AN IMMUNOHISTOCHEMICAL LOCALIZATION OF ENDOCRINAL CELLS IN THE EPITHELIUM OF THE DUODENUM MUCOSA OF TURKEY

(*Meleagaris gallpava*)

Abdulrazzaq Baqer Kadhim

Department of Anatomy and Histology and Embryology ,Collage of Veterinary Medicine, University of Al-Qadysiah, Al-Qadysiah,Iraq

(Received 13 december 2016 ,Accepted 7 january 2017)

Key words: Turkey, (Chromograinn A**,** cholecystokinin**.**

ABSTRACT

in some parts of the intestinal tract, which play an important and primary role in the secretion of certain hormones that regulate important organs of the body. The aim of this study is to examine the site endocrine cells in the mucous layer of the five male Turkey. The areas where endocrine cells in the mucous layer of the five male in the Turkish examined by using immunohistochemistry technique material. Samples were taken from five birds from the Turkish males ages ranging from 1-2 years. It was used immunohistochemistry textile technology and (ChromograinnA), which is a special detector Endocrine intestinal cells, four types of hormones. Immune chemical examination in the mucous layer duodenum and the presence of hormones, glucose insulin tropic polypeptide(GIP) results showed (GIP) and cholecystokinin (CCK) glucagon-like peptide2 (GLP-2) in epithelial cells group along the internal axis of the villi, chemical immunological results for many of the sections shown note chromogranin A detector particularly for intestinal endocrine cells, the cells that cholecystokinin(CCK) , glucose insulin tropic polypeptide contain hormones (GIP) and glucagon peptide GLP-1 \ 2)) containing this reagent indicating confirm the location of these cells, a gastric endocrine cells . The presence of these hormones in the mucous layer of the Duodenal decreases as we move away from the duodenal

INTRODUCTION

Over the years five distinct subspecies of turkey occurring in the wild have been named, all native to North America but in different habitat areas , the indigenous turkey is derived from the native wild turkey which descendant of Mexican turkey(*Meleagris gallopova*) (2).

During their long evolution ,birds have development a number of structural features that differ from those found in mammals, the distribution of endocrine cells in the gastrointestinal tract is related to species ,living environment ,feeding habits and development status rather than evolution. Extensive immunohistochemical studies have been carried out on the distribution of gastrointestinal endocrine cells in a wide variety of birds (16,21).Since the immunohistochemical method was first applied to endocrine cells in the digestive tracts of poultry(11,15) . More than 10 types of endocrine cells have been detected in the avian digestive tracts (20). Distribution of endocrine cells in the digestive tracts of bird is different from that of mammals because of the extent of intestinal maturation varies with the duration of gestational period (22) . The main gastrointestinal hormones include 5-hydroxtryptamine , gastrin , somatostatine , gluagen and substance (4). Gastrointestinal endocrine cells dispersed in the mucosa of digestive tract synthesize various types of gastrointestinal hormone which played an important role in the physiology function of alimentary tract such as nutrient ,absorption ,the secretion of intestinal and associated gland, gut motility and increased intestinal blood flow (8).Gasterointestinal hormones are regulatory peptides appear to be the major component of body integration and have an important regulatory action on physiologyical function of gasterointestinal tract (9,10) .Enteroendocrine cells such as duodenal cholecystokininare (CCK cells) generally thought to be confined to certain segments of the gasterointestinal tract and to store and release peptides derived from only a single peptide precursor (3). Glucagen (GLP) is peptide hormone ,secreted by alpha cells of the pancreas, which raises the concentration of glucose in the blood stream(5). GIP is mean glucose-dependent insulin tropic polypeptide (GIP) . Chromogranin A (ChA) is a secretary protein of

the granin family which include A,B and C .This granins are found in the secretory granules of endocrine and neuroendocrine cells (7) .

MATERIAL AND METHOD

Five adult male turkey (*Meleagris gallopova*) of different ages (1-2 years old) and trapped by peasants in Al-Diwaniyah birds market were used .Deep anesthesia of animals was induce by initial injection of ketamine (ketamine10 mg/kg i.m) followed by xylazine (rompun 0.10-0.15 mg/kg.i.m) .Tissue samples were taken from three parts of duodenum (proximal,middle,distal) and fixed 10% formalin for 48 hours .Then tissues were dehydrated through graded ethanol and embedded in paraffin. Five µm thick section were obtained and processed for immunohistochemical staining .Slides, containing wax embedded turkey duodenum tissues , were dewaxed in 100% xylene three time for 10 minutes each. The tissue was placed twice in 100% ethanol for 10 minutes. Sections were 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 two time for 5 minutes in 70% ethanol. Slides were then rehydrated twice in distilled H_2O for 5 minutes each. Slides were immersed in antigen retrieval buffer (10mMTris/HCl pH 10.0) and autoclaved two time for 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 time for 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 .For double-immuno staining, primary antibodies raised in different species were mixed with one another without changing the final required concentration and were incubated at 4°C overnight. Each slide was then washed in PBS for 5 time for 5 minutes. FITC-conjugated IgG/IgY and Cy3-conjugated IgG/IgY (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 time for 5 minutes and mounted in Vectashield Hard Set Mounting Media with DAPI. All sections of

immunohistochemistry study were visualized using an epi fluorescence microscope and the results were pictured with a canon digital camera in dark room . Images from serial sections were merged using Imaging Products Laboratory imaging software (BioVision Technologies, Exton, PA, USA). Omission of primary antibody was routinely used as a control (13)

RESULTS AND DISCUSSION

Duodenum tissues of turkey were first used as positive controls to investigate the expression of GIP, GLP-1/GLP-2 and CCK by using immunohistochemistry. The results presented that GIP, GLP-1/ GLP-2 and CCK are expressed (Fig. 1). There was no staining when the primary antibody was omitted from the control section (Fig. 2). Typical image meaning that gut hormone (GIP, GLP-1/GLP-2 and CCK) are expressed in duodenum.

This study determined the distribution pattern of GLP-2-containing intestinal L cells in duodenum of turkey .Immunoreactive cells for GLP-2 were mainly observed in the proximal duodenum (Fig 3) .This distribution pattern is similar to that of GLP-1-immunoreactive cells. Our recent investigation using immunocytochemistry with colloidal gold determined the colocalization of GLP-1 and GLP-2 in the same secretory granules (14)The similarity of the distribution patterns of GLP-1 and GLP-2 is supported by this immunocytochemi-cal study. GLP-2-immunoreactive cells are mainly located in the epithelium of the upper part of duodenum villi, while GLP-1-immunoreactive cells are located in the middle to lower parts of duodenum villi (Fig.4). The difference in localization of GLP-1 and GLP-2 reflects this finding. Biochemical and molecular biological studies of the secretary process of GLPs may be necessary to clarify this phenomenon.

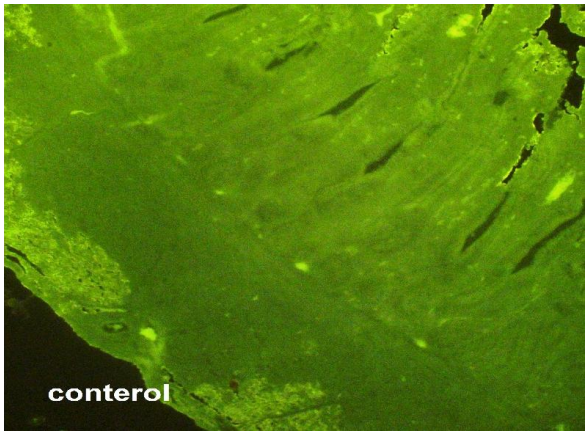
The results of immunostaining technique showed that enteroendocrine cells containing gut hormones and ChA were co-expressed in the same cells in turkey small intestine (Fig. 6). This pattern of co-expression of the gut hormones and ChA has also been shown in other mammals including human (19) . The results showed the relative frequency and distribution of endocrine cells in the small intestine of

turkey were examined by using four type of antisera. The immune reactive cells were identified in the small intestine, most of the gut hormone were located in the basal portion of glands. The IR cells were triangular or slender in shape .They appeared as close-type cells as they did not possess lamina contact with their apical cytoplasmic processes. Observed some open-type cells with apical cytoplasmic processes that reach the intestinal lumen more frequency in the duodenum region.

The relative frequency and distribution of IR endocrine cells in the duodenum on the intestinal villi, in the crypts (intestinal glands) and in Brunner's glands (duodenal glands). Generally, the endocrine cells were observed in high frequently in the villi of duodenum, The duodenum contains the greatest variety of endocrine cell types in the digestive tract. This finding supported by (17).

CCK IR- cells were observed in high frequently in the villi and the intestinal crypts of the duodenum. It is well established that the duodenal mucosa plays a very important role in digestion and influences pancreatic secretion and gall bladder emptying in higher mammals via gastrointestinal hormones released from the proximal duodenum . The proximal duodenum is thought to be protected, at least in part, from acid-pepsin entering from the stomach by secretions from Brunner's glands. Similar findings concluded that CCK have been demonstrated on turkey (6,18). In the small intestine K-cells which containing GIP and I-cells containing CCK, were detected (fig.4)

Enteroendocrine cells secrete hormones and peptides play a vital role in the function of the digestive system with enteric nervous system, whereas effect of these hormones and peptides on food intake and appetite, the regulation of glucose homeostasis, gut motility and various other physiological functions (1,12).Our results support the important digestive role for endocrine cells in the duodenum of turkey. The present study, demonstrated the expression, distribution and relative frequency of three types of enteroendocrine cells in the duodenum .



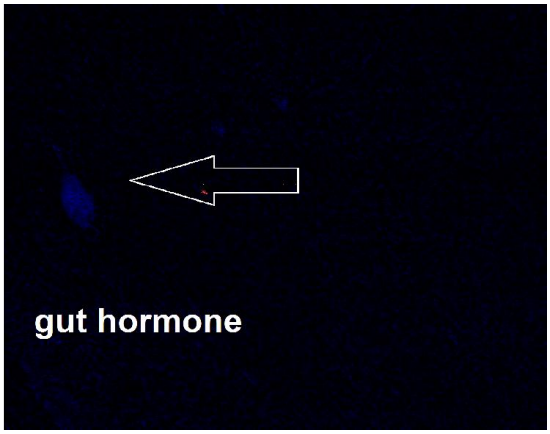


Fig.1 **fig.2**

Figure 1.2: Wax embedded intestinal tissue sections from turkey were probed with the antibodies to gut hormone (GIP, GLP-1, GLP-2 and CCK). Typical image showing that gut hormone (blue) is expressed in a subset of intestinal cells. When the primary antibody was omitted from the control section there is no labeling for gut hormones show in control image. Images are 200 X magnified. Nuclie are stained blue with 46ˈ- diamidino -2- phenylindole (DAPI).

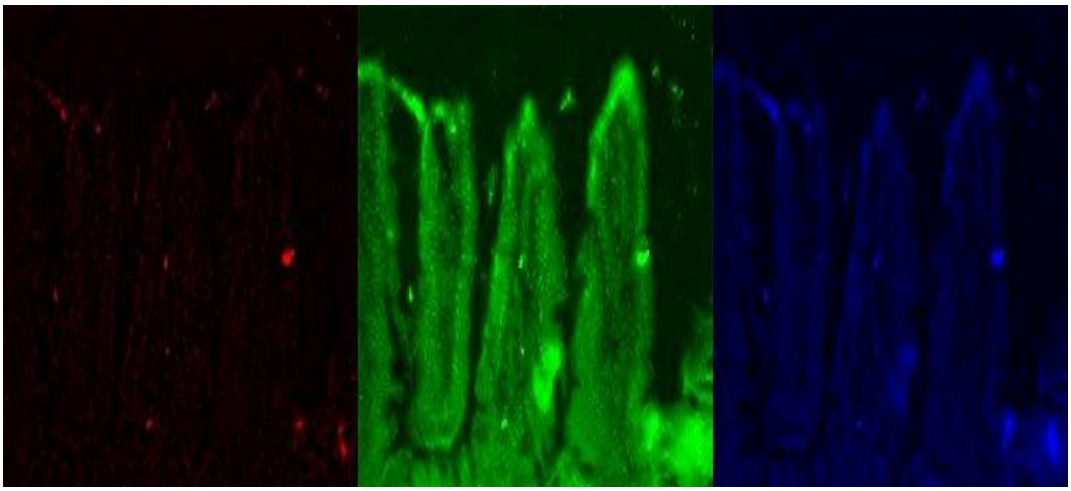


Figure 3.4.5: Typical images showing gut hormones (GLP-1 and GLP-2 in duedenum) green and red in a subset of turkey duodenum . Image Fig.5 is (200 X)

magnified Fig.4 and Fig.3 are(400 X) magnified . Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).

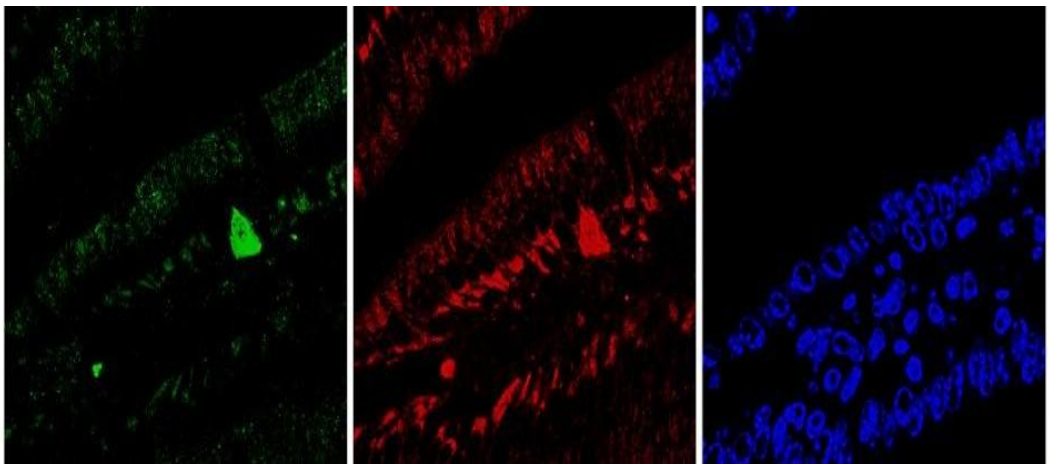


Fig. 6: typical immune fluorescence images showing co-localization of gut hormones (GIP) (green) with ChA (red) in duodenum.

الكشف عن تواجد الخلايا الصماوية في الطبقة المخاطية لاثني عشري الدجاج الرومي باستخدام تقنية الكيمياء المناعية النسيجية

عبد الرزاق باقر كاظم

فرع التشريح والانسجه والاجنة، كلية الطب البيطري،جامعة القادسية ، القادسية ،العراق.

الخلاصة

للخلايا الصماوية المعوية في بعض أجزاء القناة المعوية دور مهم ورئيسي في إفراز بعض الهرمونات التي تنظم أجهزة الجسم المهمة . أن تواجد الخلايا الصماوية في الطبقة المخاطية لاثني عشري في التركي تم الكشف عنها باستخدام تقنية الكيمياء المناعية النسيجية. تم اخذ العينات من خمس طيور من ذكور التركي بأعمار تتراوح ما بين 1-2 سنوات. استخدمت تقنية الكيمياء المناعية النسيجية وكروموكرانين (ChromograinnA)الذي يعتبر كاشف خاص للخلايا الصماوية المعوية، وأربعة أنواع من الهرمونات وهي الجلوكوز إينسولينوتروبيكالبيتيد (GIP)و كولسيتوسكينين (CCK) الجلوكاجون ببيتيد (GLP-1\2) إن الهدف من هذه الدراسة هو فحص موقع الخلايا الصماوية في الطبقة المخاطية لاثني عشري في التركي . بحيث أظهرت نتائج الفحص الكيميائي المناعي في الطبقة المخاطية ألاثني عشري وجود هرمونات، الجلوكوز

إينسولينوتروبيكالبيتيد (GIP) و كولسيتوسكينين (CCK) الجلوكاجون ببيتيد(GLP-1\2) في مجموعة الخلايا الظهارية على طول المحور الداخلي للزغابات ، أظهرت النتائج الكيميائية المناعية للعديد من المقاطع نلاحظ الكروموكرانين هو كاشف خاص للخلايا المعوية الصماوية، وان الخلايا التي تحتوي على الهرمونات الأربعة تحتوي على هذا الكاشف مما يدل على تأكيد موقع هذه الخلايا وهي خلايا معوية صماوية. أن وجود هذه الهرمونات في الطبقة المخاطية لاثني عشري تنخفض كلما ابتعدنا عن الاثني عشري .

REFERENCES

1. Ali, M.A., Nyberg, F., Chandranath, S.I. , Dhanasekaran, S., and Tariq, S. , Petroianu, G. ,Hasan,Y., Adeghate, A., and Adem, A. (2007) : Distribution of neuroendocrine cells in the small and large intestines of the one humped camel (Camelus dromedarius). Neuropeptides, 41: 293-299.

2. Cathey,J.C.,Melton,K.,Dreibebis,J.,Cavney,B.,Locke,S.L(2008):Life History physical charactistic .Biology and Mangement .Texas Institute of Renrwable Natural Resources ;4-6duodenum. Chinese Journal of Animal and Veterinary Sciences, 2004; 35: 60-63.

3. Egerod, K.L.,Engelstoft, M.S., Grunddal, K.V., Nohr, M.K., Secher, A., and Sakata, I..(2012): A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY and neurotensin but not somatostatin. endocrinology;153(12):5782–5795.

4. El-Salhy, M., Wilander, E., Lundquist, M.: Comparative studies of serotonin-like immunoreactive cell in the digestive tract of vertebrates. Biomed. Res., 1985; 6:371-375.

5. Eroschenko , V.P.(2013): Di Fiore's atlas of histology with functional correlations , 12th Edn. ,p: 341-353.

6. Fatimah S.Z. (2014) .Immunofluorescene study of enteroendocrine cells in small intestine of camel

7. Feldman, S.A., and Eiden, L.E.(2003): The chromogranins: their roles in secretion from neuroendocrine cells and as markers for neuroendocrine neoplasia. Endocr Pathol.;14(1):3-23.

8. Huang, X.G., Wu, X.B.: Immunohistochemical localization of endocrine cells in the digestive tract of Sphenomorphus indicus. Chinese Journal of Histochemistry and Cytochemistry, 2006;15:233-238.

9. Kuru, N., Cinar, K., Senol, N., Demirag, E., and Diler, D. (2010): Endocrine cells in the Gastrointestinal Tract of Garrarufa . Kafkas Univ. Vet. Fak Derg.16(Suppl-B): S235- S241.

10. Larsson, L.I. (2000): Developmental biology of gastrin and somatostatin cells in the antropyloric mucosa of the stomach. Microscopy Res. Tech., 48: 272-281.

11. Larsson, L.I., Sundler, F., Hakanson, R., Rehi eld, J.F., Stadil, F.: Distribution and properties of gastrin cells in the gastrointestinal tract of chicken. Cell Tissue Res., 1974; 154: 409-422.

12. Mellitzer, G. and Gradwohl, G.(2011): Enteroendocrine cells and lipid absorption .Curr. Opin.Lipidol.22:171–175.

13. Moran, A.W., Al-Rammahi, M., Zhang, C., Bravo, D., Calsamiglia, S.,Shirazi-Beechey, S.P. (2014): Sweet taste receptor expression in ruminant intestine and its activation by artificial sweeteners to regulate glucose absorption. J Dairy Sci.;97(8):4955-72.

14. Nishimura, K., Hiramatsu, K., Monir, M. M., Takemoto, C. and Watanabe, T. 2013. Ultrastructural study on colocalization of glucagon-like peptide (GLP)-1 with GLP-2 in chicken intestinal L-cells. J. Vet. Med. Sci. 75: 1335–1339. [Medline] [CrossRef]

15. Polak, G.M., Pearse, A.E.G., Adams, C., Garaud, J.C.: Immunohistochemical and ultrastructural studies on the endocrine polypeptide (APUD) cells of the avian gastrointestinal tract. Experimentia, 1974; 30: 564-567.proventriculus of seven species of birds. Arch. Histol. Jpn., 1985; 48: 305-314.

16. Rawdon BB. And Andrew A. nimmunocytochemical survey of endocrine cells in the gastrointestinal tract of chick sat hatching.CellandTissueResearch,220:279-292.1981.

17. Solcia E, Capella C, Vassallo G, Buffa R.(1987): Endocrine cells of the gastric mucosa. Int Revde Cytol; 42: 223-286

18. Suha R.G. Al bdere (2016).Relative Frequency and Regional Distribution of enterendocrine cells in some portion of intestine tract of camels

19. Theodorakis, M.J., Carlson,O., Michopoulos,S., Doyle,M.E., Juhaszova,M., Petraki, K. & Egan,J.M. (2006) : Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am. J Physiol. Endocrinol. Metab.290(3):E550 E559.

20. Timson, C.M., Polak, J.M., Wharton, J., Ghatei, M.A., Bloom, S.R., Usellini, L., Capella, C., Solcia, E., Brown, M.R., Pearse, A.G.E.: Bombesin-like immunoreactivity in the avian gut and its localization to a distinct cell type. Histochemistry, 1979; 61: 213-221.

21. Yamada, J., Kitamura, N., Yamashita, T.: h e relative frequency and topographical distribution of somatostatin-, GRP-, APP-, glucagon-, 5-HT-, and neurotensin-immunoreactive cells in the

22. Yang, Q., Chen, S.H., Zhao, R.X., Chen, J.: Ontogeny of somatostatin positive-cells and immunocytes in the porcine .