Scholars Journal of Agriculture and Veterinary Sciences **e-ISSN 2348–1854**
Sch J Agric Vet Sci 2017; 4(6):245-248 **p-ISSN 2348–8883**

(An International Publisher for Academic and Scientific Resources) DOI: 10.36347/sjavs.2017.v04i06.006

Confirmation of Endocrine Cells in the Pancreas of *Iguana iguana* with Immunohistochemistry

Yosua Kristian Adi[1], Teguh Budipitojo[2*]
[1]College of Magister Sain Veteriner, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Fauna street No. 2, Caturtunggal, Depok, Sleman, D.I.Y. 55821, Indonesia
[2]Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Fauna street No. 2, Caturtunggal, Depok, Sleman, D.I.Y. 55821, Indonesia.

***Corresponding Author**
Name: Teguh Budipitojo
Email: budipitojo@ugm.ac.id

Abstract: Pancreatic endocrine cells of Green iguana (*Iguana iguana*) could not be distinguished clearly with routine histology staining (hematoxylin and eosin stain). Immunohistochemical method was carried out to detect the presences of endocrine cells producing insulin, glucagon, somatostatin, and pancreatic polypeptide (PP) in the pancreas gland of Green iguana. Pancreas glands of three adult Green iguanas were fixed with Bouin's solution for 24 hour, processed with paraffin method, cut in 5 µm thicknesses, and stained by immunohistochemistry. The Immunoreactive (IR) endocrine cells were reported descriptively. Insulin- and glucagon-IR cells were showed in group of cells (islets of Langerhans) or individually dispersed in the whole pancreatic organ. Glucagon-IR cells seem more predominant than insulin-IR cells in the islets of Langerhans. Individual insulin- and glucagon-IR cells could be seen between the exocrine cells, but not as many as those found in the islets. Somatostatin-IR cells could be found in the islets of Langerhans. The amount of somatostatin-IR cells in the islets of Langerhans was less than glucogon-IR cells and insulin-IR cells. Glucagon-IR cells were the most prevalent cells compared to insulin- and somatostatin-IR cells. There was no PP-IR cells detected in the islets, around the duct of pancreatic exocrine parts, and between acinar cells. There were three major pancreatic endocrine cell types in the pancreas of Green iguana, with the most estimated amount being glucagon-IR cells, then insulin-IR cells were medium and somatostatin-IR cells were the least of all.
Keywords: Endocrine cells, Pancreas, Green iguana, Immunohistochemistry

INTRODUCTION

The Green iguana, *Iguana iguana*, inclusive to the genus Iguana in family Iguanidae is a herbivorous reptile that first domesticated by human [1]. Nowadays, Green iguana becomes popular exotic pet animal in the world, even in Indonesia. The status of Green iguana is not assessed in International Union for Conservation of Nature (IUCN) and classified as Appendix II in the Convention on International Trade in Endangered Species (CITES), so it free to be bred and trade, but must be controlled in order to avoid utilization incompatible with their survival [2]. Green iguana occurs in Mexico, Central America, South America, and distributed wide range in the world. It lives in humid tropical rainforest as arboreal reptile [3].

The pancreas is thus both an exocrine gland and an endocrine gland. Pancreatic exocrine cells are conical or pyramidal. This secretory cell of the acinus is produce digestive enzymes. The endocrine portions are the pancreatic islets (Langerhans islets). The islets are scattered masses of pale-staining cells supported by reticular connective tissue [4, 5]. There are 4 major hormones (insulin, glucagon, somatostatin, and pancreatic polypeptide (PP)) produced in endocrine pancreas [6]. Insulin and glucagon is involved in the maintaining glucose plasma level which related to diabetes mellitus. The secretion of insulin and glucagon is regulated by others factor, that are somatostatin [7] and PP [8].

In previous study, Langerhans islets of Green iguana could not be seen clearly with hematoxyline and eosin staining. Confirmation needed to evaluate the presence of endocrine cells producing four major hormones in pancreas. Identification types of endocrine cells in the pancreas of Sunda porcupine (*Hystrix javanica*) with immunohistochemistry (IHC) have been reported by Budipitojo *et al.;* [9]. This method can be used to show the Langerhans islets [10].

MATERIAL AND METHODS

Animal

Pancreatic tissues of three adult Green iguanas, *Iguana iguana*, from East Java, Indonesia, were used as samples. The pancreatic tissues were fixed in Bouin's solution for 24 hour and then moved in 70% alcohol solution before processing. Tissues were processed with paraffin method and cut serially in 5 µm thicknesses.

Immunohistochemical staining

Immunohistochemical staining was carried out with Starr Trek Universal HRP Detection System kit (Biocare Medical, Cat. No. STUHRP700 H L10, USA) to visualize the immunoreactivities. Tissue sections on the slides were deparaffinized in xylene and rehydrated in series of ethanol from absolute to low concentration. After that, slides were washed in running tab water for 10 min and ended in distilled water. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 15 min. After washing in phosphate-buffer saline (PBS), non-specific staining was prevented by incubate slide in Background Sniper for 15 min. Primary antibody (Table 1.) was applied two hours. Slide washed in PBS and incubated in Trekkie Universal Link as secondary antibody for 20 min. After washing in PBS, TrekAvidin-HRP applied for 15 min and then washed again in PBS. Batazoid DAB Chromogen was mixed with Betazoid DAB Substrate Buffer (4:100) and then applied to produce color signal in the immunoreactive site. Slide was counterstained with Harris Hematoxylin, dehydrated with series of ethanol, clearing with xylene, and mounted with deck glass. Canada balsam was used as glue. The slide was examined with a conventional light microscope and taken a photomicrograph with digital camera.

Table 1: Primary antibodies used

Primary antibody	Concentration
Insulin	1:500
Glucagon	1:2000
Somatostatin	1:2000
Pancreatic Polypeptide	1:1000

RESULTS AND DISCUSSION

Results

This research confirmed the presence of islets of Langerhans in pancreas gland of Green iguana. The islets of Langerhans distributed in all around the organ. The islets of Langerhans could be divided into three groups based on the size. There were large, medium, and small islets of Langerhans embedded within the exocrine pancreas (Fig. 1). Three endocrine cell-forms, fusiform, round, and oval, composed the islets of Langerhans of Green iguana pancreatic tissue.

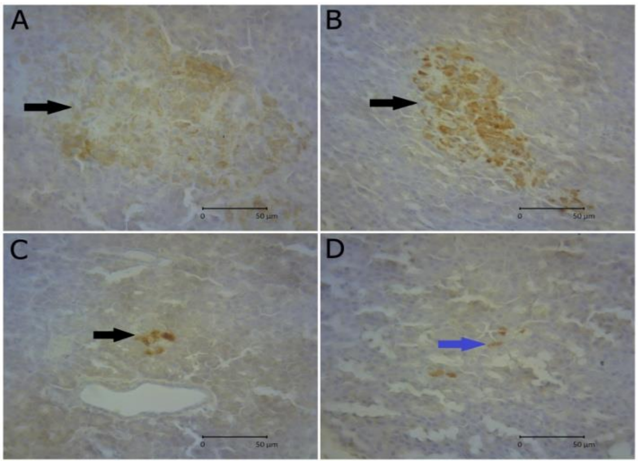

Fig 1: Islets of Langerhans in pancreas tissue of Green iguana.

There were three groups islet of Langerhans (black arrow) based on size, large (A), medium (B), and small (C), that embedded within the exocrine pancreas. Individually endocrine cell (D) (blue arrow) also detected between exocrine pancreas tissues (IHC-glucagon, scale bar 50 µm).

There were three types of pancreatic endocrine cells in the islets of Langerhans that detected with immunohistochemistry. Those were insulin-, glucagon-, and somatostatin- producing endocrine cells. There was no PP-producing endocrine cell in the islets of Langerhans. The insulin- and glucagon-IR cells formed a cluster of cells (islet of Langerhans) or separated individually between the exocrine cells. The islets of Langerhans were predominantly by glucagon- and insulin-IR cells, but some somatostatin-IR cells could be seen. The most estimated amount of endocrine cell type in the islet of Langerhans was glucagon-IR cells, followed by insulin-IR cells, and then somatostatin- IR cells were the least of all.

DISCUSSION

The endocrine part of pancreas gland produced four major hormones, include glucagon, insulin, somatostatin, and pancreatic polypeptide [6]. Differ with others reptiles, such as *Chalcides ocellatus* [11], *Caiman latirostris* [12], *Mabuya quinquetaeniata* and *Uromastyx aegyptia* [13], that have all of pancreatic major hormones, in pancreas tissue of Green iguana only possessed three type endocrine cells producing major hormones (Fig. 2). Glucagon-, insulin-, and somatostatin-producing endocrine cells were detected in islets of Langerhans or individually scattered between exocrine pancreas cells.

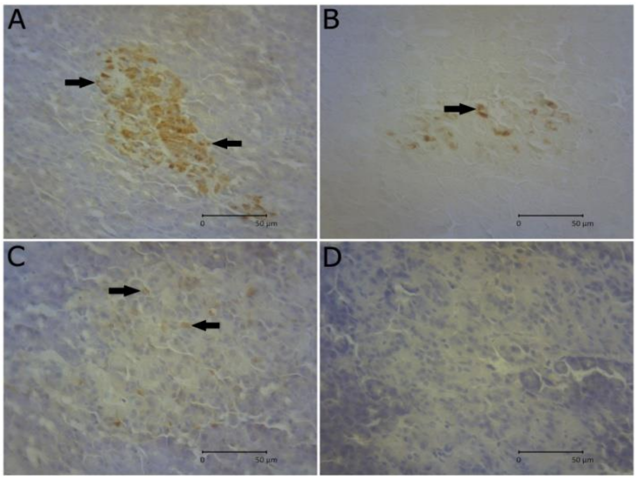

Fig 2: The glucagon-, insulin-, and somatostatin-IR cells in the islets of Langerhans of Green iguana pancreatic tissue.

Glucagon-IR cells (A) were the most predominant IR cells in the islets, followed by insulin-IR cells (B), and then somatostatin-IR cells (C) were the least of all. There was no PP-IR cell in the islets (D). The immunoreactive cells were indicated by black arrows (IHC-glucagon, -insulin, -somatostatin, and -PP, scale bar 50 µm).

Subjectively, there were more glucagon- producing cells than insulin-producing cells in the islets of Langerhans. This finding in Green iguana was differ with other lizard, *Calotes versicolor*, that reported by Chandavar and Naik [14]. The insulin-producing cells in the islets of Langerhans of *C. versicolor* always greater than glucagon-producing cells in all breeding season. The same review has been reported by Ku and Lee [15], in grass lizard (*Takydromus wolteri*). Insulin-IR cells were the most predominant cell types in pancreas islets. Other study by Chandavar and Naik [16], revealed a negligible difference in the distribution of glucagon- and insulin-IR cells in pancreas tissue of turtle *Melanochelys trijuga*. The same results as our findings have been reported by Putti *et al.;* [17] in lizard *Podarcis sicula sicula*. The two cell types were present in almost equal number with glucagon-producing cell slightly more than insulin-producing cell. Very few glucagon- and insulin-IR cells were found scattered throughout the pancreas of Green iguana (Fig. 3), same as reported in other lizard *C.versicolor* [14], turtle *Takydromus wolteri* [15], and mammalian *Hystrix javanica* [9].

Somatostatin-IR cells scattered in islets of Langerhans of Green iguana pancreas tissue. The number of somatostatin-IR cell was less compared to glucagon- and insulin-IR cells. In the pancreas of grass lizard, *Takydromus wolteri*, somatostatin-IR cells were the third most abundant cell type [15]. Somatostatin exerts its modulatory activity both in the islets of Langerhans and the exocrine part of pancreas gland [6]. Somatostatin is a potent regulator of insulin and glucagon secretion [7]. More detail observation about the presence of somatostatin-IR cell in Green iguana exocrine pancreas is needed. There was no PP-IR cell observed in the islets of Langerhans of Green iguana. This finding was same as reported by Abou-Zaid *et al.;* [18] in young pigeon. The islets of young pigeon lacked the PP-IR cells, but occurred in adult and old. Physiological effects of the PP are still unclear [6, 19].

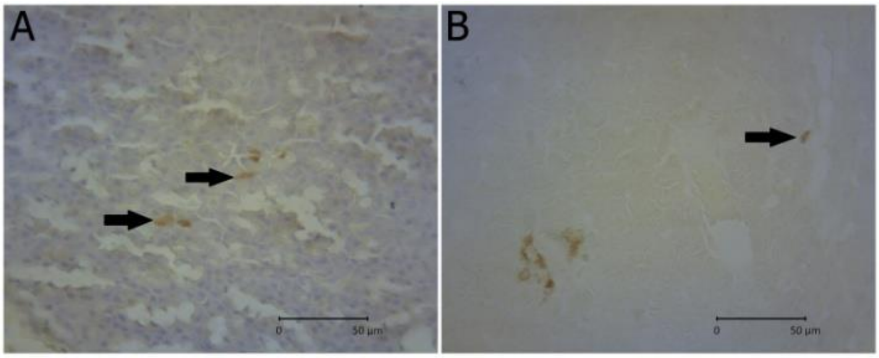

Fig 3: Individual endocrine cells. Glucagon-IR cells (A) and insulin-IR cells (B) could be found scattered between acinar cells of pancreatic exocrine part.

CONCLUSIONS

There were three major pancreatic endocrine cell types that could be detected in the pancreas of Green iguana. The most estimated IR cells amount in the islets of Langerhans being glucagon-IR cells, followed by insulin-IR cells and somatostatin-IR cells were the least of all. There was no PP-IR cells detected in the islets, around the duct of pancreatic exocrine parts, and between acinar cells. Further research about pancreatic endocrine cell of Green iguana in all of live stage or reproduction cycle is necessary to understand the role of pancreatic hormones.

REFERENCES

1. Ballard B. Exotic animal medicine for the veterinary technician. John Wiley & Sons; 2016 Sep 26.
2. Bock BC. Iguana iguana Linnaeus 1758: Common Green Iguana. 2014. Available from http://www.iucn-isg.org/species/iguana-species/iguana-iguana/
3. Csurhes S. Weed risk Assessment: Green iguana Iguana iguana. Queensland Government. Queensland. 2011.
4. Banks WJ. Applied veterinary histology. Mosby-Year Book, Inc; 1993.
5. Kardong KV. Vertebrates Comparative Anatomy, Function, Evolution. 6^{th} Ed. McGraw-Hill. USA. 2012.
6. Škrha J. Pancreatic hormones and hormonal regulation of insulin secretion. Cas Lék Ces. 2006; 145:599-605.
7. Strowski MZ, Blake AD. Function and expression of somatostatin receptors of the endocrine pancreas. Molecular and cellular endocrinology. 2008 May 14; 286(1):169-79.
8. Aragon F, Karaca M, Novials A, Maldonado R, Maechler P, Rubi B. Pancreatic polypeptide regulates glucagon release through PPYR1 receptors expressed in mouse and human alpha-cells. Biochimica et Biophysica Acta (BBA)-General Subjects. 2015 Feb 28; 1850(2):343-51.
9. Budipitojo T, Fibrianto YH, Mulyani GT. The types of endocrine cells in the pancreas of Sunda porcupine (Hystrix javanica). Veterinary world. 2016 Jun; 9(6):563.
10. Nugroho WS, Kusindarta DL, Susetya H, Fitriana I, Mulyani GT, Fibrianto YH, Haryanto A, Budipitojo T. The structural and functional recovery of pancreatic β-cells in type 1 diabetes mellitus induced mesenchymal stem cell-conditioned medium. Veterinary world. 2016 May; 9(5):535.
11. El-Salhy M, Abu-Sinna G, Wilander E. The endocrine pancreas of a squamate reptile, the desert lizard (Chalcides ocellatus). Histochemistry and Cell Biology. 1983 Sep 1; 78(3):391-7.
12. Ono K, Yamada J, Pai VD, Kitamura N, Gregório EA, Yamashita T, Campos VJ. An ultrastructural study on the endocrine pancreas of Caiman latirostris, with special reference to pancreatic motilin cells. Archives of histology and cytology. 1991; 54(3):349-57.
13. El-Salhy M, Grimelius L, Wilander E, Abu-Sinna G, Lundqvist G. Histological and immunohistochemical studies of the endocrine cells of the gastrointestinal mucosa of the toad (Bufo regularis). Histochemistry and Cell Biology. 1981 Mar 1; 71(1):53-65.
14. Chandavar VR, Naik PR. The endocrine pancreas of the lizard Calotes versicolor: An immunocytochemical and physiological study with respect to its reproductive cycle. Journal of Cytology and Histology. 2012; 3(3):143.
15. Ku SK, Lee HS. The distribution and frequency of endocrine cells in the splenic lobe of grass lizard (Takydromus wolteri): An immunohistochemical study. European journal of histochemistry: EJH. 2004 Oct 1; 48(4):429.
16. Chandavar VR, Naik PR. Immunocytochemical detection of glucagon and insulin cells in endocrine pancreas and cyclic disparity of plasma glucose in the turtle Melanochelys trijuga. Journal of biosciences. 2008 Jun 1; 33(2).
17. Putti R, Cavagnuolo A, Varano L. Morphology of the lizard endocrine pancreas in normal, insulin-and ACTH-treated Podarcis sicula sicula. Italian Journal of Zoology. 1983 Jan 1; 50(3-4):149-57.
18. Abou-Zaid FA, Salem SB, Madkour GA, Alm-Eldeen AA. Histological and immunohistochemical studies on the pigeon endocrine pancreas at different ages. Egypt. J. Exp. Biol. (Zool.). 2010; 6:385-94.
19. Williams JA. MOLECULE PAGE: Pancreatic Polypeptide. The Pancreapedia: Exocrine Pancreas Knowledge Base. 2014. Available from https://www.pancreapedia.org/molecules/pancreatic-polypeptide