new biomarkers for early diagnosis and prediction as part of prevention programs in DKD. The results may also be useful in finding novel targets for the treatment of DKD (Thomas, 2016).

Figure (12): The relationship between genetic, epigenetic and phenotypic studies in diabetic kidney disease (DKD). Genetic association studies are fundamentally important for identification of susceptibility or resistance genes (G). Epigenetic studies analyzing genomic DNA methylation changes, chromosome histone modification and ncRNA regulation are useful for dissecting the interaction of the genes with environmental factors. The combined data from genetic, epigenetic and phenotypic (Phe) studies may provide the opportunity for us to understand new pathways underlying the pathogenesis of DKD and to discover new biomarkers for early diagnosis and to find targets for prevention and treatment programs of this disease. The different sizes of the ‘G” and “Phe” represent the variation of genetic and phenotypic effects (Gu, 2019).
Susceptibility Genes in DN and ESRD:

It is well known that gene susceptibility to DN plays an important role in individuals, even with the same environmental exposure. Family clustering also supports the importance of hereditary factors in DN and ESRD (Skrunes et al., 2014). Therefore, a myriad of genetic studies has been conducted to identify potential candidate genes in large diabetic cohorts, which may facilitate the exploration of the pathogenesis of DN (McDonough et al., 2011).

Figure (13): The susceptibility genes in diabetic nephropathy (Wei et al., 2018).

The susceptibility genes in diabetic nephropathy are divided into different categories according to their main functions:

1-Lipid Metabolism-Related Genes

Previous studies have shown that an increase in renal lipid retention, which is related to the accumulation of biglycan, contributes to the development of DN. Dysregulation of genes related to lipid metabolism accounts for lipid deposition, resulting in the decline of glomerular filtration rate and inflammation. Variants in the acetyl-coenzyme A
carboxylase beta (ACACB) and adiponectin (ADIPOQ) genes are likely involved in the development of DN (*Wei et al.*, 2018).

- **Acetyl-coenzyme A carboxylase beta (ACACB):**

  The ACACB gene is located on chromosome 12q24.1 and encodes acetyl-coenzyme A (CoA) carboxylase beta (ACC2/ACACB). ACC2 is a key rate-limiting enzyme for the β-oxidation of fatty acid. (*Wakil et al.*, 2009). A single nucleotide polymorphism (SNP) in intron 18 of ACACB that was identified by a large-sample meta-analysis in Japanese individuals (rs2268388) showed a significant association with type 2 diabetes-related nephropathy (T2DN) (*Maeda et al.*, 2010).

- **Adiponectin (ADIPOQ) genes:**

  The ADIPOQ gene, which is located on chromosome 3q27, encodes adiponectin and has been identified as the susceptibility gene of cardiovascular disease, type 2 DM (T2D), obesity, and insulin resistance (*Fisman et al.*, 2014). Adiponectin is mainly secreted by adipocytes and acts as a vital modulator in insulin resistance and lipid metabolism. It is commonly believed that adiponectin is insulin-sensitizing and facilitates β-cell oxidation, which also has anti-atherogenic and anti-inflammatory effects (*Liu et al.*, 2014). The SNP rs17300539 (ADIPOQ_prom2GA) was initially found to be associated with DN in both Danish and French patients with T2D by linkage studies (*Jorsal et al.*, 2008). Reported that the A allele may increase the risk of nephropathy in T1D. They found that carriers of the minor allele A in –11387 (rs17300539) and the non-A-allele in +2033 tended to have notably increased serum adiponectin levels in T1D, which predicted the progression of ESRD (*Chung et al.*, 2014). Other SNPs, such as rs1063537, rs2241767, and rs2082940, are also related to DN (*Jorsal et al.*, 2008).
2- Glucose Metabolism-Related Genes

There is no doubt that DN, as the main complication of diabetes, is associated with glucose metabolism. Polymorphisms of glucose metabolism-related genes, including glucokinase regulatory protein (GCKR) and transcription factor 7-like 2 (TCF7L2), are believed to be related to DN (Wei et al., 2018).

- **Glucokinase regulatory protein (GCKR):**

  Large-scale GWAS have illustrated that the GCKR gene is related to a reduction of renal function and chronic kidney disease (CKD) (Köttgen et al., 2010). GCKR has been considered a susceptibility gene for diabetes and many studies have been conducted to explore the association between GCKR and renal complications in T2D (Iwata et al., 2012). The Genetics of Diabetes Audit and Research Tayside (GoDARTs) study showed that the P446 L of rs1260326 in GCKR was associated with a higher baseline eGFR, especially in those with albuminuria. Implying an association between GCKR variants and DN (Deshmukh et al., 2013).

- **Transcription factor 7-like 2 (TCF7L2):**

  TCF7L2 is a susceptibility gene that is strongly associated with diabetes, in recent years, studies have confirmed that the polymorphisms of TCF7L2 are correlated with T2DN (Jainandunsing et al., 2018). Buraczynska et al. found that rs7903146 in TCF7L2 was strongly correlated with DN in Caucasians, especially for the early onset of diabetes. Their study showed that in diabetic and nondiabetic ESRD patients, the T allele of rs7903146 increased the risk of developing DN (Buraczynska et al., 2014).
Abnormal angiogenesis is a main characteristic of DN. Genes related to angiogenesis, such as the hormone erythropoietin (EPO) promoter gene and vascular endothelial growth factor A (VEGFA), are associated with DN (Wei et al., 2018).

- **Erythropoietin (EPO) promoter gene:**

  The EPO promoter gene is located on chromosome 7q22 and encodes EPO. EPO is a key factor involved in erythrocyte production and is widely used for the treatment of chronic renal failure and anemia after chemotherapy (Coronado et al., 2015). Circulating EPO is mainly produced by fibroblasts in the adult renal peritubular interstitial (Bunn et al., 2013).

  EPO is a powerful angiogenic factor in diabetic microvascular disease. The Genetics of Nephropathy— an International Effort (GENIE) study validated the correlation in European patients with T1D. Although the meta-analysis showed that the correlation reached genome-wide significance, they believed the reason for the difference lay in the requirement to establish a statistically lower threshold. Furthermore, it is not clear whether a DN susceptibility gene has the same effect on T1D and T2D. Additionally, the mechanism of EPO gene polymorphisms in DN has not yet been investigated (Williams et al., 2012).

- **Vascular endothelial growth factor A (VEGFA):**

  The VEGFA gene is located on chromosome 6 (6p21.3). VEGFA is a cytokine that is highly correlated with diabetic microvascular diseases. It induces the proliferation of endothelial cells in the glomerulus and migrates and changes the permeability of various tissues. VEGFA is
expressed in the kidney and is mainly distributed in vascular endothelial cells and podocytes. Therefore, VEGFA may be associated with diabetic microvascular complications. VEGFA gene polymorphisms and the expression of the protein are closely related. Multiple loci are associated with DN, such as –2549 I/D/rs35569394, +405/ rs2010963, and –1499C>T/rs833061 (Yang et al., 2003).

4-Genes Related to Renal Structure and Function

Proteinuria and constant decreased kidney function in DN may be closely linked to the pathologic changes in renal structure and function. Glomerular podocyte dysfunction is extremely important for the initiation and progression of DN. Abnormalities in podocytes, such as podocyte hypertrophy or loss, are attributed to many factors. Some genes, including 4.1 protein ezrin, radixin, moesin (FERM) domain-containing 3 (FRMD3) and shroom3 (SHROOM3), which are related to renal structure and function, have been identified as susceptibility genes for DN (Wei et al., 2018).

- **SHROOM3**:

  The SHROOM3 gene is located on 4q21.1 and encodes shroom3 protein, which is related to endothelial morphology. Shroom3 protein mainly regulates the morphogenesis of epithelial cells and tissues. In the rat kidney, the shroom3 protein is expressed in the condensing mesenchyme, Baumann’s sac, and podocytes (Khalili et al., 2016). A GWAS found that the rs17319721 SNP in the intronic region of the SHROOM3 gene was associated with CKD (Köttgen et al., 2009). Due to its close correlation with renal structure function, some studies further verified that rs173197213 was related to the eGFR in T2D patients with proteinuria (Deshmukh et al., 2013).
5-Inflammation and Oxidative Stress-Related Genes

Disorders of blood glucose and lipid metabolism are another main character of diabetes and DN, which promote inflammation and oxidative stress in patients with diabetes and DN. Several genes, such as engulfment and cell motility protein 1 (ELMO1), TGF-β, and nitric oxide synthase 3 (NOS3, eNOS), participate in the processes of inflammation and oxidative stress and are all involved in the pathogenesis of DN (Wei et al., 2018).

- **Engulfment and cell motility protein 1 (ELMO1):**

  The ELMO1 gene is located on 7p14.2-p14.1 and encodes an evolutionarily conserved cytoplasmic protein with no obvious catalytic domains. ELMO1 is a crucial factor for the pathogenesis of T2DN and certain nephropathy-associated variants differ across populations (Park et al., 2007).

  Shimazaki et al. Identified ELMO1 as a susceptibility gene for DN by analyzing a large number of SNPs in Japanese populations; the strongest associated SNP is intron 18+9170 (Shimazaki et al., 2005).

  Another variation in intron 13 of the ELMO1 gene was also found to be related to DN in African-Americans (Leak et al., 2009). The severity of renal fibrosis, the amount of urinary albumin excretion and changes in the ultrastructure of the glomerular basement membrane in Akita diabetic mice paralleled the genetic levels of ELMO1 (Hathaway et al., 2016).

- **TGF-β1:**

  TGF-β1, one of three isoforms of the TGF-β family, is a multifunctional cytokine that modulates a myriad of cellular processes, including proliferation, differentiation, apoptosis, angiogenesis,
extracellular matrix (ECM) formation, and immune processes (Zhou et al., 2014). In renal diseases, elevated expression of TGF-β1 can induce renal hypertrophy and promote excessive accumulation of ECM proteins, thus leading to renal fibrosis (Mou et al., 2016). To be more specific, TGF-β1 is significantly enhanced in the renal tissues of patients with DN, especially in mesangial cells of diabetic glomeruli (El-Sherbini et al., 2013). Therefore, it is likely that these pathological changes caused by TGF-β1 may contribute to the initiation and progression of DN (Fujii et al., 1986).

6-Genes Related to the Renin-AngiotensinAldosterone System

The renin-angiotensin-aldosterone system (RAAS) regulates not merely blood pressure but also the internal pressure of the glomerulus and hypertension is an independent risk factor of DN. Therefore, polymorphisms of RAAS-related genes, such as angiotensin-converting enzyme (ACE) and angiotensin II receptor type 1 (AGTR1), are closely related to the development of DN (Wei et al., 2018).

- **Angiotensin-converting enzyme (ACE I/D):**

  The ACE gene, which is located on 17q23.3, contains 26 exons and 25 introns and mainly encodes ACE. ACE is one of the key enzymes of the RAAS and mainly transforms angiotensin I to angiotensin II, thus regulating the activity of angiotensin and bradykinin. The ACE gene is mainly expressed in the kidney, especially in the brush border of renal proximal tubules. It also exists in glomerular endothelial cells, mesangial cells, podocytes, and distal nephrons. Due to the crucial role of ACE in the RAAS, a large number of studies have linked its polymorphisms to the development of diabetic microvascular complications, such as DN (Wang et al., 2016).
ACE I/D is the most susceptible locus for DN. In the Diabetes Control and Complications Trial (DCCT) and following in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, Boright et al. found that the risk of developing refractory proteinuria and severe kidney diseases was lower in T1D patients with the II genotype than the I/D genotype \cite{Boright2005}. Therefore, they confirmed an association between the ACES I/D polymorphism and the development of DN in T1D. However, in another large-scale trial, T2D patients with the ID or DD genotype tended to have a lower incidence of end-stage renal failure, which contradicts the other 2 studies \cite{Hadjadj2008}.

- **Angiotensin II receptor type 1 (AGTR1):**

  The AGTR1 gene is located on 3q21-25 with a length of less than 55 kb, encoding the angiotensin II receptor type 1. The angiotensin II receptor mainly regulates the level of angiotensin II, a key enzyme involved in the RAAS. The RAAS regulates vasoconstriction, the reabsorption of sodium, and the inflammatory cascade, which is currently believed to exert a positive effect on the development of DN. Moreover, specific receptor blockers of AGTR1 exert a renal protective effect in DN which can lower blood pressure and reduce the occurrence of cardiovascular events \cite{Brenner2001}. Therefore, some studies have suggested that the polymorphism of AGTR1, especially the rs5186 (A1166C) mutation, is one of the potential candidate susceptibility genes for DN \cite{Mollsten2008}. 
Subjects and Methods

I. Subjects:

This study was designed as a case control study and conducted at the Internal Medicine department of Benha University Hospitals on 50 patients with type 2 diabetes during the period from January 2019 to October 2019.

The subjects were divided into 3 groups based on albuminuria and eGFR:

**Group (I):** included 20 patients with type 2 diabetes complicated with DKD with albuminuria, Their diagnosis was based on their medical records and fulfilling the diagnostic criteria of ADA (American Diabetes Association, 2014), that specifies the following; an estimated GFR (eGFR) less than 60 ml/min/1.73 m² with albumin/creatinine ratio equal or more than 30 mg/g. There were 7 males and 13 females, their mean age was $(58.4 \pm 11.4)$ years. Duration of DM was $(13.6 \pm 4.0)$ years.

**Group (II):** included 20 patients with type 2 diabetes complicated with DKD without albuminuria Their diagnosis was based on their medical records and fulfilling the diagnostic criteria of ADA (2014), that specifies the following; an estimated GFR (eGFR) less than 60 ml/min/1.73 m² with albumin/creatinine ratio less than 30 mg/g. There were 4 males and 16 females; their mean age was $(61.1\pm11)$ years. Duration of DM was $(10.8\pm3.1)$ years.

**Group (III):** included 10 patients with type 2 diabetes without DKD (Normoalbuminuric diabetic control group). The albumin/creatinine ratio of these patients was less than 30 mg/g/creatinine and
Subjects and Methods

eGFR ≥ 60 ml/min/1.73 m². There were 1 male and 9 females, their mean age was (60.9±8.9) years, and Duration of DM was (7±2.3) years.

Ethical consideration:

Ethical permission for the study was obtained from all patients after fully informed about all study procedures and their consent was obtained prior to enrollment in the study. This study was approved by the Ethical Committees of Faculty of Medicine, Benha University.

Inclusion Criteria:

All patients included in this study were selected according to the following criteria:

1. Patients with type 2 diabetes, diagnosed according to ADA criteria (2014), that specifies any of the following; fasting plasma glucose (FPG) ≥ 126 mg/dl or 2h post-prandial plasma glucose (PPG) ≥ 200 mg/dl or random plasma glucose (random blood sugar) (RBS) ≥ 200 mg/dl or hemoglobin A1c (HbA1c) level ≥ 6.5% (American diabetes association, 2014).

2. Duration of diabetes should be equal or more than 5 years.

3. All subjects were above 18 years old.

4. DKD as defined by the criteria of the ADA (2014), that specifies the following; an estimated GFR (eGFR) less than 60 ml/min/1.73 m² with albumin/creatinine ratio equal or more than 30 mg/g (American diabetes association, 2014).
Exclusion Criteria:

Any patient with the following was excluded from this study:

1. Patients with type 1 DM.
2. Patients less than 18 years old.
3. Patients with other causes of chronic kidney disease.
4. Pregnant patients.

II. Methods:

All participants were subjected to the following:

1-Full history taking with focusing on:

Baseline demographic characters including: age, sex, smoking, lifestyle, duration of diabetes, degree of glycemic control, complications of diabetes, medical comorbidities, family history and drug history.

2-Clinical examination including:

Anthropometric measurements: Body weight of subjects was measured when the participants were lightly clothed and barefoot with the help of digital weighing machine. Height was measured using stadiometer with the help of a fixed scale.

Body mass index (BMI): was calculated as weight in kilograms divided by the squared of height in meters (kg/m²).

Blood Pressure was measured by a manual mercury sphygmomanometer at the time of subject recruitment.
3-Laboratory investigations:

a- Routine studies:

1. Kidney function tests (urea, creatinine).
2. Urinary albumin creatinine ratio.
3. Lipid profile (triglycerides, cholesterol, HDL-c and LDL-c).
4. Fasting/postprandial blood glucose level.
5. Estimated GFR using The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), eGFR should be calculated from serum Cr using the Cockcroft-Gault equations (C&G):

\[
GFR(\text{ml/min}) = \left(\frac{140 - \text{age (year)}}{72 \times \text{serum Cr (mg/dL)}}\right) \times \text{weight (Kg)} \times 0.85 \text{if female}
\]

An eGFR < 60 ml/min/1.73 m² is generally considered abnormal.

- Glycated hemoglobin (HbA1c).

b- Special investigations:

Detection of SIRT1 gene polymorphism using real-time PCR.

Blood sample:

Five milliliters of venous blood were drawn under complete aseptic conditions from each patient after overnight fasting and distributed as follows:

(a) Two milliliters of blood were taken in to EDTA (ethylene diamine tetra-acetic salt (1.2mg/mL) vacutainer as an anticoagulant then divided into 2 aliquots one was used for: Glycated hemoglobin (HbA1c). And the other stored at -70ºC for subsequent DNA extraction.
(b) Three milliliters of blood were taken in plain test tubes, samples were allowed to clot for 30 minutes at room temperature, and then were centrifuged (at 3000 rpm for 15 minutes). The separated serum was used for clinical chemistry tests.

Another sample was taken for postprandial blood glucose level.

**Urine sample:**

A second voided morning urine sample was collected in a sterile urine container; ten milliliters were separated for immediate estimation of urine creatinine and albumin in all subjects.

**Analytical Methods:**

The following tests were done using Biosystems A15 auto-analyzer (Barcelona, Spain) by appropriate chemical principles:

**1- Creatinine (serum & urine):**

**Principle:**

The analysis was done by applying modified jaffé reaction.

Creatinine +picric acid + alkaline solution $\rightarrow$ creatinine picric acid complex.

As alkaline solution creatinine combines with picric acid to form orange red colored complex. The absorbance increases proportional to concentration of creatinine. The complex formation rate is measured in a short period to avoid interferences (*Fabiny et al., 1971*)
Creatinine in urine: Urinary creatinine is also measured by the same method after diluting the urine sample with distilled water (1:49), then the result is multiplied by 50.

2. Blood Urea:

**Principle:** Urease/glutamate dehydrogenase method.

\[
\text{Urea} \xrightarrow{\text{Urease}} 2\text{NH}_4\text{+CO}_2
\]

\[
\text{NH}_4\text{+NADH+H}^+2\text{-Oxoglutarate} \xrightarrow{\text{glutamatedehydrogenase}} \text{Glutamate+NAD}
\]

The rate of oxidation of NADH to NAD was measured *(Burtis et al., 2012)*.

3. Blood Glucose:

**Principle:** The analysis was done applying enzymatic colorimetric method.

Glucose in the sample originates by means of coupled reaction described below; a colored complex can be measured spectrophotometrically.

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{– aminophenazine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\text{H}_2\text{O}
\]

The intensity of the red color formed was proportional to the glucose concentration in the sample *(Trinder, 1969)*.

4. Total cholesterol (TC):

**Principle:** TC was done by applying an enzymatic colorimetric method.
In this method, cholesterol esterase (CE) hydrolyzes cholesterol esters to the free cholesterol and fatty acid.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \quad \text{Cholesterol esterase} \quad \text{cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 + \text{H}_2\text{O} \quad \text{Cholesterol oxidase} \quad \text{H}_2\text{O}_2 + \text{cholesterol 4en-3-one}
\]

\[
2\text{H}_2\text{O}_2 + \text{4-aminoantipyrine (4-AAP)} + \text{phenol} \quad \text{peroxidase quinoneimine} + 4\text{H}_2\text{O}
\]

Red color was measured at 546 nm (Dietschy et al., 1976).

5. Triglycerides:

**Principle of the method:** The analysis of TG was done applying an enzymatic colorimetric method (GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE).

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry

\[
\text{Triglycerides} + \text{H}_2\text{O} \quad \text{lipase} \quad \text{Glycerol} + \text{Fatty acids.}
\]

\[
\text{Glycerol} + \text{ATP} \quad \text{glycerol kinase} \quad \text{Glycerol – 3 – P} + \text{ADP.}
\]

\[
\text{Glycerol – 3 – P} + \text{O}_2 \quad \text{G-3-P-oxidas} \quad \text{Dihydroxyacetone – P} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{4-aminoantipyrine} + 4\text{-Chlorophenol} \quad \text{peroxidas}
\]

Quinoneimine + 4 H2O.

Red color was measured at 546 nm (McGowan et al., 1983).
6. High density lipoproteins (HDL-C):

The cholesterol from low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons is broken down by the cholesterol oxidase in an enzymatic accelerated non-color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from high density lipoproteins (HDL) in the sample. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below.

\[
\text{Cholesterol ester + H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol + fatty acids}
\]

\[
\text{Cholesterol + 1/2O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol oxidase}} \text{H}_2\text{O}_2 + \text{Cholestenone}
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine (4-AAP) + phenol} \rightarrow \text{quinoneimine} + 4\text{H}_2\text{O}.
\]

7. Low density lipoproteins (LDL-C):

- LDL-C value was calculated according to "Friedwald's equation":
  \[
  \text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C} + \text{TG/5})
  \]
  This equation is applied provided that serum TG is < 400 mg/dl; normal range <100 mg/dl (*Friedwald et al., 1972*).

**Estimation of urinary microalbumin:**

**Principle of the method:**

Albumin in the urine sample causes agglutination of the latex particles with anti-human albumin. The agglutination of the particles is proportional to the albumin concentration and can be measured by turbidimetry (*Medcalf et al., 1990*).

This test was done by using BTS-350 semi-automated analyzer using kits supplied by Biosystems (Barcelona, Spain).
Calculation of the albumin /creatinine ratio in mg/g:

If the urine microalbumin is 10 mg/L and the urine creatinine is 100 mg/dL, then the albumin/creatinine ratio is 10 mg/g. In this example, the urine creatinine value is multiplied by 10 in order to convert it into mg/L (i.e., 100 mg/dl × 10 dl/ L = 1000 mg/l). Then the urine albumin value (10 mg/l) is divided by the urine creatinine value (1000 mg/l) to arrive at the ratio (10 mg/l /1000 mg/l=0.01) Then multiply by 1000 to express the value as (mg albumin/g creatinine)

**Albumin / Creatinine ratio results:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 30 mg/g</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30-300 mg/g</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>&gt;300 mg/g</td>
</tr>
</tbody>
</table>

Glycated hemoglobin (HbA1c):

Quantitative determination of HbA1c (Hemoglobin A1c) in human whole blood by fluorescence Immunoassay (FIA).

The test was done by AFIAS-6 using HbA1C kits provided by BODITECH MED INC (KOREA).

**Principle of the method:**

The test uses a sandwich immune detection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigens in sample form the more antigen-antibody complex and leads to stronger intensity
Detection of SIRT1-related SNPs rs10823108 gene by quantitative Real Time PCR:

All subjects were genotyped for SIRT1 gene single nucleotide polymorphism rs10823108 using quantitative real time PCR which analyzed by Taqman probe assay using commercially available primers and probe in the following steps:

1. Genomic DNA was extracted from EDTA blood.
2. The isolated DNA was amplified using sets of primers designed to detect the target polymorphism.
3. Direct detection of PCR product by monitoring the increase of fluorescence of a dye-labeled DNA probe.

Principle of TaqMan®Probe Method:

TaqMan® is referred to as 5´-nucleas assays; exploit the 5´ to 3´ exonuclease activity of Taq DNA polymerase. Each reaction contains a gene specific primer and a fluorescently labeled TaqMan® probe. The probe contains a 5´ reporter dye and a 3´ quencher dye. The 3´-end is also blocked to prevent extension during PCR. The probe was designed to anneal the target sequence between the forward and reverse PCR primers. While the probe was intact, the quencher suppressed the fluorescence of the reporter dye. During amplification, Taq DNA polymerase cleaved the probe and displaced it from the target, allowing extension to continue. Cleavage of the probe separated the reporter dye from the quencher dye, resulting in an increase in fluorescence. The increased fluorescence only occurs if the target sequence is amplified and is complimentary to the
probe, thus preventing detection of non-specific amplification. For any given cycle within the exponential phase, the amount of product, and hence fluorescence signal, was directly proportional to the initial copy number. Thus, higher copy number templates was crossed a fluorescence detection threshold before lower copy templates (Kutyavin et al., 2000).

Figure (14): TaqMan probe fluorescence.

**Polymerization:** A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5’ and 3’ ends of a TaqMan® probe respectively.

**Strand displacement:** When the probe is intact the reporter dye emission is quenched.

**Cleavage:** During each extension cycle the DNA polymerase cleaves the reporter dye from the probe.

**Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluoresce

**Assay Procedure:**

1- DNA extraction:

Genomic DNA was extracted from peripheral whole blood using blood genome DNA extraction kits (Quick-DNA™ Miniprep Kit) (cat No D3024 & D3025) supplied by ZYMO RESEARCH (German), according to the manufacturer’s instructions.
Principle:

The extraction of DNA from whole blood encompassed the lysis of proteins, nucleases and contaminants by proteinase K enzyme with the lysis buffer. DNA extraction was carried out using QIAamp Spin Columns. The lysate buffering conditions allowed optimal binding of the DNA to the QIAamp membrane as soon as the sample was loaded onto the Spin Column.

DNA was adsorbed onto the QIAamp silica-gel membrane during a brief centrifugation. Salt and pH conditions in the lysate ensured that proteins and other contaminants, which could inhibit PCR were not retained on the membrane. DNA bound to the membrane was washed by two different wash buffers in two centrifugation steps to improve the purity of the eluted DNA.

Purified DNA was eluted from the QIAamp spin column in a concentrated form in elution buffer.

**Table (5):** Components of the extraction kit:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic lysis buffer*</td>
<td>50mL</td>
</tr>
<tr>
<td>DNA Pre-wash Buffer**</td>
<td>15mL</td>
</tr>
<tr>
<td>g-DNA Wash Buffer</td>
<td>50mL</td>
</tr>
<tr>
<td>DNA Elusion Buffer</td>
<td>10mL</td>
</tr>
<tr>
<td>Zymo-Spin™ IIC Columns</td>
<td>50</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>100</td>
</tr>
</tbody>
</table>

**Buffer preparation:** beta-mercaptoethanol was added to the genomic lysis buffer to a final dilution of 0.5% (v/v) i.e., 250 µL per 50 ml.
Whole blood genomic DNA purification modified protocol:

1. One hundred µl of whole blood was added to 400 µL of genomic lysis buffer and mixed completely by vortexing then stood 5-10 minutes in room temperature.

2. Mixture was transferred to a Zymo-Spin™ IIC Column in a collection tube and centrifuged at 10000 rpm for one minute then the collection tube with the flow through was discarded.

3. The Zymo-Spin™ IIC Column was transferred to a new collection tube.

4. Two hundred µl of DNA Pre-wash Buffer were added to the spin column and centrifuged at 10000 rpm for one minute.

5. Five hundred µl of g-DNA Wash Buffer were added to the spin column and centrifuged at 10000 rpm for one minute.

6. The sample was incubated at 56 °C for 10 minutes while vortexing occasionally until the cells were completely lysed.

7. The spin column was transferred to a clean microcentrifuge tube.

8. Fifty µl of DNA Elusion Buffer were added to the spin column and incubated 2-5 minutes at room temperature then centrifuged at top speed for 30 seconds to elute the DNA.

9. The eluted DNA was stored at -20 °C for subsequent steps.

Measurement of DNA concentration of the samples:

The nanodrop spectrophotometer (Thermoscientific, USA) was used to measure the concentration of each sample using 1ul of the sample.
Figure (15): shows QIAamp Spin Column procedure.
Subjects and Methods

2- Single Nucleotide Polymorphism (SNP) detection (Genotyping):

Genotyping of SIRT1 gene SNP (rs10823108) were performed using the TaqMan SNP Genotyping assays (Applied Biosystems, USA). The PCR amplification was done using Stepone Real Time PCR instrument (S/N 272005304) (Applied Biosystems, USA).

Figure (16): Amplification Plot (rs10823108).

Figure (17): Amplification Plot (rs 10823108).
Principle:

During the first step of a TaqMan SNP Genotyping Assay experiment, amplification of the target DNA was done using sequence-specific primers. TaqMan MGB probes from the SNP Genotyping Assay provide a fluorescence signal for the amplification of each allele. After PCR amplification, an endpoint plate read using an Applied Biosystems Real-Time PCR System was performed. The Sequence Detection System (SDS) Software used the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated which alleles were in each sample. StepOne plus allelic discrimination software was used for calculation of normalized dye fluorescence (ΔRn) for Allele A (wild-type) or Allele G (mutant). The software made an automatic call of either Allele G(homozygous G/G), Allele A(homozygous A/A) or heterozygous (G/A) (Marinou et al., 2007).

Figure (18): Real time PCR, 5' Nuclease assay process (Quoted from, TaqMan® SNP Genotyping Assays Protocol hand book)
Steps:

1. DNA + H2O → 11.25 ul in PCR tubes.
2. Nuclease free H2O was added to each PCR tube to reach 11.25ul.
3. The reaction mix was prepared according to the following table and added to the PCR tubes:

**Table (6):** Components used with in the centrifuge tube for gene amplification:

<table>
<thead>
<tr>
<th>Component</th>
<th>PCR tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqMan Universal PCR Master Mix (2X), No AmpErase UNG</td>
<td>12.50</td>
</tr>
<tr>
<td>20X working stock of SNP Genotyping Assay</td>
<td>1.25</td>
</tr>
<tr>
<td>Total Volume per tube</td>
<td>13.75</td>
</tr>
</tbody>
</table>

4. PCR tubes were loaded into Stepone qPCR after programming as follows:

**Table (7):** Programing stepone qPCR :

<table>
<thead>
<tr>
<th>Hold</th>
<th>PCR (40 Cycles)</th>
<th>Denature</th>
<th>Anneal/Extend</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min at 95 °C</td>
<td>15 sec at 92 °C</td>
<td>1 min at 60 °C</td>
<td></td>
</tr>
</tbody>
</table>

5. Stepone software for allelic discrimination (Applied Biosystems, USA) was used for analysis of PCR products to determine genotype of each sample.
Statistical Analysis

The collected data was revised, coded and tabulated using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

Shapiro test was done to test the normality of data distribution. Significant data was considered to be nonparametric.

Descriptive statistics:

1. Mean, Standard deviation (± SD) for parametric numerical data; median, minimum and maximum for non parametric numerical data.
2. Frequency and percentage of non-numerical data.

Analytical statistics:

Student T Test was used to assess the statistical significance of the difference between two study group means.
For the comparison of the three groups’ means, **one way** analysis of variance (ANOVA) was used.

**Chi-Square test** was used to examine the relationship between two qualitative variables.

**Fisher’s exact test** was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.

**Regression analysis:** Logistic regression analysis was used for prediction of risk factors.

**Deviations from Hardy–Weinberg equilibrium** expectations were determined using the chi-squared test.

Odds ratio and 95% confidence interval were calculated

All reported p values were two-tailed and p < 0.05 was considered to be significant.
Results

The present study was conducted on 50 diabetic patients; 40 cases had DM with DKD; out of them 20 had albuminuria and 20 without albuminuria. In addition to 10 DM without DKD were added as a control group.

Figure (20): Stratification of studied cases.
Table (8): Comparison of demographic and anthropometric data between studied groups and subgroups:

<table>
<thead>
<tr>
<th></th>
<th>DM without DKD N=10</th>
<th>Diabetic patients complicated with DKD</th>
<th>with albuminuria N=20</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DM with DKD N=40</td>
<td>without albuminuria N=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>Mean ±SD</td>
<td>59.8 ±11.1</td>
<td>61.1 ±11</td>
<td>58.4 ±11.4</td>
<td>0.764 T</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1</td>
<td>11</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>10%</td>
<td>27.5%</td>
<td>20%</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td>9</td>
<td>29</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>90%</td>
<td>72.5%</td>
<td>80%</td>
<td>65%</td>
</tr>
<tr>
<td><strong>BMI (Kg/m²)</strong></td>
<td>Mean ±SD</td>
<td>30.6 ±7.2</td>
<td>32.9 ±5.4</td>
<td>34</td>
<td>31.9</td>
</tr>
</tbody>
</table>

- Mean age of diabetics with DKD was 59.8, they were 11 males (27.5%) and 29 females (72.5%). Those without albuminuria had mean age of 61.1 years, they were 4 males (20%) and 16 females (80%). While those with albuminuria had mean age of 58.4 years. They were 7 males (35%) and 13 females (65%).
- The mean age of diabetic patients without DKD was 60.9 years. They were 1 (10%) male and 9 females (90%). They act as a control group of matched age and gender.
- There were no significance difference between studied groups and subgroups as regarding age, sex and BMI (p > 0.05).

SD, standard deviation; T, student t test; C, Chi square test; F, Fisher exact test; p¹, comparison between DM with and without DKD; p², comparison between DKD with and without albuminuria.
Table (9): Comparison of clinical data between studied groups and subgroups:

SD, standard deviation; T, student t test; p1, comparison between DM with and without DKD; p2, comparison between DKD with and without albuminuria.

- There was significance difference in duration of DM between diabetic patients with and without DKD, (P <0.05).
- Blood pressure did not differ significantly between groups and subgroups, (P >0.05).
**Table (10):** Comparison of FBG, 2hrPPG and HbA1C between studied groups and subgroups:

<table>
<thead>
<tr>
<th></th>
<th>DM without DKD N=10</th>
<th>Diabetic patients complicated with DKD</th>
<th>p&lt;sub&gt;1&lt;/sub&gt;</th>
<th>p&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM with DKD N=40</td>
<td>without albuminuria N=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>with albuminuria N=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>Mean ±SD</td>
<td>148.7 ±9.5</td>
<td>143.2 ±16</td>
<td>148.7 ±18.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>189.7 ±18.1</td>
<td>227.3 ±55.8</td>
<td>221.2 ±52.6</td>
</tr>
<tr>
<td>2hr PPG (mg/dL)</td>
<td>Mean ±SD</td>
<td>7.6 ±0.8</td>
<td>8.6 ±1.5</td>
<td>8.4 ±1.1</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>Mean ±SD</td>
<td>148.7 ±9.5</td>
<td>143.2 ±16</td>
<td>148.7 ±18.5</td>
</tr>
</tbody>
</table>

SD, standard deviation; T, student t test; p<sub>1</sub>, comparison between DM with and without DKD; p<sub>2</sub>, comparison between DKD with and without albuminuria.

- FBG was significantly higher in DKD without albuminuria when compared to DKD with albuminuria (p=0.029), while FBG did not differ significantly between diabetics with and without DKD.

- 2hrPPG and HbA1C were significantly high in diabetic patients with DKD when compared to those without DKD (p=0.042) and (p=0.044) respectively, while 2hrPPG and HbA1C did not differ significantly between those with and without albuminuria.
**Table (11):** Comparison of lipid profile between studied groups and subgroups:

<table>
<thead>
<tr>
<th></th>
<th>DM without DKD N=10</th>
<th>Diabetic patients complicated with DKD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>DM with DKD N=40</td>
<td>without albuminuria N=20</td>
<td>with albuminuria N=20</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>179.2 ±31.1</td>
<td>206.3 ±33.1</td>
<td>201.4 ±29.8</td>
<td>211.2 ±36.3</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>206.3 ±34.7</td>
<td>245 ±42.8</td>
<td>233.9 ±40.2</td>
<td>256.2 ±43.3</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.4 ±8.2</td>
<td>34.8 ±6.3</td>
<td>34.9 ±7.5</td>
<td>34.8 ±5</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>129.1 ±36.3</td>
<td>169.3 ±40.2</td>
<td>159 ±39.3</td>
<td>179.5 ±39.3</td>
</tr>
</tbody>
</table>

SD, standard deviation; T, student t test; \(p_1\), comparison between DM with and without DKD; \(p_2\), comparison between DKD with and without albuminuria.

- TG, TC and LDL, were significantly increased, while HDL was significantly decreased in diabetics with DKD when compared to those without DKD, \(P\) value was (0.023, 0.011, 0.008, 0.006) respectively.
- Lipid profile did not differ significantly between DKD with and without albuminuria.
Table (12): Comparison of renal function tests between studied groups and subgroups:

<table>
<thead>
<tr>
<th></th>
<th>DM without DKD N=10</th>
<th>Diabetic patients complicated with DKD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DM with DKD N=40</td>
<td>without albuminuria N=20</td>
<td>with albuminuria N=20</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>Mean ±SD</td>
<td>30.1 ±9</td>
<td>132 ±40.4</td>
<td>135.3 ±44.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>132 ±40.4</td>
<td>135.3 ±44.1</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>Mean ±SD</td>
<td>1 ±0.2</td>
<td>3.4 ±1.1</td>
<td>3.4 ±1.1</td>
</tr>
<tr>
<td>mg/dL</td>
<td></td>
<td></td>
<td>3.4 ±1.1</td>
<td>3.4 ±1.1</td>
</tr>
<tr>
<td></td>
<td>Median range</td>
<td>23.5-14</td>
<td>35.5-12-900</td>
<td>22.5-12-32</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>Mean ±SD</td>
<td>113.9 ±28.4</td>
<td>37.4 ±10.2</td>
<td>39.1 ±12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.4 ±10.2</td>
<td>39.1 ±12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.4 ±10.2</td>
<td>39.1 ±12.3</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>&lt;0.001</td>
<td>0.682</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td></td>
<td>0.046</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SD, standard deviation; T, student t test; M, Mann Whitney test; p1, comparison between DM with and without DKD; p2, comparison between DKD with and without albuminuria.

- Serum level of urea, creatinine and ACR were significantly increased, while eGFR was significantly decreased in diabetics with DKD when compared to those without DKD, P value was (<0.001, 0.002, 0.046, < 0.001) respectively.

- ACR was significantly higher in DKD with albuminuria when compared to those without albuminuria, (P<0.001), while serum level of urea, creatinine and eGFR didn’t differ significantly between albuminuric and non albuminuric DKD.
**Table (13):** Assessment of Hardy Weinberg equilibrium of studied SNPs in DM with and without DKD groups:

<table>
<thead>
<tr>
<th>rs10823108</th>
<th><strong>DM without DKD</strong></th>
<th><strong>DM with DKD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>N=10</strong></td>
<td><strong>N=40</strong></td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td><strong>Observed</strong></td>
<td><strong>Expected</strong></td>
</tr>
<tr>
<td><strong>GG</strong></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>GA</strong></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.213</td>
<td></td>
</tr>
</tbody>
</table>

**Table (14):** Assessment of Hardy Weinberg equilibrium of studied SNP in DM with DKD with and without albuminuria:

<table>
<thead>
<tr>
<th>rs10823108</th>
<th><strong>DM with DKD without albuminuria</strong></th>
<th><strong>DM with DKD with albuminuria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>N=20</strong></td>
<td><strong>N=20</strong></td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td><strong>Observed</strong></td>
<td><strong>Expected</strong></td>
</tr>
<tr>
<td><strong>GG</strong></td>
<td>8</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>GA</strong></td>
<td>11</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.257</td>
<td></td>
</tr>
</tbody>
</table>

This sample of individuals was selected randomly from population in Qaliobeya Governorate in Egypt. Applying Hardy Weinberg equation revealed that rs10823108 genotypes in studied groups and subgroups were in HW equilibrium.
Table (15): Comparison of rs10823108 genotypes and alleles between DM with and without DKD groups:

<table>
<thead>
<tr>
<th>rs10823108</th>
<th>Genotypes</th>
<th>DM without DKD N=10</th>
<th>DM with DKD N=40</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td>GG</td>
<td>4</td>
<td>40</td>
<td>22</td>
<td>55</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>3</td>
<td>30</td>
<td>16</td>
<td>40</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>3</td>
<td>30</td>
<td>2</td>
<td>5</td>
<td>0.047</td>
</tr>
<tr>
<td>Dominant model</td>
<td>GA+AA</td>
<td>6</td>
<td>60</td>
<td>18</td>
<td>45</td>
<td>0.397</td>
</tr>
<tr>
<td>Recessive model</td>
<td>GA+GG</td>
<td>7</td>
<td>70</td>
<td>38</td>
<td>95</td>
<td>0.038</td>
</tr>
<tr>
<td>Alleles</td>
<td>G</td>
<td>11</td>
<td>55</td>
<td>60</td>
<td>75</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>9</td>
<td>45</td>
<td>20</td>
<td>25</td>
<td>0.086</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

- GG genotype showed higher frequency, while AA showed lower frequency in diabetic patients with DKD when compared to diabetic patients without DKD.
- GG genotype showed high risk of DKD development within diabetic patients (p=0.047, OR=3.573). AA genotype showed protective effect against DKD development within diabetic patients (p=0.047, OR=0.280).
- Patients carrying G allele (recessive model) had significant risk to develop DKD within diabetic patients (p=0.038, OR=3.547).
Results

Table (16): Comparison of rs10823108 genotypes and alleles between DM with DKD with and without albuminuria subgroups:

<table>
<thead>
<tr>
<th>rs10823108</th>
<th>DM with DKD without albuminuria N=20</th>
<th>DM with DKD with albuminuria N=20</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>8</td>
<td>14</td>
<td>70</td>
<td>0.048</td>
<td>2.180</td>
</tr>
<tr>
<td>GA</td>
<td>11</td>
<td>5</td>
<td>25</td>
<td>0.049</td>
<td>0.433</td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0.707</td>
<td>0.706</td>
</tr>
<tr>
<td>Dominant model</td>
<td>GA+AA</td>
<td>12</td>
<td>60</td>
<td>30</td>
<td>0.047</td>
</tr>
<tr>
<td>Recessive model</td>
<td>GA+GG</td>
<td>19</td>
<td>38</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>27</td>
<td>67.5</td>
<td>33</td>
<td>82.5</td>
<td>0.126</td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>32.5</td>
<td>7</td>
<td>17.5</td>
<td>0.126</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

- GG showed significantly higher frequency in diabetics with DKD with albuminuria when compared to those without albuminuria (p=0.048) with risk to develop albuminuria in DKD patients (OR=2.180).
- GA showed significantly lower frequency in diabetics with DKD with albuminuria when compared to those without albuminuria (p=0.049) with protective effect against albuminuria in DKD patients (OR=0.433).
- Diabetics with DKD carrying A allele (dominant model) showed significantly lower frequency in diabetics with DKD with albuminuria when compared to those with no albuminuria (p=0.047) with protective effect against albuminuria in DKD patients (OR=0.459).
**Results**

**Figure (21):** rs10823108 genotypes in DM with and without DKD groups.

**Figure (22):** rs10823108 genotypes in DM with DKD with and without albuminuria subgroups.
Table (17): Comparison of demographic and anthropometric data between rs10823108 genotypes in all studied cases:

<table>
<thead>
<tr>
<th></th>
<th>GG N=26</th>
<th>GA N=19</th>
<th>AA N=5</th>
<th>GA+AA N=24</th>
<th>GA+GG N=45</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>26</td>
<td>19</td>
<td>5</td>
<td>24</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>9</td>
<td>34.6%</td>
<td>1</td>
<td>20.0%</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
<td>4</td>
<td>21</td>
<td>34</td>
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<td></td>
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</tr>
<tr>
<td>%</td>
<td>65.4%</td>
<td>89.5%</td>
<td>80.0%</td>
<td>87.5%</td>
<td>75.6%</td>
<td></td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>N</td>
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<td>32.6</td>
<td>32.3</td>
<td>32.6</td>
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<td></td>
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</tr>
<tr>
<td>%</td>
<td>5.9</td>
<td>6.3</td>
<td>4.1</td>
<td>5.8</td>
<td>6.0</td>
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<tr>
<td>Females</td>
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</tr>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
<td>4</td>
<td>21</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>65.4%</td>
<td>89.5%</td>
<td>80.0%</td>
<td>87.5%</td>
<td>75.6%</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

P1, comparison between GG, GA, AA; p2, comparison between GA+AA versus GG; p3, comparison between GA+GG versus AA; SD, standard deviation; A, ANOVA; T, t test.

➢ No significant associations were found between demographic and anthropometric data and rs10823108 genotypes in all studied cases.

Table (18): Comparison of clinical data between rs10823108 genotypes in all studied cases:

<table>
<thead>
<tr>
<th></th>
<th>GG N=26</th>
<th>GA N=19</th>
<th>AA N=5</th>
<th>GA+AA N=24</th>
<th>GA+GG N=45</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>133.5</td>
<td>123.4</td>
<td>130</td>
<td>124.8</td>
<td>129.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>±16.1</td>
<td>±20.1</td>
<td>±7.1</td>
<td>±18.2</td>
<td>±18.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>85.8</td>
<td>79</td>
<td>82</td>
<td>79.6</td>
<td>82.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>±11.2</td>
<td>±12.9</td>
<td>±7.6</td>
<td>±11.9</td>
<td>±12.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11.2</td>
<td>12.4</td>
<td>6.2</td>
<td>11.1</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>±3.4</td>
<td>±3.6</td>
<td>±1.5</td>
<td>±2.2</td>
<td>±3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11.2</td>
<td>12.4</td>
<td>6.2</td>
<td>11.1</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>±3.4</td>
<td>±3.6</td>
<td>±1.5</td>
<td>±2.2</td>
<td>±3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1, comparison between GG, GA, AA; p2, comparison between GA+AA versus GG; p3, comparison between GA+GG versus AA; SD, standard deviation; A, ANOVA; T, t test.

➢ No significant associations were found between clinical data and rs10823108 genotypes in all studied cases.
Table (19): Comparison of FBG, PPG, and HA1C between rs10823108 genotypes in all studied cases:

<table>
<thead>
<tr>
<th></th>
<th>GG N=26</th>
<th>GA N=19</th>
<th>AA N=5</th>
<th>GA+AA N=24</th>
<th>GA+GG N=45</th>
<th>( p^1 )</th>
<th>( p^2 )</th>
<th>( p^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mmHg)</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>GG</td>
<td>143.8 ±17.5</td>
<td>144.6 ±12</td>
<td>145.8 ±14.2</td>
<td>144.9 ±12.2</td>
<td>144.2 ±15.2</td>
<td>0.959(^A)</td>
<td>0.805(^T)</td>
<td>0.819(^T)</td>
</tr>
<tr>
<td>GA</td>
<td>227.1 ±58.2</td>
<td>218.5 ±49.4</td>
<td>187 ±7.9</td>
<td>211.9 ±45.8</td>
<td>223.4 ±54.2</td>
<td>0.299(^A)</td>
<td>0.314(^T)</td>
<td>0.143(^T)</td>
</tr>
<tr>
<td>AA</td>
<td>8.7 ±1.8</td>
<td>8.3 ±1.1</td>
<td>7.4 ±0.3</td>
<td>8.1 ±1</td>
<td>8.5 ±1.5</td>
<td>0.186(^A)</td>
<td>0.169(^T)</td>
<td>0.106(^T)</td>
</tr>
</tbody>
</table>

\( p^1 \), comparison between GG, GA, AA; \( p^2 \), comparison between GA+AA versus GG; \( p^3 \), comparison between GA+GG versus AA; SD, standard deviation; A, ANOVA; T, t test.

➢ No significant associations were found between FBG, PPG, HA1C and rs10823108 genotypes in all studied cases.

Table (20): Comparison of lipid profile between rs10823108 genotypes in all studied cases:

<table>
<thead>
<tr>
<th></th>
<th>GG N=26</th>
<th>GA N=19</th>
<th>AA N=5</th>
<th>GA+AA N=24</th>
<th>GA+GG N=45</th>
<th>( p^1 )</th>
<th>( p^2 )</th>
<th>( p^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dl)</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>GG</td>
<td>200.2 ±40.7</td>
<td>205.7 ±23.7</td>
<td>186.0 ±32.7</td>
<td>201.6 ±26.3</td>
<td>202.5 ±34.3</td>
<td>0.524(^A)</td>
<td>0.891(^T)</td>
<td>0.310(^T)</td>
</tr>
<tr>
<td>GA</td>
<td>234.8 ±46.3</td>
<td>246.5 ±41.3</td>
<td>215.0 ±37.1</td>
<td>240 ±41.7</td>
<td>239.7 ±44.2</td>
<td>0.336(^A)</td>
<td>0.680(^T)</td>
<td>0.235(^T)</td>
</tr>
<tr>
<td>AA</td>
<td>37.2 ±7.1</td>
<td>34.1 ±7.2</td>
<td>38.4 ±7.1</td>
<td>35 ±7.2</td>
<td>35.9 ±7.2</td>
<td>0.284(^A)</td>
<td>0.293(^T)</td>
<td>0.459(^T)</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>GG</td>
<td>158.1 ±42.9</td>
<td>171.2 ±41.9</td>
<td>139.4 ±38</td>
<td>164.6 ±42.4</td>
<td>163.6 ±42.5</td>
<td>0.288(^A)</td>
<td>0.595(^T)</td>
<td>0.228(^T)</td>
</tr>
</tbody>
</table>

\( p^1 \), comparison between GG, GA, AA; \( p^2 \), comparison between GA+AA versus GG; \( p^3 \), comparison between GA+GG versus AA; SD, standard deviation; A, ANOVA; T, t test.

➢ No significant associations were found between lipid profile and rs10823108 genotypes in all studied cases.
Table (21): Comparison of renal function tests between rs10823108 genotypes in all studied cases:

<table>
<thead>
<tr>
<th></th>
<th>GG (N=26)</th>
<th>GA (N=19)</th>
<th>AA (N=5)</th>
<th>GA+AA (N=24)</th>
<th>GA+GG (N=45)</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>115.7 ±37.3</td>
<td>123 ±34.3</td>
<td>47 ±15.3</td>
<td>107.2 ±35.9</td>
<td>118.8 ±29.8</td>
<td>0.031^A</td>
<td>0.614^T</td>
<td>0.009^T</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.7 ±0.9</td>
<td>3.6 ±1.1</td>
<td>1.1 ±0.4</td>
<td>3 ±1</td>
<td>3.1 ±1</td>
<td>0.025^A</td>
<td>0.869^T</td>
<td>0.009^T</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>Median range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>64.5-1200</td>
<td>26-1600</td>
<td>29-14-760</td>
<td>64.5-1200</td>
<td>27-14-760</td>
<td>0.467^K</td>
<td>0.217^M</td>
<td>0.651^M</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.8 ±13.7</td>
<td>47.1 ±15</td>
<td>89 ±16.3</td>
<td>55.8 ±17.2</td>
<td>48.7 ±12.5</td>
<td>0.025^K</td>
<td>0.877^T</td>
<td>0.013^T</td>
</tr>
</tbody>
</table>

P1, comparison between GG, GA, AA; p2, comparison between GA+AA versus GG; p3, comparison between GA+GG versus AA; SD, standard deviation; A, ANOVA; T, t test; K, Kruskal Wallis test; M, Mann Whitney test.

- Urea, creatinine and eGFR showed significant differences between GG, GA and AA genotypes. Diabetic patients carrying G allele had significantly higher urea, creatinine concentration and significantly lower eGFR when compared to those carrying AA genotype P values (0.031, 0.025, 0.025) respectively.
- ACR did not differ significantly according to genotypes.
Figure (23): Urea concentration according to rs10823108 genotypes in all studied cases.

Figure (24): Creatinine concentration according to rs10823108 genotypes in all studied cases.

Figure (25): eGFR according to rs10823108 genotypes in all studied cases
Table (22): Regression analysis for prediction of DKD development within diabetic patients:

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th></th>
<th>Multivariable</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
<td>OR</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.758</td>
<td>0.990</td>
<td>0.926</td>
<td>1.057</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>0.270</td>
<td>0.293</td>
<td>0.033</td>
<td>2.590</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.256</td>
<td>1.076</td>
<td>0.948</td>
<td>1.222</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>0.122</td>
<td>1.034</td>
<td>0.991</td>
<td>1.078</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>0.120</td>
<td>1.053</td>
<td>0.987</td>
<td>1.123</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>0.039</td>
<td>1.336</td>
<td>1.015</td>
<td>1.760</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>0.047</td>
<td>2.118</td>
<td>1.968</td>
<td>4.635</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>0.046</td>
<td>1.241</td>
<td>1.169</td>
<td>1.518</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>&lt;0.001</td>
<td>0.995</td>
<td>0.993</td>
<td>0.996</td>
</tr>
<tr>
<td>rs10823108 GG</td>
<td>0.047</td>
<td>3.573</td>
<td>1.018</td>
<td>12.927</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

- Logistic regression analysis was conducted for prediction of DKD development within diabetic patients using age, gender, BMI, BP, DM duration, HbA1C, ACR, eGFR and rs10823108 genotypes as covariates. Longer DM duration, higher HbA1C, ACR, lower eGFR, GA+GG genotypes were associated with risk of DKD development within diabetic patients in univariable analysis.

- However, taking significant covariates in univariable analysis into multivariable analysis revealed that only lower eGFR, GA+GG genotypes were considered independent risk factors for prediction of DKD development within diabetic patients.
Table (23): Regression analysis for prediction of albuminuria development within diabetic DKD patients:

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th></th>
<th></th>
<th>Multivariable</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$</td>
<td>OR</td>
<td>95% CI</td>
<td>$p$</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age(years)</td>
<td>0.437</td>
<td>0.986</td>
<td>0.951</td>
<td>1.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender(M/F)</td>
<td>0.289</td>
<td>0.620</td>
<td>0.256</td>
<td>1.501</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI(kg/m$^2$)</td>
<td>0.209</td>
<td>0.953</td>
<td>0.884</td>
<td>1.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPB(mm Hg)</td>
<td>0.703</td>
<td>1.005</td>
<td>0.981</td>
<td>1.028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP(mm Hg)</td>
<td>0.945</td>
<td>1.001</td>
<td>0.968</td>
<td>1.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of DM(years)</td>
<td>0.209</td>
<td>1.036</td>
<td>0.980</td>
<td>1.095</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1C(%)</td>
<td>0.250</td>
<td>1.170</td>
<td>0.895</td>
<td>1.530</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR(mL/min/1.73 m$^2$)</td>
<td>0.625</td>
<td>0.996</td>
<td>0.978</td>
<td>1.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs10823108 GG</td>
<td>0.048</td>
<td>2.180</td>
<td>1.976</td>
<td>4.869</td>
<td>0.016</td>
<td>1.117</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval. Logistic regression test was used.

- Logistic regression analysis was conducted for prediction of albuminuria development within diabetic DKD patients, using age, gender, BMI, BP, DM duration, HbA1C, eGFR and rs10823108 genotypes as covariates. GG genotypes were considered independent risk factors for prediction of albuminuria development within diabetic DKD patients in uni- and multivariable analyses.
Discussion

CKD has affected millions of people from all over the world, and has been identified as a worldwide public health problem. DKD is a common and serious complication of diabetes and has become the leading cause of ESRD (National Kidney Foundation, 2012).

The global prevalence of DKD has attracted more attention of scholars. A deeper understanding of the genetic and molecular basis of the development and progression of DKD has presented. DKD has been considered as a nonimmune disease, but researches have revealed a great role of immune-mediated inflammatory processes in the pathophysiology of DKD. Findings have also found that genetic variations play a crucial role in the development of DKD (Celec et al., 2012).

Both clinical and epidemiological studies have demonstrated that there is familial aggregation of DKD in different ethnic groups, indicating that genetic factors contribute to development of the disease. Furthermore, genetic risk factors in DKD interact with the environmental factors as lifestyle, diet and medication (Kato and Natarajan, 2014).

This case control study aimed to investigate the association between SIRT1 gene polymorphism and development of DKD in patients with type 2 diabetes (T2D) based on the presence or absence of albuminuria.

SIRT1 gene polymorphism was evaluated by using real time PCR on 50 patients with type 2 DM, patients were divided in to 3 groups: Group (I): included 20 patients with type 2 diabetes complicated with DKD with albuminuria (ACR more than 30 mg/g creatinine), Group (II): included 20 patients with type 2 diabetes complicated with DKD without albuminuria (ACR less than 30 mg/g creatinine)and Group (III): included
10 patients with type 2 diabetes with non-apparent DKD as a control group.

The present study showed that there is no significant difference as regarding (age, sex, and BMI) between the three studied groups.

These results were consistent with a study performed by Yue et al. (2018) who stated that the results of statistical analysis showed no significant difference in age, gender and weight in diabetics with and without DKD.

In contrary, in a study carried out by Nicola et al. (2017) they found that patients with low proteinuric chronic kidney disease (LP-CKD) were more common in female gender, older age, as compared to patients with proteinuric renal insufficiency, also Tang et al. (2017) Study in Chinese population revealed that T2DM subjects who suffered from DKD were considerably older with a higher BMI, than diabetic without DKD.

The present study demonstrated that the duration of DM is increased in patients with DKD (albuminuric and non albuminuric) while having shorter duration of DM is more frequent in patients without DKD. This was also reported in a previous Japanese study by Zhao et al. (2017) and Chinese study by Tang et al. (2017) who found that T2DM subjects who suffered from DKD were, had diabetes for a significantly longer time period than diabetic without DKD.

On the other hand, in a study carried out by Yue et al. (2018) they found that there was no significant difference in the course of diabetes between diabetic with and without DKD.

The current study showed that there is no significant difference in blood pressure between the three studied groups. These results were in
consistent with a study performed by Yue et al. (2018) who revealed that systolic and diastolic blood pressure nearly the same in both groups (diabetic with and without DKD), (P value =0.514).

In contrary, in a study made Laranjinha et al. (2016) they found that normoalbuminuric DKD (NA-DKD) group had lower hypertension prevalence than albuminuric DKD (p<0.001).

Moreover, Mottl et al. (2013) who concluded that the prevalence of normoalbuminuric chronic kidney disease (NA-CKD) is significantly lower in those with poorly controlled HTN.

Bakris et al. (2003) documented that the significant difference as regard HTN in albuminuric chronic kidney disease (ALB-CKD) is supported by the association between HTN and albuminuria. Multiple mechanisms involved in the development of HTN in patients with DKD, including inappropriate activation of the renin angiotensin aldosterone system (RAAS), volume expansion due to increased sodium reabsorption, peripheral vasoconstriction, upregulation of endothelin 1, inflammation, and downregulation of nitric oxide. These factors accelerate the development of kidney disease and increase the risk for albuminuria (Bakris et al., 2003).

As regarding lipid profile, a significant increase in (total cholesterol, LDL-c, and triglycerides) and decrease in HDL-C were found in DKD patients (with and without albuminuria) when compared to those without DKD. This was agreed by a study made by Zhao et al. (2017) who concluded that T2DN cases had significant higher total cholesterol level, and lower HDL-C level than T2DM control group, while in a study by Mottl et al. (2013) they revealed a significant increase in LDL-c in albuminuric group when compared to non albuminuric group.
Dyslipidemia tends to progress along with the declination of renal function in patients with CKD, even at the early stages of renal dysfunction and it also contributes to the association between CKD and cardiovascular disease, which is the main cause of death in patients with CKD and ESRD (*Hager et al.*, 2016).

This study showed a significant increase in HbA1c levels in DKD groups when compared to those without DKD, while no significant differences were found between albuminuric and non albuminuric group. This goes in line with a study made by *Erdogan et al.* (2011) who found that HbA1c levels were higher in patients with T2DM either with or without DN than in the healthy control group.

Moreover in another Portuguese study made by *Laranjinha et al.* (2016) who aimed to evaluate the prevalence of the demographic and clinical characteristics of type 2 diabetic patients with NA-DKD and A-DKD, they found no significant difference in HbA1c levels between the two groups.

In contrary, in a study carried out by *Retnakaran et al.* (2006) they concluded that HbA1c could be predictor for albuminuria in DKD.

This could be explained due to irreversible binding of glucose to proteins in the kidneys and circulation to form advanced glycation end products (AGEs), which can form complex cross-links over years and contribute to renal damage (*Bohlender et al.*, 2005).

Concerning kidney function a significant increase were found in serum creatinine and urea levels in DKD groups when compared to diabetic patients without DKD, while no significant differences were found between albuminuric and non albuminuric group.
These results go in line with a study made by Erdogan et al., (2011) who found that serum creatinine and serum urea levels were significantly higher in patients with type 2 diabetes mellitus either with or without DN than in the healthy control group.

The present study revealed that eGFR is significantly lower in DKD groups (with and without albuminuria) when compared to those without DKD, while it didn’t differ significantly between albuminuric and non albuminuric group.

This was approved by a study performed by Jerums et al. (2012) who reported a significant proportion of diabetic patients has decreased GFR without albuminuria and cocluded that loss of GFR may occurs before the onset of macroalbuminuria and is even seen in normoalbuminuric subjects.

Also Pavkov et al. (2012) advocated that in DKD the declining of GFR may precede the onset of albuminuria, although they still defend that the progression to CKD is strongly dependent on the appearance of albuminuria (especially albuminuria greater than 300 mg/g).

In contrary, Brenner et al. (1996) revealed a significant correlation between reduction in functional nephron number (decreased eGFR) and progressive increase in AER.

In study by Laranjinha et al. (2016) they observed a significant negative correlation between albuminuria and decreased eGFR.
A decline in glomerular filtration rate (GFR) was initially considered to occur only in patients with macroalbuminuria (Mogensen et al., 1984). Subsequent experience has shown that GFR may start to decline in subjects with type 1 diabetes at the stage of microalbuminuria (Perkins et al., 2007). Furthermore, progressive decline in GFR has also been described in normoalbuminuric subjects with type 1 or type 2 diabetes (Tsalamandris et al., 1994).

Using serial measurements of creatinine clearance, it was shown that approximately one-third of subjects with either type 1 or type 2 diabetes developed a decline in renal function without a corresponding increase in AER. Subsequent studies in type 2 diabetes using isotopic GFR showed that approximately one-quarter of subjects developed non-albuminuric renal insufficiency (GFR <60 ml/min/1.73 m²), after exclusion of patients treated with renin-angiotensin system (RAS) inhibitors. Although these studies have shown that early GFR loss is not confined to macroalbuminuric subjects, the rate of progression of GFR loss is usually more rapid in subjects with macroalbuminuria (MacIsaac et al., 2004).

The present study showed that UACR is significantly higher in DKD with albuminuria (group I) compared to DKD without albuminuria (group II). This result goes in line with The United Kingdom Prospective Diabetes Study (UKPDS,2006) who found that approximately 2% per year of DKD patients with T2DM progressed from normo- to microalbuminuria and from micro- to macroalbuminuria.

Albuminuria has been used classically as the first sign of renal involvement in diabetic patients and it is also used to evaluate the progression of DKD (ADA, 2012).
Using albuminuria as an early marker of DKD onset or progression requires a careful interpretation because in diabetic patients albuminuria has a great tendency to regress spontaneously to normal levels \((\text{Laranjinha et al., 2016})\).

\textit{Newman et al. (2005)} found that approximately 18–51% of type 2 diabetic patients (followed during 2–10 years) initially albuminurics became non-albuminurics spontaneously during follow-up time. Moreover, United Kingdom Prospective Diabetes Study (\textit{UKPDS, 2006}) detected that some diabetic patients pass directly from a normoalbuminuric stage to renal insufficiency (0.1% per year).

This finding underscores the fact that albuminuria is a dynamic, fluctuating condition rather than a linearly progressive process. Potential pathogenic alternatives to the underlying mechanisms of NA-CKD include accelerated vascular disease, renal aging and masking of albuminuria by RAAS inhibitors \((\text{Mottl et al., 2013})\).

In a study by \textit{Mark et al., (2010)} they found that NA-DKD has been noted to carry a more benign clinical course with respect to GFR loss when compared to DKD with albuminuria.

\textit{Penno et al. (2012)} showed that the majority of patients with type 2 DM and DKD had increased albuminuria (independently of decreased eGFR).

\textit{MacIsaac et al. (2006)} demonstrated that diabetic patients with decreased eGFR had a higher intrarenal resistive index, and that there was no association between albuminuria range and this index, suggesting a role for intrarenal vascular disease in renal insufficiency in NA-DKD patients. Many authors have also assumed cholesterol emboli as a
frequent cause of decreased GFR in diabetic patients \cite{Rychlik:1999}.

The presence or absence of albuminuria should therefore be interpreted as an important, but not the sole, risk marker that should be evaluated when stratifying the risk of progressive DKD in an individual patient \cite{MacIsaac:2014}.

The function of SIRT1 in the occurrence and development of renal disease has been of increasing concern in humans. SIRT1 makes a contribution towards innate cell apoptosis reduction in the kidney, kidney failure retardation caused by increase in age, inflammation reduction, renal interstitial fibrosis inhibition, blood pressure regulation, autophagy induction and protection in DKD \cite{Li:2010}.

The expressions of sirtuin families have also been observed in the kidneys, and SIRT1 has been shown to mediate a protective role of calorie restriction (CR) in the progression of the aging kidney. These observations suggest the possibility that mammalian sirtuins are a candidate for conferring susceptibility to diabetic nephropathy \cite{Kume:2010}.

SIRT1 has a protective role in limiting podocyte injury in DN. There are data that confirm that the conditional deletion of SIRT1 in the podocytes of diabetic mice results in acetylation of the p65 subunit of NF-κB and STAT3, which likely translates into increased levels of urinary protein excretion and more severe renal damage compared with mice without the genetic deletion \cite{Liu:2014}.

Since SIRT1 could affect various metabolic activities, the effects of SIRT1 polymorphisms on susceptibility to diabetic nephropathy might be
mediated by differences in the metabolic state among individuals, including glycemic control, obesity and blood pressure \((\text{Huang et al., 2015})\).

The evidence of reduced SIRT1 expression in both tubular cells and glomeruli from patients with DN provides additional clues regarding the potential involvement of SIRT1 in human DN \((\text{Zhao et al., 2017})\).

In the present study, considering GG genotype as the mutant gene and AA as the wild one, the GG genotype of SIRT1-related SNPs rs10823108 gene polymorphism was significantly associated with the occurrence of DKD.

This results were in consistent with a study by \textit{Yue et al. (2018)} who demonstrated that patients with mutant homozygous type GG in rs10823108 had a higher risk of DKD than those with wild homozygous type AA, and that GG + GA carriers had a higher risk of disease than AA carriers in Chinese patients, also \textit{Zhao et al. (2017)} suggested that rs10823108 AA genotype within SIRT1 has significant associations with about a 0.60-fold decreased risk of DN \((\text{OR} = 0.60, 95\% \text{CI:} 0.38 \text{–} 0.97)\) than GG genotype in Chinese patients.

In Japanese study conducted by \textit{Maeda et al. (2011)} they identified that SNPs within SIRT1 were nominally associated with susceptibility to diabetic nephropathy. Also identified one haplotype consisting of the 11 SNPs in SIRT1 had a stronger association with diabetic nephropathy than single SNPs alone, whereas SNPs in other sirtuin families did not show any association with diabetic nephropathy.

In the present study GG showed higher frequency in DKD with albuminuria when compared to those without albuminuria (risk to
develop albuminuria in DKD patients), while GA and AA showed low frequency in DKD with albuminuria when compared to those without albuminuria (protective effect against albuminuria in DKD patients).

These results go in line with a study by Yue et al. (2018) who revealed that patients with GG in the rs10823108 locus had a higher risk of proteinuria than those with AA.

According to the result of the present study, Diabetic patients carrying G allele (GG and GA) had significantly higher urea, creatinine concentration and significantly lower eGFR when compared to those carrying AA genotype.

Also in a study by Faradonbehwe et al. (2019) who investigated the association of the rs3758391 polymorphism of the SIRT1 gene with the risk of diabetic nephropathy. They found elevated BUN, creatinine and albuminuria, and decreased eGFR (as markers of kidney damage) in patient with the TT genotype as compared with other variants. This might show the higher risk of micro-vascular complications of T2DM for homozygous risk allele carriers.

In this study no significant associations were found between demographic and anthropometric data (age, sex and BMI), clinical data (blood pressure and duration of DM), laboratory parameters (FBG, PPG and HbA1C) and lipid profile (TG, TC, HDL and LDL) and rs10823108 genotypes in all studied cases.

Logistic regression analysis was conducted for prediction of DKD development within diabetic patients using age, gender, BMI, BP, DM duration, HbA1C, ACR, eGFR and rs10823108 genotypes as covariates. Longer DM duration, higher HbA1C, higher ACR, lower eGFR, GA+GG
genotypes were associated with risk of DKD development within diabetic patients in univariable analysis. However, taking significant covariates in univariable analysis into multivariable analysis revealed that only lower eGFR, GA+GG genotypes were considered independent risk factors for prediction of DKD development within diabetic patients.

However in a logistic regression analysis by Yue et al. (2018) they showed that TC, LDL, BMI and high HbA1C level were contributing factors for DKD development.

The step-by-step regression analysis by Tang et al. (2017) was utilised to analyse the effects of the different environmental factors on DKD in T2DM patients, The binary logistic regression analysis first revealed these variables (age, fasting blood glucose and HDL-C) as autonomous risk factors of DKD. Then BMI, HbA1c, diastolic blood pressure and mean arterial blood pressure were identified as autonomous risk factors for DKD progression.

While using logistic regression analysis in the current study for prediction of albuminuria development within diabetic DKD patients, using age, gender, BMI, BP, DM duration, HbA1C, eGFR and rs10823108 genotypes as covariates. GG genotypes were found to be independent risk factors for prediction of albuminuria development within diabetic DKD patients in uni- and multivariable analyses.

On the other hand, multivariable logistic regression analysis by Laranjinha et al. (2016) adjusted to gender, age, GFR, hypertension and CAD prevalence, showed that developing NA-DKD was positively associated with age and female gender and negatively associated with GFR but not associated with hypertension and CAD.
Moreover, in the Swedish National Diabetes Register (*NDR*) and U.K. Prospective Diabetes Study 74 (*UKPDS*) studies in diabetic patients advocated that female gender is a risk factor for renal impairment (GFR ≤ 60 mL/min), while males are associated with the development of albuminuria.

Although, there is a nephron loosing related to age, older ages have been related not only with lower GFR but also with higher albuminuria (*Afghahi et al., 2011*). The female tendency for NA-DKD is a consistent finding in other studies by *MacIsaac et al. (2004)*.

**Conclusion:**

From the results of this study rs10823108 GG genotype was risky while AA genotype was protective against DKD within diabetic patients. Moreover, rs10823108 GG genotype was found to be risky while GA genotype was protective against albuminuria development in diabetic patients with DKD.

It could be concluded that SIRT1 (rs10823108) gene polymorphism was found to be significantly associated with increased risk of DKD development and can be used in early detection of the disease especially if the study applied to large scale.
Summary and conclusion

Sirtuins (SIRTs) are members of the silent information regulator 2 family. SIRT1 exerts anti-apoptotic, anti-oxidative, and anti-inflammatory effects against cellular injury, and protects the cells through the regulation of mitochondrial biogenesis, autophagy, and metabolism. SIRT1 also promotes vasodilation and protects vascular tissues. In humans with diabetic kidney disease (DKD), its expression tends to be decreased in renal cells.

SIRT1 gene polymorphism was detected using real-time PCR in a total number of 50 subjects, 40 type 2 diabetic patients with diabetic kidney disease (DKD) and 10 diabetics without DKD as a control subjects. The subjects were divided in to 3 groups; (Group I), included (20) type 2 diabetic patients with DKD, with albuminuria [A/C ratio > 30 mg/g], (Group II), included (20) type 2 diabetic patients with DKD, without albuminuria [A/C ratio < 30 mg/g], and (Group III), (10) type 2 diabetic patients as a controls without DKD. The following investigations were done: fasting blood glucose (mg/dl), total cholesterol (mg/dl), triglycerides (mg/dl), HDL-c (mg/dl), LDL-c (mg/dl), serum creatinine (mg/dl), HbA1c (%), ACR (mg/g), eGFR (ml/min/1.73m²).

As regards age, sex, BMI and blood pressure there were no significant difference between the three studied groups, while there was significant increase in duration of DM, HbA1c, 2hr PPG, TC, TG, LDC,Urea,Creatinine and ACR and significant decrease in HDL-C and eGFR in diabetics with DKD when compared to those without DKD.

Comparing (Group I) with (Group II) FBG and ACR were significantly higher in DKD with albuminuria when compared to those
without albuminuria, while duration of DM, HbA1c, 2hr PPG, Lipid profile urea, creatinine and eGFR did not differ significantly between DKD with and without albuminuria.

The genotype distribution of SIRT1 gene polymorphism was different between the control group (AA 30%, GA 30%, GG 40%) and the DKD patients (AA 5%, GA 40%, GG 55%). The frequency of the mutant G allele in control group was 55%, while it was 75% in DKD group.

There was statistical significant increase in GG and decrease in AA frequency in patients with DKD when compared to patients without DKD P values were 0.047 and 0.047 respectively, OR (95% C.I) were 3.573 (1.018-12.927) and 0.280 (0.080-.983) respectively. Moreover, there was statistical significant increase in G allele frequency in patients with DKD when compared to patients without DKD (P value =0.086), OR (95% C.I) = 2.455 (0.889-6.779) and 0.594 (0.328-1.076) respectively.

The genotype distribution of SIRT1 gene polymorphism in DKD patients with albuminuria (group I), was (AA 5%, GA 25%, GG 70%) versus (AA 5%, GA 55%, GG 40%) in those without albuminuria (group II). The frequency of the mutant G allele in group I was 82.5%, while it was 67.5% in group II.

There was statistical significant increase in GG and decrease in GA frequency in DKD patients with albuminuria when compared to those without albuminuria P values were 0.047 and 0.049 respectively, OR (95% C.I) were 2.180 (1.976-4.869) and 0.433 (0.188-0.998)respectively.

According to the result of the present study, diabetic patients carrying G allele (GG and GA) had significantly higher urea, creatinine
concentration and significantly lower eGFR when compared to those carrying AA genotype.

As regarding the eGFR, it was significantly lower in DKD groups (with and without albuminuria) when compared to those without DKD but didn’t differ significantly between albuminuric and non albuminuric DKD, while UACR is significantly higher in DKD with albuminuria (group I) compared to DKD without albuminuria (group II).

Logistic regression analysis was conducted for prediction of DKD development within diabetic patients revealed that longer DM duration, higher HbA1C, ACR, lower eGFR, GA+GG genotypes were associated with risk of DKD development within diabetic patients in univariable analysis However, multivariable analysis revealed that only lower eGFR, GA+GG genotypes were considered independent risk factors for prediction of DKD development within diabetic patients.

Logistic regression analysis was conducted for prediction of albuminuria development within diabetic DKD patients revealed that GG genotypes was considered independent risk factors for prediction of albuminuria development within diabetic DKD patients in uni- and multivariable analyses.
Conclusion

This study concluded that the SIRT1(rs10823108) gene polymorphism was found to be associated with the risk of development of diabetic kidney disease and albuminuria and the mutant G allele of SIRT1(rs10823108) gene polymorphism is associated with an increased risk of DKD compared to the wild A allele in the studied type 2 diabetic patients. Moreover, the G allele is significantly associated with higher urea, creatinine concentration and significantly lower eGFR when compared to those carrying AA genotype.
Recommendations

1. Further studies on wider scale are needed for better assessment of role of SIRT1 rs10823108 gene polymorphism with DKD in Egyptian patients.

2. Future studying of SIRT1 (rs10823108) gene polymorphism could be done together with studying other SNPS within the gene, which might have synergistic effects on increasing the risk of developing DKD, as well as the progression of the disease in type 2 diabetic patients.

3. More studies about gene–gene and gene–gene–environment interactions are recommended which could be more powerful than SNP-by- SNP approaches.

4. Using DNA Sequencing technique for detection of different SIRT1 gene polymorphisms will increase accuracy of the results.
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الملخص العربي

المقدمة:

ان تشخيص مرض الكلي المزمن يكون من خلال زيادة نسبة افراز الألبومين بالبول وانخفاض معدل الترشيح الكبيبي. ويحدث ذلك بسبب مرض السكري، حيث يتطور حوالي 40% من مرضى السكري إلى المراحل الأخيرة للمرض الكلي.

كان قديماً لزيادة نسبة الألبومين في البول دور مركزي في تشخيص وعلاج مرض الكلي السكري. لكن على مدى العقد الماضي، لوحظ أن مرض الكلي المزمن بدون زيادة نسبة الألبومين بالبول شائع، ومازال حتى الآن لم يتم توضيح أسباب ذلك، وقد تم تفسير انخفاض معدل الترشيح الكبيبي لدى مرضى السكري من النوع الأول بدون زيادة نسبة الألبومين بالبول إلى أصابات الكلي بسبب السكري، ولكن في السكري من النوع الثاني، يعتبرارتفاع ضغط الدم الكلوي وأمراض الأوعية الدموية وشيخوخة الكلي المرتبطة بقدم العمروافاء الألبومين في البول بمثبطات رأس من الأسباب المفترضة لحدث مرض الكلي المزمن بعده الألبومين طبيعي.

كما أظهرت دراساتعينات من نسيج الكلى تغييرات مرضية كبيرة تتفق مع تأثير السكري على الكبيبات في مرض السكري من النوع الأول. وتتناقض البيانات فيما يتعلق بفروق ضغط الدم بين مرضى الكلي السكري بعده الألبومين طبيعي والمرضى مع زيادة الألبومين في البول في مرض السكري.

وقد أظهرت العديد من الدراسات أن العوامل الوراثية والبيئية تلعب دوراً كبيراً في عملية حدوث وتطور مرض الكلي المزمن. حيث أن مرض الكلي السكري لديه صفة التجميع العائلي، والعامل الوراثي يلعب دوراً هاماً في التسبب في مرض الكلي السكري. إن تنظيم تعديلات الهستون له تأثير معين على آلية التخليقية في مرض الكلي السكري. ويعتبر جين SIRT1 نوع من الإنزيمات الفاعلة لـ NAD حيث أن الهستون منظماً للعديد من الوظائف الحيوية في الجسم.

وحدثاً أن تأثير جين SIRT1 يزيد أهميته في حدوث وتطور أمراض الكلي. ويساهم جين SIRT1 في التقليل من موت الخلايا المبرمج في الكلي وتأخير حدوث الفشل الكلوي الناتج عن SIRT1.
الملخص العربي

عن التقدم في السن وتقليل الالتهابات والتليف الخلوي وتحل الالتهاب الذاتي في مرض الكلى السكري بالإضافة إلى ذلك فإنه يقوم بتنظيم الأيض في أوقات الصوم وتفادي السعرات الحرارية.

الهدف من الدراسة

تهدف هذه الدراسة إلى التعرف على علاقة تعدد الأشكال لجين SIRT1 وقابلية الإصابة

بمرض الكلى السكري عند مرضى النوع الثاني للسكري استنادًا إلى وجود أو عدم وجود زيادة

نسبة الألبومين بالبول.

المريضي وطرق الدراسة

تم تصميم هذه الدراسة كدراسة مقطعية بعد موافقة لجنة أخلاقيات البحث العلمي بكلية طب

بها. وتم تسجيل مجموعه مكونه من 50 مريضا مصاباً بأمراض السكري من النوع الثاني من

المرضى المتعددين على قسم الباطنة العامة بمستشفى بنها الجامعي في هذه الدراسة.

تم تقسيم الحالات بناءً على نسبة النزول في البول

المجموعة (1): وتشمل 20 مريضاً مما يعانون بدء الكلى السكري ومعهم زيادة نسبة

الألبومين في البول.

المجموعة (2): وتشمل 20 مريضاً مما يعانون بدء الكلى السكري بدون زيادة نسبة

الألبومين في البول.

المجموعة (3): وتشمل 10 مرضى مصابين بالسكري بدون الكلى السكري (كمجموعة

ضابطة)، مماثلة لنفس العمر والجنس.

معايير الدمج: المرضى بالسكري من النوع الثاني على أن تكون فترة الإصابة بالسكري

تعادل أو تزيد عن 5 سنوات، مرضى من الجنسين فوق عمر ال18 عام.

معايير الاستبعاد: المرضى بالسكري من النوع الأول الأقل من 18 عام، المرضى الذين

يعانون من أمراض كلى أخرى، ومرضى الحوامل.

2
الملخص العربي

جميع المرضى خضعوا لما يلي:

1- اخذ التاريخ المرضي متضمنا الاسم والسن والجنس ومدة الإصابة بداء السكري.

2- الفحص الاعتيادي ... ويتضمن الوزن والطول، وميؤشر كتلة الجسم، متوسط ضغط الدم الانقباضي والانقباضي.

3- الفحوصات العملية وتشمل:

- نسبة الجلوكوز في مصل الدم أثناء الصوم، نسبة الهيموجلوبين السكري بالدم.

- اختبارات وظائف الكلى.

- نسبة الألبومين للكرياتينين في البول.

- الكولستيرول والدهون الثلاثية والبويولا والكرياتينين في مصل الدم والترشيح الكببي.

- نسبتة الألبومين للكرياتينين في البول اعلي احصائياً في مرضى الكلي السكري (سواه زياده أو بدون زيده نسبه الألبومين بالبول) عن مرضى السكري بدون الإصابه بهاء الكلي السكري.

- تفاعيل البلمرة المتسلسل لمعرفة التعدد الشكلي لجين SIRT1.

وقد أسفرت هذه الدراسة عن الاستنتاجات التالية:

أن طول مدة الإصابة بالسكري ونسبه السكر فاطر بالدم والهيوموجلوبين السكري والكولستيرول الالي، والدهون الثلاثية والبويولا والكرياتينين في مصل الدم والترشيح الكببي ونسبه الاليومين للكرياتينين في البول اعلي احصائياً في مرضى الكلي السكري (سواه زياده أو بدون زيده نسبه الألبومين بالبول) عن مرضى السكري بدون الإصابه بهاء الكلي السكري.

ان نسبة السكر صائم بالدم ونسبه الاليومين للكرياتينين بالبول اعلي احصائياً في مرضى الكلي السكري مع زيادة نسبة الاليومين بالبول.

ان النمط الجيني GG للتمدد الشكلي لجين SIRT1 يوجد بنفسه اعلي احصائياً في مرضى الكلي السكري مع زياده نسبه الاليومين بالبول ويتوفيق مع حدوث مرض الكلي السكري وزيادة نسبه الاليومين بالبول وبالتالي فان الالي GG يزيد من خطر الإصابة بهاء الكلي السكري.
الملخص العربي

بينما النمط الجيني AA يوجد بنسبة أقل في مرضى الكلى السكري (سواء بزيادة أو بدون زيادة نسبة الالبومين بالبول) وGA يوجد بنسبة أقل في مرضى الكلى السكري مع زيادة نسبة الالبومين بالبول وبالتالي فإن الالبيل A له تأثير وقائي لمنع زيادة نسبة الالبومين في البول في مرضى الكلي السكري.

كما أظهرت الدراسة أن الأليل G مرتبط بزيادة نسبة البوريا والكرياتينين في مصل الدم وانخفاض معدل التشريح الكبيبي.

إن تحليل الانحدار اللوجستي أوضح أن طول هذه الاستجابه بالسكري وزيادة نسبة الهيموغلوبين السكري بالدم وانخفاض معدل التشريح الكبيبي مرتبط بحدوث مرض الكلى السكري في المرضى المصابين بالسكري.

وإن انخفاض معدل التشريح الكبيبي النمط الجيني GG+GA هما من العلامات الهامة للتنبؤ بحدوث مرض الكلى السكري في المرضى المصابين بالسكري.

تحليل الانحدار اللوجستي للتنبؤ بحدوث زيادة نسبة الالبومين بالبول في مرضى الكلى السكري أوضح أن النمط الجيني علامه هامة لحدوث زيادة نسبة الالبومين بالبول. 

G
العلاقة بين تعدد الأشكال في جين SIRT1 وقابلية الأصابة بمرض الكلى السكري

الرسالة

للحصول على درجة الماجستير في الباثولوجيا الاكلينيكية والكيميائية

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الطبية / بسمه جمال إسماعيل نور الدين
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