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RESEARCH ARTICLE

POSTNATAL DEVELOPMENTAL HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDY OF THE DUODENUM IN THE DOMESTIC CAT (*FELIS CATUS*)

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ABSTRACT

The present study was conducted to investigates the histomorphological and histochemical developmental changes established in the duodenum of the domestic cats at three different postnatal ages, that were one week (suckling kittens), 4-6 weeks (weaned immature cats) and adult of one year and up cats. Macromorphometric measurements of duodenum were conducted and listed in tables. Histological sections prepared and stained by general and special stains. Gross findings revealed that the duodenum is U-shaped of longer descending part and shorter ascending part. The beginning of the duodenum contains duodenal papilla in which found central orifice for the exits of bile and pancreatic secretions. The internal mucosal surface of the organ showed gross circular folds called plicae circularis. Histologically, the wall of duodenum in suckling kittens possessed thick tunica muscularis and thin mucosa, but it changed in weaned immature and adult cats to become thin tunica muscularis and thick mucosa. At all ages the submucosa remains thin layer, but slightly thickened in the first part of descending duodenum due to the presence of Brunner's glands. Characteristically goblet cells in cat's duodenum were rounded or circular in shape rather than globular shape as usually found. Their number in the villi was higher than those counted in the duodenal crypts at one week, but approximately equal in number in the 4 weeks and were conversely changed in adult in which the percentage of goblet cells was higher in crypts than in the villi. Histochemically they were stained faintly with PAS stain showed moderate amount of neutral mucin in their cytoplasm. Paneth cells were detected in the duodenal crypts in 4 weeks aged cats and subsequent adult cat but not after birth in one week aged kittens. It could be concluded that the duodenum was not fully developed in cats at birth.

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INTRODUCTION

Cats are commonly suffered pancreatitis and tumors in both pancreas and duodenum. They were usually affected by obstruction of the pancreatic duct in the exocrine portion associated with the inflammation of bile duct and adenocarcinoma of the duodenum. The obstruction of the duodenum cause subsequently crohn's disease in the animal. The latter characterized by dehydration, loss of appetite, loss of body weight, abdominal pain, vomiting and diarrhea (De Cock *et al*., 2007; Bossche *et al*., 2010). In fact, the commonest sites of the intestinal adenocarcinoma in man and dog are the colon and duodenum, whereas gastrointestinal one of the common form of neoplasm recorded in cats is lymphoma which was occurred primarily in the intestinal tract (Louwerens *et al*., 2005; Daniaux *et al*., 2014). The epithelium of the small intestinal mucosa is invaginated to form small crypts continuous with large evaginating villi, the crypts are more numerous and smaller than the villi. These crypts contain lysozyme and cryptdin-positive producing paneth cells at their bases, columnar absorptive cells, goblet cells, enteroendocrine cells in addition to the stem cells (Karam, 1999). One decade before, Leedham *et al* (2005) mentioned that the mammalian intestine constructed by simple columnar lining epithelium rested on non epithelial stromal tissue which in turn laying on a layer of smooth muscle bundles. The epithelium is invaginated to form protruded finger-like structures which are extending toward the lumen of intestine called crypts of Lieberkühn and villi, respectively. The epithelial lining is continuously face renewal processes in a constant manner and dead cells are regularly discarded into the intestinal lumen. The renewal and loss of cells processes must be strictly regulated in order to avoid irregular accumulation of cells or epithelial ablation (Potten *et al*., 1997). The renewal processes was thought to be directed by pluripotent stem cells, existed in the bases of the intestinal crypts (Ponder *et al*., 1985; Winton, 1990). In mice, it was found that the stem cell were divides

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slowly in which approximately once every 24 h (Potten, 1990). Whereas, subsequently the latter investigator found such process eight folds slower in humans (Potten, 1992), producing progenitor cells known as transit amplifying cells. These are progenitor cells proliferated so fast when they move up toward the crypt-villus axis (Deheragoda and Wright, 2006). Cells exit this zone and they differentiate and terminate into one of the four mature epithelial cell types (enterocytes, goblet cells, enteroendocrine cells, or Paneth cells) (Leedham *et al*., 2005). The Paneth cells are reside at the bases of the crypts, but the other epithelial cell types migrate up the crypt–villus axis and at the end of the villus, they undergo apoptosis before being shed into the intestinal lumen (Deheragoda and Wright, 2006). There is paucity of work on the Histomorphological study of duodenum in the domestic cat and to our knowledge there is no local study up to date investigated the postnatal developmental histomorphological changes of this organ in the local domestic cats.

MATERIALS AND METHODS

Cat's collection and study design

Clinically healthy pregnant queens were collected by hunting method and caged in the animal house till their delivery to obtain at least twelve kittens from them. Six kittens of one week of age were removed from their mothers and euthanized and considered suckling kittens group. Other six kittens were left for not less than four weeks. They were euthanized and considered weaned immature group. Six adult healthy cats of more than one year were hunted and kept under supervision in cages for one week and then euthanized as they were considered adult group.

Preparation of specimens

Each of the selected kittens, immature or adult cats were euthanized prior to their dissection by intra-cardiac injection of over dose of sodium pentobarbital (100 mg/kg) (AVMA, 2013). After that, the animal was fixed to be dissected on a dissecting board. The abdominal wall opened to view the abdominal viscera, then the duodenal loop was pointed out and the organ location and relationship with other digestive organs was photographed in situ. The topography and shape of the organ was studied and documented with aid of digital camera. Macroscopic measurements of the duodenal loop such as relative weight, length were measured after its extirpation from the intestinal tract. The data on such macromorphometric and subsequent micromorphometric were presented in tables.

Histological procedures

The representative specimens of one cm were cut from the descending (divided into three parts, that were proximal, middle and distal parts) and ascending limbs of the duodenum. Specimens were immersed in 10% neutral buffered formalin for 72 hrs for general histological study or in Bouin's solution for histochemical staining for 48 hrs. Specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90% and 100%) each for 2 hrs, then cleared with xylene for two times each of ½ hr. Processed specimens were infiltrated with paraffin wax on 55 ºC then embedded with new paraffin wax to obtain blocks of paraffin. Paraffin sections of 6 µm were obtained by using rotary microtome (Bancroft and Gamble, 2008).

Staining procedures

The sections were stained with either one of the following stains: Hematoxylin and Eosin (H&E) as routine stain for general features identification, Masson trichrome (MTC) stain for the staining of the connective tissue of the lamina propria, submucosa and serosa of the duodenal wall. The stain can also stain the smooth muscle fibers present in the muscularis mucosa and muscularis externa of the duodenal wall. Periodic acid Schiff stain for the goblet cells in the duodenal mucosa (Luna, 1968). For the identification of paneth cells, both H&E and MTC were successfully used.

Micromorphometric measurements

The tissue sections were analyzed using Olympus light microscope and were photographed and analyzed by Dino-eye piece camera provided with Image software. Micromorphometric data collect on the duodenal wall was comprised the following parameters: epithelial height, number of goblet cells, number of villi, length of villi, width of villi, depth of crypts, thickness of each tunic of the wall of the duodenum, percentage of mucosa thickness wall thickness and percentage of muscularis thickness wall thickness.

Statistical analysis

Statistical calculations were carried out with the SPSS 15.0 for windows software package. All numerical values were expressed as the mean ± standard error (SE). The statistical significance set at $p < 0.05$ which was assessed by ANOVA.

RESULTS

Gross Findings

Macroscopic findings showed that in the cat the duodenum was noticeably short U-shaped tubular organ (Fig. 1). It was located at the right side of abdominal cavity in which it was suspended together with the pancreas by the duodenal mesentery to the dorsal wall of the abdominal cavity. It joined cranially the pylorus of stomach where it formed the first duodenal flexure. Caudally, its ascending limb which was very short joined to the jejunum when the organ reflected to the left side of the abdominal cavity forming the third duodenal flexure. The site of joining descending with the ascending limb structured the second flexure. The descending limb was longer and larger in diameter compared to that of ascending limb. Post longitudinal dissection in the wall of the duodenum, the organ showed characteristically many circular folds (Fig. 2). Duodenal papilla was found just 4 to 5 cm distance below the gastro-duodenal opening. The papilla view small opening to discharge the secretion of both bile and major pancreatic ducts through which they convey their secretions to the proximal part of the descending duodenum. The ascending limb of duodenum appeared very short crossed by the distal colon where it turns to the left side of abdominal cavity to join the jejunum. Macromorphometric measurements such as length, weights and relative weights of the duodenal parts were listed in Table 1. The data obtained revealed that the mean of length of the duodenum of one weak kittens, 4 weeks weaned cats and adults was increased with the progress of age of the animals. The mean of length of descending duodenum was 6.5 cm ± 0.01 SE and that of the ascending one was shorter obviously (3.5 cm ± 0.05 SE) in the kittens of one week of age. Next, the

length was increased in the 4 weeks aged premature weaned cats up to 11.4 cm ± 0.10 SE and 6.1 cm ± 0.04 SE in the descending and ascending duodenum, respectively. Similarly the data showed an increase in the lengths of these two parts of the duodenum to become 12.1 cm ± 0.22 SE and 6.3 cm ± 0.11 SE, respectively in the adult cats. The relative length of descending part (length of descending part / total length of duodenum) was 65%, whereas the relative length of ascending was 35% in one week kittens. These percentages were slightly changed into 65.14% and 34.86% at 4 weeks of age and changed into 65.76% and 34.24% at adulthood in the ascending and descending parts, respectively (Table 1).

The mean of weight of descending duodenum was 00.06 gm ± 0.02 SE and that of the ascending one was lighter (00.03 gm ± 0.15 SE) in the kittens of one week of age. In the 4 weeks aged premature weaned cat, the weight was increased up to 12.4 gm ± 0.40 SE and 4.6 gm ± 0.07SE in the descending and ascending duodenum, respectively. Once more the data showed an increase in the weight s of the two parts of the duodenum to become 13.25 gm ± 0.02 SE and 5.31 gm ± 0.04 SE, respectively in the adult cats. The relative weight of descending part (weight of descending part / total weight of duodenum) was 66.67%, whereas the relative weight of ascending was 33.33% in one week kittens. These percentages were changed into 71.39 % and 28.61% at 4 weeks of age and changed into 65.76% and 34.24% at adulthood in the ascending and descending parts, respectively (Table 1).

Histological findings

Microscopic examination of the duodenal wall revealed that its structure was formed of four major tunicae that were tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Most typical structures were recorded in the wall of duodenum of all studied postnatal ages of cats.

Descending duodenum

The wall of duodenum of one week kittens showed thin mucosa, thinner submucosa compared to thickest tunica muscularis (Fig. 3). The mucosa revealed numerous villi which were finger shaped projections covered with typical simple columnar epithelial cells associated with abundant goblet cells interspersed between them (Fig. 4). Intestinal crypts were similarly covered with the same epithelium of villi. These glands were detected in the lamina propria of one week kittens with the underneath muscularis mucosa of many circular bundles of smooth muscle fibers (Fig. 5). The goblet cells were prominently circular in shape with their nuclei enforced toward the bases of these unicellular glandular cells. These cells were more numerous in the villi compared to their number in the crypts. Numerous folds were observed which were structured from both mucosa and submucosa called plicae circularis. These folds were evidently observed grossly when the duodenum dissected longitudinally (Fig. 2). Microscopic examination revealed complete absence of paneth cells in the lining epithelium of the crypts at this age of cats. Submucosa was thinner than mucosa and was constructed of irregular dense bundles of connective tissue fibers richly supplied with blood vessels. Submucosal or Brunner's glands were detected characteristically in the duodenal sections selected from the proximal part of this organ only (Fig. 5). Accordingly, the submucosa of proximal part of duodenum was relatively thicker than those of the middle and distal parts as they miss these glands. These Brunner's glands were observed as aggregated mucous secretory units invested in the connective tissue which was stained moderately with PAS stain. They were distributed as 2 or 3 to 8 aggregated units lined with columnar cells with obvious lumina. The submucosal nerve plexuses were identified as small group of ganglion cells in the deeper part of the submucosa adjacent to the inner circular layer of tunica muscularis which may called Henle's plexus. These plexuses were smaller and finer than those of Auerbach's (myenteric) nerve plexuses which were identified in the tunica muscularis. The Henle's nerve plexuses were identified in one week aged kittens and the subsequent studied ages. Tunica muscularis found obviously thick in this age of cats compared to the other structural tunicae of the wall of duodenum (Fig. 3). It was constructed of two layers that were thicker inner circular and thinner outer longitudinal layers of smooth muscle bundles. Connective tissue fibers were interspersed between bundles of inner circular layer of muscle. The connective tissue also separated the two layers of tunica muscularis which included Auerbach's nerve plexuses associated with rich blood vessels. Tunica serosa, which is the outer most layer of the intestinal wall, composed of thin layer of loose connective tissue blended with tissue of the mesentery.

Examination of the descending duodenum of 4 weeks postweaned cats revealed some histological changes. The thickness of tunica mucosa was increased relatively compared with that of one week aged kittens. In another aspect, its thickness increased relatively compared with other tunicae which were constructed the wall of this organ (Fig. 6). The mucosal villi were increased in length and width. Intestinal glands were well developed. The number of goblet cells was increased in the lining epithelium of both villi and crypts (Fig. 7). Characteristically, these cells were also spherical in shape as in the organ of one week aged kittens. Similarly to the mucosa, the thickness of submucosa was also increased relatively compared to that of one week aged kittens because of the increased blood vessels surrounding more mucous secretory units of the Brunner's glands at the proximal part and fewer scattered number at the middle part of the descending duodenum. Secretory units were increased in number and size with large lumina (Fig. 8). Total absence of these glands was monitored at the distal part of the organ. Few number of paneth cells were recorded in the bases of the intestinal crypts which were possessed fine red colored granules in their cytoplasm when the sections of the duodenum were stained with Masson's trichrome stain (Fig. 9). Tunica muscularis decreased in thickness compared to the previous one week aged kittens, especially the inner circular layer. In another aspect, its thickness decreased relatively compared to the other tunicae of the wall of this organ. The Auerbach's plexuses were increased in number and size. They look like beads distributed linearly in the connective tissue existed between the inner and outer layers of tunica muscularis. In the adult cats, the structure of the descending duodenum showed structural changes out of which the increase of lengths of both the villi and intestinal glands in the mucosa. The latter glands were deeper compared to those found in the previous mentioned ages of cats and the number of goblet cells was highly increased in them. In fact, many histological sections appeared fully lined with these unicellular glandular cells. Many mitotic figures were recorded in the intestinal crypts which were showed different mitotic stages (Fig. 10).

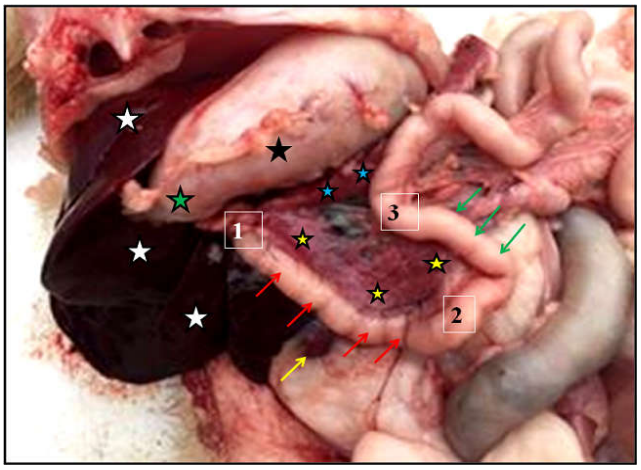

Fig. 1. Pancreas and duodenum of cat in situ. It showed the followings: left lobe (blue stars), right lobe (yellow stars) of the pancreas, first, second, third flexures of duodenum (1, 2, 3, respectively), descending (red arrows) and ascending duodenum (green arrows), hepatic lobes (white stars), stomach (black star)

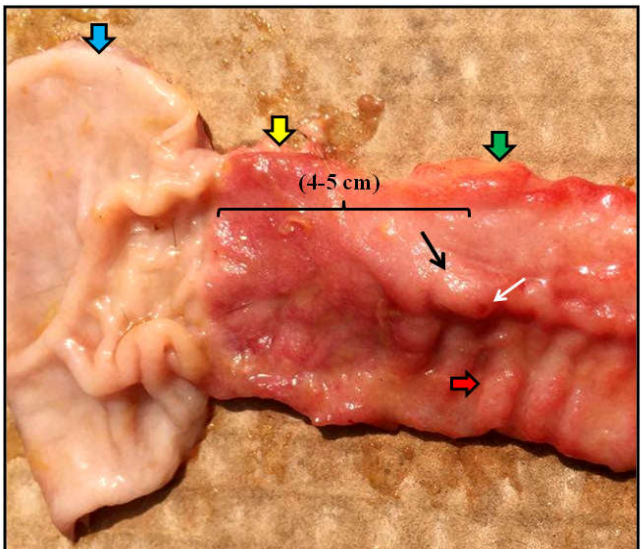

Fig. 2. Duodenal papilla (black arrow) inside the lumen of proximal part of the duodenum (green arrow). It showed its opening (white arrow) around 4 to 5 cm distally to the duodeno-pyloric junction (yellow arrow), circular folds (red arrows). Blue arrow represents the pylorus

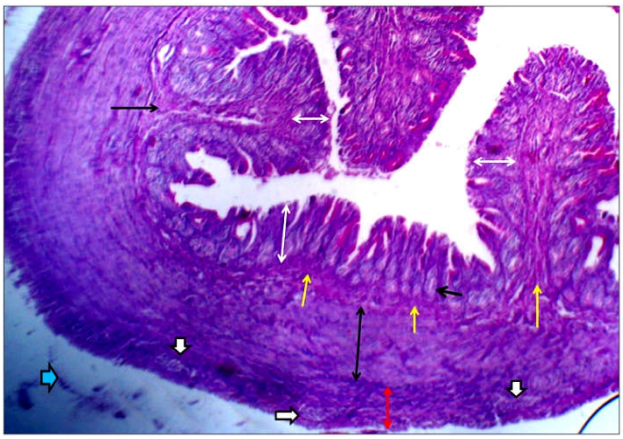

Fig. 3. Cross section in the descending duodenum of one week aged kitten. It showed tunica mucosa (double heads white arrow), submucosa (yellow arrows), inner layer (double heads black arrow) and outer layer (double heads red arrow) of tunica muscularis. Many plicae circularis (black arrows), Auerbach's plexuses (white arrows) are present. The organ surrounded by mesentery (blue arrow). H&E, X100

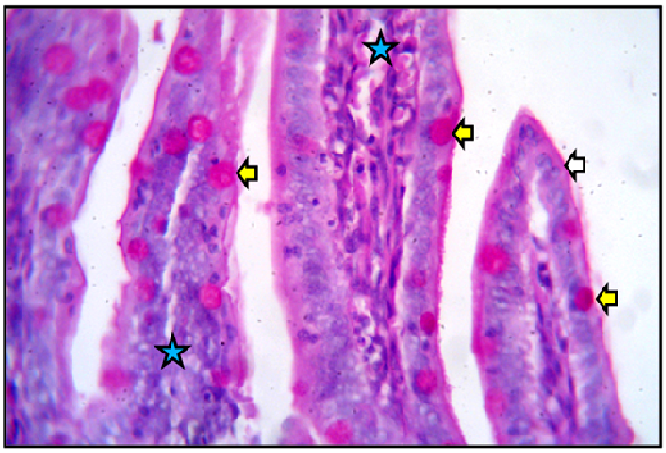

Fig. 4. Mucosa of the descending duodenum of one week aged kitten. It showed villi (white arrows) that are lined by simple columnar epithelium with goblet cells (yellow arrows).Blue star represent connective tissue core of the villi. PAS, X100

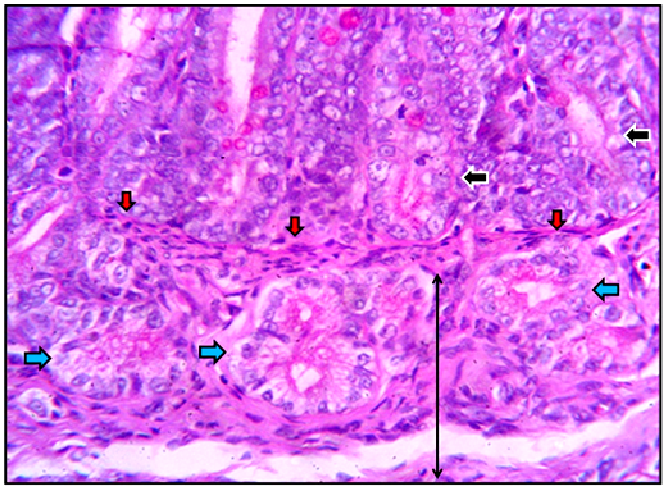

Fig. 5. Mucosa-submucosa of the descending duodenum of one week aged kittens. It showed crypts of Lieberkühn (black arrows), muscularis mucosa (red arrows), Brunner's glands (blue arrows) in the submucosa (double heads black arrow). PAS, X400

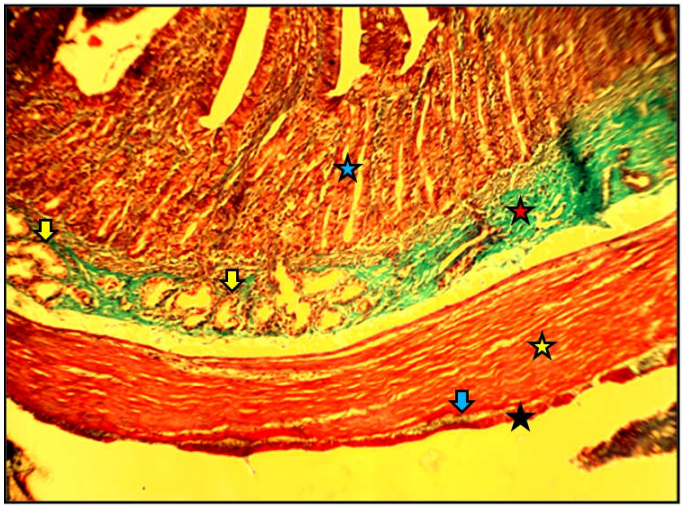

Fig. 6. Cross section in the wall of descending duodenum of 4 weeks aged weaned cat. It showed tunica mucosa (blue star), tunica submucosa (red star) and inner layer (yellow star) and outer layer (black star) of tunica muscularis. Auerbach's plexuses (blue arrow) are present. Many Brunner's gland present in submucosa (yellow arrows). Masson's Trichrome, X100

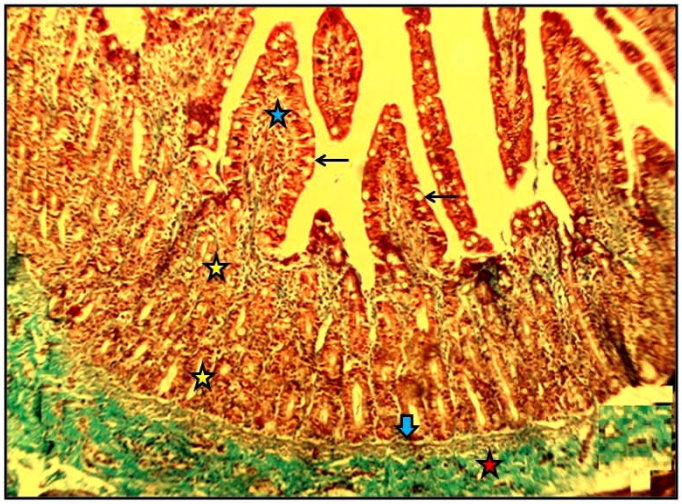

Fig. 7. Mucosa-submucosa of the descending duodenum of 4 weeks weaned cat. It showed thick mucosa (yellow stars), muscularis mucosa (blue arrow), submucosa (red star), villi core (blue star) and goblet cells (black arrows). Masson's Trichrome, X100

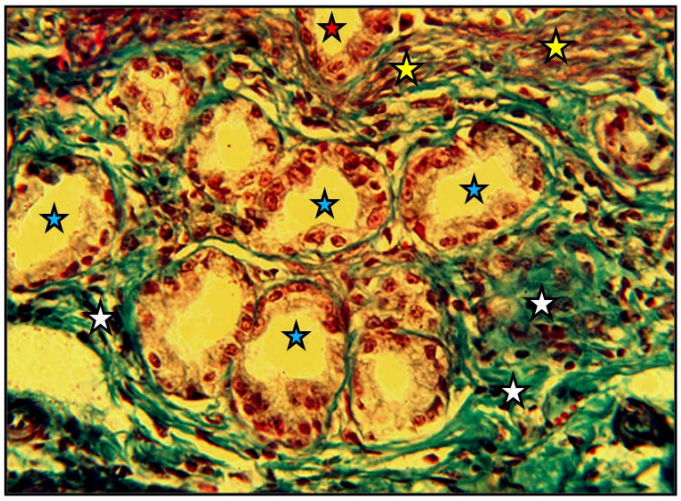

Fig. 8. Submucosa of the descending duodenum of 4 weeks weaned cat. It showed Brunner's glands (blue stars) surrounded by the submucosal connective tissue (white stars). The figure also showed crypt of Lieberkühn (red star) and muscularis mucosa (yellow stars). Masson's Trichrome, X400

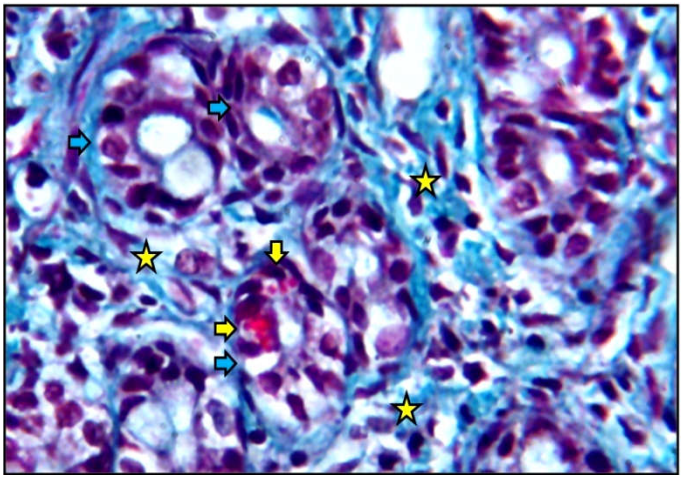

Fig. 9. Mucosa of the descending duodenum of 4 weeks weaned cat. It showed paneth cells (yellow arrows) present in the crypt's of Lieberkühn (blue arrows). Yellow stars represent connective tissue of lamina propria. Masson's Trichrome, X400

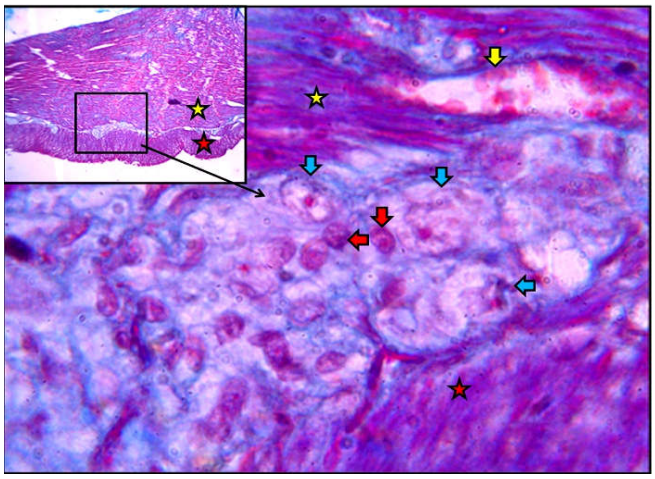

Fig. 10. Mucosa of the descending duodenum of adult cat. It showed mitotic figures (blue arrows) in the crypts which are lined with simple columnar epithelium (yellow arrows) with goblet cells (black arrows). Yellow stars represent submucosa and blue stars represent lamina propria. H&E, X400

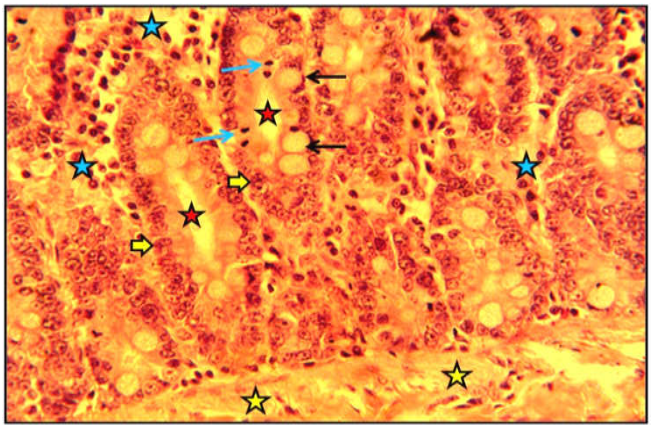

Fig. 11. Auerbach's nerve plexuses in the wall of descending duodenum of adult cat. The plexuses present between inner (yellow star) and outer layers (red star) of tunica muscularis surrounded by blood vessels (yellow arrow). The plexuses consists of group of large ganglion cells (blue arrows) supported by neuralgia (red arrows). Masson's Trichrome, X1000, X100 (small rectangle)

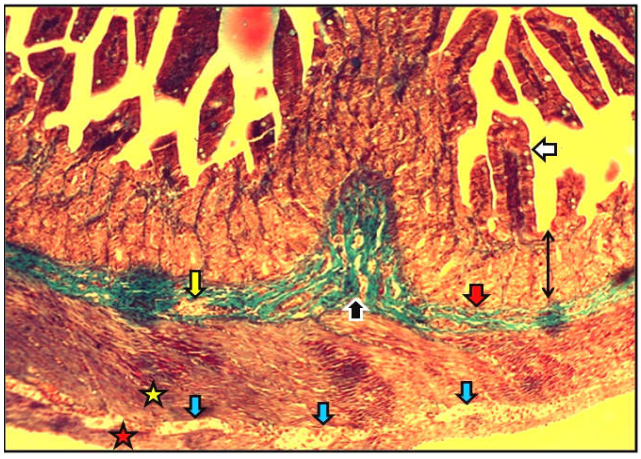

Fig. 12. Cross section in the wall of ascending duodenum of one week aged kitten. It showed tunica mucosa (double heads black arrow), submucosa (red arrow), inner layer (yellow star) and outer layer (red star) of tunica muscularis. It showed also plicae circularis (black arrows), Villi (white arrow), submucosal nerve plexuses (yellow arrow) and Auerbach's plexuses (blue arrows) are present. Masson's Trichrome, X100

The muscularis mucosa evidently observed and they were well developed separating the mucosa from the underneath submucosa. The mucosa and submucosa together formed many folds (plicae circularis). The latter folds showed small aggregations of lymphocytes forming small lymphatic nodules in the connective tissue of their submucosal constituents. Prominent submucosal nerve plexuses were observed. The thickness of the tunica muscularis was relatively lesser compared to that of mucosa in the adult cat's duodenum. The latter tunica showed distinct Auerbach's plexuses distributed between their two inner and outer muscular layers (Fig. 11).

Ascending duodenum

In general, the histological structure of the ascending duodenum in the kittens was similar to that described to the descending duodenum but characteristically the Brunner's glands were totally absent in the submucosa. Similarly, its mucosa with the submucosa was constituted the plicae circularis. Auerbach's plexuses were found larger and more numerous between inner circular and outer layers of tunica muscularis in this part of the duodenum compared to those described in the structure of the descending one. The submucosa appeared thinner due to the absence of submucosal glands in its structure (Fig. 12). Microscopic examination of the ascending duodenum of 4 weeks aged premature cats revealed similar structures to that of the descending one with few differences. It showed thinner submucosa compared to that of the descending part due to total absence of Brunner's glands in its structure. Few number of paneth cells recorded in the middle part of crypts instead of the base as in the descending part. In the adult cat the ascending duodenum showed similar structures of that of descending part in which the villi and crypts were increased in lengths. Obviously, large number of blood vessels recorded around the intestinal glands. The submucosa was totally devoid of Brunner's glands.

Micromorphometric measurements

Descending duodenum

A. Tunica Mucosa

Measurements that were conducted on the wall structures of the descending duodenum revealed critical changes in 4 weeks aged cats. In fact this age represent weaned cats that were separated from their dams and were fully feed on available food other than milk (Table 2). Measurements showed that the mean of tunica mucosa thickness was 233.26 µm ± 4.6 SE in one week aged kittens. It was increased significantly ($P < 0.05$) in 4 weeks aged weaned premature cats up to 802.11 µm ± 8.3 SE (increased more than three times). The thickness again increased up to 998.73 µm ± 11.3 SE in the adult cats which was again significant ($P < 0.05$). The data showed significant development of the mucosa after weaning period in 4 weeks aged premature cats compared to the kittens and adult cats because the relative thickness was 36.04%, 66.14% and 70.17% in 1 week, 4 weeks and adult cats, respectively. The epithelial height was 15.08±0.21 SE at one week of age, which was increased significantly at 4 weeks aged cats (30.94±0.56). The height not significantly increased in the subsequent adult cats into 33.49±0.54. In parallel with these findings, measurements showed that the length (467.70±6.5 SE) and width (125.47±1.96 SE) of villi as well as depth of crypts (282.10±1.6 SE) at 4 weeks of age were elevated significantly compared to both one week kittens (142.29±5.1 SE, 34.27±0.87 SE and 92.27±1.8 SE, respectively) and adult cats (538.47± 5.5 SE, 122.54±2.50 SE and 408.86±6.1 SE, respectively).

B. Tunica Submucosa

Submucosa was thinner compared to the mucosa at all length of the duodenal parts. The mean was 68.15 µm ± 2.35 SE in one week aged kittens. The mean of thickness was increased significantly up to 148.96 µm ± 1.16 SE in the 4 weeks aged weaned premature cats. The mean thickness in adult was not obviously changed (140.62 µm ± 1.18 SE).

C. Tunica muscularis

It was found thickest significantly at one week of age compared to those recorded in the 4thweek and adult aged cats. The relative thickness of tunica muscularis was 53.42%, 21.57% and 19.31% in one week, 4 weeks and adult cats, respectively. In fact in one week kittens, tunica muscularis was the thickest one relatively out of the total tunicae of the wall, whereas, the submucosa was the thinnest one. In 4 weeks aged and adult cats, tunica mucosa was the thickest one relatively and again the submucosa was the thinnest one.

Ascending duodenum

A. Tunica Mucosa

Measurements that were conducted on the wall structures of the ascending duodenum revealed that the mean of tunica mucosa thickness was 344.20 µm ± 4.6 in one week aged kittens increased significantly to 972.39 µm ± 12.7 SE in age of 4 weeks (approximately 3 times) and up to 1022.24 µm ± 10.2 SE in the adult cats. In fact, relative thickness was lowest (52.33%) increased up to 71.09% and 77.47% in 1 week, 4 weeks and adult, respectively (Table 3). The epithelial height was 17.27±0.38 at one week of age, which was increased significantly at 4 weeks aged cats (25.36±0.64). The height significantly increased in the subsequent adult cats into 29.97±0.54. The length and width of villi as well as depth of crypts at one week aged kittens were 278.05±1.7 SE, 57.95±0.91 SE and 99.65±1.05 SE, respectively. These measurements were elevated significantly into 586.30±4.1 SE, 130.50±1.10 SE and 323.71±2.31 SE, respectively at 4 weeks of age. At adulthood period, the villi heights (587.83±5.2 SE) not significantly changed compared to the 4 weeks of age, but both the width of villi (109.47±1.18 SE) and depth of crypts (452.54±9.4 SE) were significantly changed (Table 3).

B. Submucosa

It was thinner compared to the mucosa at all length of the duodenal parts. The mean of thickness was 55.75 µm ± 1.01 SE in one week aged kittens. It was increased significantly up to 116.00 µm ± 1.16 SE in the 4 weeks aged weaned premature cats. The mean thickness in adult was slightly changed (88.1 µm ± 1.38 SE). In fact, the relative thickness of submucosa during different postnatal ages of cats was 8.47%, 8.48% and 6.67% at 1 week, 4 weeks and adult, respectively. It indicated that the tunica was relatively very thin compared to the total wall thickness. In another aspect, it showed thinner thickness in the adult compared to younger ages (Table 3).

Table 1. Macromorphometric measurements of duodenum of cats at different postnatal ages

Age of Animal	Parameters	Descending Limb	Ascending Limb
One week	Length (cm)	6.5±0.01 SE	3.5±0.05SE
	Relative length	65%	35%
	Weight (gm)	0.06±0.02 SE	0.03±0.15 SE
	Relative weigh	66.67%	33.33%
4 weeks	Length (cm)	11.4±0.10 SE	6.1±0.04 SE
	Relative length	65.14%	34.86%
	Weight (gm)	12.4±0.40 SE	4.6±0.07 SE
	Relative weigh	72.94%	27.06%
Adult	Length (cm)	12.1±0.22 SE	6.3±0.11 SE
	Relative length	65.76%	34.24%
	Weight (gm)	13.25±0.02 SE	5.31±0.04 SE
	Relative weight	71.39%	28.61%

SE: slandered error

Table 2. Micromorphometric measurements of descending duodenum at different postnatal aged cats

Ages Parameters (μm)		1 Week	4 Weeks	Adult
Epithelium height		15.08±0.21 [a]	30.94±0.56 [b]	33.49±0.54 [b]
Villi length		142.29±5.1 [a]	467.70±6.5 [b]	538.47±5.5 [c]
Villi width		34.27±0.87 [a]	125.47±1.96 [b]	122.54±2.50 [b]
Crypt depth		92.27±1.8 [a]	282.10±1.6 [b]	408.86±6.1 [c]
Mucosa thickness		233.26±4.6 [a]	802.11±8.3 [b]	998.73±11.3 [c]
Submucosa thickness		68.15±2.35 [a]	148.96±1.16 [b]	149.62±1.18 [b]
Tunica muscularis thickness	Inner	312.70±4.2 [a]	222.69 ±2.4 [b]	186.44±2.6 c
	Outer	33.07±0.99 [a]	38.96±1.01 [b]	88.39±1.46 [c]
	Total	345. 77 ± 2.6	261. 65 ± 1.7	274. 83 ± 1.75
Total wall thickness		647.18	1212.72	1423.18
Mucosa/ wall		36.04%	66.14% *	70.17%
Muscularis/wall		53.42%**	21.57%	19.31%

The similar letters in rows means there is no significant ($p < 0.05$) difference among ages.
The different letters in rows means significant ($p < 0.05$) difference among ages.
** Significant ($p < 0.05$) thickened muscular tunic at 1 week of age
* Significant ($p < 0.05$) change in mucosa thickness at 4 weeks of age

Table 3. Micromorphometric measurements of ascending duodenum at different postnatal aged cats

Age Parameters		1 Week	4 Week	Adult
Epithelium height		17.27±0.38 [a]	25.36±0.64 [b]	29.97±0.30 [c]
Villi length		278.05±1.7 [a]	586.30±4.1 [b]	587.83±5.2 [b]
Villi width		57.95±0.91 [a]	130.51±1.10 [b]	109.47±1.18 [c]
Crypt depth		99.65±1.05 [a]	323.71±2.31 [b]	452.54±9.4 [c]
Mucosa thickness		344.20±4.6 [a]	972.39±12.7 [b]	1022.24±10.2 [c]
Submucosa thickness		55.75±1.01 [a]	116.00±1.16 [b]	88.10±1.38 [c]
T. muscularis	Inner	203.27±3.33 [a]	239.11±1.36 [b]	168.06±2.51 [c]
thickness	Outer	54.50±1.2 [a]	40.16±0.98 [b]	41.10±1.1 [b]
	Total	257.77±2.26	279.27±1.17	209.16±1.35
Total wall thickness		657.72	1367.66	1319.5
Mucosa/ wall		52.33%	71.09% *	77.47%
Submucosa/wall		8.47%	8.48%	6.67%
Muscularis/wall		39.19% **	20.41%	15.85%

The similar letters in rows means there is no significant ($p < 0.05$) difference among ages.
The different letters in rows means significant ($p < 0.05$) difference among ages.
** Significant ($p < 0.05$) thickened muscular tunic at 1 week of age
* Significant ($p < 0.05$) change in mucosa thickness at 4 weeks of age

Table 4. Ratios of Goblet Cells / Epithelial Cells of Villi and crypts

	1 week		4 weeks		Adult	
	Ascending duodenum	Descending duodenum	Ascending duodenum	Descending duodenum	Ascending duodenum	Descending duodenum
Villi	8.4%	6.6%	8.2%	7.6%	14.2%	11.4%
Crypts	3.4%	2.8%	7.2%	6%	33%	23%

* Goblet cells percentage at the villi was higher than those present in the crypts at one week, but approximately equal in the 4 weeks and conversely changed in adult in which the percentage of goblet cells was higher in crypts than in the villi
* Goblet cells percentage at ascending duodenum was higher than those present in the descending duodenum at all studied ages

C. Tunica muscularis

Similarly to the descending duodenum, the tunica muscularis was relatively the thickest at one week of age, which decreased by the progress of cat's age. The measurements showed that thickness of tunica muscularis was 257.77± 2.26 SE in kittens, changed to 279.27±1.17 SE and 209.16±1.35 SE at 4 weeks and adult cats, respectively. In contrary to mucosa, tunica muscularis thickness was thickest relatively at one week kittens (39.19%) compared to 4 weeks (20.41%) and adult cats (15.85%) (Table 3).

Goblet cells

Micromorphometric data revealed that the percentage of goblet cells in the villi of the descending and ascending was 6.6% and 8.4%, respectively. These percentages were recorded for a lower extent into 2.8%and 3.4%, respectively in the crypts in the duodenum of one week aged kittens (Table 4). At 4 weeks of age, the percentage of goblet cells in the villi was increased in the descending duodenum more than in the descending. The percentage of goblet cells in the villi of the descending and ascending was 7.6% and 8.2%, respectively. These percentages which were recorded in the crypts were significantly higher that what was recorded in the crypts of the duodenum of one week aged kittens. The percentage of goblet cells in the crypts was 6% and 7.2% in descending and ascending duodenum, respectively (Table 4). At adulthood age, the percentages of goblet cells in the villi and crypts were higher in the ascending duodenum than in the descending. The percentage of goblet cells in the villi of the descending and ascending was 11.4% and 14.2%, respectively. The percentage of goblet cells in the crypts of the descending and ascending was 23% and 33%, respectively.

DISCUSSION

Duodenum morphology was critically changed at 4 weeks of age. Characteristically morphometrical measurements were showed changed ratio of mucosa/wall thickness and muscularis/ wall thickness. Accordingly, the thinner mucosa and thicker muscularis tunicae were changed into thicker mucosa and thinner muscularis. These critical changes indicated the higher functional mucosa of duodenum of weaned cats compared to those newly borne kittens of one week of age. These changes were parallel to those occurred in digestion and subsequent absorption processes in the duodenum luminal contents. Weaning stage of the animals appeared critical period through which morphological changes occurred in the digestive organs such as the duodenum (Al-Saffar and Al- Haik, 2016). The latter were recorded obvious changes in this organ which were between 15 and 40 days of age in rabbits. Actually, the recorded changes post weaning period in the present study confirmed the previous postulations that weaning is a critical stage for the young mammals, because the digestive processes are highly maturating in association with shifting of feeding behavior of the animal as in young pigs (Lalles *et al*., 2004); piglets (Montagne *et al*., 2007) and in the rabbits (Gidenne *et al*., 2007). The thickness of tunica mucosa was increased relatively at 4 weeks weaned cats compared with that of one week aged kittens. In another aspect, it was thickest one compared with the other tunicae constructed the wall of this organ. The mucosal villi were increased in length and width associated with well developed intestinal glands. Such changes revealed enhanced functional digestive processes of the lining epithelium of the duodenum. These changes were in consistence with those detected in small intestine in rats during their postnatal developmental periods which were extended from birth till adulthood period (Wołczuk *et al*., 2011). But the present findings and most of previous researches have described changes of the villus height around the time of weaning (Toloza *et al*., 1992; Ginneken *et al*., 2002; Paulsen *et al*., 2003). The goblet cells were prominently circular in shape with their nuclei enforced toward the bases of these unicellular glandular cells. Goblet cells percentage at the villi was higher than those present in the crypts at one week, but approximately found equal in both of them at the 4 weeks and conversely changed in adult in which the percentage of goblet cells was higher in crypts than those in the villi. Goblet cells percentage at ascending duodenum was higher than those present in the descending duodenum at all studied ages. The changes could be due to structural prominent development and elongation of intestinal crypts at 4 weeks and adult cats and functional due to the demand of their secretion in the processes of digestion of the food contents in the duodenum.

Paneth cells were few in number compared to the total epithelial lining of the crypts. They were recorded at 4 weeks of age present in the bases of the intestinal crypts which were possessed fine red colored granules in their cytoplasm when the histological sections of the duodenum were stained with Masson's trichrome stain**.** They are previously considered to be important in innate intestinal defense as regulators of microbial density in the small intestine and in the protection of nearby stem cells (Elphick *et al*., 2005). In fact, paneth cells which are specialized cells present in the epithelium of the small intestine considered an important foundation of antimicrobial peptides in the bowel (Bevins and Salzman, 2011). Morphological changes in the duodenal mucosa around the weaning time were probably related to the change in the supplemented diet to these animals and the transition from milk to solid food which indicated intestinal maturation and the adaptation of mucosa to the new intake of a new type of food or diet. Brunner's glands were existed in the submucosa of the first part of descending duodenum only after birth in all studied ages that were one week, 4 weeks and adult cats. This property was dissimilar to those recorded in rabbits in which these glands only present before the weaning time of these animals. Although, these glands were well developed in all parts of the duodenum they were absent in the duodenum of one-day aged rabbits (Al-Saffar and Al- Haik, 2016). Similarly, Vigueras *et al* (Vigueras *et al*., 1999) observed the Brunner's glands at all studied ages of rats even at one day of age.

Similarly to the present findings, the submucosal glands were recorded previously in the duodenal submucosa of human beings (Macéa *et al*., 2006). In fact this reference observed these glands at all over the duodenal submucosa with massive present at the first part of duodenum and decreases in the other portions of the duodenal submucosa towards the jejunum. The greater number in the beginning of duodenum suggests the need for greater amounts of secretion from these glands, aiming to neutralizing the greater acidity of the food coming from the stomach. Brunner's glands are distinctive glands to mammalian species which are restricted mainly to the submucosa of the proximal duodenum. In most species they were found at the gastrointestinal junction and extend for variable distances distally. Secretion of these glands contributes to a layer of mucus which constructs a slimy,

viscoelastic gel lubricating the lining of the proximal part of the small intestine (Krause, 2000). This feature in cat duodenum indicated considerable development of these glands even at their birth. However, the number of these glands was significantly increased at 4 weeks aged cats. The duodenum in all studied ages of cats revealed the presence of well developed Auerbach's plexuses located in the connective tissue interposed between inner and outer layers of tunica muscularis. For a lesser extent, submucosal plexuses were detected in the deep part of this tunic which was called Henle's plexuses. In fact, submucosal plexuses that was present in the superficial part of submucosa called Meissner's nerve plexuses which were absent in cat. Auerbach's (myenteric) nerve plexuses which were recorded in the present study were similar to those recorded previously in the intestine of rabbit, rat, guinea pig and mouse (Furness *et al*., 2006). Henles type of plexuses in cats was similarly observed in humans (Trautmann and Fiebiger,1952; Hoyle *et al*., 1989). It could be concluded that the duodenum was not fully developed in cats at birth and those critical morphological changes can occur after weaning time of the animal. Owing to the significance present findings, wakefulness should be taken by both veterinarians and owners of cats on the management and feeding program particularly the period around weaning of the animals to avoid nutritional diseases and cover their vaccine programs and subsequently caused growth with well health condition.

Conflict of Interests

The authors have not declared any conflict of interests.

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