Abstract
Diabetic nephropathy is a leading cause of end-stage renal failure worldwide. Its morphologic characteristics include glomerular hypertrophy, basement membrane thickening, mesangial expansion, tubular atrophy, interstitial fibrosis and arteriolar thickening. All of these are part and parcel of micro vascular complications of diabetes. Previous study evidences indicates that oxidative stress is the common denominator link for the major pathways involved in the development and progression of diabetic micro- as well as macro vascular complications of diabetes. SIRT1 deacetylates target proteins using the coenzyme NAD⁺ and is therefore linked to cellular energy metabolism and the redox state through multiple signalling and survival pathways. SIRT1 deficiency under various stress conditions, such as metabolic or oxidative stress or hypoxia, is implicated in the pathophysiology of age-related diseases including diabetes, cardiovascular diseases, neurodegenerative disorders and renal diseases.

Methodology
In the present study, 30 cases presenting with diabetic Nephropathy and 30 ageand sex matched controls with Type 2 diabetes were included in the study.

Results
We found there was significant increase in the levels of all parameters such as MDA, SOD, GPx, GR, and SIRT1 in Diabetic Nephropathy patients when compared with Type 2 diabetes Mellitus subjects.

Conclusion
This study revealed that Sirt 1 plays a role in susceptibility to diabetic nephropathy patients with type 2 DM. Therefore the activation of SIRT1 in the kidney may be a new therapeutic target to increase resistance to many causal factors in the development of renal diseases, including diabetic nephropathy.

Keywords: Diabetes, Nephropathy, SIRT 1, Superoxide dismutase, Glutathione peroxidase,
vascular diseases. Currently, more than 347 million people worldwide are suffering from DM (1). The increased prevalence of DM has led to a significant increase in the prevalence of diabetic kidney disease (DKD) with estimates that 44% of all new end stage renal disease (ESRD) cases in US are due to DKD (2, 3). Several factors including hyperglycemia, insulin resistance, renal lipid accumulation, inflammation, and activation of the renin–angiotensin system (RAS) are involved in the pathogenesis of DKD (4) and they activate multiple signaling pathways resulting in kidney cell injury and the development and progression of the disease (5, 6). Since the discovery of the silent information regulator 2 (Sir2) family and its beneficial effects on aging (7, 8), scientists have shown that the homologs of Sir2 in higher eukaryotic organisms, known as Sirtuins (SIRTs), are a conserved family of a nicotinamide adenine dinucleotide (NADC)-dependent deacetylases/mono-ADP ribosyltransferases that are associated with numerous cellular signaling pathways that include senescence (9–12), apoptosis (13), DNA damage repair (14), and autophagy (12, 15). By far, SIRT1 is the most studied member of this family and its protective roles against kidney injury are well established, making it a promising candidate for targeted therapies to halt disease progression.

Diabetic nephropathy is a serious microvascular complication of diabetes, and is a leading cause of end-stage renal disease in Western countries (16). The escalating prevalence and limitation of currently available therapeutic options highlight the need for a more accurate understanding of the pathogenesis of diabetic nephropathy. According to world health organization it is the seventh leading cause of death by 2030 (17). The prevalence of diabetic nephropathy was higher in Asians, Africans and Americans. In India, the prevalence of diabetic nephropathy is 2.2% (5). As per International Diabetes Federation (IDF), total number of people with diabetes are about 69.2 million and it may raise to 123.5 million by 2040 (18).

Role of SIRT 1 in diabetic nephropathy
Sirtuin is a nicotinamide adenine dinucleotide–dependent deacetylase. One of its isoforms, Sirt1, is a key molecule in glucose, lipid, and energy metabolism. The renal protective effects of Sirt1 are found in various models of renal disorders with metabolic impairment, such as diabetic nephropathy. Protective effects include the maintenance of glomerular barrier function, anti–fibrosis effects, anti–oxidative stress effects, and regulation of mitochondria function and energymetabolism. Various target molecules subject to direct deacetylation or epigenetic gene regulation have been identified as effectors of the renal protective function of sirtuin. Recently, it was demonstrated that Sirt1 expression decreases in proximal tubules before albuminuria in a mouse model of diabetic nephropathy, and that albuminuria is suppressed in proximal tubule–specific mice over expressing Sirt1. These findings suggest that decreased Sirt1 expression in proximal tubular cells causes abnormal nicotine metabolism and reduces the supply of nicotinamide mononucleotide from renal tubules to glomeruli. This further decreases expression of Sirt1 in glomerular podocytes and increases expression of a tight junction protein, claudin-1, which results in albuminuria. Activators of the sirtuin family of proteins, including resveratrol, may be important in the development of new therapeutic strategies for treating metabolic kidney diseases, including diabetic nephropathy.

Oxidative Stress plays a major role in pathogenesis of diabetic nephropathy. It is caused by an imbalance between a relative overload of oxidants and a depletion of antioxidants (12). Sirtuin 1 expression is decreased in conditions like chronic metabolic stress, oxidative stress or hypoxia that drives the pathophysiologies of age related diseases which includes CVS, diabetes and renal diseases. As the disease progresses, antioxidant potential decreases, and the plasma lipid peroxidation products increase depending upon the level of glycemic control. Increased oxidative stress has been associated with aging, and SIRT1 has been shown to combat oxidative...
stress by modulating transcriptional activities of several key proteins involved in oxidative stress response and mitochondrial biogenesis.

The aim of the present study is to find the relation between SIRT 1 level and antioxidant status in diabetic nephropathy in South Indian population.

Materials and Methods

Study design and Ethical clearance

The present study was conducted in the Department of Biochemistry in collaboration with Department of Nephrology in Saveetha medical College, Thandalam, Chennai. The study was conducted on patients with Type 2 Diabetes mellitus and Diabetic Nephropathy admitted in the nephrology unit in Saveetha Hospital and Medical College. This study was approved by Institutional Human ethics Committee.

Type of study
It is a Case –Control Study.

Sample size
Study population consisted of 30 patients with Diabetic nephropathy (Age range 40-75 yrs) and control group consisted of 30 patients with Type 2 Diabetes Mellitus who are on medical treatment without any complications.

Inclusion criteria
- Cases : Known diagnosed patients of Diabetic Nephropathy attending the department of nephrology of saveetha medical college. (defined as patients having arterial hypertension less than 200/160,eGFR > 45 and <90 mL/min/1.73 m2 and/or urinary albumin:creatinine ratio >3 mg/mmol(15)
- Controls: Known diabetes mellitus patients who are on medical treatment without any complications as controls
- Age Group of 40-70yrs for both cases & controls

Exclusion criteria
Patients will be excluded if they have any of the following:
- a history of cardiovascular disease, defined as having a clinical record of ischemic heart disease (angina, myocardial infarction, coronary artery revascularization and or heart failure),
- peripheral vascular disease (intermittent claudication or peripheral artery revascularization) or
- cerebrovascular disease (transient ischemic episodes or stroke),
- a history of malignancy or any other life threatening illness, current pregnancy, systolic blood pressure >200 mmHg, diastolic blood pressure >160 mmHg, hemoglobin A1c > 10 %,
- Significant renal impairment (eGFR< 45 mL/min 1.73 m2) and nephrotic range urine protein excretion (total protein excretion rate >3 g/day or albumin:creatinine ratio >300 mg/ mmol).
- Patients with age <40 and >70 are excluded (15).

Sample collection and storage : 5ml of venous whole Blood and EDTA samples were collected from both Type 2 Diabetes Mellitus and Diabetic Nephropathy.

Biochemical analysis: Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Sirtuin 1 (Sirt1) levels were estimated by ELISA (Enzyme linked Immunosorbent Assay) using Robonik ELISA reader instrument.

Statistical analysis : Statistical analysis were done using student t – test and p-value significance. P-value <0.01 were considered as significant.

Results
In the present study a total number of 60 subjects comprising of 30 Type 2 Diabetes Mellitus patients ((Control) Group-I ) and 30 Diabetic Nephropathy cases (group-II) were included.

In the present study, we identified that association between SIRT1 and oxidative stress
is nominally associated with susceptibility to diabetic nephropathy.

In the present study, there was significant increase in the Microalbuminuria excretion ratio in the Diabetic nephropathy patients when compared with diabetic patients. (Table 1).

We found that, the levels of lipid peroxidation product Malondialdehyde were significantly high in diabetic nephropathy cases (8.06) when compared with diabetes mellitus (3.71) patients.

In the present study, the levels of antioxidant enzymes statistically significantly decreased in diabetic nephropathy patients (SOD-58 ± 11; GPx-45 ± 18.2; GR-10 ± 2.54) when compared with normal diabetic cases (SOD-60±13.5; GPx-49±13.4; GR-12± 2.60).

In the present study, we observed SIRTUIN 1 levels were also significantly decreased in diabetic neprhopathic patients When compared with diabetic patients.(D-3.0±0.7; DN-2.0±0.66).

**Discussion**

Diabetic nephropathy is characterized by albuminuria (>300mg/day) and a reduced GFR (19).

The present findings revealed that there was significant increase in albumin levels in DN cases when compared with type 2 diabetic patients (Table 1).

It should be considered that the albuminuria is sometimes present at the moment when DM is diagnosed, after the kidney has been exposed to chronic hyperglycemia since the prediabetic phase. The mechanisms implicated in the pathogenesis of DN are multiple and complex. The first hemodynamic changes of glomerular hypoperfusion and hyper filtration favour the leakage of albumin from the glomerular capillaries.

Oxidative stress results from the link with the majority of molecular events that underline the pathological process in DN. It is related to alterations in the redox state caused by the persistent hyperglycemic state and the increase in AGEs. These events affect the renin-angiotensin system and the signalling of the transforming growth factor-beta (TGF-β), producing chronic inflammation and glomerular and tubular hypertrophy. The renal fibrosis is due primarily to the

<table>
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<th>Clinical Parameter</th>
<th>Diabetic Nephropathy patients (Mean±SD)</th>
<th>Type 2 diabetic patients (Mean±SD)</th>
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<td>Microalbuminuria</td>
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<th>S.NO</th>
<th>Antioxidant Enzymes</th>
<th>GROUP-I n = 30 Mean ± SD n</th>
<th>GROUP-II n = 30 Mean ± SD n</th>
<th>p-Value</th>
</tr>
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<td>1</td>
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<td>8.06 ± 6.19</td>
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<tr>
<td>2</td>
<td>SOD</td>
<td>60±13.5</td>
<td>58 ± 11</td>
<td>0.5318</td>
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<td>GR</td>
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<td>10 ± 2.54</td>
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</tr>
<tr>
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<td>SIRT1</td>
<td>3.0±0.7</td>
<td>2.0±0.66</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
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accumulation of the mesangial cells, favouring the depositing of extracellular matrix (ECM), the thickening of the tubular and glomerular membranes, the dysfunction of podocytes, and the appearance of apoptosis.

Oxidative stress in DN has the ability to act as a trigger, modulator, and link within the complex web of pathological events that occur in DN. There are various molecular events that underlie and connect the metabolism, inflammation, and the oxidation in DN. It is demonstrated that the main cause of morbidity and mortality in patients with CKD is due to CVD and that the oxidative stress together with the subclinical inflammatory state is ultimately responsible for the generation of atherosclerotic plaque (20).

In the present study revealed that there was significant decreased levels were observed in diabetic nephropathy cases.

The various body organs, particularly the kidney, suffer from different degrees of age-related damage. The kidney is vulnerable to specific age-related injuries. Therefore, the incidence of chronic kidney diseases develops along with age. Aging often leads to increased oxidative stress, free radical generation, and decreased antioxidant and free radical-scavenging activities.

These findings suggest that oxidative stress is a significant cause of chronic kidney diseases. In the present study there were significant increased levels of MDA in DN cases compared with Type 2 diabetic cases (Table 2).

MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress (21). Significant changes in lipid metabolism and structure have been reported in diabetes, particularly in patients with vascular complications (22). Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defence mechanisms of enzymatic and non-enzymatic antioxidants (23).

MDA increase confirms that it is associated with increased production of reactive oxygen species and free radicals. Our study correlated with previous findings. (1, 13, 14).

Superoxide Dismutase, a superoxide scavenging enzyme which is considered the first line of defence against deleterious effect of oxygen radical in the cells. Which is decreased in diabetic nephropathy when compared to type 2 diabetes and it is not statistically significant (Table 2).

A selenium containing enzyme, Glutathione Peroxidase is also decreased in diabetic nephropathy when compared to type 2 diabetes and it is not statistically significant. GR levels in diabetic nephropathy are decreased when compared with T2D and it is statistically significant (Table 2).

SIRT1 expression changes under different physiological and morbid conditions. It is decreased in conditions of chronic metabolic stress, oxidative stress, or hypoxia that drives the pathophysiology of age related diseases including diabetes, cardiovascular, and renal diseases. In aging kidneys both the expression and activity of SIRT1 is decreased due to age associated reduction in systemic NAD biosynthesis (12). Similarly, reduction in SIRT1 expression was observed in kidney glomeruli and tubule interstitial compartments of patients with mild to severe DKD, which was inversely correlated with the histopathological severity of the renal disease and with the amount of proteinuria (24, 25).

SIRT1 is a member of NAD+ -dependent histone deacetylase, which involves in various nuclear events such as transcription, DNA replication, and DNA repair. SIRT1 plays an important role not only in the regulation of aging and longevity, but also in the development and/or progression of age-associated metabolic diseases, such as type 2 diabetes. 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, blood pressure.

A comparative of sirt 1 and antioxidant status in type 2 diabetic and diabetic nephropathic patients
As sirtuin 1 is involved in several energy homeostasis pathways it is considered as master regulator. Prior studies showed the associations between SIRT1 and oxidative stress.

SIRT1 can protect cells from apoptosis induced by oxidative stress. Hao and Haase (26) observed that SIRT1 is over expressed when renal medullary interstitial cells are exposed to high-permeability and low oxygen environments. Down regulated SIRT1 expression significantly reduces oxidative stress resistance and triggers massive apoptosis. Conversely, activated SIRT1 promotes cell survival. This finding was verified in an in vitro unilateral urethral obstruction model. SIRT1 directly or indirectly controls the activation of FOXO1, FOXO3, and FOXO4 through deacetylation and regulates cell response to oxidative stress (27).

Oxidative stress is hypothesized to play a role in the development of diabetes with and without nephropathy. Oxidative stress has been considered to be a pathogenic factor of diabetic complications including nephropathy.

High intracellular glucose concentration has been suggested to be a prerequisite for the development of functional and structural changes in the kidney typical of diabetic nephropathy. Under the conditions of intracellular hyperglycemia, the cellular NADH/NAD⁺ ratio is decreased.

Antioxidant therapy is one of the most important treatment strategies for diabetic patients without nephropathy for the prevention and slowing of diabetic nephropathy before reaching to End Stage Renal Disease.

Vascular endothelial growth factor (VEGF) is a protein secreted by the podocytes and the mesangial renal cells under oxidative stress situation which plays a role in the extension of diabetic kidney disease.

Reactive oxygen species are reduced by Sirtuin, by modulating the acetylation of respiratory chain and by stimulating mitochondria superoxide dismutase and isocitrate dehydrogenase which generates NADPH for glutathione pathway.

The decreased levels of antioxidants which in turn decreases the level of SIRT 1 in diabetic nephropathy when compared to Type 2 Diabetes. It is statistically significant (Table 2).

Conclusion
In recent decades, numerous investigators have made efforts to identify the molecular mechanisms involved in the initiation and progression of diabetic nephropathy to develop new therapeutic strategies. However, end-stage renal failure due to diabetic nephropathy continues to increase worldwide. There is an urgent need to identify new therapeutic targets to prevent diabetic nephropathy. The present findings revealed that there was significant decrease in antioxidant enzymes and SIRT 1 in Diabetic nephropathy patients. These studies revealed that further investigation into the targets and functions of other sirtuins will help develop new strategies for protection against renal diseases.

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Figure 1: Biochemical Variations in cases (Diabetic Nephropathy) and Controls (Type 2 diabetic patients).
Conflict of Interest

The authors declare that there is no conflict of interest in this study.

References


