CHAPTER 1

INTRODUCTION

Economic progression and altered lifestyle along with genomic susceptibility provides a comprehensive threat for higher prevalence of diabetes in Indian population where 72,946,400 million individuals are already affected by the disease. (Cho, N., *et al*. 2018). Four fifty one million individuals acquire diabetes and it was projected that the incidence may increaseupto 693 million by 2045 globally. (Cho, N., *et al*. 2018). Impaired glucose tolerance (IGT) or pre-diabetic is a major challenge where in 2017; 374 million people were living with the same. About 145.7 and 279.2 million population with diabetes is living in rural and urban area respectively and about 4 million deaths worldwide were attributable to diabetes in 2017(Cho, N., *et al*. 2018). USD 727 billion in 2017 was estimated for the global healthcare expenditure on people with diabetes (Cho, N., *et al*. 2018, Baena-Diez, J. M., *et al*. 2016).Worldwide, direct and indirect losses in gross domestic product (GDP) due to diabetes during 2011 to 2030, will incur a total cost of US$ 1.7 trillion, US$ 900 billion for high-income countries and US$ 800 billion for low- and middle-income countries respectively

(Cho, N., *et al*. 2018). Prevalence of diabetes and prediabetes in 15 states of India as estimated by ICMR–INDIAB study was 7·3 per cent (95 per cent; CI: 7·0–7·5) and 10·3 per cent (95 per cent; CI: 10·0–10·6) respectively (Anjana, R. M., *et al*. 2017). Assam has 5.5 per cent and 11.9 per cent diabetes and prediabetes according to the ICMR-INDIAB study (Anjana, R. M., *et al*. 2017). Altered lifestyle along with genomic susceptibility provides a comprehensive threat for Diabetes associated morbidity and mortality among Indian population. According to International Diabetes Federation (IDF) the prevalence of diabetes in adults is diverse in South East Asia (SEA) and the range is 4.0 per cent in Nepal to 8.8 per cent in India. (Hills, A. P., *et al.*2018). Diabetes contributed to 3·1per cent of all deaths in India from 1990 to 2016. It is predicted that approximately 98 million people in India will be suffering from type 2 diabetes by 2030 (Tandon, N., *et al*. 2018).

Hesy-Ra, an Egyptian physician described for the first time in 1552 B.C. the symptom of frequent urination of a mysterious disease (Krisha 2009). The term diabetes was used for first time to denote an excessive passage of urine and also ascribed as the aetiology to the kidney by an Egyptian physician named Apollonius Memphites around 230 BC (Papaspyros 1964). The word “Diabetes” is a Greek word meaning to siphon or pass through and the “mellitus” is a Latin word which means honey or sweet (Diabetes: Past treatments, new discoveries, Krisha 2009). In 1675, the word "mellitus" was added to the name "diabetes" (Krisha 2009). The Indian synonym of diabetes was “madhumeha” (‘honey urine’) because urine of patients is attracted by ants. Sushruta and the surgeon Charaka (400–500 A.D.) identified the two types of diabetes, i.e; Type I and Type II diabetes (Frank 1957, Tipton 2008 and Lakhtakia 2013). Various breakthrough have been achieved across the centuries by scientific community for the understanding the disease and starting from F.G. Banting and J.J.R. Macleod for the discovery of Insulin in 1923 (Polonsky 2012). Seven Nobel Prizes were awarded to scientists for commendable works in this field (Polonsky 2012).

Type I, Type II, Gestational Diabetes and Maturity Onset Diabetes of the Young (MODY) are the major types of diabetes. Weight loss, polyuria (increase urination), polydipsia (increase thirst) and polyphageia (increase hunger) are the major sign and symptoms of untreated diabetes (Cooke, D. W. and Plotnick, L. 2008). Incase of type 1 diabetes symptoms may develop rapidly (weeks or months) and may be subtle or absent in

type 2 DM (Cooke, D. W. and Plotnick, L. 2008). Blurred vision, headache, fatigue, slow healing of wounds and itchy skin are commonly associated symptoms related to diabetes (Symptoms: American Diabetic Association).

Pathologically, diabetes mellitus (DM) is a complex heterogeneous metabolic disorder characterized byhyperglycemia resulting from defects of insulin secretion, insulin resistance, or both (American Diabetes Association. 2013). Type I diabetes is an autoimmune disorder where insufficient insulin production occurred due to destruction of insulin-producing beta cells in the islets of the pancreas gland by host's immune system (International Diabetes Federation. IDF Diabetes Atlas, 9th edition, 2019). Type 1 diabetes is commonly associated with genetic risk factors. Type 2 diabetes is more common than type 1 DM and account for more than 90per cent of all cases of diabetes. Type 2 diabetes develops when there is relative deficiency of insulin due to insufficient insulin production or insulin resistance. Previously it has been referred as non insulin dependent diabetes mellitus also. Defective insulin secretion is the prime factor for the pathophysiology of type 2 diabetes. Insulin resistance leads to condition termed as hyperinsulimaemia, further increased insulin destroy the beta cells function and leads to hyperglycemia (Baynest 2015, Hackett, E. and Jacques, N. 2009). Untreated diabetes irrespective of types is the major risk factor for developing the chronic complications, although rates of progression may differ.

A special form of diabetes is the gestational diabetes mellitus (GDM) which is associated with high blood glucose levels during pregnancy. It develops in one in 25 pregnancies worldwide and is associated with complications to both mother and baby. Usually GDM disappears after pregnancy but women with GDM and their children are prone to development of type 2diabetes in later life (Melchior, H. *et al*. 2017)**.** Maturity Onset Diabetes of the Young (MODY) is a rare form of diabetes associated with genomic variation of certain genes that actively involve insulin catabolic and metabolic pathways (American Diabetes Association. 2017). Several reports postulate that autosomal dominant genomic variation may be critical determinant for the same that was established at adolescence or early adulthood (Rubio-Cabezas, O. 2014). MODY accounts for up to 2 per cent of all cases of diabetes in the United States among younger people aged upto 25 (Pihoker 2013).

Criteria to diagnose of Diabetes is mainly based on plasma glucose either fasting plasma glucose test (FPG) or 2 hours oral glucose tolerance test (OGTT). American Diabetes Association (ADA) emphasizes about HbA1c (glycated heamoglobin) test for diagnosis of diabetes (ADA. 2018). HbA1c measurement provides the more reliable results about the glucose control over a period of 3 months. It is recommended to be under 7per cent of HbA1c results for good glycemic control (Kilpatrick 2004). Diabetes are diagnosed based on random or plasma glucose level after oral consumption of 75gm of glucose is more than 200 mg/dL and fasting plasma glucose is more than 126 mg/dl with symptoms of hyperglycemia or hyperglycemic crisis (American Diabetes Association. 2017).

Metformin is the preferred pharmacological agent for type 2 diabetes (George, M. M., and Copeland, K. C. 2013, Nasri, H. and Rafieian-Kopaei, M. 2014). Upon resistance to metformin monotherapy a combination of metformin with sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, sodium–glucose cotransporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) agonists, or insulin therapy are used for management of diabetes (George, M. M. and Copeland, K. C. 2013, Nasri, H. and Rafieian-Kopaei, M. 2014, Inzucchi, S. E. *et al* 2015).

Persistent hyperglycaemia frequently leads to several microvascular and macrovascular complications in different organs. Microvascular complications (due to damage to small blood vessels) include diabetic retinopathy (DR) diabetic nephropathy (DN) and diabetic neuropathy (DN) (Forbes, J. M., and Cooper, M. E. 2013, Papatheodorou, K. *et al*. 2018). The major macrovascular complications (due to damage to the arteries) include cardiovascular disease including hypertension and stroke (Nathan 1993).

Studies estimated that about 47 per cent of retinopathy, 17 per cent for nephropathy, and 14 per cent for cardiovascular diseases are associated with type 1diabetes mellitus (T1DM) (Nathan, D. M. *et al.* 2009) Data for type 2 diabetes mellitus (T2DM) is relatively enigmatic across the population but it has been documented that about 20per cent of T2DM cases develop several micro and macro vascular complications after 10 years of establishing the T2DM (Fowler 2008). Asians have higher prevalence of nephropathy but a lower

incidence of cardiovascular disease than Caucasians among the T2DM subjects (Chan 2004, Moore, D. J. *et al*. 2009).

Poor glycemic control seems the crucial accelerator for various complications including end-stage renal disease (ESRD) which increases the risk upto 10 folds (Bash, L. D., *et al*. 2008). Parving, H. H. *et al.* in 2006 revealed the prevalence of normo, micro and macroalbuminuria was 51, 39, and 10per cent respectively among T2DM individuals (Parving, H. H. *et al.* 2006). Study reported that, after being diagnose with T2DM 2.0, 2.8 and 2.3percent persons per year progress to microalbuminuria, microalbuminuria to macroalbuminuria and from macroalbuminuria to overt nephropathy respectively (Adler, A.I. *et al.*2003). DN is highest (30.3per cent) among chronic renal failure patients followed by chronic interstitial nephritis (23per cent) and chronic Glomerulonephritis (17.7per cent) (Dabla 2010). It was estimated that the prevalence of overt nephropathy and microalbuminuria was 2.2 and 26.9per cent, respectively among urban Asian (Unnikrishnan, R. *et al.* 2007). The prevalence of chronic kidney disease (CKD) was estimated by the International Society of Nephrology's Kidney Disease Data Center was 17per cent and the range from 1per cent to 13per cent in different region of India (Varughese, S. and Abraham, G. 2018). Epidemiological studies postulates that, rate of development of DN is comparatively less among Caucasians than the population like African Americans, American Indians and Hispanics or Latinos (Dabla 2010). DN rarely develops more frequently among T2DM patients than T1DM before the age of 10 years (Gheith, O. *et al*. 2015). Population based study revealed that Diabetic kidney disease is the single most common cause of End Stage Renal Disease (ESRD) in many parts of the world including Europe, Japan, USA, India along with other south east Asian countries (Persson, F., and Rossing, P. 2018). DN is characterized by progressive kidney damage with presence of increasing albuminuria, impairment in renal function leading to decline in glomerular filtration rate (GFR) (Dabla 2010, Persson, F., and Rossing, P. 2018). Micro and macroalbuminuria is the main clinical phenotypeof DN which is based on the amount of urinary albumin excreation (UAE) on timed, 24-hrs, and spot urine samples (Persson, F., & Rossing, P. 2018, Care 2004).

Third National Health and Nutrition Examination Survey (NHANES III) observed that low GFR (<60 ml min^{-1} 1.73 m^{-2}) was present in 30 per cent of patients in the absence of

micro- or macroalbuminuria and retinopathy (Garg, A. X. *et al.* 2002, Kramer, H. J., *et al.* 2003). In clinical practice, GFR can be estimated based on serum creatinine, age and weight by Cockroft and Goult formula [CCr={((l40–age) x weight)/(72xSCr)}x 0.85 (if female)] (Cockcroft, D.W. and M.H. Gault. 1976). Annual monitoring of urinary albumin-to-creatinine ratio, estimated GFR (eGFR), and blood pressure is also recommended to assess the progression of DN (Dabla 2010, Garg, A. X. *et al.* 2002). Several new biomarkers or profiles of biomarkers have been investigated to improve prognostic and diagnostic precision, but none have yet been implemented in routine clinical care.

Anatomical changes in glomeruli due to diabetes can be seen using various techniques like staining with hematoxylin and eosin, periodic acid–Schiff (PAS), Masson trichrome, and periodic acid methenamine silver stains for light microscopy (Movat, H. Z., and Mcgregor 1959, Jain 2012). Immunofluorescence requires the use of antibodies against IgA, IgG, IgM, C3, C1q, and kappa and lambda light chains to rule out other renal diseases (Tervaert, T. W. C *et al*. 2010). Glomerulosclerosis due to diabetes is characterized by increased glomerular basement membrane width, diffuse mesangial sclerosis, hyalinosis, microaneurysm, and hyalinearteriosclerosis (Mauer S.M. *et al.*1981). Tubular and interstitial changes are also present (Brito, P. L., *et al.,* 1998, Katz, A., *et al.*, 2002). Kimmelstiel-Wilson nodules or nodular mesangial expansion characterized by expansion of extreme mesangial areas are observed in 40–50per cent of patients developing proteinuria (Kimmelstiel, P., and Wilson, C. 1936). It is well documented that type 2 diabetes has more structural heterogeneity than patients with type 1 diabetes with Micro and macroalbuminuria (Osterby, R. *et al*. 1993, Fioretto, P. *et al.*1996).

Risk factor for the progression of DN can be distinguished as susceptibility factors, initiation factors and progression factors (Gross, J. L. *et al*. 2005, Staples, A., and Wong, C. 2010). Susceptibility factors includes, age, sex, race or ethnicity and family history i.e; genetic susceptibility. Hyperglycemia is the principal factor responsible for occurrence of DN through structural alterations and renal damage directly or through hemodynamic modifications (Kanwar, Y. S. *et al*. 2011). Previous studies documented that, increasing systolic blood pressure by 10mmHg was associated with a 15per cent increases of both micro and macroalbuminuria and impaired kidney function (Retnakaran, R. *et al.* 2006). Among the

genetic factors, Uromodulin, APOL1 and renin–angiotensin system genes are associated with DN progression (Kazancioglu 2013).

Various pathways involving metabolic and hemodynamic alterationare associated with histological changes in kidney in DN. M.Brownlee in 2001 first described hyperglycemia as metabolic pathway for DN (Brownlee 2001, Toth-Manikowski, S., and Atta, M. G. 2015). Persistent hyperglycemia induced several independent biochemical pathways such as, glucose-induced activation of protein kinase C (PKC) isoforms; increased formation of glucose-derived advanced glycation end products; and increased glucose flux through the aldose reductase pathway, polyol pathway, hexosamine pathway, oxidative stress and accumulation of proinflamatory molecules (Shiju, T. M., and Pragasam, V. 2012, Sharma, V., and Sharma, P. L. 2013). Reactive oxygen species (ROS) is a potent activator for aldose reductase, induce diacylglycerol, activate PKC, induce advanced glycation end product formation, and activate the pleiotropic transcription factor nuclear factor-kappa B (NF-κ;B) (Giacco, F., and Brownlee, M. 2010, Sharma, V., and Sharma, P. L. 2013). A number of studies have demonstrated that hyperglycemia induced the development of DN by inducing endothelial dysfunction, excessive extracellular matrix (ECM) production, podocyte abnormality and tubulointerstitial fibrosis (Lee, H. B. *et al*. 2003, Kawanami, D., Matoba, K., and Utsunomiya, K. 2016). Key factors that are involved in diabetic kidney damage are oxidative stress, overproduction of advanced glycation end products (AGE), apoptosis and inflammation due to the local release of proinflammatory cytokines (Sharma, V., and Sharma, P. L. 2013 , Behl, T. *et al*.2014,).

Over last decades considerable progress has been made in the understanding of the pathogenesis of DN with the aid of genomics as well as proteomics and several factors have been implicated in its pathogenesis including growth factors and cytokines (Viswanathan V, *et al*. 2012, Duran-Salgado, M. B., and Rubio-Guerra, A. F. 2014). It has been postulated that persistent hypoxia and hyperglycemia may activate inflammation cascade which ultimately leads to cellular damage, endothelial dysfunction and matrix degradation during the adverse prognosis of DN (Giacco, F., and Brownlee, M. 2010, Leong, Wen Bun, *et al*. 2014, Tesch 2017). Associations between elevated levels of proinflammatory cytokines and homeostasis model assessment–insulin resistance (HOMA-IR) suggest that inflammation is an important

etiological factor for the development of both insulin resistance and its associated complication including DN (Wang, X. *et al*. 2013). The innate immune mediators are associated with T2DM and its complications (Xiao, J. *et al*. 2014, Donate-Correa, J. *et al.* 2015). The innate immune system is regulated by activation of Pattern Recognition Receptors (PRRs) by Pathogen Associated Molecular Patterns or Damage Associated Molecular Patterns (DAMPs) in response to stress, tissue injury or cell death (Schroder and Tschopp, 2010). DAMPs trigger innate immune molecules by activating Inflammasome (Anders and Muruve, 2011; Anders and Schaefer, 2014; Harijith, Ebenezer, and Natarajan, 2014; Rosin and Okusa, 2011). Activation of Inflammasome complex is a necessary and critical defense mechanism for the clearance of pathogens or damaged cells. Overt inflammasome activation is also a major driver of several autoimmune and metabolic disorders (Sharma, D. and Kanneganti, T. D. 2016). Inflammasome formation or activation requires a pattern recognition receptor (PRR) as the sensor (He, Y. *et al.* 2016). Recent understanding on intracellular multiprotein inflammatory machinery viz. inflammasome complex (NLRP3, Caspase 1 and PYCARD) and its crucial involvement has been documented on varied vascular diseases in mouse model as well as in human subjects (Donate Correa, J. *et al.* 2015, Anders and Schaefer, 2014, Qiu, Y. Y., and Tang, L. Q. 2016, Sakai, N., and Wada, T. 2015). Recent studies reveal that prolonged inflammation may be crucial for pathogenesis of DN but yet to be explored comprehensively atleast in human (Qiu, Z., *et al.* 2017). It has been documented that treatment naive DM subjects are associated with NLR-dependent inflammasome complex (IC) (Lee, H. M., *et al.* 2013). Down regulation of IC upon anti-diabetic administration have been documented, in-vitro (Qiu, Z., *et al.* 2017). The potent role NLRP3 inflammasome complex (NLRP3, Caspase-1 and PYCARD) for the pathogenesis of DN has been recently documented in animals but limited for human (Qiu, Z., *et al.* 2017). Activation of Inflammasome leads to pyroptosis, a distinct form of programmed cell death characterized by cellular lysis, release of intracellular components and an inflammatory response (Sakai, N., and Wada, T. 2015, Shahzad, K. *et al.* 2016, Qiu, Z., *et al.* 2017). Caspase-induced pyroptosis has been demonstrated in macrophages, dendritic cells, enterocytes and hematopoietic progenitors (Shahzad, K. *et al.* 2016). Pyroptosis is therefore a critical feature of inflammasome activation in a wide variety of cells leading to adverse prognosis of disease including DN (Sharma, D., and Kanneganti, T. D. 2016, 90. Zheng, Z., and Zheng, F. 2016). Significant relation has been established

between hyperglycemic and NLRP3-inflammasome in mediating caspase-1 activation in primary human adipocytes which leads to pyroptosis.

Elevated serum levels of innate immunity inflammatory cytokines such as IL-6 (Akbari, M., & Hassan-Zadeh, V. 2018), IL-18 (Fujita, T. *et al.* 2012) and TNF-α (Lampropoulou, I. T., *et al.* 2014) have been reported for T2DM and it's certain vascular complications. Therefore, it has been hypothesized that the T2DM is immune dependent. As the crucial relationship between innate immunity and T2DM has been already documented, the involvement of innate immune receptors and their signalling molecules need to be studied to understand the patho-mechanism for genesis of DN.

Toll-like receptors (TLRs), the family of pattern recognition receptors activate the innate immune response upon interaction with various pathogen-associated molecular patterns (PAMPs), including lipids, lipoproteins, proteins and free fatty acids (Suresh, R., and Mosser, D. M. 2013). After establishing its active role on PAMPs, recent human and animal model studies have postulated the potent role of TLRs on recognition of damage-associated molecular patterns (DAMPs) for the genesis of T2DM and its associated complication. Limited studies show that treatment naive T2DM patients has elevated level of TLR2 whereas in animal model TLR4 exhibits its crucial role in T2DM etiology (Dasu, M. R. *et al.* 2010, Pal, D. *et al.* 2012, Gupta, S. *et al.* 2017). Impaired renal function due to glomerular damage after prolonged hyperglycaemia is postulated to be due to cumulative effect of metabolic aberrations, inflammation and insulin resistance (Zeni, L., *et al.* 2017). Although the activation of pro-inflammatory cytokine have been documented both in animal and human, the mechanisms by which these metabolic abnormalities and inflammation cause DN through TLR mediated pathways still remains enigmatic.

It has been also evident that epigenomic alteration including microRNAs (miRNAs) are crucial regulator for gene expression and recognized as potential player for metabolic diseases including type2 diabetes mellitus (T2DM) (Carthew, R. W. 2006, Wu, H. *et al*. 2014, Simpson, K., *et al.* 2016). Earlier studies postulates clear links between altered miRNA expression and certain diabetes complications in mouse model but studies are completely or substantially lacking in human subjects.

MicroRNAs (miRNAs) are non coding smallRNA molecules approximately 20-22 nucleotides long that regulate other genes in the human genome (Boyd 2008). miRNA

mediated gene regulationis mainly based on the complementarities between the mRNA and bases 2–8 of the miRNA (seed sequence) and thermodynamic stability to ensure the binding with the miRNA 3′end (Wilczynska, A., and Bushell, M. 2015). It was assumed that miRNAs control approximately 30% of all human proteincoding genes (Carthew 2006). miRNA play a distinct role in the gene regulation in mammal and thus a potential novel class of therapeutic targets as well as biomarker (Wahid, F., *et al.* 2010)**.** miRNA mediated regulation of gene expression represents a novel epigenetic mechanism that may alter the homeostatic process and pathological condition within the cells (Riffo-Campos, A. L. *et al*. 2016). Recent studies suggested the role of miRNA in the pathogenesis or association of various noncommunicable diseases like arthritis, kidney disease, cardiovascular diseases, etc. (Denby, L., and Baker, A. H. 2016, Hackfort, B. T., and Mishra, P. K. 2016). Role of miRNA in cancer has been extensively studied over the recent years and postulates as either tumor suppressors or inducers. The role of miRNA in diabetic microvascular complication such as retinopathy, nephropathy, woundhealing and myocardial injury in insulin relevant tissue has been reviewed in earlier studies (Zhang, Y., *et a*l. 2017). miRNAs are the best-studied among small noncoding RNAs, post-transcriptionally regulate mRNA levels and translation, and there is an increasing awareness of their role in disease pathophysiology, their use as biomarkers, and their potential as therapeutics (Voskarides, K., and Felekkis, K. 2015). Genetic associations along with the systemic relation with miRNA levels and their transmitting role for m-RNA regulation may yield novel insights into molecular mechanisms of disease pathogenesis, particularly for the hyperglycaemia mediated multifactorial disease like DN and DR as the interpretation of miRNA association studies is almost substantially lacking. Use of miRNA offers new targets for early detection and therapeutic intervention of various diseases. Extensive researches are going on to overcome the obstacle in RNA-based therapeutics will soon enter into the clinic as next generation drugs. miRNA based therapeutics definitely has the potential to contribute significantly to the futureof medicine as targeted therapy. The roles of miRNAs are just beginning to be understood, but the study of miRNA function has been limited by poor understanding of the general principles of gene regulation by miRNAs. miRNA mediated regulation on NLRP3 Inflammasome and TLR pathways under hyperglycemic condition is substantially lacking. Through computational analysis, miRNAs

were predicted as putative regulators of these inflammatory cascade genes. But it was also observed previously that computational analysis is not always replicative.

Gut microbiome (GM) seems a crucial regulator for host metabolism through intestinal glucose absorption as well as energy balance (Parekh, P. J., *et al*. 2014). Apart from temporal and spatial alteration of GM architecture, several metabolic and lifestyle disorder like diabetes-mellitus (DM) and hypertension associated with the same (Qin, J., *et al*. 2012). Host-GM interactions play a central role in bile acid (BA) metabolism which is essential for metabolic homeostasis (Parekh, P. J., *et al*. 2014). Studies postulates that prolonged hyperglycemia associated biochemical aberration and chronic inflammation associated with severity of T2DM where inflammation also coined as mediator of GM dysbiosis (Parekh, P. J., *et al*. 2014, Qin, J., *et al.* 2012, Demirel, I., *et al*. 2018, Bhattacharjee, C. K. *et al*. 2019). Hyperglycemia, retarded carbohydrate hydrolysis, inflammation and GM dysbiosis may impair glucose absorption to alter microbial fermentation in gut which ultimately altered intestinal environment (Parekh, P. J., *et al*. 2014, Qin, J., *et al.* 2012). Shift of GM architecture may potentially contribute to metabolic endotoxemia which may damage the prolonged glomerular cells but yet to explore. Identification of microbial colonization profiles and characterization associated with health and disease state still remains a challenge due its complexity and inter-individual differences (Lazar, V., *et al*. 2018). Animal model studies revealed the significant correlation of *Bifidobacterium ssp*. with improved glucose tolerance and low-grade inflammation in prebiotic treated mice (Cani, P. D., *et al*. 2008, Larsen, N. *et al.* 2010). It is also reported that increased hepatic production of triglycerides and increase uptake of monosaccharide from the gut has been directly associated with the development of insulin resistance (Membrez, M . *et al*. 2008, Larsen, N. *et al.* 2010).

Aetiology of DN is multi-factorial and innate immunity plays a crucial role for adverse prognosis of T2DM. The present study was aimed to quantitate the difference of messenger RNA (m-RNA) expression of Inflammasome complex (NLRP3, CASP1, PYCARD), TLRs (TLR1 to TLR10) and pro-inflammatory cytokines (IL1β, IL18, TNF-α) on NLR pathway among DN patients with control subjects for deeper understanding the disease pathogenesis and severity of DN in terms of reduce eGFR. As the m-RNA expression is crucially modulated through epigenomic modification, the present study also assessed the expression of

epigenomic regulator like mi-RNA that targets the genes of NLRP3 and TLR pathway. The scenario necessitates to quantities the predictive role of inflammasome complex (NLRP3, CASP1, PYCARD), TLRs receptors (TLR1 to TLR10) and pro-inflammatory cytokines (IL1β, IL18, TNF-α) and their targeting miRNAs among T2DM patients. Futher, we aimed quantified the shift of GM architecture in a subset of T2DM with DN in reference to healthy control (HC) and its relation with the expression of host inflammatory machinery that includes NLRP3, CASP1, TLR4 and IL1β.