ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Genomic Approach to Identify Reference Basic Genes in Glucose Homeostasis along with the Immunohistochemical Profile of Diabetic and Non-Diabetic Human Pancreases in the Indian Population

Nithyakalyani Mohan[1]*, Anusha Sunder[2], Kandappa Himakar Reddy[3] and Ponnuraja C[4]

[1]*Transplant and Research, Apollo Hospitals, Chennai, Tamilnadu, India*

[2]*Research Associate, Apollo Hospital and Research Foundation, Chennai, Tamilnadu, India*

[3]*Molecular Biologist Associate, Siruseri Biotech Park for Women, Chennai, Tamilnadu, India*

[4]*Statistics, ICMR -National Institute for Research in Tuberculosis, Chennai, Tamilnadu, India*

***Corresponding Author:** Nithyakalyani Mohan, Senior Scientist, Transplant and Research, Apollo Hospitals, Chennai, Tamil Nadu, India.

Received: June 16, 2023; **Published:** July 12, 2023

Abstract

The pathogenesis of hyperglycemia observed in most forms of diabetes is intimately tied to the islet β cell. Impairments in pro-peptide processing and secretory function, along with the loss of these vital cells, is demonstrable not only in those in whom the diagnosis is established but typically also in individuals who are at increased risk of developing the disease. Due to ethical and practical difficulties, genomic or pathological data in Indian population are remarkably missing. Here we report genome transcript analysis validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and correlated with immunohistological observations for 21 pancreases. We analysed clinical-pathological features of 21 pancreas with the expression pattern of various genes which were involved in the glucose homeostasis in order to understand their prognostic value. We also investigated the pathological differences between diabetic and non-diabetic samples and the expression of insulin in each case along with pathological changes associated with beta cell density, expression, size, diameter etc. This study provides an insight into the complex pattern of the gene expression disturbances that occur in the diabetic and non-diabetic pancreas. Further on, we have observed the immunopathological and histochemical mechanisms that highlight a number of- inflammatory, immunoregulatory and regenerative pathways, some of which have received relatively little attention so far.

Keywords: *Diabetes; Beta Cells; Islets; Genes; Pathological; Glucose Homeostasis; Human Pancreas; Immunohistochemistry*

Introduction

In all vertebrates, maintenance of normal blood sugar levels is vital for life. The hormone, insulin, regulates glucose homeostasis at major metabolic sites like liver, muscle and adipose tissue. The pancreatic endocrine cells (α- and β-cells) predominantly regulate glucose homeostasis in an antagonistic manner. While α- cells respond to hypoglycemia with the release of glucagon resulting in the elevation of circulating glucose, β-cells secrete insulin at hyper-glycemic conditions leading to the reduction of plasma glucose levels. The efficiency of α- and β-cells to regulate plasma glucose levels relies on their ability of detecting changes in the extracellular glucose levels. The α- and

β-cells have special glucose transporters which continuously monitor extracellular concentrations of glucose and aid in quick adaptation during hormone secretion [1-3]. A substantial insight on the regulation of insulin secretion and beta-cell mass through various signaling pathways is essential in developing novel approaches for type 1 diabetes. And such therapeutic interventions include beta cell maintenance and replacement, islet transplantation, and stem cell therapy.

Beta cells are unique endocrine cells that synthetize, store and secrete insulin under the control of multiple and integrated signals, thus tightly regulating blood glucose concentrations. In the beta cell, insulin secretion stimulated by glucose is a multistep process. Initially the process requires transport and oxidation of glucose, followed by electrophysiological changes and culminating in the fusion of insulin- containing secretory granules into the beta-cell plasma membrane. The rate-limiting step of glucose metabolism starts when the Glucose enters the cell by facilitated diffusion mediated by glucose transporters (GLUT1 in humans). Inside the cell, glucose gets phosphorylated by glucokinase to form glucose-6-phosphate. The enzyme, glucokinase, is like a glucosensor for the pancreatic beta cell as it initiates glucose-stimulated insulin secretion. The kinetics of glucokinase tags it as a 'deciding factor' of glucose phosphorylation. Glucose phosphorylation activates and triggers an increase in Ca^{2+} inside the cell by mobilizing its intracellular stores [3-6]. This increase in Ca^{2+} causes a fusion of insulin-containing secretory granules with the plasma membrane, eventually leading to insulin release into the circulation. Direct assessment of expression of genes from freshly isolated islets in relation to some of the major underlying mechanisms and simultaneous examination of pancreatic tissue will provide us some clue for the future research for conceiving better prevention and treatment of diabetes by targeting pancreatic beta cells.

Evidence mounts in support of transplanting cadaveric human islets as an effective therapeutic mode for type 1 diabetes. Then again, evaluating the suitability of islet samples in a clinical setting is quite challenging. We hypothesized that islet quality can be reflected in the expression of specific genes which can be used as pre-transplant bio-signatures. In this research paper, we propose to understand the expression, regulation and function of normal and diabetes beta-cell function and beta-cell mass regulation, and related signals in the beta-cell milieu. To interpret the pathology more clearly and to advance in our understanding of the processes that leads to expression and regulation, it is necessary to have an extensive description of every change at the molecular level *in situ* [5-8].

Materials and Methods

Twenty-one human pancreases were collected according to protocols approved by Apollo Hospital and Velammal Medical College and Hospital. The protocols were designed according to the guiding principles approved by the Indian Council of Medical Research without affecting the Indian organ procurement program. Human adult pancreases were provided by Velammal Medical College and Hospital with consent to use for research purposes.

As soon as the tissue samples reached the laboratory they were divided into different portions depending on the weight of the pancreas received. Approximately three-quarters of each gland underwent digestion, while one-quarter was cut into small cubes in which one part was snap-frozen and kept in liquid nitrogen until RNA extraction. The other part was used for immunohistology studies.

Immunohistology

A surgically removed human tissue specimen was obtained and washed with saline. The specimens were fixed in 10% buffered formalin (pH 7.4). The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 4 μm paraffin sections by a rotator microtome. Hematoxylin and Eosin (H&E stain manufactured by Sigma-Aldrich Pty Ltd, an affiliate of Merck KGaA, Darmstadt, Germany) were used to stain these sections.

Immunohistochemistry

Three-micrometer-thick paraffin sections were mounted on positively charged slides and subjected to the immunohistochemical (IHC) procedure using a direct detection system (BioScience Bio SB, CA, USA). Sections were incubated with insulin antibody (manufactured

by Cell Signaling Technology, Inc. Alexa Fluor, a registered trademark of Life Technologies Corporation, headquartered in Danvers, Massachusetts, USA) for 30 minutes at room temperature followed by secondary HRP antibody. The IHC procedure was performed by manual method following the manufacturer's instructions. A light microscope (BX51, Olympus, Tokyo, Japan) with an in-built 'Olympus' digital camera was used to examine and photograph the slides.

Real time RT-PCR

Total RNA from the tissue was extracted and isolated with TRI Reagent (Sigma), We assessed RNA quantity and purity by using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific) and RNA was measured. We synthesized cDNA templates from RNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). TaqMan Fast Advanced Master Mix (Applied Biosystems) was used to perform real-time PCR, using 10-ng cDNA and 1 µL of TaqMan Gene Expression Assay (Applied Biosystems) in each well. 100 ng of RNA was used to perform RT-PCR on a Bio-Rad Gene Cycler. For each sample, we performed triplicate amplifications and used average measurements for data analysis.

Statistics

All the statistical analysis was performed using Graph-pad prism (Version 6). All the data that followed normality were expressed as Mean ± standard deviation, while Median was also considered for analysis where data deviated from normality. To determine the statistical significance between two unrelated groups (diabetic and non-diabetic), inferential tests (both parametric and non-parametric) like Independent T test, Mann-Whitney U test, Fishers' test and Linear by linear association test were used. The p-value has been chosen at 0.05 to test for significance in all tests, which means we established the 95% confidence intervals and 5% level of significance. Pearson correlation was done between the measured variables with $p < 0.05$ being considered as statistically significant. Rstudio version 2023.03.0+386 'Cherry Blossom' was used for the analysis.

Results

General profile of the study population

In the study population, non-diabetic subjects out-numbered their counterparts, and the following table (Table 1) and figure (Figure 1) support this statement.

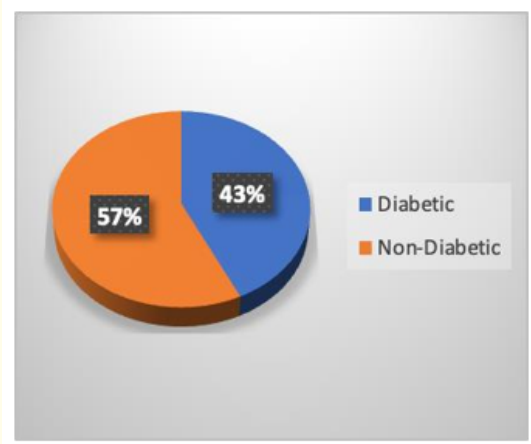

Figure 1: *Distribution of diabetic and non-diabetic subjects in the study population.*

Figure 1 shows the distribution of diabetic and non-diabetic subjects in the study population, clearly stating a majority of non-diabetes.

Category	Non-Diabetic	Diabetic	Total
Number	12	9	21
Percentage (%)	57.1	42.9	100

Table 1: *Distribution of diabetic and non-diabetic subjects in the study population.*

As shown in table 2, a majority of the study population (38%) were in the age group of '46 to 60' years with second-in-line being the 'less than 30' age group (33.3%). With regard to gender, males were predominant in the study population (71.4%).

Category	Age (years)					Gender		
	< 30	31 to 45	46 to 60	> 60	Total	Male	Female	Total
Number (total = 21)	7	4	8	2	21	15	6	21
Percentage (%)	33.3	19	38.1	9.5	100	71.4	28.6	100

Table 2: *Age and gender distribution of the study population.*

With regard to blood group, it is explicit with table 3, that, a majority (42.9%) were B+ve followed by O+ve (28.6%) and A+ve (23.8%). The following table (Table 3) and figure (Figure 2) reveal that a majority of the study population (47.6%) were having HbA1C levels between 5.6% to 8.0%; followed by 38% having a value of less than 5.5%; while 14.4% had the levels in the range of 8.1% to 10.0%.

Category	Blood group				HBA1C (%)			
	A+ve	B+ve	O+ve	Total	< 5.5	5.6 - 8.0	8.1 - 10.0	Total
Number (total = 21)	5	9	7	21	8	10	3	21
Percentage (%)	23.8	42.9	33.4	100	38	47.6	14.4	100

Table 3: *Characteristics like blood group and HbA1c of the study population.*

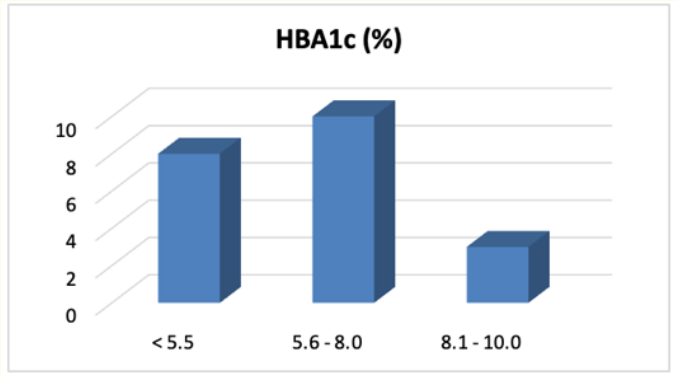

Figure 2: *HbA1c levels of the study population.*

Figure 2 reveals that a majority of the study population (47.6 %) were having HbA1C levels in the range of 5.6% to 8.0%.

Comparison between the groups for general profile

There was no statistically significant difference between the diabetic and non- diabetic groups with regard to parameters like, age, gender and blood group. This observation is supported by the following table (Table 4).

Variables		Diabetic	Non- Diabetic	P-Value
Age	Mean (SD)	42.1 (16.07)	42.8 (15.64)	.92@
	Median (Min, Max)	40 (18, 67)	41 (24, 76)	
	Standard Error (SE)	5.08	4.18	
Gender	Male	6	9	.393*
	Female	4	2	
Blood Group	O+ve	5	2	.176#
	A+ve	2	3	
	AB-ve	0	0	
	B+ve	3	6	

@: Independent t-test

*: Fishers' test

#: Linear by linear association test

Table 4: *Comparison between the groups for general profile.*

Comparison between the groups on gene expression

The assessment of islet functionality vitally considers the circadian regulation of glucose homeostasis and insulin secretion. A group of core clock genes inter-relate to from a transcription-translation complex. This in turn drives the molecular clock mechanism of various cells in a highly conserved manner. Therefore, insights on the molecular mechanisms that underlie islet function, as well as its disruption, are fundamental in disease prevention and transplantation.

A glucose sensor in pancreatic beta cells controls glucose homeostasis. Currently little evidence is available on the studies of human pancreatic beta cells and role of GLUT1, Glucokinase in mechanisms leading to diabetes. To understand and to have a better insight into the insulin secretory activity of beta cells in human pancreas, the expression of glucose transporter 1 (GLUT-1), Glucokinase and calcium signaling channels were studied. The following table (Table 5) and figure (Figure 3) shows that the expression of Glut1, Glucokinase and insulin were increased in human pancreas of non-diabetic samples when compared to diabetic samples, this observation was statistically significant ($p < 0.001$).

Calcium-binding proteins regulate transcription and secretion of pancreatic islet hormones. In islet cells, calcium signaling has a pivotal role as it dually regulates the secretion of insulin and glucagon. Thus, we investigated the presence of the calcium- binding protein DREAM and related family members in the human pancreas. To determine if DREAM is expressed in the pancreatic islet cells, total RNA from human pancreas were examined for DREAM (KChIP3) gene expression together with other similar KChIP family members via RT-PCR. The RTPCR of human pancreas and islet RNA with KChIP1, KChIP2, DREAM, and KChIP4 specific primers resulted in the dominant expression of genes in the non-diabetic samples when compared to diabetic samples. As shown in table 5 and figure 3, gene expressions

Variables		Non-Diabetic	Diabetic	P-Value
Insulin	Mean (SD)	31.76 (3.77)	24.9 (3.74)	<0.001
	Median [min, max]	29.8 [27.3, 39.3]	24.5 [17.4, 29.9]	
	Standard Error (SE)	1.26	1.08	
Glut 1	Mean (SD)	31.09 (4.50)	23.42 (3.26)	<0.001
	Median [min, max]	31.7 [23.3, 38.5]	23.8 [17.3, 28.8]	
	Standard Error (SE)	1.5	0.94	
GK	Mean (SD)	30.04 (2.49)	23.90 (3.27)	<0.001
	Median [min, max]	29.8 [27.3, 34.7]	24.2 [19.3, 31.5]	
	Standard Error (SE)	0.83	0.94	
KChiP 1	Mean (SD)	28.72 (4.23)	23.29 (3.41)	<0.01
	Median [min, max]	28.8 [22.1, 35.6]	23.8 [17.1, 27.9]	
	Standard Error (SE)	1.41	0.99	
KChiP 2	Mean (SD)	27.86 (5.64)	23.79 (3.62)	0.082
	Median [min, max]	28.7 [21.7, 36.8]	24.4 [17.6, 29.9]	
	Standard Error (SE)	1.88	1.04	
KChiP 4	Mean (SD)	29.42 (5.16)	23.23 (2.58)	<0.01
	Median [min, max]	29.9 [23.1, 39.9]	23.8 [18.1, 26.8]	
	Standard Error (SE)	1.72	0.74	
Dream	Mean (SD)	32.33 (4.71)	23.33 (2.99)	<0.001
	Median [min, max]	32.7 [26.2, 39.9]	22.9 [18.9, 28.7]	
	Standard Error (SE)	1.57	0.87	

Table 5: *Comparison between the groups on gene expression (Insulin, Glut1, Glucokinase/GK, insulin, KChIP1, KChIP2, DREAM/KChIP3, and KChIP4).*

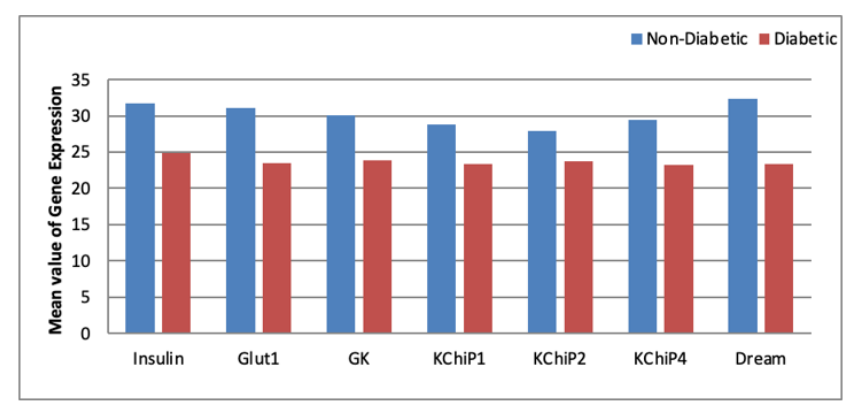

Figure 3: *Comparison between the groups on gene expression.*

Figure 3 shows that the expression of Glut1, Glucokinase, insulin, KChIP1, KChIP2, DREAM, and KChIP4 was higher in human pancreas of non-diabetic samples when compared to diabetic samples.

of KChIP1 ($p < 0.01$), DREAM ($p < 0.001$), and KChIP4 ($p < 0.01$) were significantly higher in the non-diabetic group, while KChIp2, though higher didn't show statistical significance. Based on table 5, the results presented here demonstrate the expression of the calcium-binding protein and its importance as a calcium-dependent transcriptional regulator within the pancreatic islet. This study has shown the basal levels of these genes in both non-diabetic and diabetic conditions for the first time in the Indian population.

Findings on immunohistology and immunohistochemistry

Studies on immunohistology were done to identify pancreatic beta cells morphology, location, characteristics, while related insulin expression was done using immunohistochemistry. We also assessed mean islet size, beta cell number, density, insulin positive area, and percentage of insulin positive area/islet cell. Figure 4 is a non-diabetic pancreas, which shows normal morphology of the acini and ducts. The Islets of Langerhans appear adequate and show normal morphology and seen dispersed among the pancreatic acini.

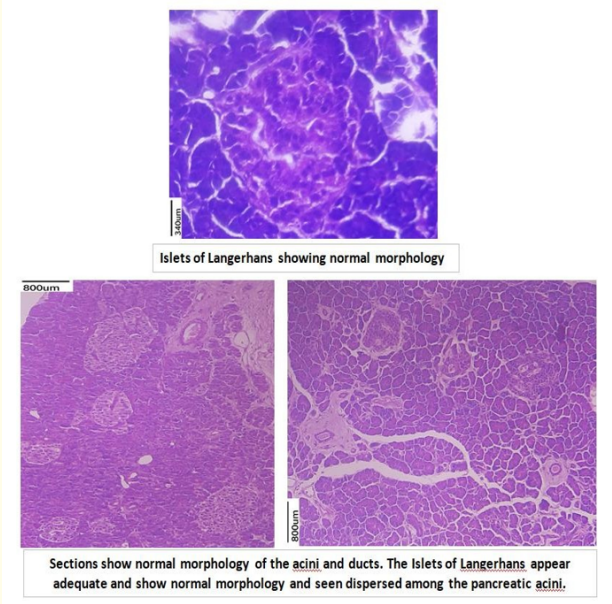

Figure 4: *Islet characteristics, and morphology of the acini and ducts in non- diabetic pancreas.*

Figure 4 shows normal morphology of the acini and ducts in non-diabetic pancreas, with adequacy of the islets as seen dispersed in the pancreatic acini.

The following figure (Figure 5) is a diabetic pancreas, showing normal morphology of the pancreatic acini and ducts, few ducts are dilated. The islets of langerhans are reduced in number and size. Only scattered Islets are seen.

Insulin expression on the diabetic and non-diabetic pancreases was done by immunohistochemistry.

Figure 6 explains on the increased percentage of positive cells in the non-diabetic samples.

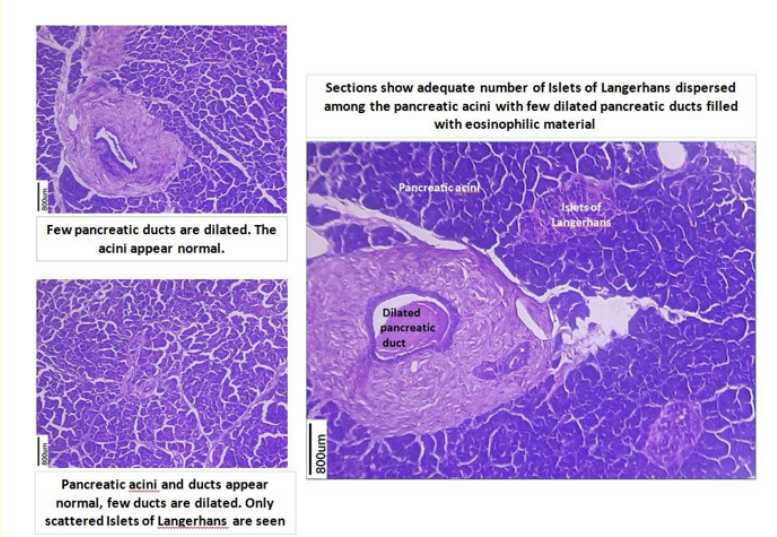

Figure 5: *Islet characteristics, and morphology of the acini and ducts in diabetic pancreas.*

Figure 5 shows diabetic pancreas, characterized by few ducts, and scattered islets being reduced in number and size.

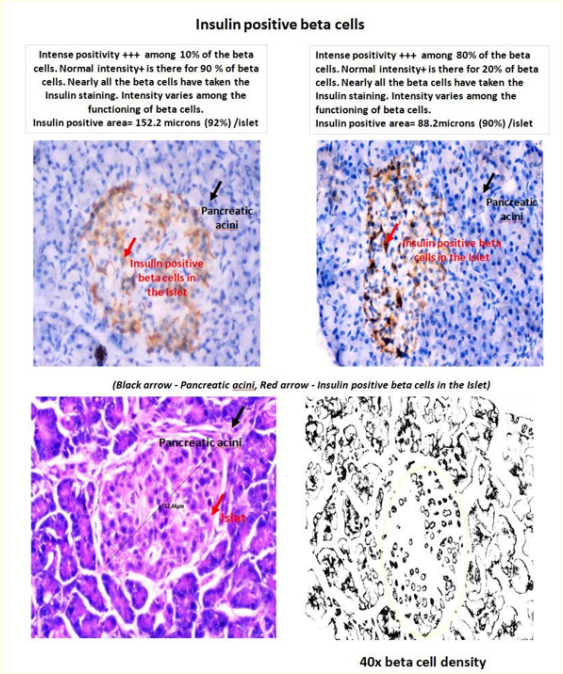

Figure 6: *Increased percentage of insulin positive cells in the non-diabetic samples.*

Figure 6 shows high percentage of insulin positive cells in the non-diabetic samples.

The following figure (Figure 7) shows the decreased percentage of positive cells in the diabetic population. It was interesting to note as the diameter of islet cell size increases the percentage of insulin positive area was decreased.

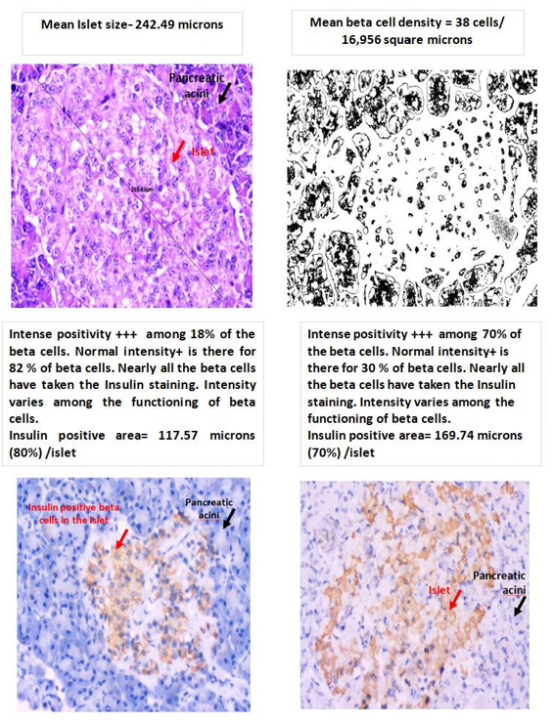

Figure 7: *Decreased percentage of insulin positive cells in the diabetic samples.*

Figure 7 shows decreased percentage of insulin positive cells in the diabetic samples due to their increased islet cell diameter.

Comparison between the groups on immunohistology and immunohistochemistry

Table 6 supported by figure 8, shows the mean islet size, beta cell number, density, insulin positive area, and percentage of insulin positive area/islet cell in both diabetic and non-diabetic population. Though mean islet size/diameter was increased in the diabetic group, the rest of the parameters, including beta cell number, insulin positive area and percentage of insulin positive area/islet cell were higher in the non-diabetic group. This could be explained by the fact that, the insulin positive area decreases as islet cell size increases; Figure 9 supports this negative correlation. A comparison between the groups on mean islet size/diameter and percentage of insulin positive area/islet cell showed statistical significance ($p < 0.01$).

Discussion and Conclusion

Our current knowledge regarding the transcriptional changes in the regulation of human pancreas and the changes associated with morphological events are unavailable in both diabetic and non-diabetic conditions for the Indian population. It is unanimously accepted that, there is a lot of variability in islet preparation, which in turn affects the islet quality. Islet quality consequently gets reflected in the expression of certain genes. Currently there is paucity in published research to explain about the basal gene expression of glucose homeostasis in the human pancreatic tissues [9-14].

Variables		Diabetic	Non-Diabetic	P-value
Mean Islet size/diameter	Mean (SD)	191.22 (48.6)	134.17 (40.54)	<0.01@
	Median [min, max]	176.7 [145.98, 289.32]	140.7 [63.23, 189.2]	
	Standard Error (SE)	15.37	12.22	
Beta cell number	Mean (SD)	0.018 (0.016)	0.016 (0.018)	0.92$
	Median [min, max]	0.015 [0.001, 0.044]	0.004 [0.002, 0.045]	
	Standard Error (SE)	0.004	0.005	
Insulin Positive area	Mean (SD)	152.78 (20.4)	166.42 (117.6, 178.6]	0.29@
	Median [min, max]	152.76 [117.6, 178.6]	175.34 [88.2, 199.3]	
	Standard Error (SE)	6.79	10.3	
Percentage of insulin positive area/islet cell	Mean (SD)	78.0 (9.3)	92.64 (2.1)	<0.01@
	Median [min, max]	80 [65, 95]	93 [90, 96]	
	Standard Error (SE)	3.1	0.62	
@: Independent t-test $: Mann-Whitney U test				

Table 6: *Comparison between the groups on immunohistology and immunohistochemistry.*

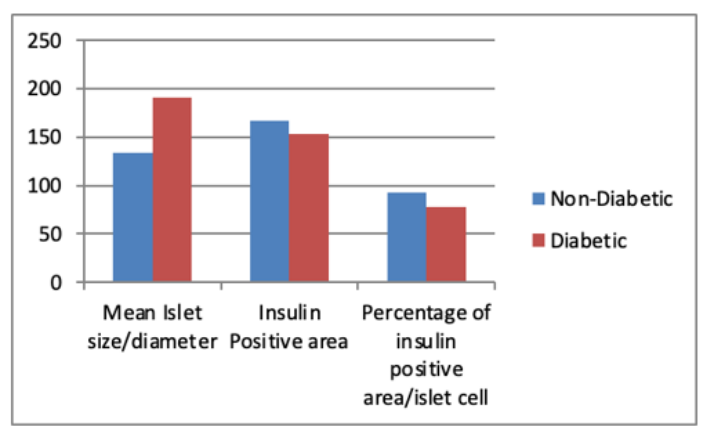

Figure 8: *Comparison between the groups on immunohistology and immunohistochemistry.*

Figure 8 shows higher mean islet size/diameter in the diabetic group. While beta cell number, insulin positive area and percentage of insulin positive area/islet cell were higher in the non-diabetic group.

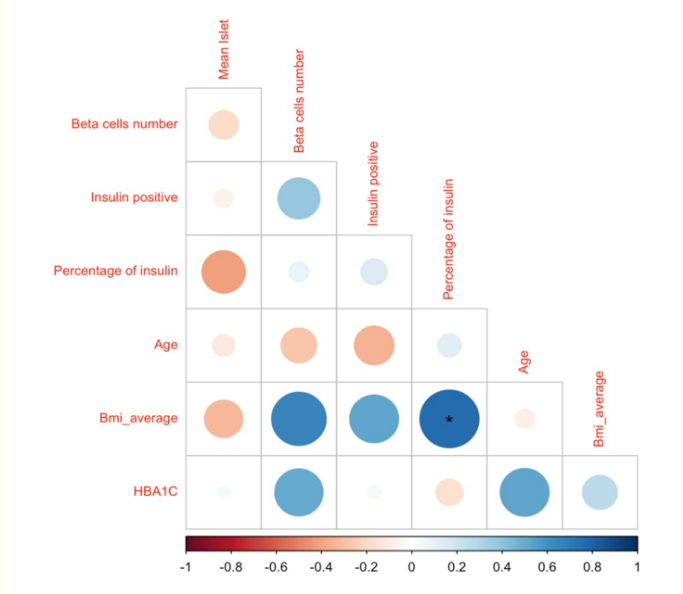

Figure 9: *Negative correlation between mean islet cell diameter and insulin positive area (shades of red show negative correlation). Figure 9 shows a significant negative correlation signifying a decrease in the insulin positive area as islet cell size increases.*

We hypothesized that beta cell functionality and the interaction of graft with host determine the effectiveness of islet graft, and these are governed by specific gene expressions in islet cells. Therefore, gene expression in the human pancreases was analyzed on functional basis so that this can be correlated for their functions with the clinical characteristics in our future studies. We have aimed to build a diagnostic tool to assess the quality of cell preparations prior to their use in clinical islet transplantation. To create islet gene signature it was also necessary to know about the gene expression even in the tissue levels before the isolation process. Thus, we have evaluated the gene expression by RT-PCR analysis with a subset of specific genes involved in the glucose homeostasis. Equally it is important to know the pathological conditions of this tissue and expression of insulin by immunohistochemistry during the diabetic and non-diabetic conditions.

Here we report whole genome transcript analysis validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) along with the immunohistological observations for these pancreases. We have found the basal level of expression in the given diabetic and non-diabetic pancreases. It was also interesting to see the pathological conditions and insulin expression in these pancreases. It was evident that insulin expression in diabetic samples has a direct role and there were some changes both in the mRNA and protein levels. The morphology, count of beta cells, percentage of insulin positive area in the diabetic sample varied significantly when compared to the non-diabetic sample. Altogether, we believe our current understanding of how beta cells can rebound from inflammatory damage provides reason for cautious optimism. It is now established that the majority of patients maintain at least a fraction of their functional beta-cell population, pointing towards the existence of powerful beta-cell survival pathways. Some of the studies have shown results similar to our study mentioning that the gene expression of glucose transporters and glucokinase has been found to be lower in human type 2 diabetic islets than in control preparations [15-19].

Gene expressions underlie molecular mechanisms, which in turn govern the harmony of cellular functions. A number of interlocked transcriptional and posttranslational feedback loops are responsible for generation and maintenance of rhythms. The expression of func-

tional and regulatory genes by islet cells is a key determinant for the success of islet transplantation. The aim with which the study was initiated is twofold: first, to characterize the cluster of genes expressed in human pancreases; and second in the future, to validate the capability of same gene panel to assess with accuracy and expression of various transcripts before the islet transplantation. In future, this data will highlight the importance of understanding the molecular and physiological basis governing islet functionality, immediately after isolation as well as before transplantation. For the clinician, the basal expression levels of relevant molecular parameters will notify on the islet functionality prior to its transplantation. Nevertheless, post-transplantation, these levels would vary based on the success of transplant surgery and also the circadian regulation.

There are several studies that highlights that beta cell compartments, functions, structures and molecules involved in the mechanisms leading to glucose-stimulated insulin release are impaired in human diabetes which is likely due to the interplay between the genetic architecture of the β cells and the role of environmental factors [16-24]. The pathology of type 2 diabetes in humans and certain features of beta cell dysfunction have shown how major *in vivo* insulin secretion defects can be recapitulated *ex vivo* through direct study of isolated islets. Islet study also gives the scope for assessing crucial biochemical and molecular traits which relate with functional impairment. Pathology studies have revealed fundamental beta cell characteristics in type 2 diabetes, such as alterations of β cell quantity and turn over as well as the presence of inflammatory traits. These are suggestive of unexpected β cell plasticity (comprising of trans- and de-differentiation) as a possible attempt for adaptation to metabolic stress. However, it has to be kept in mind that type 2 diabetes clinically shows heterogeneity as several variables may impact the quality of the pancreatic tissue and its isolated islet cells. Considering pancreatic tissue as the source, heterogeneity can arise from autoptic, surgical, bioptic variables from organ donors. Notably, there is a biological variation in isolation of human islets from donors with and without diabetes. While for isolated islet cells, the islet preparation itself can show variability with parameters such as, isolation stress, culture conditions and duration [20-27]. Noteworthy is a fact that, the outcome of islet transplantation in humans is significantly affected by collagenases as they decide the yield and quality of isolated pancreatic islet cells. A study conducted by Nithyakalyani., *et al*. 2022 concluded that the post-purification of islets in the Indian population, yielded better results with Serva collagenase NB1 compared to Vitacyte and Roche liberase. Islet cells' morphology (both free and intact), the islet yield and proportion per gram of pancreas were higher using serva collagenase compared to the other two enzyme blends [28]. Therefore, correct reporting of islet data may be challenging leading to potential inconsistencies in the experimental results. These unavoidable limitations, provides pathophysiological evidences and clues to conceive new strategies for better prevention and treatment of diabetes by targeting the β cells.

Our body's insulin levels as well as its efficiency of utilizing insulin are major influencers of diabetes pathophysiology. For the purpose of prediction, diagnosis or prognostics, biomarkers and genetics are used to understand the state of a biological process, severity of a pathological condition, and response to an intervention. This holds true for both research and clinical settings. Glucose homeostasis and insulin secretion are important factors to assess the functionality of islets. And thus, an insight on the molecular mechanism of islet functionality is pivotal in disease prevention and transplantation [29]. Hence it was interesting to initiate a study to assess the basal levels of islet functionality indices such as, GLUT receptor, Ca^{2+}, glucose kinase and Insulin expression immediately after the purification of islets are done. Such an understanding will give the clinician an idea on functionality of islets even before it is transplanted.

This study provides an insight into the complex pattern of the immune gene expression disturbances in addition to confirming the predicted immunopathological mechanisms that highlight a number of natural immunity- inflammatory, immunoregulatory and regenerative pathways, some of which have received relatively little attention so far. In conclusion, the markers presented here may also serve as a potential target in the management and seem to be playing a role in diabetes as well as in clear-cell pathogenesis. As such, it seems to be a good diagnostic and perhaps even a prognostic marker in the future islet cell isolation, function before the clinical transplantation, but warrants further investigation.

Conflict of Interest

We hereby declare that we have no conflict of interest of any form pertaining to our research study titled, 'Genomic Approach to Identify Reference Basic Genes in Glucose Homeostasis along with the Immunohistochemical Profile of Diabetic and Non-Diabetic Human Pancreases'.

Funding Support

We would sincerely thank the Indian Council for Medical Research (ICMR) [ICMR- 5/3/8/76/2020-ITR, 24-12-2020] for the encouragement and support extended to bring this process technology to India. Special Permission was given to use the pancreatic tissue for research after the ethical approval.

Acknowledgements

Indian Council for Medical Research (ICMR), New Delhi.

Apollo group of Hospitals, Chennai.

Apollo Hospital and Research Foundation, Chennai.

Velammal Hospital and Medical College, Madurai.

Biotechnology Industry Research Assistance Council (BIRAC).

Dr. Viswanadham D, Vice President, IKP.

IKP Knowledge Park, Telangana.

MS Swaminathan BioIncubation Centre.

Crescent Innovation and Incubation Council.

Bibliography

1. Chandra Rashmi and Rodger A Liddle. "Neural and hormonal regulation of pancreatic secretion". *Current Opinion in Gastroenterology* 25.5 (2009): 441-446.

2. Göke B. "Islet cell function: alpha and beta cells--partners towards normoglycaemia". *International Journal of Clinical Practice Supplement* 159 (2008): 2-7.

3. Cabrera Over., *et al*. "The unique cytoarchitecture of human pancreatic islets has implications for islet cell function". *Proceedings of the National Academy of Sciences of the United States of America* 103.7 (2006): 2334-2339.

4. Komatsu Mitsuhisa., *et al*. "Glucose-stimulated insulin secretion: A newer perspective". *Journal of Diabetes Investigation* 4.6 (2013): 511-516.

5. Khan AH and J E Pessin. "Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways". *Diabetologia* 45.11 (2002): 1475-1483.

6. Agius Loranne. "Hormonal and Metabolite Regulation of Hepatic Glucokinase". *Annual Review of Nutrition* 36 (2016): 389-415.

7. Affourtit Charles., *et al*. "Control of pancreatic β-cell bioenergetics". *Biochemical Society Transactions* 46.3 (2018): 555-564.

8. Rorsman Patrik and Matthias Braun. "Regulation of insulin secretion in human pancreatic islets". *Annual Review of Physiology* 75 (2013): 155-179.

9. Fadista João., *et al*. "Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism". *Proceedings of the National Academy of Sciences of the United States of America* 111.38 (2014): 13924-13929.

10. Wood Andrew R., *et al*. "A genome-wide association study of IVGTT-based measures of first-phase insulin secretion refines the underlying physiology of type 2 diabetes variants". *Diabetes* 66.8 (2017): 2296-2309.

11. Bae Jin-Sik., *et al*. "Transcriptional regulation of glucose sensors in pancreatic β- cells and liver: an update". *Sensors* 10.5 (2010): 5031-5053.

12. Dai C., *et al*. "Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets". *Diabetologia* 55.3 (2012): 707-718.

13. Del Guerra Silvia., *et al*. "Functional and molecular defects of pancreatic islets in human type 2 diabetes". *Diabetes* 54.3 (2005): 727-735.

14. Dorrell C., *et al*. "Transcriptomes of the major human pancreatic cell types". *Diabetologia* 54.11 (2011): 2832-2844.

15. Efrat S. "Making sense of glucose sensing". *Nature Genetics* 17.3 (1997): 249-250.

16. Eizirik DL., *et al*. "Major species differences between humans and rodents in the susceptibility to pancreatic beta-cell injury". *Proceedings of the National Academy of Sciences of the United States of America* 91.20 (1994): 9253-9256.

17. Ohtsubo Kazuaki., *et al*. "Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport". *Nature Medicine* 17.9 (2011): 1067- 1075.

18. Jamiolkowski Ryan M., *et al*. "Islet transplantation in type I diabetes mellitus". *The Yale Journal of Biology and Medicine* 85.1 (2012): 37-43.

19. Barton Franca B., *et al*. "Improvement in outcomes of clinical islet transplantation: 1999-2010". *Diabetes Care* 35.7 (2012): 1436-1445.

20. Roden Michael and Gerald I Shulman. "The integrative biology of type 2 diabetes". *Nature* 576.7785 (2019): 51-60.

21. Stumvoll Michael., *et al*. "Type 2 diabetes: principles of pathogenesis and therapy". *Lancet* 365.9467 (2005): 1333-1346.

22. Weyer C., *et al*. "The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus". *The Journal of Clinical Investigation* 104.6 (1999): 787-794.

23. Chatterjee Sudesna., *et al*. "Type 2 diabetes". *Lancet* 389.10085 (2017): 2239-2251.

24. Franks Paul W., *et al*. "Gene-environment and gene-treatment interactions in type 2 diabetes: progress, pitfalls, and prospects". *Diabetes Care* 36.5 (2013): 1413-1421.

25. Cerf Marlon E. "Beta cell dysfunction and insulin resistance". *Frontiers in Endocrinology* 4.37 (2013).

26. Srinivasan Shanthi., *et al*. "Glucose promotes pancreatic islet β-cell survival through a PI 3-kinase/Akt-signaling pathway". *American Journal of Physiology- Endocrinology and Metabolism* 283.4 (2002): E784-E793.

27. Nithyakalyani M., *et al*. "The establishment of pancreatic islet isolation in India – an update on human pancreatic islet transplantation". *Life Research* 5.4 (2022): 27.

28. Nithyakalyani M., *et al*. "Isolation of Human Pancreatic Islets from the Indian Population - Unveiling the Synergistic Interplay between different blends of Collagenases and Neutral Proteases". *Cardiometry* (2022): 243-251.

29. Nithyakalyani M and Anusha S. "Molecular Challenges and Advances in Clinical Islet Transplantation". Type 1 Diabetes Mellitus [Working Title], Intech Open (2022).

Volume 19 Issue 7 July 2023