CHAPTER 3

AIMS AND OBJECTIVES

3.1: RESEARCH QUESTION(S)/HYPOTHESIS:

Anti-inflammatory agents are gaining attention for their promising therapeutic role in the pathogenesis and preventing DN. Through *insilico* approach, miRNAs viz. 'has-miR 22-3p and has-miR 223' targeting NLRP3, 'has- miR4291' targeting Caspase1 and 'has-miR 185-3p' targeting PYCARD, hsa-miR-561-3p targeting TLR2, hsa-miR-4307 targeting TLR3, hsa-miR-448 targeting TLR4 and hsa-miR-4760-3p targeting TLR7 were predicted as putative regulators with a target score more than 80%. Pre-therapeutic marker against different angiogenic disorder provides the new opportunity to personalize the therapeutic strategy. With this background we aimed to assess the impact of miRNAs targeting Inflammasome and TLRs which may be later identified as pre-therapeutic markers for management of diabetic microvascular complications including diabetic nephropathy. Shift of gut microbiome (GM) architecture may potentially contribute to metabolic endotoxemia which may damage the glomerular cells, which is yet to be explored. It is hypothesized that insulin resistance is result from the action of metabolites from the gut microbes which compromises the gut barrier integrity further leading to leakage of inflammatory mediators into systemic circulation. As GM reflect the metabolic cooperation between different phylotypes and hyperglycemia, the

present study was aimed to quantify the shift of GM architecture among T2DM in reference to DN and healthy control (HC) and its relation with the expression of host inflammatory machineries including NLRP3 Inflammasome and TLRs.

3.2 OBJECTIVES OF THE STUDY:

Primary objectives

A. **To assess the expression of inflammasomes molecules viz. NLRP3, Caspase 1 and PYCARD in subjects with or without diabetic nephropathy (DN).**

   Level of NLRP3, Caspase 1 and PYCARD to be determined in blood from the subjects with DN and diabetic control

B. **To understand the correlation between mi-RNA targeting inflammasomes (NLRP3, Caspase 1 and PYCARD) and inflammasomes in the study participants with or without Diabetic Nephropathy (DN).**

   Level of mi-RNA 'has-miR 22-3p and has-miR 223' targeting NLRP3, 'has- miR4291' targeting Caspase 1 and 'has-miR 185-3p' targeting PYCARD to be determined in patients with different degree of DN. Further the inflammasome expression also to be correlated with their targeted miRNA.

Secondary objectives

A. **To assess the expression of Toll like receptor molecules such as TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and TLR10 in subjects with or without Diabetic Nephropathy (DN).**

   Level of TLR1-TLR10 was determined from blood from the subjects among different degree of DN and the diabetic control

B. **To understand the correlation between mi-RNA targeting TLRs (TLR2, TLR3, TLR4 and TLR7) and TLRs in the study participants with or without Diabetic Nephropathy (DN).**

   Level of mi-RNA 'hsa-miR-561-3p targets TLR2, hsa-miR-4307 targets TLR3, hsa-miR-448targets TLR4 and hsa-miR-4760-3p targets TLR7 was determined from blood

of patients with different degree of DN. Further the inflammasome expression to be correlated with their targeted miRNA.

C. To assess the expression of downstreaming cytokine molecules of Inflammasome complex includes ILβ, IL18 and TNFα in the study participants with or without Diabetic Nephropathy (DN).

D. Present study also aimed to quantify the shift of GM architecture among T2DM in reference to subjects with DN and healthy control (HC) and its relation with m-RNA expression of inflammatory genes.