CHAPTER 7

CONCLUSION AND FUTURE PROSPECTS

Inflammation associated diabetic complication has been postulated in various animal model studies but the comprehensive data still enigmatic to understand the same in human subjects. Observation of the study indicates the alteration of gene expression related to inflammation like NLRP3 Inflammasome as well as TLR4 that has been replicated with other population data. Expressional alteration of NLRP3, CASP-1, TLR4 and TLR7 revealed the higher expression significantly associated with the severity of DN with the aid of reduced eGFR. Our findings substantiate the previous in vitro studies suggesting glomerular apoptosis may contribute to diabetic nephropathy (DN), through activation of NLRP3 Inflammasome and CASP-1. Possible role of NLRP3 Inflammasome complex in pathogenesis of DN with the involvement of proinflammatory cytokines insist researcher to develop the inhibitors for the

complex prevent further pathogenesis of DN and other inflammatory diseases. Studies also reported that, potential anti-inflammatory anti diabetic agents such as insulin, biguanides, Sodium-glucose co-transporter-2 (SGLT2) inhibitors, sulfonylureas, thiazolidinediones, and dipeptidyl peptidase-4 (DPP-4) inhibitors may modulates NLRP3 Inflammasome activity in renal tissues. Aberrant glucose concentration and inflammatory cytokines secretion through TLR4 activation on mouse mesangial cells and its contribution to develop DN have been postulated. Potent detrimental effect of inflammation in DN is yet to be understood but previous studies demonstrated the deleterious effect of TLR4/NF-κB inflammatory signaling mediators for nuclear translocation in glomeruli and proximal tubules. Animal model data and our present observation i.e. significant elevation of TLR4 m-RNA expression and its pathway derived inflammatory cytokine like IL1 β may crucial for pathogenesis of DN. Recent development of TLR4 antagonist called "Eritoran" has been developed to treating inflammation in various communicable as well as noncommunicable diseases including insulin resistance in experimental animal models. miRNA mediated epigenomic alteration play a distinct role in the gene regulation in mammal and thus to be a potential novel class of therapeutic targets as well as biomarker but the data are extremely limited. 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 through miRNAs. mi-RNA directed regulation on NLRP3 Inflammasome complex and TLRs pathways under hyperglycaemia is substantially lacking. Studies documented that hyperglycemia activate inflammatory cytokines (IL-1β and IL18) through the activation of NLRP3 inflammasome complex.Therefore we may postulate that altered homeosatasis of NLRP3 Inflammasome and TLRs and their potent targeting miRNAs may actively involved for the adverse prognosis of DN. Inhibition of inflammation is crucial to prevent tissue damage, and miRNAs has the potential to regulate inflammation as it is speculated as fine-tune signaling regulators to prevent uncontrolled progress of inflammatory reactions. Various miRNA has been discovered to regulate inflammatory response and their expression levels may offer promising diagnostic value and severity prediction of different inflammatory diseases. miRNA microarrays and qRT-PCR arrays are the most sensitive and specific method to detection and measure miRNAs from human blood possibly to develop new therapeutic methods in the future. Further insilico prediction revels that, miRNAs not only targets single gene and our insilico predicted miRNAs

also targets several sets of genes including GAN, KCTD9, VASH2, GRHL3 may be encountered by the same miRNA with higher specificity. Hence the magnitude of upregulation of a miRNA to downregulate the specific gene expression may not enough due to its multigenic specificity with other sets of genes along with the multigenic crosstalk.Discovery of miRNA associated disease pathogenesis of DN insist scientists to generate the data on miRNA that target specific m-RNA to restore normal cellular function. miRNA as biomarker has been recognized for the disease including cancer, cardiovascular disease, metabolic and immune disorder. Urinary miRNA as potential noninvasive biomarker for the early detection and progression of kidney diseases including DN (Simpson, K., *et al.* 2016). Inhibition of inflammation is crucial to prevent tissue damage, and miRNAs has the potential to regulate inflammation as it is speculated as fine-tune signaling regulators to prevent uncontrolled progress of inflammatory reactions. Various miRNA has been discovered to regulate inflammatory response and their expression levels may offer promising diagnostic value and severity prediction of different inflammatory diseases.

Increasing evidence based on animal and cell culture model along with case control studies suggested naturally occurring endogenous miRNA are important antisense therapeutic molecules. Preclinical and clinical trial for treatment hepatitis C, liver cancer, and other diseases are going on by correcting the expression of miRNAs by their mimics or inhibitors to develop the same as potential therapeutic approaches (Walayat, A. *et al.* 2018). In cancer research the utility of miRNA based therapeutics emphasized on their stability and optimized delivery system as targeted therapy. Antisense oligonucleotides, miRNA sponges, miRNAmask and small RNA inhibitors are the approaches to achieve the blocking of miRNA expression. Restoring downregulated miRNA expression can be achieved by using synthetic miRNA (miRNA mimic) or by inserting genes coding for miRNA into viral constructs (Walayat, A. *et al.* 2018). With the aid of previous observation, present study postulates that elevated level of TLR4 may potentially activate inflammatory cytokines production and the hypoxia induced has-miR-448 targeting TLR4, to activate apoptosis via activation of Caspase-1 of NLRP3 inflamasome complex among T2DM which ultimately accelerates the adverse microvascular complication like DN.

Our observation may postulate that NLRP3, CASP1, TLR4 and TLR7 may serve as better explanatory marker to understand the adverse prognosis of DN as there expression was

significantly associated with the severity of DN. This study may shed light on the role of the inflammatory cascade in the pathogenesis and severity of DN. Further validation of our findings in a different cohort is required.

We have also focused to assess the impact of miRNAs targeting inflammasome which may deliver as diagnostic and therapeutic biomarkers for DN in future. Previous reports and present findings may postulate that persistent hyperglycemia may alter the homeostasis of mRNA and mi-RNA of inflammasome complex and seems crucial for deeper understanding the pathogenesis of DN. Present study may open a new horizon for deeper understanding of DN patho mechanism with the aid of interaction between micro RNA and Inflammasome complex to find out better disease explainable marker. Association of NLRP3 Inflammasome and TLRs with the pathogenesis of DN found in this study strengthens the postulation of various in-vitro and in-vivo studies where activation of NLRP3 and TLRs established as a key mechanism to induce inflammation and insulin resistance in diabetic complications including DN. Recent experimental data advocates the use of miRNAs as new therapeutics for repression of inflammation and further reduction of disease severity. miRNA inhibitors or mimic to change expression of target genes is the main clinical importance of miRNA therapy. Further study is required to investigated the molecular mechanisms of miRNAs in DN pathogenesis and possibly to develop new therapeutics in future for the better prognosis of DN. Significant association of NLRP3, CASP1 and TLR4 expression with the reduced eGFR for the severity of DN found in this study insist for further research to inhibit inflammation to reduce further pathogenesis of DN.

Present study from north east India may postulate the potent role of Inflamosome complex for the severity of DN where low-grade inflammation associated with GM dysbiosis. T2DM and DN are associated with significant shift of gut microbial diversity compared to healthy control and *Escherichia* identified as key genus that concomitantly increased with the severity of DN. The phenomenon may interact with functions underlying inflammation, energy extraction as well as the gut-barrier. North East India population along with other population around the globe possesses lesser abundance *Proteobacteria* in healthy individual compared diabetic population. Inflammasome is dependent on various extracellular priming including the virulence factors like α-hemolysin and type-1 fimbriae that derived from *Escherichia* which regulate intracellular Inflammasome signaling cascades. The present study

documented that the increment of the proportion of the genus *Escherichia* significantly associated with host inflammatory gene expression atleast for NLRP3 and CASP1. Shift of gut microbiota may cause chronic inflammation in T2DM associated micro vascular complications including DN severity. Some probiotic like *Lactobacillus* and *Bifidobacterium*can provide immune-modulatory effects to counter chronic inflammation by inducing IL10 production, which is an anti-inflammatory cytokine reported downregulates IFN- IFN-γ and IL-2/IL-1β in mice.

Extent of the severity of nephropathy including DN, eGFR identified as a potent marker for kidney function in humans and reduced eGFR is associated with severity of DN. The abundance of bacterial genus *Escherichia* seems a better explanatory marker for retarded eGFR to explain the diseases severity. Beneficial effects of gut microbiota for development of protection against promoting T2DM in obese and prediabetes depends on healthy dietary pattern and increase population of growth promoting microbiota (Diaz-Rizzolo, D. A., *et al*. 2019). Human gut microbiota plays a crucial role in maintaining homeostasis of intestine and metabolizing the xenobiotics including drugs. Advance in research provides the use of probiotics species like *Lactobacillus* and *Bifidobacterium* into a new height and extensively in use for a long time in various diseases including diabetes.

Metagenomics, transcriptomic and metabolomics approaches are required to explore the molecular basis of metabolic interactions between specific microbes in healthy or patients with diabetes related disorders including DN. Though studies bridging the link between GM with the diseases including inflammatory bowel disease, cancer, obesity and T2DM, asthma and multiple sclerosis but GM became useful for diagnostics, prognostics and therapeutics of the same also (Allin, K. H., *et al.* 2015, Sharma, S., and Tripathi, P. 2019). To establish causality related to GM, statistically well-powered prospective studies, intervention studies and randomized clinical trials are required (Allin, K. H., *et al.* 2015, Sharma, S., and Tripathi, P. 2019). Prolonged hyperglycemia associated differential carbohydrate hydrolysis, inflammation and GM dysbiosis impair glucose absorption in the intestine to alter microbial fermentation in the gut which ultimately altered intestinal environment (Qin, J., *et al*. 2012, Parekh, P.J., *et al*. 2014). Though the study limits with its sample size but the observation of increment of gut derived *Escherichia* abundance matched with the clinical outcome like retarded eGFR among T2DM may postulate that probiotic supplement may be useful to reduce

down the *Escherichia* associated inflammation. Hence further study may aim to quantities the shift of GM architecture among T2DM upon the probiotic supplement and its subsequent impact on inflammation and metabolome.

Further comprehensive research is required to address underlying mechanisms of causes, inhibitors and confounders for the severity of T2DM microvascular and macrovascular complications including DN with the aid of GM architect, Inflammasome, TLRs and their targeting miRNAs. Technological advancement can be utilize for the better prognosis of type 2 diabetic related micro and macro vascular complication especially by modulating innate immune regulatory gene expression especially for NLRP3 inflammasome and TLR4 through their targeting miRNAs. Present study may provide substantial information on regulatory miRNAs on Inflammasome complex and TLR mediated inflammation pathway and the association of GM architect for pathogenesis and prognosis of diabetic microvascular complication including DN subjects that are substantially or completely lacking in present day.