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Original Research Article

A Histological Study of Beta Cell Mass in Head, Body and Tail Region of Cadaveric Pancreas among Diabetic and Nondiabetic People of Assam

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Abstract

Background: Pancreatic islets of Langerhans are characterized by spherical or ellipsoid clusters of cells embedded in the exocrine tissue. About 1-2 millions of islets present in different parts of human pancreas whereas maximum numbers present within the tail area. The beta cells are the most common and account for 50-80% of the cells in the islets. Beta cells mainly secrete insulin and alfa cells secret glucagon. Alteration in functioning of insulin and glucagon hampers the glucose homeostasis which leads to development of diabetes mellitus(DM). IHC technique applied to study beta cell mass in different parts of pancreas.

Materials and methods: The study was conducted in the Department of Anatomy, Gauhati Medical College, Guwahati. A total of 103 specimens of human pancreas were collected after taking institutional ethical clarification from both male and female cadavers using simple random sampling method.

Results & Observation: Distribution of beta cell mass in head, body and tail region of pancreas w.r.t. surface area of beta cell mass in IL of pancreas was done. Variable number of IL in different region of pancreas were analyzed which was more in tail region. ANOVA& F-value has proved that there is a highly significant difference among the 3 variables (head, body & tail). The 't'-value was highly significant in tail part and significant among head and body part.

Conclusion: The data generated in our study with respect to beta cell mass provides the understanding the pathogenesis of diabetes mellitus. Though the present study was done on 103 CPs, further studies including a greater number of samples and advanced stereological procedure will provide better information and knowledge. With the application of newer molecular technique, the detailed investigation of islets of Langerhans cells can be possible.

Keywords: Islets of Langerhans, Beta cells, Cadaveric pancreas, Pancreas

Introduction

Pancreatic islets of Langerhans are characterized by spherical or ellipsoid clusters of cells embedded in the exocrine tissue. About 1-2 millions of islets present in different parts of human pancreas whereas maximum numbers present within the tail area. [1]

The hormones secreted directly into the blood by the endocrine parts i.e. islets of Langerhans of pancreas control the energy metabolism and storage throughout the body. The islets of Langerhans contain seven specific hormone secreting cells namely Alfa, Beta, Delta, F (or PP-Pancreatic Polypeptide), D1, EC (enterochromaffin cells) and G1 (gastrin) cells. These are complex micro-organs involved in glucose homeostasis. [2] The beta cells are the most common and account for 50-80% of the cells in the islets. [3] Specifically, the total mass of pancreatic beta cells is a critical factor in the regulation of glucose homeostasis. Beta cells mainly secrete insulin and islet amyloid polypeptide (IAPP). Alfa cells secret glucagon. [4,5] Alteration in functioning of insulin and glucagon hampers the glucose homeostasis which leads to development of diabetes mellitus(DM). The pathologic basis of development of insulin dependent diabetes mellitus(IDDM) is based on the autoimmune damage to the β-cells of islets of Langerhans (IL) of endocrine pancreas. [6] The autoimmune destruction of beta cells leads to the development of gradual progressive endogenous insulin deficiency. [7] Specialized staining procedures or immunohistochemical (IHC) techniques are necessary to distinguish the three major types of cell namely alpha, beta and delta. [8] Hence IHC technique along with conventional H&E staining procedure applied to study beta cell mass in different parts of pancreas.

In obese and insulin resistant subjects the compensatory capacity of beta cells is credited to prevent from developing type-2 diabetes mallitus (T2DM). [9,10] In these individuals, an increase in insulin output via enhanced secretion and/or expanded beta cell mass counteracts the development of hyperglycemia and glucose intolerance. [10] Hyperglycemia and T2DM may develop if the adaptive response of beta cells to increased insulin resistance is insufficient or fails. In T2DM, similar to compensation the relative insulin deficiency involves changes in both beta cell mass and function. Although a few reports did not detect any difference in the beta cell mass of T2DM patients and nondiabetic (ND) [11,12], most studies agree on a significant reduction (24%-65%) in beta cell mass. [13-20]

Literature on study of human pancreas is not available from Assam as well as other part of the North-Eastern states of India. The study was done considering the seriousness of pancreatic diseases and the utmost importance of its correct diagnosis and treatment. The results of the present study are expected to be helpful in correlating its functional capacity for further study in basic science and in decision making in clinical settings.

Materials & Methods

The present study was conducted in the Department of Anatomy, Gauhati Medical College, Guwahati from 16th May, 2016 to 15th May, 2022. A total of 103 specimens of human pancreas were collected from both male and female cadavers in the age group of 13 to 78 years. Simple random samplings method was used for sampling.

The study was conducted after obtaining ethical approval from the Institutional Ethical Committee of Gauhati Medical College, Guwahati. Written informed consent was sought from all guardians or attendants of the eligible individual after explaining the purpose and detailed procedures of the study before collecting the specimens. All the guardians or attendants of the cadavers were assured confidentiality of data obtained from the study.

Inclusion criteria:

1. Normal specimens of cadaveric and autopsy pancreas with intact viscera.

Exclusion criteria:

1. Any visible signs of pathological changes of the viscera

2. Any doubtful injury in pancreas

3. Death due to known poisoning

4. Pancreatic diseases

5. Specimens of medico legal cases.

Collection was done within 12 to 36 hours of death. During collection, approximate age, sex and cause of death were noted from record book. Then each specimen was marked with a code number for individual identification. The specimens were collected along with duodenum and spleen. After removal from the body, unwanted tissues were cleared and gently washed out in normal saline.

A midline incision was done to expos the abdominal contents. The coils of intestine were retracted and the pancreas was identified. Then the tail and body of the pancreas were turned to the right, stripping the splenic artery and vein from its posterior surface. After identifying the superior mesenteric vessels, portal vein and gastroduodenal artery they were detached from the pancreas. [21]

The pancreases were washed with tape water to clean the debris and the fatty tissue. All the blood and clots were removed from the surface of pancreas. The specimens were again washed in normal saline. The pancreases were preserved in 10% formalin solution. Selection of the tissue was done according to Wolfe-Coote & duToit[22] as head, body and tail. Each part of the fixed tissue was again sectioned into 3 mm thickness. Then, sectioned tissues were cut into 3 mm × 3 mm size with scalpel. The sections were processed following the standard operating procedure. Mounting with DPX were done after both Hematoxylin and Eosin (H&E) and Immunohistochemistry (IHC) staining of the same tissue section. [22]

Measurement of islets of Langerhans in different regions:

The tissue sections were taken from head, body and tail regions (total 3 regions) from the CPs. The average diameter and surface area of islets of Langerhans was measured by using Nikon 80i Binocular microscope with image analyzing software and their mean was recorded and entered in the MS excel sheet. The standard methods of statistical analysis were performed by adopting the statistical software Graph Pad InStat Data which were shown as mean ±SD, unless it was stated otherwise.

Results & Observation

Distribution of beta cell mass in head, body and tail region of pancreas w.r.t. surface area of beta cell mass in IL of pancreas was done. Therefore, variable number of IL in different region of pancreas (head, body and tail) were analyzed (table 1).

Table 1: Mean & SD values of the variable number of IL (head, body and tail) of 103 samples of CPs according to Diabetic Status

Diabetic Status	Number	IL (Head) Mean±SD	IL (Body) Mean±SD	IL (Tail) Mean±SD
DM	18	1.06±0.54	1.61±0.78	3.11±1.323
NDM	85	1.35±0.50	2.04±0.57	4.22±0.76

It was more in tail region than head and body region in both DM and ND CPs. Then, it was analyzed whether there was any significant difference among the variables in the 18 DM and 85 ND CPs. So, One Way Analysis of Variance (ANOVA) i.e., F-test has been applied (table 2).

Table 2: ANOVA in 3 variables (head, body and tail) of IL among 18 CPs with DM status and 85 CPs with ND status& their F-Value

Sources		Degrees of freedom (df)	Sum of Squares (SS)	Mean Squares (MS)	F-value
Between variables	18 CPs with DM	3-1=2	40.704	20.352	18 CPs with DM: 23.06** (p<0.0001)
	85 CPs with ND	3-1=2	382.34	191.17	
Within Variables	18 CPs with DM	53-2=51	45.000	0.8824	85 CPs with ND:496. 34** (p<0.0001)
	85 CPs with ND	254-2=252	97.059	0.3852	
Total	18 CPs with DM	54-1=53	85.704	-	-
	85 CPs with ND	255-1=254	479.399	-	-

**→ Highly significant among the 3 variables in both DM and ND.

ANOVA& F- value has proved that there is a highly significant difference among the 3 variables (head, body & tail) of IL among 18 CPs with DM and 85 CPs with ND. So, 't' - test has been applied to test the significant differences of all these 3 variables one by one according to DM status (table 3).

Table 3: Mean & SD values of number of IL (in head, body and tail) among 103 samples of CPs according to DM status & their 't'-value.

Parts of pancreas	DM Status	Number	Mean	SD	df	t-value	Significant/Non significant
Head	DM	18	1.06	0.54	101	2.244*	*Significant
	ND	85	1.35	0.50			
Body	DM	18	1.61	0.78	101	2.695*	*Significant
	ND	85	2.04	0.57			
Tail	DM	18	3.11	1.32	101	4.862**	**Highly Significant
	ND	85	4.22	0.76			

*&** →The 't'-value was highly significant in tail part and significant among head and body part

To analyze the distribution of beta cell mass in head, body and tail region among entire 103 CPs it was evaluated the mean & SD values with 95% CI of beta cell and mean & SD values with 95% CI of beta cell surface area (Table 4). In both the beta cell mass diameter(dm) and surface area among the entire 103 CPs the highest mean and 95% CI were in tail region and lowest mean and 95% CI were in body region.

Table 4: Mean & SD-values of Beta Cell mass diameter(dm) and surface area among the entire 103 CPs as per mean dm in µ /LPF(10X) and µ²/LPF (10X) respectively

Beta Cell in		Mean	SD	95% CI
Head	dm in µ /LPF (10X)	99.86µ	48.31 µ	90.41 – 109.31
	Surface area in μ^2/LPF (10X)	10610.44 μ^2	8871.23 μ^2	8874.50 –12346.00
Body	dm in µ /LPF (10X)	93.26 µ	38.09 µ	85.81 – 100.72
	Surface area in μ^2/LPF (10X)	7738.44 μ^2	5426.61 μ^2	6676.50 – 8800.40
Tail	dm in µ /LPF (10X)	143.80 µ	37.98µ	136.37 – 151.23
	Surface area in μ^2/LPF (10X)	18758.87 μ^2	8126.81 μ^2	17169.00-20349.00

‘t’-test was applied whether there was any significant difference in various parameters of beta cell w.r.t. diameter among the 103 CPs a between the diabetic (DM) and non-diabetic (ND) CPs (table 5).

Table 5: Mean & SD values of beta cell in head, body and tail w.r.t. diameter among the 103 samples of CPs according to diabetic status & their ‘t’-value

Parts of pancreas	Diabetic Status	N	Mean ± SD	d.f	t-value	Significant/ Non-significant
Head	DM	18	75.31 ± 43.99	101	2.430*	Significant
	ND	85	105.06 ± 47.81			
Body	DM	18	81.44 ± 28.77	101	1.457	Not Significant
	ND	85	95.76 ± 39.48			
Tail	DM	18	114.62 ± 36.54	101	13.308**	Highly Significant
	ND	85	149.98 ± 35.50			

df’ and ‘t’-value between DM and ND which were significant, not significant and highly significant in the head, body and tail part respectively. The surface area(μ^2/LPF (10X)) of beta cell were found to be highly significant, not significant and highly significant respectively (table 6).

Table 6: Mean & SD values of surface area of beta cell mass in head, body and tail among the entire 103 samples of CPs in µ2/LPF (10X) according to diabetic status & their ‘t’-value

Parts of pancreas	Diabetic Status	N	Mean± SD	df	t-value	Significant /Not significant
Head	DM	18	5723±7208.50	101	2.647**	**Highly Significant**
	ND	85	11645.31 ± 8879.50			
Body	DM	18	5884.98 ± 3197.40	101	1.607	**Not significant**
	ND	85	8130.34 ± 5726.90			
Tail	DM	18	11975.38± 7317.60	101	4.205**	**Highly Significant**
	ND	85	20195.37 ± 7577.10			

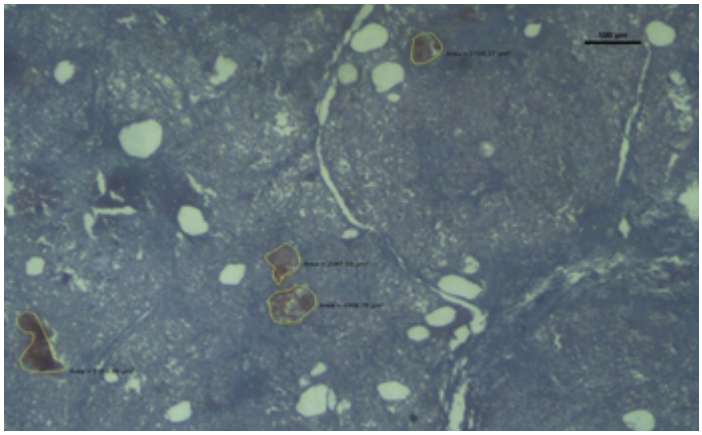

Figure 1: Photomicrograph of pancreatic lobule showing IHC stained beta cells (tail). Islets of Langerhans are demarcated with yellow lines whereas beta cells are brown in color. Scale bar, 100 µm.

Discussion

The mean & SD values of beta cell per mean diameter in µ /LPF(10X) were 99.86±48.31 µ, 93.26±38.09 µ, 143.80±37.98 µ and surface area in µ2 /LPF (10X) were 10610.44±8871.23 µ2 , 7738.44±5426.61 µ2, 18758.87±8126.81 µ2 among entire 103 CPs in head, body & tail region respectively. The 't'- value between DM and ND of beta cell w.r.t. diameter is significant, not significant and highly significant respectively in different region. Again, surface area of beta cell mass was highly significant in head and tail region, whereas it is not significant in body region. The present study was comparable with Ravi PK et al. [23], Ahmed HH et al. [24], Wang X et al. [25], Inaishi J et al. [26], Kilimnik G et al. [27]. Ravi PK et al. [23] mentioned in their study that majority of the islets were within the range of 25-50 µm. The mean effective islet diameter in the head, body and tail were 35.80 ± 0.1µm, 35.21 ± 0.1 µm and 43.87 ± 0.14 µm respectively. The paired t-test showed that the islet diameter in the tail was significantly higher than that of head and body ($P < .0001$). Ahmed HH et al. [24] reported in their study among Iranian (Turkish) population the diameter in µm were 102±2, 107±3 and 103±12 in head, body and tail region in 31- 40 years age group. The diameter was maximum in every age group in tail region than head and body region. In the study done by Ravi PK et al. [23], the endocrine proportion of the tail was 40% more in Indian population and Wang X et al. [25] reported 100% more than that of the head in the non-diabetic American population. Ravi PK et al. [23] again mentioned that reduction of the islet in the tail region may be one of the reasons for the increased susceptibility of Indians to T2DM. Inaishi J et al. [26] commented that even though Indians have 20% more beta cell area proportion and 7% more beta cell percentage than Americans, still Indians are more susceptible to diabetes. Thus, beta cell dysfunction might be a reason for the increased susceptibility of the Indians to DM. Kilimnik G et al.[27] reported the preferential loss of larger islets in T2DM pancreas when compared to non-diabetic pancreas. Therefore, Ravi PK et al.[23] mentioned that reduction in larger islet proportion might be one of the factors responsible for the increased susceptibility of the Indians to T2DM even though the beta cell percentage are higher than the American population.

In the present study, beta cell mass loss was studied and tried to grade accordingly. Hence, it was studied as number of IL in diabetic status and beta cell mass loss and grade accordingly. In the present study, the mean and SD values of the variable number of IL in head, body and tail region

among 103 CPs according to DM and ND status. The values of DM were 1.06±0.54, 1.61±0.78 and 3.11±1.323 and of ND were 1.35±0.50, 2.04±0.57 and 4.22±0.76 respectively. It is more in tail region than head and body region in both DM and NDM CPs. The F- value proved that there was a highly significant difference among the 3 variables (head, body & tail) among 18 DM CPs and highly significant among 85 ND CPs. Again 't'- value between DM and ND in the head and body region were significant and it was highly significant in tail region. The present study is comparable with Shahriah S et al.[28], Wang X et al.[25], Ravi PK et al.[23], Wittingen J et al.[29], Kilimnik G et al.[27], Kim A et al.[30], Unger RH et al.[31] Shahriah S et al.[28] studied among Bangladeshi population and reported that number of islets per unit area of microscopic field were more in tail part all in all age group than the head and tail region. In their study the maximum number 3.20±0.45 were in tail region among ≥70 years age group and 1.00±0.00 were in head region among 60-69 years age group. Wang X et al.[25] while studied among American population found larger islets (>50 μm) in head, body and tail percentage wise whereas in the tail region was more than the head and body region which was comparable with the study done by Ravi PK et al. [23]among Indian population. Wittingen J et al. [29] also reported in their study as the number of IL on a circular cross section 6 mm in diameter among DM population as head (17.1), body (26.9) and tail (39.3) region and among ND population were 25.544±9.888 in head, 28.067±8.795 in body region and 45.700±17.037 in tail region. In their study it has shown that the islet concentration of the tail is significantly greater than the concentration in the head and body. Kilimnik G et al. [27], Kim A et al [30], Unger RH et al. [31] also found significantly more number of islets in the tail region than head and body region in their study.

A significant difference among the 3 variables (head, body & tail) among the DM status one way Analysis of Variance (ANOVA) i.e., F-test has been applied which was highly significant among the 3 variables. The present study has been compared with the study done by Rahier J et al. [32], Saito K et al. [33], Shahriah S et al. [28], Wang X et al. [25], Ravi PK et al. [23], Butler AE et al. [34], Yoon KH et al. [35]. Rahier J et al. [32] revealed that the average beta cell mass is about 39% lower in type 2 diabetic subjects compared with matched controls. They found that beta cell mass do not correlate with age but it decreases with duration of clinical diabetes. They also mentioned as 24 % lower than controls in subjects <5 years and 54% lower than controls in subjects >15 years of overt diabetes respectively. Saito K et al.[33] studied 28 samples of non-diabetic cases. The total beta cell volumes do not decrease with age. However, when the ratio between alpha and beta cells were examined, it tends to reduce with age, though it was not significant statistically. Shahriah S et al.[28] reported that the number of beta cells gradually increased up to the 4th decade of life in head and body region and to the 6th decade in the tail region, then decreased as age increases. In comparison to the American population as studied by Wang X et al. [25] an Indian study by Ravi PK et al. [23] showed a 45% reduction of larger islet proportion in the head and body region with a 31.9% reduction in the tail region. Butler AE et al. [34] have reported that beta cell mass as well as beta cell function is decreased in patients with T2DM. They have reported an approximately 65% beta cell mass decrease in people with T2DM compared with ND for age and BMI. Yoon KH et al. [35] and Rahier J et al.[32] have also reported that beta cell mass in patients with T2DM is reduced by 30-40%.

Conclusion

The data generated in our study with respect to beta cell mass provides the understanding the pathogenesis of diabetes mellitus. Though the present study was done on 103 CPs, further studies including a greater number of samples and advanced stereological procedure will provide better information and knowledge. With the application of newer molecular technique, the detailed investigation of islets of Langerhans cells can be possible.

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