## CONTENTS

CHAPTER	1		1-12
	GENE	ERAL INTRODUCTION	
CHAPTER	CHAPTER 2		
	REVIEW OF LITERATURE		
	2.1	Disease Burden	13-16
		2.1.1 Disease Burden: Globe	
		2.2.2 Disease Burden: India	
	2.2	The Disease Diabetes	17-20
	2.3	Pathophysiology of Diabetes	21-22
		2.3.1 Type 1 Diabetes	
		2.3.2. Type 2 Diabetes	
		2.3.3. Gestational Diabetes (GDM)	
		2.3.4. Maturity Onset Diabetes of the Young (MODY)	
	2.4	Diagnosis of Diabetes	22-23
	2.5	Complication of Type 2 Diabetes	23-26
		2.5.1 Diabetic Nephropathy	
		2.5.2. Diabetic Retinopathy (DR)	
		2.5.3 Diabetic Neuropathy	
		2.5.4. Macrovascular Complications of Diabetes:	
		Cardiovascular Disease	
		2.5.5 Cerebrovascular Disease	
	2.6	Diabetic Therapy	27-27
	2.7	Diabetic Nephropathy	27-32
		2.7.1 Disease Burden and Epidemiology	
		2.7.2 Pathology and Diagnosis of DN	
	2.8	Anatomical and Structural Changes of Glomeruli in	32-33
		Diabetes	
	2.9	Classification of Diabetic Nephropathy	33-38
		2.9.1 Class I: Glomerular Basement Membrane Thickening.	
		2.9.2 Class II: Mesangial Expansion, Mild (IIa) or Severe	

	(IIb).	
	2.9.3 Class III: Nodular Sclerosis (Kimmelstiel- Wilson	
	lesions)	
	2.9.4 Class IV: Advanced Diabetic Glomerulosclerosis	
	2.9.5 Tubulointerstitial and Vascular Lesions in DN	
2.10	Therapy for DN	37-38
2.11	Risk Factor for Diabetic Nephropathy	38-39
2.12	Pathways involved in the pathogenesis of DN	39-45
	2.12.1 Hyperglycemia increase superoxide production and	
	role of oxidative stress in DN	
	2.12.2 Polyol pathway	
	2.12.3 Advanced Glycation End Products (AEG) pathway	
	2.12.4 Protein kinase C pathway	
	2.12.5 Hexosamine Pathway	
	2.12.6 Inflammatory Pathways of DN	
2.13	Inflammasome Complex	45-54
	2.13.1 The NLR Family	
	2.13.2 NLR family pyrin domain containing 3(NLRP3	
	2.13.3 NLRP3 gene function	
	2.13.4 CASPASE1	
	2.13.5 General function and association with disease	
	2.13.6 PYCARD	
	2.13.7 Functions of PYCARD and association with disease	
2.14	Toll Like Receptors and inflammation	55-60
	2.14.1 Toll Like Receptors (TLRs)	
	2.14.2 Cellular Localization of TLRs and Cell-Specific	
	Expression	
	2.14.3 TLR Signaling Pathways	
2.15	Inflammatory cytokines: Interleukin 1ß (IL1ß), Interleukin	60-64
	18 (IL18) and Tumor Nacrosis Factor $\alpha$ (TNF $\alpha$ )	
	2.15.1 Interleukin 1beta (IL1β)	
	2.15.2 Interleukin 18 (IL18)	
	2.15.3 Tumour Necrosis Factor alpha (TNFα)	

	2.16	Damage-associated molecular patterns (DAMPs)	64-69
		2.16.1 DAMP mediated Inflammasome activation	
		2.16.2 DAMP mediated TLRs activation	
	2.17	Non coding RNAs: Overview	69-85
		2.17.1 Micro RNAs	
		2.17.1.1 Biogenesis, structure and functions of	
		miRNA	
		2.17.1.2 miRNA-mediated silencing	
		2.17.1.3 Location of mature miRNA	
		2.17.1.4 Association of miRNA with disease	
	2.18	Human gut microbiome	85-90
		2.18.1: Human gut microbiome and disease	
		2.18.2: Human gut microbiome and host immunity	
CHAPTER	3		91-93
	AIMS	S AND OBJECTIVES	
	3.1	Research question(s)/hypothesis:	91-92
	3.2	Objectives of the Study	92-93
CHAPTER	CHAPTER 4		94-128
	MAT	ERIAL AND METHODS	
	4.1	Study design	94
	4.2	Site sites	95
	4.3	Ethical approval to recruit study subjects	95
	4.4	Recruitment of cases and controls	95-98
		4.4.1 Diagnosis of Diabetes case	
		4.4.2 Diagnosis of DN	
	4.5	Inclusion Criteria:	98
	4.6	Exclusion criteria	98
	4.7	Collection of socio-demographic and baseline information	98-99
	4.8	Sample collection and preparation for analysis	99
		4.8.1 Procedure	
	4.9	Total RNA isolation from PBMC	100-101
		4.9.1 Procedure	
	4.10	Quantification of total RNA	101-102

	4.10.1 Procedure	
4.11	Preparation of c-DNA from eluted RNA	102-103
	4.11.1 Procedure	
4.12	Real Time PCR for mRNA expression assay	104-109
	4.12.1 Introduction	
	4.12.2 Procedure for real time PCR	
4.13	Reverse Transcription and Real Time PCR for miRNA	109-113
	expression assay	
	4.13.1 miRNA selection	
	4.13.2 Reverse Transcription for miRNA expression assay	
	4.13. 3 Real Time PCR for mi-RNA expression assay	
	4.13. 4 Procedure of real-time PCR	
4.14	Calculation of relative quantification	113-114
4.15	Cell Culture Technique for Validation of miRNA-mRNA	114-118
	Interaction for The Proposed Study:	
	4.15.1 Principle	
	4.15.2 Subculture of INT407 Cell line	
	4.15.3 Cell counting	
	4.15.3.1 Procedure	
	4.15.4 Cryopreservation of cell lines	
	4.15.4.1 Principle	
	4.15.4.2 Procedure	
	4.15.5 Induction of Glucose in Cultured cells	
4.16	Estimation of IL1 $\beta$ , IL18 and TNF $\alpha$ by commercially	119-123
	available ELISA kit	
	4.16.1 Estimation of IL1β	
	4.16.1.1 Procedure	
	4.16.1.2 Calculation of results	
	4.16.1.3 Sensitivity and reproducibility and	
	specificity of the IL1 $\beta$ ELISA kit	
	4.16.2 Estimation of IL18	
	4.16.2.1 Procedure	

4.16.2.2 Calculation of results

		4.16.2.3 Sensitivity and reproducibility and	
		specificity of the IL18 ELISA kit	
		4.16.3 Estimation of TNFα	
		4.16.3.1 Procedure	
		4.16.3.2 Calculation of results	
		4.16.3.3 Sensitivity and reproducibility and	
		specificity of the TNFa ELISA kit	
4.	.17	Estimation of Gut microbiota from studied participants	123-128
		4.17.1 Methodology for DNA isolation from stool samples	
		for gut microbiota estimation from studied participants	
		4.17.1.1 Procedure	
		4.17.2 16S Metagenome Sequencing	
		4.17.2.1 Gene-specific sequences	
		4.17.2.2 Metagenominc sequence Analysis	
4.	.18	Statistical Analysis	128
CHAPTER 5	CHAPTER 5		
R	ESUI	LTS	
5.	.1	Distribution of Demographic and clinical variables of study	129-133
		subjects	
5.	.2	Expression of mRNA for NLRP3, CASP-1 and PYCARD in	134-135
		peripheral blood mononuclear cells (PBMC) among cases	
		and controls:	
5.	.3	Expression of miRNA hsa-miR 223, hsa-miR 22-3p, hsa-	136-138
		miR4291 and hsa-miR 185-3p in PBMC from DN, DC and	
		HC study population.	
5.	.4	Expression of m-RNA for TLRs (TLR1-10) in PBMC	138-141
		among diabetics and controls:	
5.	.5	Expression of hsa-miR-561-3p, hsa-miR-4307, hsa-miR-448	141-143
		and hsa-miR-4760-3p in PBMC from Type2 DN patients,	
		DC and HC study subjects.	
5.	.6	Expression of m-RNA for cytokines IL1 $\beta$ , IL18 and TNF- $\alpha$	143-146
		in PBMC among diabetics and controls	
5.	.7	Linear regression of relative quantification of	146-148

Inflammasome complex mRNA that includes NLRP3, CASP-1 and PYCARD for DN severity based on estimated glomerular filtration rate (eGFR).

- 5.8 Linear regression of relative quantification of hsa-miR-223, 149 hsa-miR-22-3p, hsa-miR-4291 and hsa-miR-185-3p miRNAs targets Inflammasome complex mRNA with DN severity based on eGFR.
- 5.9 Linear regression of relative quantification of TLR m-RNA 150-153 that includes TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and TLR10 for DN severity based on estimated glomerular filtration rate (eGFR).
- 5.10 Linear regression of relative quantification of hsa-miR-561- 153-154
   3p, hsa-miR-4307, hsa-miR-448 and hsa-miR-4760-3p
   miRNAs targets TLRs with DN severity based on eGFR
- 5.11 Linear regression of relative quantification of inflammatory 154-155 cytokines IL1β, IL18 and TNFαwith DN severity based on eGFR
- 5.12 Correlation of mRNAs and its targeted mi-RNA expression 155-164 among study subjects
- 5.13 Distribution of IL1 $\beta$ , IL18 and TNF- $\alpha$  from plasma of HC, 165-166 DC and DN patients
- 5.14 Linear regression of plasma IL1β, IL18 and TNFα cytokine 166 with DN severity based on estimated glomerular filtration rate (eGFR).
- 5.15 Validation of miRNA-mRNA interaction in INT407 cell 167-173 lines

5.15.1 Expression of Inflammasome complex gene includes NLRP3, CASP-1 and PYCARD in INT407 cells upon induced with different concentration of glucose

5.15.2 Expression of miRNAs includes hsa-miR-223, hsamiR-22-3p, hsa- miR-4291 and hsa-miR-185-3p targets NLRP3, CASP-1 and PYCARD genes in INT407 cells upon induced with different concentration of glucose.

		5.15.3 Expression of m-RNA for TLRs (TLR1-10) in	
		INT407 cells upon induced with different concentration of	
		glucose.	
		5.15.4 Expression of miRNAs includes hsa-miR-561-3p,	
		hsa-miR-4307, hsa-miR-448 and hsa-miR-4760-3p targets	
		TLRs genes in INT407 cells upon induced with different	
		concentration of glucose	
		5.15.5 Expression of IL1 $\beta$ , IL18 and TNF $\alpha$ genes in INT407	
		cells upon induced with different concentration of glucose.	
	5.16	Expression of NLRP3, CASP-1, TLR4 and TLR7 form	174-178
		single cell expression experiment and network analysis of	
		studied genes of inflammation cascade	
	5.17	Microbial architecture of study subjects	179-196
CHAPTER	6		197-219
	DISC	CUSSION	
CHAPTER	7		
	CON	ICLUSION AND FUTURE PROSPECTS	220-225
CHAPTER	8		
	BIBI	LIOGRAPHY	226-275
Appendix 1		Abbreviations Used	276-277
Appendix 2		Bio-data	278-288
Appendix 3	,	Transcript of the Ph. D. Course Work	289
Appendix 4		List of Publications	290-291
Appendix 5		Ethical Clearance certificate	292
Appendix 6		Awards and Honors	293
Appendix 7		Socio demographic and clinical information sheet and Patients	294-297
		Consent Form	

LISTS	OF	FIG	URES
LISIS	OF	FIG	URES

Figure	Description	Page
No.		No.
2.1	Global prevalence of diabetes estimated in 2017 inner circle and	15
	predicted for 2045 in outer circle	
2.2	Burden of diabetes in India	16
2.3	The Blue circle: universal symbol for diabetes	19
2.4	Sign and symptom of diabetes	20
2.5	Complications of Type 2 diabetes	24
2.6	Staging of chronic kidney disease	32
2.7	Morphology and structural changes in normal and diabetic kidney	33
	glomerulus	
2.8	Morphologic lesions in DN	35
2.9	Pathologic classification of DN based on glomerular changes	36
2.10	Various therapeutic agents identified in the metabolic and alternative	38
	pathways of DN	
2.11	Various pathways involve in the pathogenesis of DN	40
2.12	Hyperglycemia mediated activation of various pathways for the	42
	progression of DN	
2.13	Inflammatory pathway in DN and schematic summary of the	45
	therapeutic targets	
2.14	Represents the Human and Mouse NLR Family Members	47
2.15	Chromosomal position of NLRP3 protein (1q44)	48
2.16	Gene tree of NLRP3	49
2.17	Differential expression of NLRP3	49
2.18	Assemble and activation of NLRP3 Inflammasome	50
2.19	Chromosomal location of CASP1 gene	52
2.20	Gene tree of CASP1	52
2.21	Chromosomal location of PYCARD	53
2.22	Gene tree of PYCARD	54
2.23	History of Toll Like Receptors related significant achievements	56

2.24	Evolutionary relationships of all 10 human TLRs	56
2.25	Structure of TLRs	57
2.26	Toll-like receptor (TLR) protein structure, location and amino acid	58
	variations with Gene Bank Accession numbers.	
2.27	TLR signaling pathways	60
2.28	Chromosomal location of IL1β	61
2.29	Chromosomal location of IL18	62
2.30	Chromosomal location of TNFa	63
2.31	List of DAMPs and their receptors	65
2.32	DAMP associated activation of immune response	66
2.33	The NLRP3 Inflammasome activation through mitochondrial reactive	68
	oxygen specie	
2.34	Types of non coding RNAs	70
2.35	Generation of miRNA	72
2.36	Structure of miRNA genes. (a) intronic miRNAs in coding transcription	73
	units (TUs), for example, the mir-101-2 cluster (b) Intronic miRNAs in	
	noncoding TU, such as the mir-135a-2 cluster (c) Exonic miRNAs in	
	coding TU (d) Exonic miRNAs in noncoding TU	
2.37	Biogenesis and function of miRNA	75
2.38	Mechanism of action of miRNA	77
2.39	Mechanisms of miRNA-Mediated Gene Silencing	79
2.40	Figure represents the crosstalk between microbes and host	89
4.41	Image of INT407 cells	116
4.42	Induction of glucose on INT407 cells	118
5.43	Distribution of Demographic and clinical veriables among study	133
	subjects. [A]Weight of the study population significantly higher among	
	HC subjects than DC and DN. [B] Distribution of BMI among HC was	
	significantly higher than DC and DN. [C] Duration of Diabetes (DOD)	
	was significantly higher in DN than DC.[D]Random blood sugar (RBS)	
	in mg/dl was higher in DC than DN and HC. [E]Serum creatinine level	
	(mg/dl) was significantly higher in DN subjects compared toDC and	
	HC. [F]Distribution of Serum urea level (mg/dl) of the study	
	populationrevealed that DN subjects has significantly higher S.	

creatinine than DC and HC. All data is presented as mean±SD and significant level was determined by P<0.05..

- 5.44 Melting curves results of GAPDH, NLPR3, CASP-1 and PYCARD 134
   expression in SYBR green based realtime PCR. A single melting peak
   correspond to each amplicon generated using specific set of primers.
- 5.45 Gene expression of NLRP3, CASP-1 and PYCARD from DC and DN 135 patients after normalizing to HC as reference subjects. Bars represent the mean relative expression of gene  $\pm$  SD of [A] NLRP3 [B] CASP-1 and [C] PYCARD gene expression in PBMC from DC and DN patients (n = 60 per group). m-RNA expression (fold change) of [A] NLRP3, p=0.009 and [B] CASP-1,P<sub>mw</sub>=0.02 were significantly higher among DN subjects than DC subject. P value considered significant at P< 0.05, P< 0.01 and P< 0.001 between DC and DN.
- 5.46 Melting curves results of miRNA, hsa-miR-223 and hsa-miR-22-3p 136 targeting NLRP3, hsa-miR-4291 targeting CASP-1 and hsa-miR-185-3p targeting PYCARD expression in SYBR green based realtime PCR. A single melting peak corresponds to each amplicon generated using specific set of primers for each miRNA.
- 5.47 mi-RNA expression of hsa-miR-223, hsa-miR-22-3p, hsa-miR-4291and
  137-hsa-miR-185-3pfrom DC and DN patients after normalized to HC as
  138 reference subjects. Bars represents the mean relative expression of
  miRNA± SD [A] hsa-miR-223,[B] hsa-miR-22-3p, [C] hsa-miR4291and [D] hsa-miR-185-3p expression in PBMC from DC and DN
  patients (n = 60 per group). Expression (fold change) of [D] hsa-miR185-3p targeting PYCARD was significantly (P<sub>mw</sub>=0.04) high among
  DN subjects compared to DC.
- 5.48 Melting curves results of TLR1,TLR2,TLR3,TLR4,TLR5,TLR6,TLR7, 139
   TLR8, TLR9 and TLR10 mRNA expression in SYBR green based
   realtime PCR. A single melting peak corresponds to each amplicon
   generated using specific set of primers for each mRNA.
- 5.49 Gene expression of TLR1-10 from DC and DN patients after 140 normalized to HC as reference subjects. Bars represent the relative expression of gene ± SD of (A) TLR1 (B) TLR2 (C)TLR3 (D) TLR4

	(E)TLR5 (F) TLR6 (G)TLR7 (H)TLR8 (I) TLR9 and (J)TLR10 gene	
	expression in PBMC from DC and DN patients ( $n = 60$ per group). m-	
	RNA expression (fold change) of TLR4 (D) was significantly (p=0.04)	
	elevated among DN subjects than in DC subjects. P value considered	
	significant at P< 0.05, P< 0.01 and P< 0.001 between DC and DN.	
5.50	Melting curves results of hsa-miR-561-3p, hsa-miR-4307, has-miR-448	141
	and hsa-miR-4760-3p miRNA expression in SYBR green based	
	realtime PCR. A single melting peak corresponds to each amplicon	
	generated using specific set of primers for each miRNA.	
5.51	miRNA expression of hsa-miR-561-3p, hsa-miR-4307, hsa-miR-448	142
	and hsa-miR-4760-3p from HC, DC and DN patients after normalizing	
	to HC as reference subjects. Bars represents the mean relative	
	expression of miRNA± SD [A] hsa-miR-561-3p [B] hsa-miR-4307 [C]	
	hsa-miR-448 and [D] hsa-miR-4760-3p expression in PBMC from DC	
	and DN patients ( $n = 60$ per group). Expression (fold change) of (C)	
	hsa-miR-448 targeting TLR4 was significantly ( $P_{mw}=0.01$ ) high among	
	DN subjects compared to among DC. P value considered significant at	
	P < 0.05, $P < 0.01$ and $P < 0.001$ between DC and DN of miRNAs.	
5.52	Melting curves results of IL1 $\beta$ , IL18 and TNF- $\alpha$ mRNA expression in	144
	SYBR green based realtime PCR. A single melting peak corresponds to	
	each amplicon generated using specific set of primers for each mRNA.	
5.53	mRNA expression of cytokines IL1 $\beta$ , IL18 and TNF- $\alpha$ from DC and	145
	DN patients after normalizing to HC as reference subjects. Bars	
	represents the mean relative expression of mRNA $\pm$ SD [A] IL1 $\beta$ [B]	
	IL18 and [C] TNF- $\alpha$ expression in PBMC from DC and DN patients (n	
	= 60 per group). Expression (fold change) of [A] IL1 $\beta$ was significantly	
	$(P_{mw}=0.03)$ high among DC subjects compared to among DN. P value	
	considered significant at P< 0.05, P< 0.01 and P< 0.001 between DC	
	and DN of mRNAs.	
5.54	Figure represents the linear regression of the interaction of NLRP3 gene	147
	expression with eGFR among the Type2 Diabetic subjects. Elevated	
	level of NLRP3 associated with reduced eGFR level (P:0.05). The	

estimate of the regression is -0.77 with the SE:0.39. Residual standard

	error: 91.92, Multiple R-squared: 0.06, Adjusted R-squared: 0.048	
5.55	Figure represents the linear regression of the interaction of CASP-1	148
	gene expression with eGFR among the Type2 Diabetic subjects.	
	Elevated level of CASP-1 associated with reduced eGFR level (P:0.04).	
	The estimate of the regression is -2.16 with the SE:1.02. Residual	
	standard error: 87.62, Multiple R-squared: 0.07, Adjusted R-squared:	
	0.06	
5.56	Figure represents the linear regression of the interaction of TLR4 gene	151
	expression with eGFR among the Type2 Diabetic subjects. Elevated	
	level of TLR4 associated with reduced eGFR level (P:0.04). The	
	estimate of the regression is -1.38 with the SE:0.66. Residual standard	
	error: 90.69, Multiple R-squared: 0.1, Adjusted R-squared: 0.08	
5.57	Figure represents the linear regression of the interaction of TLR7 gene	152
	expression with eGFR among the Type2 Diabetic subjects. Elevated	
	level of TLR7 associated with eGFR level (P: 0.008). The estimate of	
	the regression is 0.45 with the SE: 0.15. Residual standard error: 83.19,	
	Multiple R-squared: 0.3, Adjusted R-squared: 0.2	
5.58	Figure represents the regression of NLRP3 expression with the hsa-	156
	miR-223 expression. NLRP3 expression inversely related with hsa-	
	miR-223 with an estimate -0.21 with the SE: 0.3, Residual standard	
	error: 28.76, Multiple R-squared: 0.01, Adjusted R-squared: -	
	0.01,P:0.05	
5.59	Figure represents the regression of NLRP3 expression with the hsa-	157
	miR-22-3p expression. NLRP3 expression was positively related with	
	hsa-miR-223 expression. The estimate of regression was 0.8, SE: 1.3,	
	Residual standard error: 28.64, Multiple R-squared: 0.007, Adjusted R-	
	squared: -0.007,P:0.5	
5.60	Figure represents the regression of CASP-1 expression with its targeting	158
	miRNA, has-miR-4291 expression. CASP-1 expression was positively	
	related with has-miR-4291 expression. The estimate of regression was	
	0.004, SE: 0.03, Residual standard error: 11.36, Multiple R-squared:	
	0.00003, Adjusted R-squared: -0.01, P: 0.9.	
5.61	Figure represents the regression of PYCARD expression with its	159

targeting miRNA, has-miR-185-3p expression. PYCARD expression was inversely related with has-miR-185-3p expression. The estimate of regression was -0.0005 with the SE: 0.001, Residual standard error: 83.05, Multiple R-squared: 0.002, Adjusted R-squared: -0.01, P: 0.7 5.62 Figure represents the regression of TLR2 expression with its targeting 160 miRNA, hsa-miR-561-3p expression. TLR2 expression was positively related with hsa-miR-561-3p expression. The estimate of regression was 0.31 with the SE: 0.2, Residual standard error: 9.09, Multiple Rsquared: 0.1, Adjusted R-squared: 0.09, P: 0.1 5.63 Figure represents the regression of TLR3 expression with its targeting 161 miRNA, hsa-miR-4307 expression. TLR3 expression was positively related with hsa-miR-4307 expression. The estimate of regression was 45.72, with the SE: 51.54, Residual standard error: 169.8, Multiple Rsquared: 0.07, Adjusted R-squared: -0.02, P: 0.4. 5.64 Figure represents the regression of TLR4 expression and the hsa-miR-162 448 expression. TLR4 expression inversely related with hsa-miR-448 but the association was insignificant (P: 0.8). The estimate of regression was -0.03, SE: 0.17, Residual standard error: 23.25, Multiple Rsquared: 0.001, Adjusted R-squared: -0.03. 5.65 Figure represents the regression of TLR7 expression and the hsa-miR-163 4760-3p expression. TLR7 expression positively related with hsa-miR-4760-3p but the association was insignificant (P: 0.1). The estimate of regression was 70.06, SE: 40.70, Residual standard error: 121.1, Multiple R-squared: 0.2, Adjusted R-squared: 0.1. 5.66 Figure represents the distribution of plasma level of cytokines  $IL1\beta$ , 165 IL18 and TNF-α from HC, DC and DN patients. Bars represents the plasma level of [A] IL18 [B] IL1  $\beta$  and [C] TNF- $\alpha$  as mean  $\pm$  SD (n = 60 per group). Statistically significant difference observed for IL18 level which was higher in DN ( $1957\pm771.0$  pg/ml) and DC ( $1247\pm624.5$ 

pg/ml) compared to HC (930.6±480.9 pg/ml), (P<sub>ANOVA</sub>< 0.0001).

Significantly higher plasma level of IL18 was observed among DN patients than DC ( $P_{TUKEY'S} < 0.0001$ ). P value considered significant at p < 0.05, p < 0.01 and p < 0.001.

xxiii

- 5.67 Figure represents the relative quantification of Inflammasome complex 168 gene expression in INT407 cell upon glucose induction. [A] NLRP3,
  [B] CASP-1 and [C] PYCARD gene was upregulated in INT407 cells induced with different concentration of glucose compared to untreated cells.
- 5.68 Figure represents the relative quantification of miRNAs targets 169 Inflammasome complex gene expression in INT407 cell upon glucose induction. [A] hsa-miR-223 was upregulated gradually with higher glucose concentration. [B] hsa-miR-22-3p, [C] hsa- miR-4291 and [D] hsa-miR-185-3p miRNA was downregulated in INT407 cells induced with different concentration of glucose compared to UT.
- 5.69 Figure represents the relative quantification of TLRs gene expression in 171 INT407 cell upon glucose induction.
- 5.70 Figure represents the relative quantification of miRNAs targets TLRs in 172 INT407 cell upon glucose induction. [A] hsa-miR-561-3p was upregulated with higher glucose concentration. [B] hsa-miR-4307 and [C] hsa-miR-448 was upregulated in cells treated with 125mg/dl glucose. [D] hsa-miR-4760-3p miRNA was downregulated in INT407 cells induced with different concentration of glucose compared to UT.
- 5.71 Figure represents the relative quantification of IL1β, IL18 and TNFα 173 genes gene expression in INT407 cell upon glucose induction. [A] IL1β was upregulated gradually with higher glucose concentration. [B] IL18 expressed highly in cells treated with 125mg/dl glucose, [C] TNFα expression shows downregulation in cells cultured with 500mg/dl glucose.
- 5.72 Expression of NLRP3, CASP-1, TLR4 and TLR7 form single cell
   175 expression experiment of Pancreatic Islets cells from healthy and
   T2DM patients
- 5.73 Expression of NLRP3, CASP-1, TLR4 and TLR7 form single cell 176 expression experiment of Immune cells
- 5.74Expression of NLRP3, CASP-1, TLR4 and TLR7 form single cell177expression experiment from organs and tissues from individual mice

5.75	Insilico net work analysis were done by Gene mania to understand the	178
	interaction like physical interactions, co-expression, prediction, co-	
	localization, pathways, genetic interactions and shared protein domains	
	between [A] NLRP3 Inflammasome, TLRs ,TNF $\alpha$ , IL1 $\beta$ , IL18 and	
	other inflammatory molecules [B] NLRP3 and other inflammatory	
	molecules [C] TLR4 and other inflammatory molecules	
5.76	Bacterial abundance of Phylum among study individuals	179
5.77	Represents the abundance of Actinobacteria, Bacteroidetes, Firmicutes	180
	and Proteobacteria in HC, DC and DN subjects. [A] Abundance of	
	Actinobacteria is significantly lower in DN (0.19±0.31) than HC	
	(3.07±3.37) subjects. B] Abundance of <i>Proteobacteria</i> is significantly	
	increased in DN (41.36±14.66) than HC (9.84±7.67), p<0.05.	
	Statistically significant was considered P<0.05, P<0.001 & P<0.0001	
5.78	Bacterial abundance of Class among study individuals	181
5.79	Bacterial abundance of Order among study individuals	181
5.80	Bacterial abundance of Family among study individuals	182
5.81	Represents the abundance of Escheichia among HC, DC and DN study	183
	participants. Abundance of Escherichia significantly increased in DN	
	subjects than HC and DC. Statistically significant difference for	
	abundance of <i>Escherichia</i> observed between HC (0.18 $\pm$ 0.25) and DN	
	(32.53±16.84), P<0.01.	
5.82	Figure represents the linear regression of the interaction of Escherichia	186
	with the NLRP3 gene expression among the Type2 Diabetic subjects.	
	Elevated level of Escherichia associated with increased NLRP3	
	expression (P:0.0004). The estimate of the regression is 1.64 with the	
	SE:0.33. Residual standard error: 16.04, Multiple R-squared: 0.53,	
	Adjusted R-squared: 0.50, F-statistic: 25.47	
5.83	Figure represents the linear regression of the interaction of Escherichia	187
	with the CASP1 gene expression among the Type2 Diabetic subjects.	
	Elevated level of Escherichia associated with increased CASP1	
	expression (P:0.0009). The estimate of the regression is 1.04 with the	
	SE:0.27. Residual standard error: 18.26, Multiple R-squared: 0.37,	
	Adjusted R-squared: 0.35, F-statistic: 14.38	

5.84	Figure represents the linear regression of the interaction of <i>Escherichia</i>	188
	with the phylum Bacteroidetes among the Type2 Diabetic subjects.	
	Elevated level of Escherichia associated with decreased Bacteroidetes	
	(P:0.03). The estimate of the regression is $-0.75$ with the SE:0.33.	
	Residual standard error: 20.98, Multiple R-squared: 0.17, Adjusted R-	
	squared: 0.1, F-statistic: 5.08.	
5.85	Figure represents the linear regression of the interaction of Escherichia	189
	with the genus Acinetobacter among the Type2 Diabetic subjects.	
	Elevated level of Escherichia associated with increased Acinetobacter	
	(P:0.001). The estimate of the regression is 5.56 with the SE:1.52.	
	Residual standard error: 18.43, Multiple R-squared: 0.37, Adjusted R-	
	squared: 0.34, F-statistic: 13.47.	
5.86	Figure represents the linear regression of the interaction of Escherichia	190
	with the genus Wautersiella among the Type2 Diabetic subjects.	
	Elevated level of Escherichia associated with increased Wautersiella	
	(P:0.0009). The estimate of the regression is 2.29 with the SE:0.61.	
	Residual standard error: 18.22, Multiple R-squared: 0.38, Adjusted R-	
	squared: 0.36, F-statistic: 14.31.	
5.87	Figure represents the linear regression of the interaction of Escherichia	191
	with the genus Prevotella among the Type2 Diabetic subjects. Elevated	
	level of Escherichia associated with decreased Prevotella (P:0.04). The	
	estimate of the regression is -0.48 with the SE:0.23. Residual standard	
	error: 21.2, Multiple R-squared: 0.16, Adjusted R-squared: 0.12, F-	
	statistic: 4.49.	
5.88	Figure represents the linear regression of the interaction of Escherichia	192
	with the eGFR status among the Type2 Diabetic subjects. Elevated level	
	of Escherichia associated with decreased eGFR status (P:0.02). The	
	estimate of the regression is -0.2 with the SE:0.08. Residual standard	
	error: 20.63, Multiple R-squared: 0.20, Adjusted R-squared: 0.17, F-	
	statistic: 6.08	
5.89	Bray-Curtis diversity index (BCDI) of inter and intra group of study	195
	subjects. BCID reveals larger inter-individual distance among the DN	
	whereas BCDI significantly reduced for HC and DC. (A) Intergroup	

	BCDI significantly significantly higher among diabetic subjects (DC	
	and DN) compared to HC (P<0.0001). (B) Intra individual BCDI was	
	higher in HC/DN and DC/DN than HC/ DC	
5.90	Frequency of genus in Gut microbiota among study subjects	196
5.91	Principal component analysis of Diabetic and non diabetic subjects	196

## LISTS OF TABLES

Table No.	Description	Page
		No.
2.1	Global estimation of Diabetes: Estimated from 2017 and	14
	predicted for 2045	
2.2	Nobel Prize in the field of diabetes related work	17-18
2.3	Criteria for the diagnosis of diabetes	23
2.4	Epidemiology of diabetic nephropathy	28
2.5	Diagnosis and main clinical characteristics of Diabetic	29
	nephropathy based on urine albumin excretion	
2.6	Severity and diagnosis of DN according to Glomerular	31
	Filtration Rate and the clinical renal manifestation.	
2.7	Scoring system of interstitial and vascular lesions of DN	37
2.8	The most relevant web-based tools for miRNA sequence-	81-82
	based prediction.	
2.9	List of miRNAs involved in Vascular complications	83-84
2.10	Highly-abundant bacterial species in different disease	87
	conditions	
4.10	ADA/ICMR guidelines for diagnosis of diabetes and	96
	intermediate hyperglycaemia	
4.11	Diabetic nephropathy diagnosis and main clinical	97
	characteristics based on urine albumin excretion	
4.12	Severity and stages of DN according to GFR	98
	(ml/min/1.73m2)	
4.13	Spectrophotometer reading of total RNA	102
4.14	Genomic DNA elimination reaction components	103
4.15	Reverse transcription reaction components	104
4.16	Primers used for the detection of expression level for the	105-107
	targeted genes and housekeeping gene	
4.17	Preparation of reaction mix for real-time PCR for mRNA	108
	expression	
4.18	The amplification protocol of real-time PCR for mRNA	109

	expression	
4.19	List of miRNA and their target genes with target score from	110
	miRDB	
4.20	Preparation of reverse-transcription master mix for cDNA	111
	synthesised	
4.21	Reaction setup for real-time PCR	112
4.22	Thermal cycling conditions for real-time PCR	113
5.23	Distribution of Demographic and clinical variables of study	131-132
	subjects	
5.24	Relative quantification of Inflammasome complex and their	138
	targeting miRNA expression from Diabetic Control and	
	Diabetic Nephropathy patients taking healthy subjects as	
	reference.	
5.25	Relative quantification of TLRs and their targeting miRNA	143
	expression from DC and DN patients taking healthy subjects	
	as reference.	
5.26	Relative quantification of cytokines IL1 $\beta$ , IL18 and TNF- $\alpha$	145
	expression from Diabetic Control and Diabetic Nephropathy	
	patients taking healthy subjects as reference subject.	
5.27	Linear regression of relative quantification of Inflammasome	148
	complex m-RNA that includes NLRP3, CASP-1 and	
	PYCARD for DN severity based on estimated glomerular	
	filtration rate (eGFR).	
5.28	Linear regression of relative quantification of hsa-miR-223,	149
	hsa-miR-22-3p, hsa- miR-4291 and hsa-miR-185-3p miRNAs	
	targets Inflammasome complex mRNA with DN severity	
	based on eGFR.	
5.29	Linear regression of relative quantification of TLR m-RNA	153
	that includes TLR1, TLR2, TLR3, TLR4, TLR5, TLR6,	
	TLR7, TLR8, TLR9 and TLR10 for DN severity based on	
	estimated glomerular filtration rate (eGFR).	
5.30	Linear regression of relative quantification of hsa-miR-561-	154
	3p, hsa-miR-4307, hsa-miR-448 and hsa-miR-4760-3p	

	miRNAs targets TLRs with DN severity based on eGFR.	
5.31	Linear regression of relative quantification of inflammatory	155
	cytokines IL1 $\beta$ , IL18 and TNF $\alpha$ with DN severity based on	
	eGFR	
5.32	Correlation of mRNAs and its targeted mi-RNA expression	164
	among study subjects	
5.33	Linear regression of plasma IL1 $\beta$ , IL18 and TNF $\alpha$ cytokine	166
	with DN severity based on estimated glomerular filtration rate	
	(eGFR).	
5.34	Bacterial abundance of Genus Acinetobacter, Bacteroides,	184
	Bifidobacterium, Dialister, Escherichia, Faecalibacterium,	
	Lactobacillus, Prevotellaand Wautersiella among HC, DC	
	and DN study subjects	
5.35	Bacterial abundance of Gammaproteobacteria (Class),	185
	Enterobacteriales (Order), Enterobacteriaceae (Family) and	
	Escherichia (Genus) among HC, DC and DN study subjects	
5.36	Linear regression to predict the relative abundance of phylum,	193
	gene expression and clinical parameters with the genus	
	Escherichia	
5.37	Represents the Operational Taxonomic Units (OUT) and	194
	Alpha diversity (Shannon -diversity-index and Simpsion)	
	among HC, DC and DN study subjects	
5.38	Bray-Curtis dissimilarity index among intra-group and	194
	intergroup of study plot	