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Original Research Article

A Histological Study of Number of Islets of Langerhans in Head, Body and Tail Region of Cadaveric Pancreas among Diabetic and Nondiabetic Assamese People

Baneswar Baro[1], Dipali Hazarika[2], Bornali Hazarika[3], Usha Sarma[4], Gunamani Rabha[5]

[1]Associate Professor, Department of Anatomy, Diphu Medical College, Diphu, Assam, India.
[2]Associate Professor, Department of Anatomy, Assam Medical College, Dibrugarh, Assam, India.
[3]Associate Professor, Department of Anatomy, Gauhati Medical College, Guwahati, Assam, India.
[4]Professor, Department of Pathology, Gauhati Medical College, Guwahati, Assam, India.
[5]Assistant Professor, Department of Anatomy, Diphu Medical College, Diphu, Assam, India.

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Corresponding author: Dr. Gunamani Rabha
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Abstract

Background: The pancreas is a specialized organ composed of both exocrine and endocrine glandular tissue. The exocrine pancreas occupies most of its parenchyma in which pancreatic islets of Langerhans (endocrine cells) are embedded. An islet is a mass of polyhedral cells, located in the vicinity of fenestrated capillaries and a rich autonomic innervation. Beta cells mainly secrete insulin and islet amyloid polypeptide (IAPP). Alfa cells secret glucagon. Alteration in functioning of insulin and glucagon hampers the glucose homeostasis which leads to development of diabetes mellitus.

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

Results & Observation: In different region (head, body, and tail) of pancreas variable number of Islets of Langerhans (IL)are analyzed and the mean and SD values among 103 samples of cadaveric pancreas(CP)s according to diabetic status. It is more in tail region than head and body region in both diabetic(DM) and nondiabetic (NDM) CPs. The ANOVA i.e. F- value has a highly significant among 18 DM & 85 NDM CPs.

Conclusion: More number of samples and advanced stereological procedure will provide better information and knowledge. The data generated in our study with respect to beta cell mass provides the understanding of the pathogenesis diabetes mellitus. With the application of newer molecular technique the detailed investigation of islets of Langerhans cells can be possible.

Keywords: Pancreas, Islets of Langerhans, Beta Cells, Cadaveric Pancreas.

Introduction

The pancreas is a specialized organ composed of both exocrine and endocrine glandular tissue. The exocrine pancreas occupies most of its parenchyma in which pancreatic islets of Langerhans (endocrine cells) are embedded. [1,2,3,4] The endocrine pancreas occupies approximately 1-2% of the entire parenchyma. [5] It is composed of pancreatic islets of Langerhans, characterized by spherical or ellipsoid clusters of cells embedded in the exocrine tissue which is a mass of polyhedral cells, located in the vicinity of fenestrated capillaries rich autonomic innervation. Though the numbers of islets in human pancreas is about or more than 1-2 millions, its distribution within the different parts of pancreas varies with maximum numbers present within the tail area. [6]

The endocrine pancreas secretes hormones directly into the blood to control energy metabolism and storage throughout the body. These are complex micro-organs involved in glucose homeostasis. The IL contain seven specific hormone secreting cells namely Alfa, Beta, Delta, F (or PP-Pancreatic Polypeptide), D1, EC (enterochromaffin cells) and G1 (gastrin) cells. [7] The Beta cells are the most common and account for 50-80% of the cells in the islets. [8] Pancreatic islets are comprised of a few cells or many hundreds of polygonal cells arranged in short irregular cords that are abundantly invested with a network of fenestrated capillaries. 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. [9,10] Alteration in functioning of insulin and glucagon hampers the glucose homeostasis which leads to development of diabetes mellitus. The pathologic basis of development of IDDM development is based on the autoimmune damage to the β-cells of islets of Langerhans (IL) of endocrine pancreas. [11] The autoimmune destruction of beta cells leads to the development of gradual progressive endogenous insulin deficiency. [12] Inflammation, beta cell destruction and alpha cell expansion are the key pathologic change noted in diabetes mellitus. Insulitis characterized by infiltration of inflammatory cellular infiltrate affecting the islets of Langerhans has been regarded as the characteristic lesion of recent onset Type I (insulin-dependent) diabetes mellitus. Recent studies have stated that pathological changes in the pancreatic islets like hyaline degeneration [13,14], inflammation [15,16], beta cell loss [17,18] and alpha cell expansion [19] were associated with the DM. Hence localization of alpha & beta cell mass within the islets of Langerhans provided a detailed status of glucose homeostasis on this background, a cross sectional descriptive study has been undertaken with the aim & objective to study the expression of anti-insulin antibody in islets of Langerhans of pancreas with the help of immunohistochemical staining also to look for age related change of endocrine pancreas. Specialized staining procedures or immunohistochemical techniques are necessary to distinguish the three major types of cell, designated alpha, beta and delta. [1]

Obesity and insulin resistance remain major risk factors for Type-2 diabetes mellitus (T2D) [20], the compensatory capacity of beta cells is credited to prevent most obese and insulin resistant subjects from developing T2D. [21,22] When the adaptive response of beta cells to increased insulin resistance is insufficient or fails hyperglycemia and T2D develop. Similar to compensation, relative insulin deficiency in T2D involves changes in both beta cell mass and function. By now, ample data is available that describes the occurrence of beta cell loss in T2D. Although a few reports did not detect any difference in the

beta cell mass of T2D patients and nondiabetic controls [23,24] but most studies agree on a significant reduction in beta cell mass that ranging from 24% to 65%. [25-32]

Materials & Methods

Place of study: The present study has been conducted in the Department of Anatomy, Gauhati Medical College, Guwahati from 16th May, 2016 to 15th May, 2022.

Study Design: An appropriate choice in study design helps the researchers to execute the study in a way that help the researchers to arrive at an appropriate study conclusion. [33,34]

Simple random samplings method is used for sampling.

Ethical approval for conducting the study: The study is conducted after obtaining ethical approval from the Institutional Ethical Committee of Gauhati Medical College, Guwahati. Written informed consent is sought from all guardians or attendants of the eligible individual after explaining the purpose and detailed procedures of the study before collecting the specimens with assurance confidentiality of data obtained from the study.

Inclusion criteria:

1. Well preserved specimens of cadaveric and autopsy pancreas with intact viscera.
2. Diabetic status of the cadaver were collected from the legal guardian or party who donated the cadaver to the department and the data was entitled in the proforma .

Exclusion criteria:

1. Putrefacted or autolyzed viscera and pancreas
2. Any doubtful injury in pancreas
3. Death due to known poisoning.
4. Patient having known history of pancreatic diseases.
5. Specimens of medico legal cases.

Collection of specimen:

A total of 103 specimens of human pancreas are collected from Department of Anatomy and Department of Forensic Medicine, Gauhati Medical College, Guwahati with male:female ratio being 80:23 cadavers in the age group of 13 to 78 years.

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

Instruments and Materials:

1. Dissecting box (with pointed, plain and toothed forceps, scalpels and scissors).
2. Trays and surgical gloves.
3. 10% buffered formaline.
4. Jars and buckets (for storage of specimen).
5. Normal saline (0.9% w/v sodium chloride).

Process of dissection:

A midline abdominal incision is made to expose the abdominal contents. The intestinal coils are retracted and the pancreas is identified in the epigastric and left subphrenic region. Then the tail and body of the pancreas are 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 are detached from the pancreas. [35]

The pancreases are washed with tape water to clean the debris and the fatty tissue. All the blood and clots are removed from the surface of pancreas. The specimens are again washed in normal saline.

The pancreases are preserved in 10% formalin solution (Formalin = 40% solution

of formaldehyde in water, 10% Formalin = 10 parts formalin with 90 parts water)

Procedure for histological study:

Selection of the tissue is done according to Wolfe-Coote & duToit [36] as head, body and tail. Each part of the fixed tissue is again sectioned into 3 mm thickness. Then, sectioned tissues are cut into 3 mm × 3 mm size with scalpel. The sections are processed following the standard operating procedure.

Tissue Processing & Sectioning:

(i) Dehydration: The removal of unbound water and aqueous fixatives from the tissue components are carried out by passing the tissue through ascending grades of alcohol (50%, 70%, 95% and finally 100% alcohol).

(ii) Clearing: Removal of dehydrating agent is done by passing the tissue through xylene. The purpose of this step is to make the tissue components receptive to the infiltrating medium.

(iii) Impregnation: The tissue is impregnated with wax, which forms matrix preventing tissue structure distortion during microtomy.

(iv) Embedding tissues in paraffin blocks: Paraffin embedding of the impregnated tissue is done which involves the enclosing of properly processed & correctly oriented specimens in a support medium that provides external support during microtomy.

Microtomy & staining of tissue section:

The paraffin block is placed in the block holder and it is advanced closer to the blade. The ruff trimming of paraffin section has been started until a complete section is seen in the block. Utilizing a clean piece of a microtome blade, a ribbon is obtained at a thickness of 3 µm; the ribbon is picked up with a paint brush (Camel hair) and transferred to water bath at 35-37°C. Then the ribbon is laid on the water bath and the sections are allowed to stretch for a few seconds. Carefully the sections are separated and each section is picked up on to an albumenized glass slide. The slides are tilted at an angle to allow the water to exit the slide and section. The glass slides are then placed in a warm oven for about 15 minutes to help the section adhere to the slide.

Staining of tissue section:

The embedding process is reversed in order to get the paraffin wax out of the tissue and to allow water soluble dyes to penetrate the sections. Therefore, any staining can be done after the slides are "deparaffinized" by running them through xylenes (or substitutes) to alcohols and water. As per protocol few sections were stained with Hematoxylin and Eosin (H&E) and few good slides were stained with Immunohistochemistry (IHC) Staining Technique.

Mounting:

Using forceps, slides from the rack are removed and surplus xylene is drained onto a tissue paper; gently slides containing the section are lowered onto the drop of DPX so that the section is sandwiched between its slide and the cover-slip. Slide is turned over and DPX is allowed to spread between the section and cover-slip. Using a paper tissue, surplus DPX is wiped off the slide, around the cover-slip and left to dry.

For the purpose of counting the number of islets, the slides are viewed under low magnification (×10 objectives, ×10 eyepiece) using compound microscope (Olympus CH20, Singapore). The average number of islets per unit area of microscopic field for each slide is calculated and their mean is recorded and entered in the MS excel sheet.

The Statistical Analysis:

The cadaver‘s demographic and clinical data are collected from the departmental record book and data entry is carried out by using Microsoft office excel 2010. The standard methods of statistical analysis is

performed by adopting the statistical software Graph Pad In Stat Data are shown as mean±SD, unless it is stated otherwise.

Results & Observation

In different region(head, body, and tail) of cadaveric pancreas(CP)s variable number of Islets of Langerhans (IL) are analyzed. Following table is being prepared for this purpose.

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

Interpretation: Table 1 showing the mean and SD values of the variable number of IL in head, body and tail region among 103 samples of CPs according to diabetic status. It is more in tail region than head and body region in both diabetic (DM) and non-diabetic (ND) CPs.

To test whether there is any significant difference among the variables of IL in head, body and tail among the 18 DM and 85 NDM, One Way Analysis of Variance (ANOVA) i.e., F-test has been applied.

Table 2: ANOVA in 3 variables of IL (head, body and tail) among 18 CPs with DM & 85

Among 18 CPs with DM status	**Sources**	**Degrees of freedom (df)**	**Sum of Squares (SS)**	**Mean Squares (MS)**	**F-value**	**Significant/ Non significant**
	Between variables	3-1=2	40.704	20.352	23.06** (p<0.0001)	**Highly significant among the 3 variables
	Within Variables	53-2=51	45.000	0.8824		
	Total	54-1=53	85.704			
Among 85 CPs with NDM status	Between variables	3-1=2	382.34	191.17	496.34** (p<0.0001)	**Highly significant
	Within Variables	254-2=252	97.059	0.3852		
	Total	255-1=254	479.399			
CPs with NDM & their F-Value						

Interpretation: The ANOVA table 2 & F-value has proved that there is an highly significant difference among the 3 variables(head, body, tail) of IL among 18 CPs with DM status as well as 85 CPs with NDM status.

As there we found highly significant differences of IL among 3 variables like head, body and tail among diabetes mellitus(DM) status CPs. So, the researchers were interested to test the significant differences of all these 3 variables one by one among the CPs according to DM status. 't' - test has been applied for this specific purpose. Following tables have been prepared to fulfil this purpose.

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

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

Interpretation: Table 3 have shown the mean & SD values of number of IL in head and body region among 103 samples of CPs were significant and highly significant in tail region of CPs according to DM status.

Table 4: Mean & SD values of the variable IL (head, body & 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

Interpretation: Table 4 has shown the mean & SD values of the variable IL (head, body & tail) of 103 samples of CPs according to diabetic status whereas DM and ND CPs are 18 and 85. Mean IL is more in tail region than head and body region.

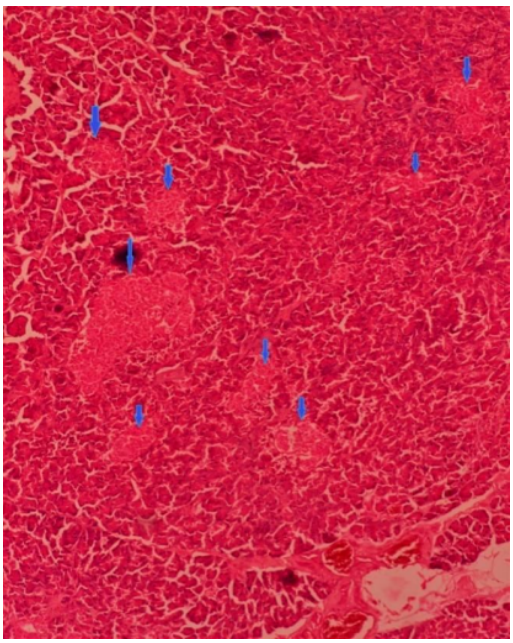

Figure 1: Photomicrograph of the pancreas (tail). This H&E – stained specimen shows pancreatic lobules. The pancreatic lobule consists of exocrine acini. Lobules exhibit multiple lighter staining IL (blue arrow) of variable size. X100.

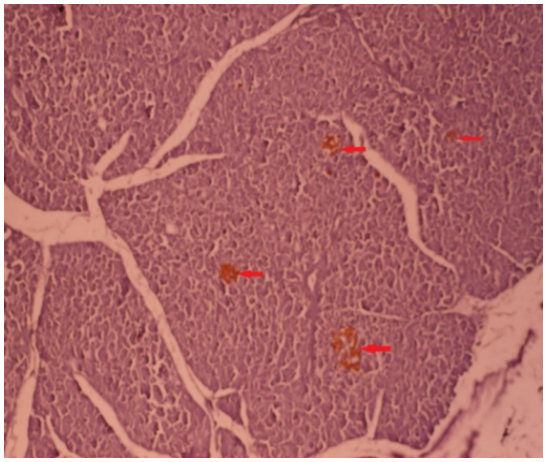

Figure 2: Photomicrograph of pancreas showing reduced number of IL (red arrow) stained with immunostaining using a specific marker for beta cell, the anti-insulin antibody (IHC technique). X100.

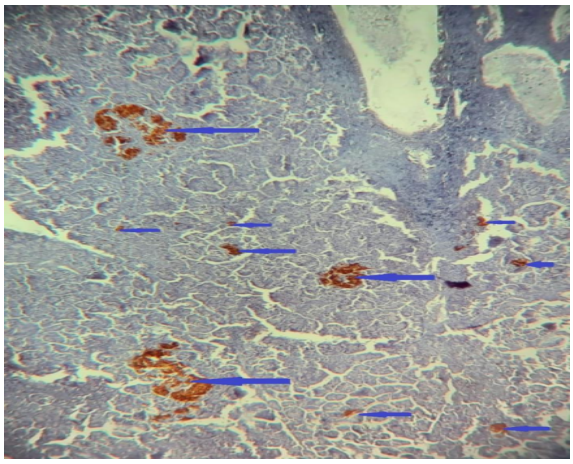

Figure 3: Photomicrograph of pancreas showing reduced beta cell mass in IL (blue arrow) stained with immunostaining using a specific marker for beta cell, the anti-insulin antibody (IHC technique). X400.

Discussion

Out of 103 CPs, numbers of DM pancreas are 18 and number of NDM pancreases are 85. 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 NDM status. The values of DM are 1.06±0.54, 1.61±0.78 and 3.11±1.323 and of NDM are 1.35±0.50, 2.04±0.57 and 4.22±0.76 in head, body and tail region respectively. It is more in tail region than head and body region in both DM and NDM CPs. The F- value has proved that there is an highly significant difference among the 3 variables (IL head, IL body, IL tail) among 18 CPs with DM status and highly significant among 85 CPs with NDM status. Again ‗t'- value between DM and NDM in the head and body region are significant and in tail region it is highly significant. The present study is comparable with Shahriah S et al.[37], Wang X et al. [38], Ravi PK et al.[39], Wittingen J et al. [40], Kilimnik G et al.41, Kim A et al. [42], Unger RH et al. [43]

Shahriah S et al. [37] studied among Bangladeshi population and reported that number of islets per unit area of microscopic field were more 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.

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.[41] , Kim A et al1 [42], Unger RH et al.[43] also found significantly more number of islets in the tail region than head and body region in their study.

In the study done by Ravi PK et al.[39], the endocrine proportion of the tail was 40% more in Indian population and Wang X et al.[38] reported 100% more twice) than that of the head in the non-diabetic American population. Ravi PK et al. [39] 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. [44] 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 Indians to DM. Ethnic variations as reported in the literature further confounds the comparison with the American population.

Kilimnik G et al. [41] reported the preferential loss of larger islets in T2DM pancreas when compared to non-diabetic pancreas. Therefore, Ravi PK et al. [39] 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.

Conclusion

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

References

1. Standring S. The Pancreas, Abdomen and Pelvis. In: Borley NR, Healy JC ed. Gray's Anatomy, The Anatomical Basis of Clinical Practice. 40th ed. London: Churchill Livingstone, Elsevier; 2008; 1183-90.
2. Datta AK. The Pancreas, The Alimentary System, Thorax, Abdomen and Pelvis, Essentials of Human Anatomy. 8th ed. Kolkata: Current Books International; 2008;258-265.
3. Mehta V, Suri RK. Pancreas, The Abdominal Cavity, Snell's Clinical Anatomy by Regions. South Asian 9th ed. New Delhi: Wolters Kluwer; 2018; 233.
4. Romanes GJ. The Pancreas, The digestive system, Cunningham's Textbook of Anatomy. 12th ed. New York; Oxford; 2019; 485-488.
5. Longnecker D. Anatomy and Histology of the Pancreas, Pancreapedia: Exocrine Pancreas Knowledge Base, Version 1.0, March 21, 2014.
6. Waugh A, Grant A. Pancreas, The Digestive System, Ross and Wilson Anatomy and Physiology in Health and Illness. 13th ed. London: Elsevier; 2018; 336.
7. Eroschenko VP. Pancreas, Digestive System, diFiore's Atlas of Histology with Functional Correlations. 13th ed. Philadelphia: Wolters Kluwer; 2018; 388.
8. Marchetti P, Ferrannini E. Beta cell mass and function in human type 2 diabetes, in International Textbook of Diabetes Mellitus, eds DeFronzo R. A., Ferrannini E., Keen H., Zimmet P. New York, NY: John Wiley & Sons Ltd; 2015; 354–370.
9. Standring S. The Pancreas, Abdomen and pelvis. In: Borley NR, Healy JC ed. Gray's Anatomy, The Anatomical Basis of Clinical Practice. 39th ed.

Edinburg, London, New York: Churchill Livingstone; 2005;1231-7.
10. Standring S. The Pancreas, Abdomen and pelvis. In: Stringer MD, Rela M, Reddy MS eds. Gray's Anatomy, The Anatomical Basis of Clinical Practice. 41st ed. London: Elsevier; 2016;1179.
11. Burrack AL, Martinov T, Fife BT. T Cell-Mediated Beta Cell Destruction: Autoimmunity and Alloimmunity in the Context of Type 1 Diabetes, REVIEW article Front. Endocrinol. 2017 Dec 5. Available from URL: https://doi.org/10.3389/fendo.2017.00343.
12. Iki K, Pour PM. Distribution of Pancreatic Endocrine Cells, Including IAPP-expressing Cells in Non-diabetic and Type 2 Diabetic Cases. J.Histochem. Cytochem. 2007 Feb. [2006]. Available from: URL: https://doi.org/10.1369/jhc.6A7024.2006
13. Westermark P, Wernstedtt C, Wilander E, Hayden DW, O'brient TD, Johnsont KH. Amyloid fibrils in human insulinoma and islets of langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. Proc Nati Acad Sci USA.1987;84:3881-3885.
14. Kamata K, Mizukami H, Inaba W, Tsuboi K, Tateishi Y, Yoshida T et al. Islet amyloid with macrophage migration correlates with augmented βcell deficits in type 2 diabetic patients. Amyloid. 2014; 21:191-201.
15. S, Inaba W, Mizukami H. Dynamic pathology of islet endocrine cells in type 2 diabetes: β-cell growth, death, regeneration and their clinical implications. J Diabetes Investig. 2016; 7:155-165.
16. Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes.2007;56:2356–2370.
17. Saito K, Nobuhisa Y, Tohbu T. Differential volumetry of A, B and D cell in the pancreatic islets of diabetic and nondiabetic subjects. Tohoku J Exp Med. 1979; 129:273–283.
18. Gepts W. Pathologic anatomy of the islet cell system in juvenile diabetes mellitus. Diabetes. 1965; 14:619–633.
19. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH et al. Selective βcell loss and α-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab. 2003; 88:2300-2308.
20. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-846.
21. Meigs JB, Wilson PWF, Fox CS, Vasan RS, Nathan DM, Sullivan LM et al. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. Journal of Clinical Endocrinology & Metabolism. 2006;91(8):2906-12.
22. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes. 1993;42(11):1663-72.
23. Rahier J, Goebbels RM, Henquin JC. Cellular composition of the human diabetic pancreas. Diabetologia. 1983; 24(5):366-371.
24. Guiot Y, Sempoux C, Moulin P, Rahier J. No decrease of the beta-cell mass in type 2 diabetic patients. Diabetes. 2001; 50(Suppl 1):S188.
25. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH et al. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. Journal of Clinical Endocrinology & Metabolism. 2003; 88(5):2300-2308.
26. Hanley SC, Austin E, Assouline-Thomas B, Kapeluto J, Blaichman J, Moosavi M et al. {beta}-Cell mass dynamics and islet cell plasticity in human type 2 diabetes. Endocrinology. 2010;151(4):1462-72.

27. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler, PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003;52(1):102-110.
28. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, Obesity & Metabolism. 2008;10(Suppl 4):32-42.
29. Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. Diabetologia. 2002; 45(1):85-96.
30. Inaishi J, Saisho Y, Sato S, Kou K, Murakami R, Watanabe Y et al. Effects of Obesity and diabetes on alpha- and beta-cell mass in surgically resected human pancreas. Journal of Clinical Endocrinology & Metabolism. 2016;101(7):2874-2882.
31. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU. Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Survey and Synthesis of Pathology Research. 1985;4(2):110-125.
32. Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR et al. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Research. 1988;9(4):151-159.
33. Ranganathan P, Agarwal R. Study designs: Part -1 An overview and classification. Perspect Clin Res. 2018; 9(4): 184-6.
34. Theise MS. Observational and interventional study design types: An overview. 2, 2014, Biochem Med(Zagreb). 2010;24(2): 199-210.
35. Rachel K. Pancreas, The Abdominal Cavity, Thorax and Abdomen, Cunningham's Manual of Practical Anatomy. 16th ed. New York: Oxford; 2007. p. 180-181.
36. Wolfe-Coote SA, DuToit DF. Distribution of cell types of the islets of Langerhans throughout the pancreas of the Chacma baboon. Anat Rec. 1987;217(2):172-7.
37. Shahriah S, Nurunnabi ASM, Begum GN, Rayhan KA, Perven HA. A postmortem histological study on the alpha and beta cells of the islets of Langerhans. Bangladesh Med J. 2014 Sep;43 (3).
38. Wang X, Misawa R, Zielinski MC, Cowen P, Jo J, Periwal V, Ricordi C et al. Regional differences in islet distribution in the human pancreas - preferential beta-cell loss in the head region in patients with type 2 diabetes. PLoS One. 2013; 8:1-9.
39. Ravi PK, Purkait S, Agrawal U, Patra S, Patnaik M, Singh SR et al. Regional variation of human pancreatic islets dimension and its impact on beta cells in Indian population. ISLETS. 2019; 11(6):141-151.
40. Wittingen J, Frey CF. Islet concentration in the head, body, tail and uncinate process of the pancreas. Ann Surg. 1974;179(4):412-414.
41. Kilimnik G, Jo J, Periwal V, Zielinski MC, Hara M. Quantification of islet size and architecture. Islets. 2012;4 (2):167-172.
42. Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M. Islet architecture: a comparative study. Islets. 2009; 1:129-136.
43. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci. 2010; 107:16009-16012.
44. Inaishi J, Saisho Y. Ethnic Similarities and Differences in the Relationship between Beta Cell Mass and Diabetes, J. Clin. Med. 2017;6(12):113.