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Research Article

Human Pancreatic Islet Gene View in glucose homeostasis of Diabetic and Non-Diabetic Pancreases in the Indian population

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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, are 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) for 21 pancreases. We analysed clinical-pathological features of 21 pancreases 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. This study provides an insight into the complex pattern of the gene expression disturbances that occur in the diabetic and non-diabetic pancreas.

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

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 Ca2+ inside the cell by mobilizing its intacellular stores [3-6]. This increase in Ca2+ 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. Our protocol design accorded with the guidelines of the Indian Council of Medical Research, and it abided by 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 (the results of which are presented in a separate paper).

The pancreata were harvested from the sacrificed rats after dissection, and were weighed and washed with saline. The specimens were stretched on filter paper and 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. The sections were stained withHematoxylin and Eosin (H&E) and with Masson trichrome stains. The pancreata were harvested from the sacrificed rats after dissection, and were weighed and washed with saline. The specimens were stretched on filter paper and 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. The sections were stained with Hematoxylin and Eosin (H&E) and with Masson trichrome stains.

Real time RT-PCR

Total RNA from the tissue was extractedand 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). We performed Real-time Polymerase Chain Reaction/ PCR with TaqMan Fast Advanced Master Mix (Applied Biosystems), 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. Triplicate amplifications were performed and the average values were used for analyzing individual sample data.

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.

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.

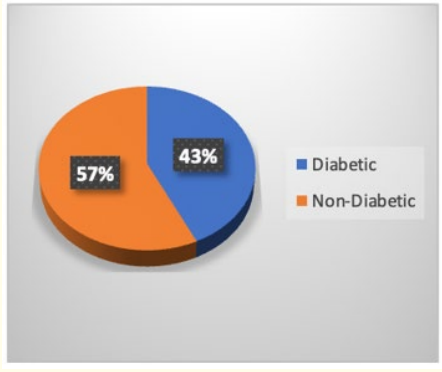

Figure 1: Distribution of diabetic and non-diabetic subjects in the study population.

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.

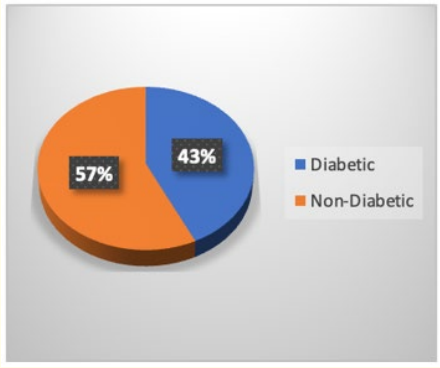

Figure 2: HbA1c levels of the study population.

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).

Comparison between the groups on gene expression

The assessment of islet functionality vitally considers the circadian regulation of glucose homeostasis and insulin secretion.

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.

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 ($p < 0.001$).

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	
KChiP1	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	
KChiP2	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	
KChiP4	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).

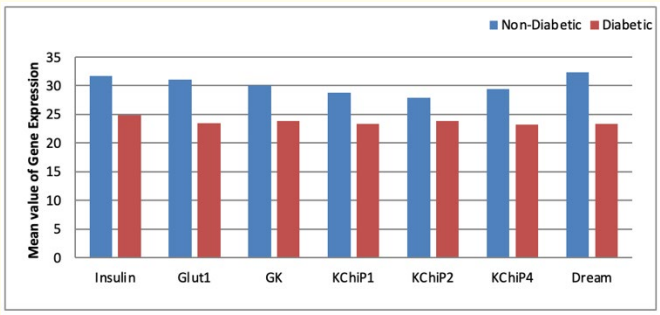

Figure 3: Comparison between the groups on gene expression.

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 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.

Discussion

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].

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.

The expression of functional and regulatory genes by islet cells is a key determinant for the success of islet transplantation.

Here we report whole genome transcript analysis validated by quantitative reverse transcription–polymerase chain reaction (qRT–PCR) 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 [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 functional 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.

Enormous research on beta cell structure and function has highlighted their pivotal role in diabetes through the impairment of glucose-stimulated insulin release. This impairment is likely due to the interplay between the genetic architecture of the β cells and the role of environmental factors [16-24]. 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 [25-29]. Hence it was interesting to initiate a study to assess the basal levels of islet functionality indices such as, GLUT receptor, Ca2+, 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, "Human Pancreatic Islet Gene View in glucose homeostasis of Diabetic and Non-Diabetic Pancreases in the Indian population".

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