Original Article

A postmortem histological study on the alpha and beta cells of the islets of Langerhans

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Abstract

A cross-sectional, descriptive type of study was done was done in the Department of Anatomy, Dhaka Medical College, Dhaka, from August 2005 to December 2006, based on collection of 75 postmortem male human pancreas, to determine the number of alpha and beta cells per islet of Langerhans in different parts of the pancreas with advancing age. The collected samples were divided into seven age groups, i.e. A (10-19 years), B (20-29 years), C (30-39 years), D (40-49 years), E (50-59 years), F (60-69 years) and G (≥70 years), for convenient description of their various age related changes. Histological slides were prepared by using Gomori's chromium haematoxylin phloxine stain. The number of alpha and beta cells per islet of Langerhans was measured by point counting technique on a grid square drawn by Visio basic 2000 software with high magnification under the compound light microscope. The mean number of alpha cells per islet in the head of the pancreas were 17.60±2.51, 21.40±2.07, 27.40±3.13, 20.80±2.59, 15.40±2.51, 14.60±3.58 and 11.40±1.67; in the body of the pancreas were found 24.60±1.67, 31.40±2.60, 40.00±2.45, 26.80±2.05, 22.60±2.51, 19.20±2.68 and 20.00±2.45; in the tail region were 37.80±4.09, 42.20±4.82, 53.20±2.94, 55.60±6.69, 43.60±6.69, 39.20±8.01 and 35.60±1.34 in group A, B, C, D, E, F and G respectively. The mean number of beta cells per islet in the head of the pancreas were 32.60±2.51, 36.40±2.07, 42.40±3.13, 35.80±2.59, 30.40±2.51, 29.60±3.58 and 26.40±1.67; in the body of the pancreas were found 39.60±1.67, 46.40±2.07, 55.00±2.45, 41.80±2.05, 37.60±2.51, 34.20±2.68 and 35.00±2.45; in the tail region were 52.80±4.09, 57.20±4.82, 68.20±2.94, 69.50±6.58, 70.60±6.69, 58.20±8.01 and 50.60±1.34 in group A, B, C, D, E, F and G respectively. In the present study, the number of alpha cells of islets of Langerhans gradually increased up to the 4th decade of life in head and body of the pancreas and up to the 5th decade in the tail region, later it decreased. Besides, the number of beta cells gradually increased up to the 4th decade of life in head and body of the pancreas and up to the 6th decade in the tail region, then decreased. Here, females were excluded due to less availability of the female cadaveric pancreas during study period.

Key words: Pancreas, islets of Langerhans, alpha cell, beta cell

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Introduction

The Endocrine pancreas is a diffuse organ scattered as small nests of cells (numerous in tail) called Islets of Langerhans.[1] Islets secrete hormones that regulate blood glucose levels.[2] Specifically, the total mass of pancreatic beta cells is a critical factor in the regulation of glucose homeostasis.[3] Besides, the total beta cell mass consists of a dynamic cell population that either expands or declines to adapt to altered physiological conditions.[4] Individual pancreatic islet may contain a few cells or many hundreds of polygonal cells arranged in short irregular cords that are profusely invested with a network of fenestrated capillaries. Here, usually alpha (A) cells form approximately 20%, located in the periphery region, while, beta (B) cells constitute 75%, concentrated in the centre of the islets; and minor islet cells constitute about 5% of pancreatic islets.[1,2,5] In various pathological conditions affecting the islets, including diabetes mellitus, proportion of different cell types varies.[5] The number of these cells probably changes with age and in association with injury to pancreatic tissue, pancreatitis and diabetes.[6] Hence, the pancreatic islet function is closely associated with the morphologic changes in islet cells. The most common disease of the endocrine pancreas is diabetes mellitus associated with changes in the size and cell population of islets[5,7] and based on this pathophysiology, scientists found that the most promising of developments in research for diabetes is in stem cells.[8] Recently, islet cells were successfully generated in vitro

from human pancreatic stem cells.[9] Detail knowledge on normal cell population, especially alpha and beta cells, of pancreatic islets play a crucial role for researchers engaged in pancreatic stem cell unit. However, only few studies have been done in this field in our country. Therefore, we proposed this study to determine the number of alpha and beta cells per islet of Langerhans in different parts of the pancreas in different age groups in a Bangladeshi population. The results of the present study are expected to be contributory to the information pool on normal cell population of the islets of Langerhans of Bangladeshi people with age related changes and correlation of its functional capacity with proportion of cells.

Methods

This cross-sectional, descriptive type of study was done in the Department of Anatomy, Dhaka Medical College, Dhaka, from August 2005 to December 2006, based on collection of 75 postmortem male human pancreas aging from 10 to 76 years. The study was approved by the ethical review committee of Dhaka Medical College, Dhaka. The collected samples were divided into seven age groups, i.e. A (10-19 years), B (20-29 years), C (30-39 years), D (40-49 years), E (50-59 years), F (60-69 years) and G (≥70 years), for convenient description of their various age related changes.[10] Any apparent signs of decomposition, any injury in pancreas, death due to poisoning and death due to known pancreatic disease were excluded from the study.

Selection of the tissue was done according to Wolfe-Coote & duToit,[11] and 3mm × 3mm were cut with scissors for histological study (Table-I). The histological slides were prepared by using standard procedure with stain.

Table-I: Location of the tissues collected from each sample11

Part	Tail	Body	Head
Area	1 inch proximal to tail end from horizontal portion of pancreas lateral to superior mesenteric vessels.	Apparently central region.	Portion lying between inner curve of second part of duodenum and superior mesenteric vessels.

Complete transverse sections of the head, body and tail were fixed in Bouin-Allen's solution and embedded in paraffin. From these paraffin blocks, two consecutive 5µm thick sections were cut. For the purpose of counting the number of alpha and beta cells per islet of Langerhans, the slides were stained with Gomori's chromium haematoxylin phloxine stain. Colours of alpha cells were red and the beta cells were blue.[12] (Figure-1)

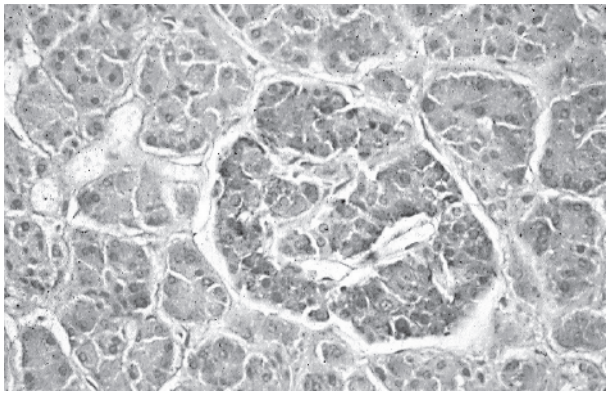

Figure-1: *Photomicrograph of the islet of Langerhans of tail region of the human pancreas showing that the centre is rich in insulin-secreting beta cells (blue) and glucagon-secreting alpha cells (red) are located mostly in the periphery (Gomori's chromium haematoxylin phloxine stain), taken from group C (30-39 years) (× 100 magnification).*

The total number of cells was counted from 3 randomly chosen sectioned islets. From each slide a special counting chamber was devised for counting the cells. The cell counting device was as followings - the outline of a "6mm × 6mm" square was drawn by Visio basic 2000 software then divided into twenty five (5 × 5) smaller squares. The print of this grid square has been taken on a transparent plastic sheet size to fit the aperture of the eyepiece. The circular sheet was in round shape and placed in the eyepiece. When any islet was observed under the microscope, its cells were in view with the above mentioned counting chamber superimposed on them. A simple method of counting all the cell nuclei fallen in each small square in the chamber was undertaken. For the nuclei fallen on the dividing lines of the chamber, those falling on the lower and right margin of each square were included in the square, while those falling the upper and left margins were excluded.[13]

Data were collected and appropriate statistical analyses were done by using SPSS version 11.0, as ANOVA test and multiple comparisons were done by Posthoc in LSD (least significance difference).

Results

The mean number of alpha cells per islet in the head of the pancreas were 17.60±2.51, 21.40±2.07, 27.40±3.13, 20.80±2.59, 15.40±2.51, 14.60±3.58 and 11.40±1.67 in group A, B, C, D, E, F and G respectively. The difference between the number of alpha cells in the head of the

pancreas was statistically significant in A vs B, A vs C, A vs G, B vs C, B vs E, B vs F, B vs G, C vs D, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G, and E vs G group (Table-II).

Table-II: Number of alpha cells per islet of Langerhans in the head, body and tail of the pancreas in different age group

Group (n)	Number of alpha cells per islet of Langerhans		
	Head Mean±SD	Body Mean±SD	Tail Mean±SD
A	17.60±2.51	24.60±1.67	37.80±4.09
(5)	(15.0-20.0)	(23.0-27.0)	(35.0-44.0)
B	21.40±2.07	31.40±2.60	42.20±4.82
(5)	(20.0-25.0)	(30.0-35.0)	(37.0-47.0)
C	27.40±3.13	40.00±2.45	53.20±2.94
(5)	(24.0-30.0)	(38.0-44.0)	(50.0-56.0)
D	20.80±2.59	26.80±2.05	55.60±6.69
(5)	(18.0-23.0)	(25.0-30.0)	(46.0-62.0)
E	15.40±2.51	22.60±2.51	43.60±6.69
(5)	(13.0-18.0)	(20.0-25.0)	(35.0-51.0)
F	14.60±3.58	19.20±2.68	39.20±8.01
(5)	(10.0-20.0)	(16.0-22.0)	(33.0-42.0)
G	11.40±1.67	20.00±2.45	35.60±1.34
(5)	(9.0-13.0)	(16.0-23.0)	(35.0-38.0)
	Head P value	Body P value	Tail P value
A vs B	<0.05*	<0.001***	>0.10^{ns}
A vs C	<0.001***	<0.001***	<0.001***
A vs D	>0.05^{ns}	>0.10^{ns}	<0.001***
A vs E	>0.10^{ns}	>0.10^{ns}	>0.05^{ns}
A vs F	>0.05^{ns}	<0.01**	>0.50^{ns}
A vs G	<0.01**	<0.01**	>0.10^{ns}
B vs C	<0.01**	<0.001***	<0.01**
B vs D	>0.50^{ns}	<0.01**	<0.001***
B vs E	<0.01**	<0.001***	>0.50^{ns}
B vs F	<0.001***	<0.001***	>0.10^{ns}
B vs G	<0.001***	<0.001***	<0.05*
C vs D	<0.001***	<0.001***	>0.10^{ns}
C vs E	<0.001***	<0.001***	<0.01**
C vs F	<0.001***	<0.001***	<0.001***
C vs G	<0.001***	<0.001***	<0.001***
D vs E	<0.01**	<0.01**	<0.001***
D vs F	<0.01**	<0.001***	<0.001***
D vs G	<0.001***	<0.001***	<0.001***
E vs F	>0.50^{ns}	<0.05*	>0.50^{ns}
E vs G	<0.05*	>0.05^{ns}	<0.05*
F vs G	>0.05^{ns}	>0.50^{ns}	>0.10^{ns}

Figures in parentheses indicate range. Statistical analysis done by one-way ANOVA (PostHoc) test, ns = not significant, */**/*** = significant

The mean number of alpha cells per islet in the body of the pancreas were 24.60±1.67, 31.40±2.60, 40.00±2.45, 26.80±2.05, 22.60±2.51, 19.20±2.68 and 20.00±2.45 in group A, B, C, D, E, F and G respectively. The difference between the number of alpha cells in the body of the pancreas was statistically significant in A vs B, A vs C, A vs F, A vs G, B vs C, B vs D, B vs E, B vs F, B vs G, C vs D, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G and E vs F group (Table-II).

The mean number of alpha cells per islet in the tail region were 37.80±4.09, 42.20±4.82, 53.20±2.94, 55.60±6.69, 43.60±6.69, 39.20±8.01 and 35.60±1.34 in group A, B, C, D, E, F and G respectively. The difference between the number of alpha cells in in the tail of the pancreas wasstatistically significant in A vs C, A vs D, B vs C, B vs D, B vs G, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G and E vs G group. (Table-II)

In the present study, the number of alpha cells in Islets of Langerhans gradually increased up to 4th decade of life in head and body of the pancreas and up to 5th decade in the tail region; later it decreased.

The mean number of beta cells per islet in the head of the pancreas were 32.60±2.51, 36.40±2.07, 42.40±3.13, 35.80±2.59, 30.40±2.51, 29.60±3.58 and 26.40±1.67 in group A, B, C, D, E, F and G respectively. The difference between the mean number of beta cells in the head of the pancreas was statistically significant in A vs B, A vs C, A vs G, B vs C, B vs E, B vs F, B vs G, C vs D, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G and E vs G group. (Table-III)

The mean number of beta cells per islet in the body of the pancreas were found 39.60±1.67, 46.40±2.07, 55.00±2.45, 41.80±2.05, 37.60±2.51, 34.20±2.68 and 35.00±2.45 in group A, B, C, D, E, F and G respectively. The difference between the number of beta cells in the body of the pancreas was statistically significant in A vs B, A vs C, A vs F, A vs G, B vs C, B vs D, B vs E, B vs F, B vs G, C vs D, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G, and E vs F group. (Table-III)

The mean number of beta cells per islet in the tail region of pancreas were found to be 52.80±4.09, 57.20±4.82, 68.20±2.94, 69.50±6.58, 70.60±6.69, 58.20±8.01 and 50.60±1.34 in group A, B, C, D, E, F and G respectively. The difference between the number of beta cells in the tail region of the pancreas was found to be statistically significant in A vs C, A vs D, B vs C B vs D, B vs G, C vs E, C vs F, C vs G, D vs E, D vs F, D vs G and E vs G group. (Table-III)

In the present study, the number of beta cells in Islets of Langerhans gradually increased up to 4th decade of life in head and body of the pancreas and up to 6th decade in the tail region, then decreased.

Table-III: Number of beta cells per islet of Langerhans in the head, body and tail in different age group

Group (n)	Number of beta cells per islet of Langerhans		
	Head	Body	Tail
	Mean±SD	Mean±SD	Mean±SD
A	32.60±2.51	39.60±1.67	52.80±4.09
(5)	(30.0-35.0)	(38.0-42.0)	(50.0-59.0)
B	36.40±2.07	46.40±2.07	57.20±4.82
(5)	(35.0-40.0)	(45.0-50.0)	(52.0-62.0)
C	42.40±3.13	55.00±2.45	68.20±2.94
(5)	(39.0-45.0)	(53.0-59.0)	(65.0-71.0)
D	35.80±2.59	41.80±2.05	69.50±6.58
(5)	(33.0-38.0)	(40.0-45.0)	(61.0-71.0)
E	30.40±2.51	37.60±2.51	70.60±6.69
(5)	(28.0-33.0)	(35.0-40.0)	(61.0-77.0)
F	29.60±3.58	34.20±2.68	58.20±8.01
(5)	(25.0-35.0)	(31.0-37.0)	(55.0-60.0)
G	26.40±1.67	35.00±2.45	50.60±1.34
(5)	(24.0-28.0)	(31.0-38.0)	(50.0-53.0)
	Head P value	Body P value	Tail P value
A vs B	<0.05*	<0.001***	>0.10^{ns}
A vs C	<0.001***	<0.001***	<0.001***
A vs D	>0.05^{ns}	>0.10^{ns}	<0.001***
A vs E	>0.10^{ns}	>0.10^{ns}	>0.05^{ns}
A vs F	>0.05^{ns}	<0.01**	>0.50^{ns}
A vs G	<0.01**	<0.01**	>0.10^{ns}
B vs C	<0.01**	<0.001***	<0.01**
B vs D	>0.50^{ns}	<0.01**	<0.001***
B vs E	<0.01**	<0.001***	>0.50^{ns}
B vs F	<0.001***	<0.001***	>0.10^{ns}
B vs G	<0.001***	<0.001***	<0.05*
C vs D	<0.001***	<0.001***	>0.10^{ns}
C vs E	<0.001***	<0.001***	<0.01**
C vs F	<0.001***	<0.001***	<0.001***
C vs G	<0.001***	<0.001***	<0.001***
D vs E	<0.01**	<0.01**	<0.001***
D vs F	<0.01**	<0.001***	<0.001***
D vs G	<0.001***	<0.001***	<0.001***
E vs F	>0.50^{ns}	<0.05*	>0.10^{ns}
E vs G	<0.05*	>0.05^{ns}	<0.05*
F vs G	>0.50^{ns}	>0.50^{ns}	>0.10^{ns}

Figures in parentheses indicate range. Statistical analysis done by on-way ANOVA (PostHoc) test, ns = not significant, */**/*** = significant

Discussion

Woolf-Coote & duToit[11] stated that most of the mammals were found to contain significantly higher numbers beta cells and lower numbers of alpha cells. Alpha cell distribution was significantly smaller than that of beta (insulin) cells, especially in the tail compared with other regions. Our study revealed similar results; however, the difference was not statistically significant.

In our study, variability in the cellular composition of islets was evident in the cadaveric pancreas. Alpha cells sometimes formed a discontinuous peripheral rim around the core of beta cells, but were more often localized in the centre of the islets, usually close to vessels. In some slides, large islets with a vast majority of alpha cells were occasionally observed. These findings are very similar to those of Henquin & Rahier.[14] Karim[13] and Firoz[15] found that the lowest mean numbers of alpha and beta cells per islet of Langerhans in humans was in head region and highest number in the tail region of the pancreas, which are in agreement with the present study.

Firoz[15] studied forty cadaveric pancreas and found the mean number of alpha and beta cells respectively 95.25±22.68 (39.85%) and 143.75±34.54 (60.15%) in male and 64.66±12.23 (36.43%) and 111.00±30.66 in female in group A (15-20 years), while 108.00±34.74 (41.14%) and 154.50±42.27 (58.86%) in male 75.33±15.73 (39.93%) and 113.33±27.28 (60.07%) in female in group B (21-40 years) and 68.75±16.59 (39.40%) and 105.75±26.65 (60.60%) in male and 59.00±15.52 (38.81%) and 93.00±26.58 (61.18%) in female in group C (41-65 years). Henquin & Rahier[14] stated that the alpha cell mass was virtually identical normal healthy and diabetic subjects, and was not influenced by age, sex or basal metabolic index (BMI). Rahier et al.[16] found that beta cell mass did not correlate with age but decreased with duration of clinical diabetes (24 % and 54% lower than controls in subjects with <5 and >15 years of overt diabetes respectively). They also revealed that pancreatic insulin concentration was 30% lower in patients and the average beta cell mass is about 39% lower in type 2 diabetic subjects compared with matched controls. Saito et al[17] studied 28 samples of non-diabetic cases where the total beta cell volume did not decrease with aging, nor did the total alpha cell volume. However, when the ratio is examined, it tends to reduce with aging, though not significant statistically. Our study revealed some age related changes in number of alpha and beta cells (as the difference in both alpha and beta cells was found significant in between age groups in head, body and tail regions of the pancreas), which exclusively differ with the previous investigators.[14-17]

However, in our study, females were excluded due to less availability of the female cadaveric pancreas during the study period.

In the present study, the number of alpha cells of islets of Langerhans gradually increased up to the 4th decade of life in head and body of the pancreas and up to the 5th decade in the tail region, later it decreased. Besides, the number of beta cells gradually increased up to the 4th decade of life in head and body of the pancreas and up to the 6th decade in the tail region, then decreased. Further studies with large sample in both sexes including different ages, height, body weight, body surface area, basal metabolic index (BMI) are recommended. Besides, comparison between the number of beta cells in diabetic and non diabetic cases, dietary predisposition on beta cell count, analysis of the viability of cadaveric alpha and beta cells are suggested for the further research as well.

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