

## Immunofluorescence Identification of the Endocrine Cells in the Pancreatic Islets of the Camel (*Camelus dromedarius*)

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### Abstract

The aim of the study and characterize the spread of two types of endocrine cells of the pancreatic alpha and beta with hormones in one hump camel (*Camelus dromedaries*) by using immunofluorescence technique and compositions histological study of the pancreas. Conducted the study on the pancreas to twelve of the camels from AL-Dwaynia province slaughter house, six animal for histological study and six other for immunofluorescent study. To identify two types of endocrine cells in the pancreas with endocrine hormones. By use specific antisera multiple glucagon peptide-1 and Glucose-dependent insulinotropic peptide. The results showed that the distribution of these types of immune cells in the pancreas difference. Immunoreactive cells (beta) cells, the most numerous and take a central position and surround the pancreatic islets. The most distinct delineation of the islets from the surrounding acini. Glucose-dependent insulinotropic peptide-immunoreactive cells ( $\beta$ -cells) were most numerous followed by Glucagon like peptide-1 ( $\alpha$ -) immunoreactive cells in decreasing order. Alpha cells were mostly observed as clumps in the periphery area. The result concluded from the current study, the difference in the distribution of these cells depends on the type of species when compared to the results of this study with other results.

**Keywords:** immunoreactive cells, endocrine cells, pancreas, camel.

تمييز المناعي الاستشعاعي للخلايا الصماوية في جزر لانكرهانز في بنكرياس الجمال وحيدة  
السنام

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### الخلاصة :

الهدف من الدراسة تمييز وانتشار نوعين من خلايا الغدد الصماوية البنكرياسية الالفا والبيتا مع هرموناتهما في الجمال وحيدة السنام *Camelus dromedarius* باستخدام تقنية الاستشعاع المناعي ودراسة التراكيب النسيجية للبنكرياس. ان لهذا التمييز والانتشار اهمية في العلوم التطبيقية الاخرى. اجريت الدراسة على البنكرياس لاثنا عشر من الجمال المحلية, استخدم (6) لغرض الدراسة النسيجية (6) اخرى لغرض دراسة التقنية الاستشعاعية. تم البحث عن نوعين من خلايا الغدد الصماء في البنكرياس مع هرموناتها. استخدمت مضادات متعددة النسل ل الجلوكاجون بيتيد-1 و الجلوكو اينسولينو تروبيك بيتيد.

وقد أظهرت النتائج اختلاف توزيع هذه الأنواع من الخلايا في البنكرياس. خلايا بيتا الأكثر عددا وتأخذ الموضع المركزي والمحيطي في الجزر البنكرياسية. الجلوكاجون ببنيدي-1 الموجود في الخلية (خلية ألفا) أظهرت أقل عدد في محيط الجزر البنكرياسية.

نستنتج من الدراسة الحالية ان الاختلاف في توزيع هذه الخلايا يعتمد على نوع الحيوان وذلك عند مقارنة نتائج هذه الدراسة مع النتائج الأخرى .

**كلمات البحث:** الخلايا المناعية، خلايا الصماء ، البنكرياس والجمال.

## Introduction

The pancreas is a composed of exocrine and endocrine gland. The pancreas is made up of mammals areas the head and body and tail regions(1). The endocrine cells found in the small islands of cells called the islets of Langerhans (2). The islet of Langerhans in mammalian pancreases consists of few to several thousand cells (3) The function of each of the endocrine secretions and interactive parts of particular importance to the natural functioning of the body. Endocrine cells produce hormones that are indispensable, such as Glucose-dependent insulinotropic peptide and glucagon which is consider critical to the optimal performance of the body metabolism (4). Glucagon is a peptide hormone, produced by alpha cells of the pancreas and it raises the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers the glucose concentration (5). When the concentration of glucose in the blood stream falls too low , the pancreas releases glucagon(6) . Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood-glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level. Glucagon belongs to a family of several other related hormones(7). The purpose of this work to clarify the basic regional distribution of two types

of endocrine cells in the pancreas of the one humped camel by immunofluorescent study .

## Material and method

Twelve Samples were obtained from pancreas of dromedaria camels (*Camelus dromedarius*; of both male and female , aged 1-5 year; weight 400-450kg), were collected from Al-Qadisiyah provina slaughter house . Sections were rinsed in ice-cold 0.9% (w/v) NaCl pH 7.4 and fixed in 10% formaldehyde, dehydrated through an ethanol-xylene series then that embedded in paraffin for histological examinations (8) and immunofluorescent studies(9). Sections were cut at 5mm in thickness. Slides, containing wax embedded camel pancreas tissue, were dewaxed in 100% xylene for 3 x 10 minutes each. The tissue was placed twice in 100% ethanol for 2 x 10 minutes and removed, allowed to air dry for 10 minutes and were circled with ImmEdge Hydrophobic Pen and allowed to dry for 10 minutes. Subsequently, they were placed 2 x 5 minutes in 70% ethanol. Slides were then rehydrated twice in distilled water for 5 minutes each. Slides were immersed in antigen retrieval buffer (10mM Tris/HCl pH 10.0) and autoclaved (Series A1200086, LMS CONS. Ltd, Germany) 2 x 15 minutes at 121°C and 15 psi. Subsequently, slides were allowed to cool in antigen retrieval buffer for 30-60 minutes at room temperature and washed for 3 x 5 minutes in phosphate buffer saline (PBS). Nonspecific antibody binding sites were blocked by incubating the

tissue sections for 1 hour in the blocking solution 10% (v/v) donkey serum in a humidified chamber at room temperature. Sections were incubated overnight at 4°C with primary antibodies (Table 1). Each slide was then washed in PBS for 5 x 5 minutes. FITC-conjugated IgG/IgY (Table 1) (Stratech, Scientific Limited, Suffolk, UK) were used at a dilution of 1:500 for 1 hour incubation at room temperature. Finally, slides were

washed with PBS for 5 x 5 minutes and mounted in Vectashield Hard Set Mounting Media with DAPI (Vector Laboratories Ltd, Peterborough, UK). Sections were visualized using an epifluorescence microscope (MEIJI TECHNO, Model MT4300, Japan) and images were captured with a Canon digital camera (DS126371, Canon INC, Japan). Omission of primary antibody was routinely used as negative control

Table1. Primary and secondary antibodies were used.

Primary antibody	Host	Dilution	Clonality and Source
Anti-GIP	Goat	1:100	Polyclonal, GIP (Y-20): sc-23554, Santa Cruz Biotechnology, INC., Santa Cruz, CA, USA.
Anti-GLP-1	Goat	1:100	Polyclonal, GLP-1 (C-17): sc-7782, Santa Cruz Biotechnology, INC., Santa Cruz, CA, USA.

Secondary antibody	Label	Dilution	Source
Secondary anti-goat IgG	FITC	1:500	Fluorescein-conjugated IgG (705-095-147), Stratech Scientific Limited, Suffolk, UK.

The relative frequency of IR cells was placed into one of four categories, not detected (-), few (++; mean values were below 5/one field); moderate (+++; mean values were below 10/one field) and high (++++; mean values were up to 20/one field), according to their observed mean numbers as seen under one field of

epifluorescence microscope (200X) and the observation of camel pancreas was conducted as triplet by three histologists.

**Result**

**Histology of camel pancreas**

Camel pancreas in this study consisted of endocrine islets of different sizes and shapes were scattered throughout the pancreatic tissue(fig.1).

The beta and alpha cells could be identified in the islets of Langerhans by the shape of their nuclei, the nucleus of the beta cell is round in shape, while the nucleus of alpha cell is oval in shape(fig.2) .

**Expression of GIP and GLP-1 receptors in the pancreas of camel**

The result of the specific immunofluorescent study revealed to regional distribution of types of cells in the endocrine pancreas of the one

humped camel. Glucagon like peptide-containing cells(Alpha cells )sort about 28% of total population endocrine cells in a pancreatic islet. They are located in the periphery of the islets in camel (fig.3.A and figs.4.A.B and C).GIP-positive cells (Beta cells) are the most numerous cell types in the one humped camel pancreas, Are located in both the central and peripheral parts of the islet and sort for about 60-70% of the total cell population of islet of Langerhans (table.1) (fig.3.B and fig.5).The immunoflurcent examination of GLP-1- and GIP -(Fig.3.A and B) immuno-positive endocrine cells were disclosure of the pancreatic islets. Also, GIP-and GLP-1- immunopositive cells were found within the acini as both single and ductular areas (figs.3.E, F) .

Antibody	Pancreatic Islet	
	Peripheral	center
GIP-positive cells	++++	+++
GLP-1 containing cells	++	-
Relative frequency :		
_ : not detected , ++: afew , +++ moderate , ++++: high		

**Discussion**

In the present study the islets of Langerhans of the camel display different sizes and shapes and make up of irregular clumps of cells. This confirms the findings of Alani (1987) and Sultan(1999) in the camel and Mukherjee *et al.*, (1988) in sheep. Sultan (1999)claimed that there are

interlobular islets of Langerhans in the camel pancreas.

In the present study and the study of Sultan (1999) in the camel, who reported that the nucleus of the beta cell is oval in shape and located in center and peripheral of islets of Langerhans, while the nucleus of the alpha cell is round and located in peripheral of islets of Langerhans ; this disagrees with the observation of

Mukherjee et al. (1988) in sheep, the beta and alpha cells could be identified in the islets of Langerhans by the shape of their nuclei, the nucleus of the beta cell is round in shape, while the nucleus of alpha cell is oval in shape. On the other hand, the alpha cells were located peripherally in the islets of the camel, this is similar to the finding of Erlandsen *et al.* (1976) in human and rat and Dellmann (1981) in the cattle and Sultan (1999) in the camel. but disagree with Furuoko *et al.* (1989) in horse due to found in centrally in the islets of . In the present study and the study of Mukherjee et al. (1988) in sheep, the beta and alpha cells could be identified in the islets of Langerhans by the shape of their nuclei. The nucleus of the beta cell is round in shape, while the nucleus of alpha cell is oval in shape; this disagrees with the observation of Sultan (1999) in the camel, who reported that the nucleus of the beta cell is oval in shape, while the nucleus of the alpha cell is round.

Camel pancreas, which consisted of endocrine islets of different sizes and shapes were scattered throughout the pancreatic tissue, the variation of size of the islets have been noted before in various species ; including the human (Kim *et al.*, 2009).

The study reveal to distribution of GIP- and GLP-1-immunopositive endocrine cells in one humped camel pancreas using immunofluorescent methods. This finding similar reported in adeghate, 1997 and A different diagram of distribution was reported in Khatim *et al.* (1985).

The number of islets vary greatly in number, with large islets being replaced near the arterioles and smaller islets being replaced in the deeper pancreatic parenchyma. Endocrine islets show different distributions, locations, and characteristics among mammals species (Kim *et al.*, 2009). A high number of alpha- and beta-releasing cells are located in the pancreatic endocrine islets of horse (Furuoka *et al.*, 1989).

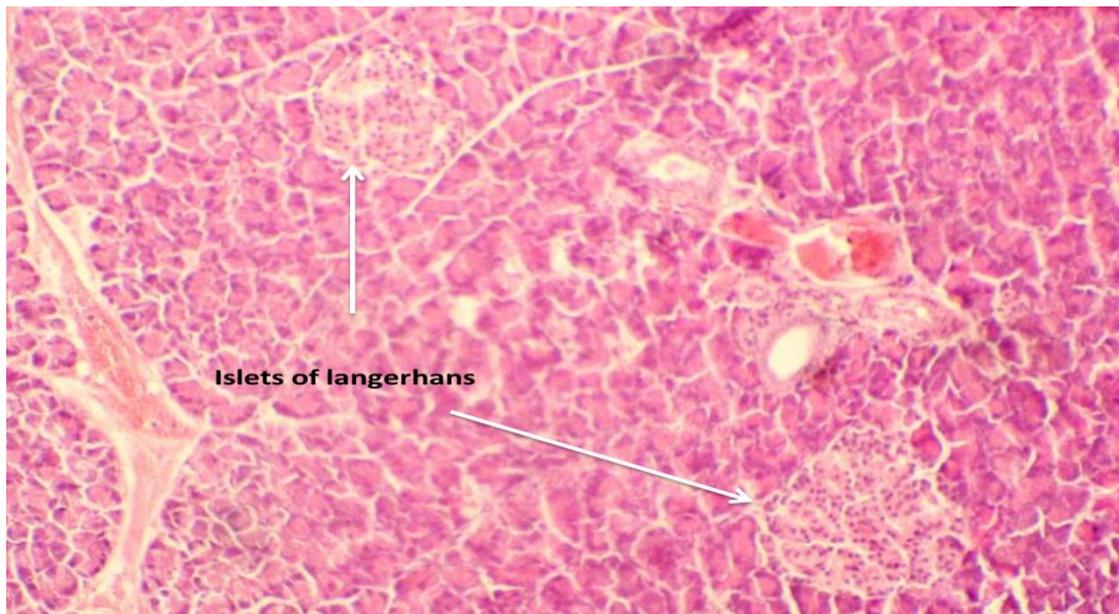


Fig.1: histological microscopic image of the pancreas of the camel,using (H&E) X200

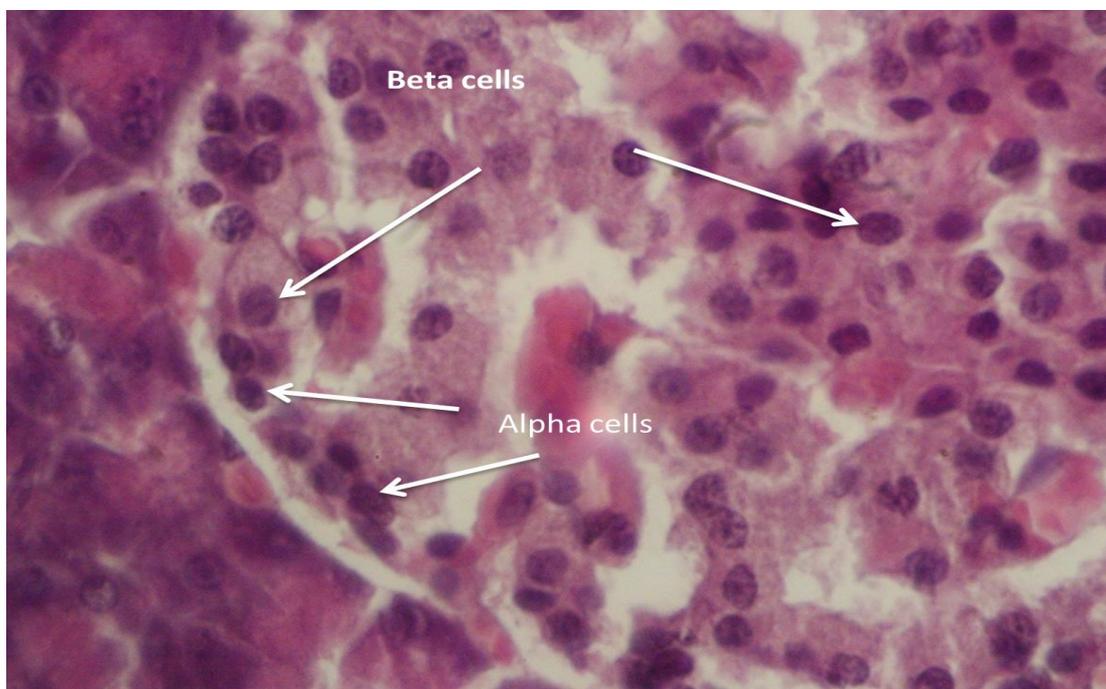


Fig.2: histological microscopic image of the pancreas of the camel, using (H&E) X400 .

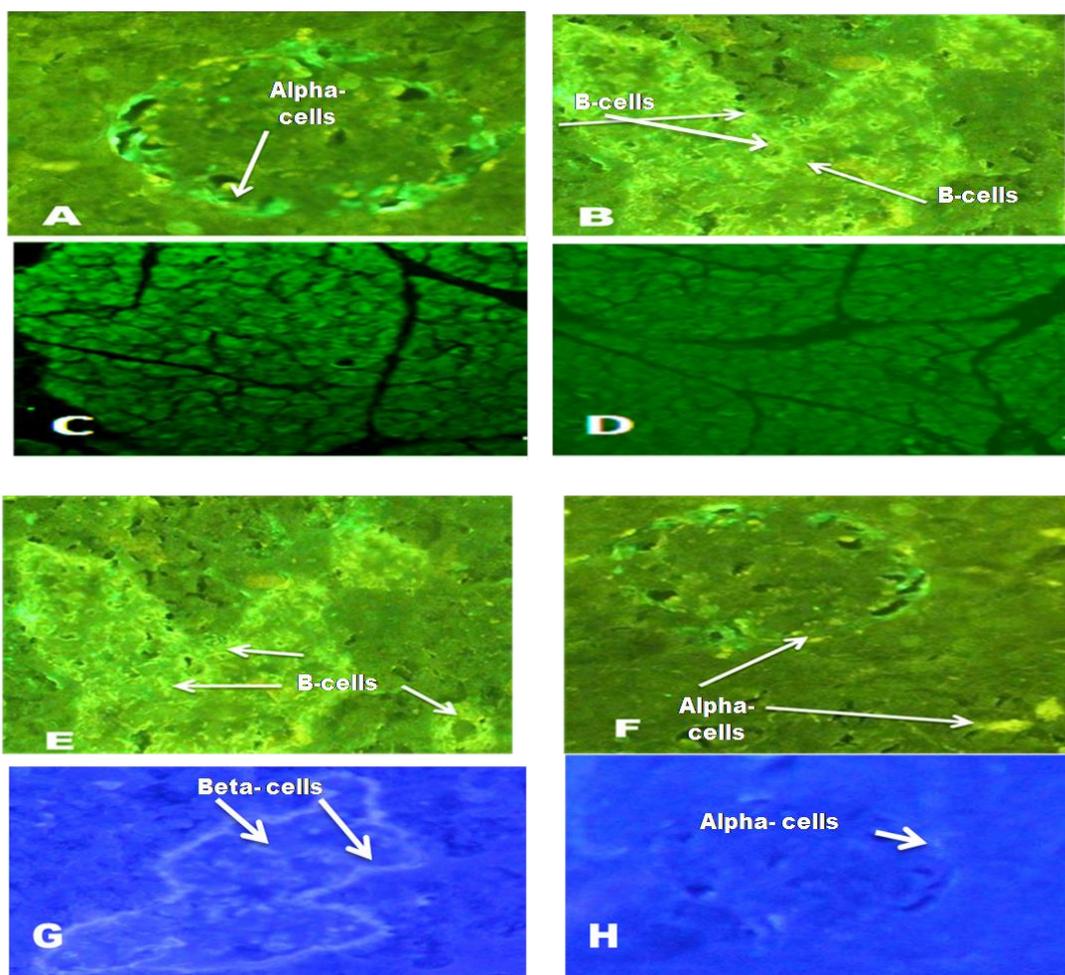


Fig.3:fluorescent microscopic images of the pancreas of the camel, using immunostaining of Glucose dependent insulinotropic peptide-producing cells (GIP) and glucagon Like peptide-1-producing cells(GLP-1), Beta cells were distributed throughout the islet in the center and the periphery(figs.B&E). Alpha cells were mostly observed as clumps in the periphery area(fig.A&F).(figs.C&D) negative control .(figs.G&H) Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).(figs. E&F) GIP and GLP-1- immunopositive cells were found within the acini as both single and ductular areas .

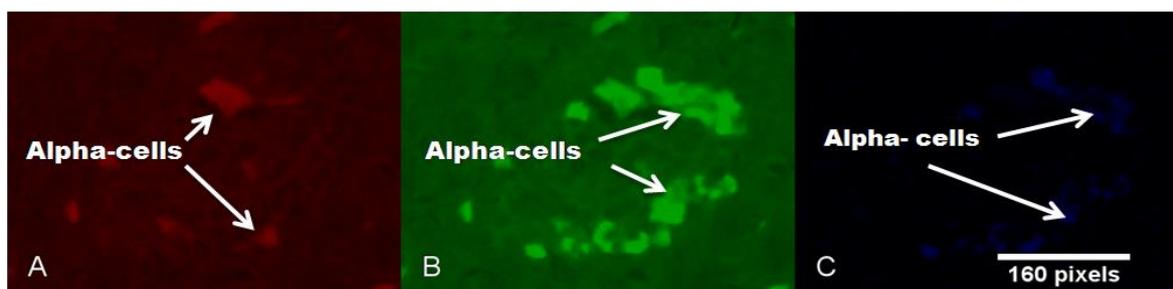


Fig.4:fluorescent microscopic images of the pancreas of the camel, using immunostaining of glucagon Like peptide-1- producing cells(GLP-1), Alpha cells were mostly observed as clumps in the periphery area(A with CY3&B with FITC and figC. Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI) (X200).

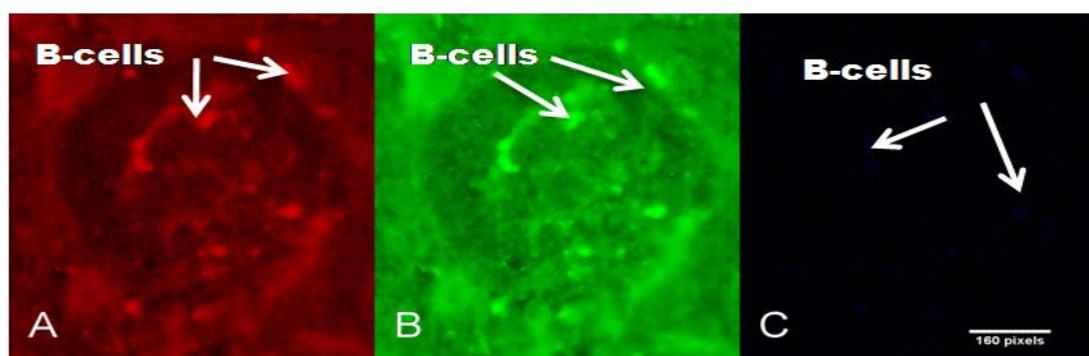


Fig.5: fluorescent microscopic images of the pancreas of the camel, using immunostaining of GIP- producing cells, Beta cells were mostly observed as clumps in the periphery area and center(A- with CY3 , B- with FITC and C- Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI) . (X200) .

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