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Evaluation of adrenal gland at pre and post maturation in domestic cats: A comparative histological study

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ABSTRACT

Object of this study was to describe the histological composition of the adrenal gland in immature and adult local domestic cats. Twenty four healthy cats, as twelve immature aged 2-3 months and twelve adult aged 11-12 months were used. Post euthanized of all cats, their abdominal dissected, identified their adrenal glands and taken carefully. Proper specimens for histological assessment from glands were obtained and after fixation and processing routinely, thin sections prepared and stained. Histological results illustrated, in both ages of the cat, a connective tissue capsule encircled the gland. Two zones of tissues were represent the gland parenchyma including medullary region that hold chromaffin cells, and outer cortex which composed of glomerular, fasciculate, and reticular zones, which contain cells that differ in shape and arrangement. According to data analysis that related to measurements of basic structures of gland, it was revealed that significant differences in most them between the left and right gland as well as between both ages of cats. In conclusion, the study confirmed that the adrenal gland in immature cats showed a distinguish in formation of all basic structure of the gland despite the difference in the thickness of those structure from that of adult cats.

KEY WORDS:

Local cat, Cortex, Zone, Histology

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تقييم الغدة الكظرية قبل وبعد النضج في القطط المنزلية: دراسة نسيجية مقارنة

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الخلاصة

هدفت الدراسة الى مقارنة التركيب النسيجي للغدة الكظرية في القطط المنزلية المحلية البالغة وغير الناضجة. تم استخدام أربعة وعشرون من القطط المنزلية المحلية السليمة، منها اثني عشر قط غير ناضجة بأعمار 2-3 أشهر، واثني عشر بالغة بأعمار 11-12 شهر. بعد القتل الرحيم لجميع القطط، تم تشريح البطن وحددت الغدد الكظرية الخاصة بها وأخذها بعناية. تم الحصول على عينات مناسبة للتقييم النسيجي من تلك الغدد وبعد التثبيت والتبريد بشكل روتيني، تم تحضير المقاطع النسيجية الرقيقة وصبغها. أظهرت النتائج النسيجية، في كلا عمري القط، وجود محفظة من النسيج الضام تحيط بالغدة. يتميز النسيج المتني للغدة إلى النخاع الداخلي الذي يحتوي على خلايا الكرومافين، والقشرة الخارجية التي تتكون من مناطق كبيبية وحزمة وشبكية، والتي تحتوي على خلايا تختلف في الشكل والترتيب. ومن خلال تحليل البيانات المتعلقة بقياسات التراكيب الأساسية للغدة، تبين وجود اختلافات معنوية في معظمها بين الغدة اليسرى واليمنى وكذلك بين العمرين في القطط. وفي الختام أكدت الدراسة أن الغدة الكظرية في القطط غير الناضجة أظهرت تميزاً في تكوين جميع التراكيب الأساسية للغدة على الرغم من اختلاف قياسات سمك تلك التركيبات عن تلك الموجودة في القطط البالغة.

الكلمات المفتاحية: قط محلي ، محفظة، منطقة.

INTRODUCTION

The mammalian adrenal glands are important structures contribute to a prominent work in maintaining the vital activities inside the body through the secretion of multiple kinds of hormones from different tissue structures that make them up, such as regulating metabolism, balance of salts, sexual maturity, response to stress and immunity, and homeostasis of glucose (Mitani, 2014; Pignatti and Fluck, 2021). The two glands anatomically found at the cranial to medial aspect for every kidney (Yilmaz and Girgin, 2010).

The domesticated cat (*Felis catus*) is a small species from the Felidae family which also involve about other thirty eight kind that are widespread all over the world (Montague *et al.*, 2014). For promote regular develop, growth and breeding; they are demand large quantity of amino acid in their food (Che *et al.*, 2021). *Felis catus* has been considered by many studies as a good model for checking the state of histology, physiology and metabolic disease (Hoenig, 2006). The shape of adrenal gland in cat in most cases is bean like and the oval to elongated form is less frequent. The means length of right and left gland in cat through ultrasonographic measurement were 10.45 mm and 9.88 mm respectively (Silva *et al.*, 2016)

Microscopically, the cortex and medulla were constituted the glandular parenchyma of adrenal, as well as all gland covered with capsule. Embryologically, the ectoderm layer give the

origin of the medulla, while the mesoderm participate in the formation of the cortex (Nama *et al.*, 2015; AL-Zubaidi and Nasif, 2020). Due to the scarcity of data over the microscopic composition in adrenal glands of domestic cats as well as the rare of informations regarding its development with age, so the current study was aimed to give a histological description of the adrenal gland in immature and mature locally domestic cats.

MATERIALS AND METHODS

Animals

Twenty four healthy domestic cats with age ranged between 2-3 months (twelve animals) and 11-12 months (twelve animals) were purchased from local animals owners, and placed in a cages of the animal house at Diyala University - College of Veterinary Medicine.

Animal Approval

The protocol of this study was approved by the scientific ethics committee of College of Veterinary Medicine/Diyala University.

Study design

The cats were categorized into pr-mature or juvenile group and adult group based on their aged. An overdose of xylazine and ketamine were injected intravenously for euthanized all experimental animals (Hussein, 2023). The ventral surface of abdominal wall was incised longitudinally with surgical scalpel to observe the abdominal viscera and to identified the adrenal gland in its location above the kidneys. Each gland was take out and irrigated carefully with a solution of saline. For histological assessment, a specimens from each gland were obtained and fixed for 48 hours with 10% Neutral buffered formalin (Alkhazraji *et al.*, 2021). After the requited fixation period passed, the specimens washed with tap water, then processed routinely through dehydration, clearing, and embedding in paraffin. A series sections at thickness of 5-6µm were taken from the paraffin block by using rotary microtome. Hematoxylin and Eosin, Masson trichrom, and PAS were used for stained the thin sections on slides (Bancroft and Stevens, 2010; Ali Alshammary, 2021). A light microscope was used to examined the histological sections of the gland, and all microscopic measurements as thickness of different

structural layers of both gland in each animal of the two groups were achieved using software of image processing (image scope) with digital camera (Alkhazraji and Naser, 2024).

Statistical analysis

All data were represent (mean \pm SE), and the analysis achieved by using the SAS (Version 9.4, institute Inc.) (Karim and Abdulla, 2024). The t-test was used for determined the differences between means. The level at $p < 0.05$ considered the significant of differences (Noraldin and Sabow, 2023).

RESULTS AND DISSCUSION

Microscopic examination of adrenal gland in cats showed that it whether in immature and adult animal were encompassed by capsule. This structure was composed primarily of connective tissue that sends trabeculae inside the parenchymal tissue of the gland. The connective tissue was irregular type (Fig. 1&2). These finding agree with those reported in dolphin by (Clark *et al.*, 2007), in bison by (Barszcz,2016) and in rabbit by (Fahmy *et al.*, 2008). Alkhazraji *et al.* (2023) observed that the collagen and elastic fibers presnt richly in the adrenal gland capsule of dogs. In the present study, the capsule thickness in adult cat recorded mean of $4.93 \pm 0.32 \mu\text{m}$ and $5.61 \pm 0.21 \mu\text{m}$ respectively in left and right organ, whereas measures of the two sides of this gland in immature cat were $3.76 \pm 0.17 \mu\text{m}$ and $4.12 \pm 0.13 \mu\text{m}$ respectively (Table. 1). Differences in measuring of capsule were insignificant between the right and left side, but significant variation in measurements was detected between adult and immature cats. This difference could be related to age variation. This interpretation is coincide with Mughal *et al.*, (2004) who explained that the expansion of the inner layers of the gland with increasing age rises the thickness of capsule.

Adrenal gland parenchyma in the adult and immature cats was differentiated into two compartments as cortex and medulla. The cortical tissue showed a layers of glomerulosa, fasciculata and reticularis, from outer to inner orientation. Cells in polyhedral shape were found in glomerulosa layer which appeared in small size in immature cat when compared with those in adult cat (Fig. 1, 2 &3). These findings correspond with previous studies by (Hafez *et al.*2005; Fahmy *et al.*, 2008; and Pignatti *et al.*, 2017). Our study reported thickness measuring of this layer which were $25.10 \pm 0.15 \mu\text{m}$ and $28.43 \pm 0.28 \mu\text{m}$ in left and right gland in immature cats, while mean of $30.16 \pm 0.60 \mu\text{m}$ and $32.26 \pm 0.36 \mu\text{m}$ were recorded in adult cat, for left and right

gland respectively (Table.1). The data of measurements showed significant differences in this layer whether between the left and right gland or between the immature and adult cats.

The second layer, fasciculata, in adult cat was took wide place of the cortex and their cells had rounded to oval form, look foamy, and coordinated as cords from first to third layer of cortex. Similar observation was detect in immature cat except those cells had rounded shape mostly (Fig. 1,2,3 &4). This result agree with prior report in mice by (Bielohuby et al., 2007). On other hand, this layer recorded mean thickness of $51.31 \pm 0.27 \mu\text{m}$ and $54.62 \pm 0.43 \mu\text{m}$ in immature cat and $58.57 \pm 0.93 \mu\text{m}$, $63.89 \pm 0.72 \mu\text{m}$ in adult cat, respectively for left and another right adrenal (Table.1). It appeared differences as significant for those in left and right gland, and between the two groups of animals. The differences could related in variation in age.

The last layer of cortex, reticularis, appeared in adult cat like a network of irregularly ordered cells, disperse in various orientations and separated by a number of sinusoids (Fig. 5). This zone had less area in immature cats in compared with those in adult cat (Fig. 4). The result is consistent with what was mentioned previously by (Xing *et al.*, 2015). This layer in left and right organ reported average thickness of $15.92 \pm 0.53 \mu\text{m}$ and $17.50 \pm 0.22 \mu\text{m}$ in immature cat, while were $18.36 \pm 0.24 \mu\text{m}$ and $20.18 \pm 0.41 \mu\text{m}$ in adult cat (Table. 1). It was detected significant difference between immature and adult cats, which may related to age differences. The midst portion of the gland was the medulla, which contained large cells called chromaffin as well as found of sinusoids (Fig.6). Similar finding was too said by (Rosol *et al.*, 2001). In previous study by Batah and Mirhish (2019) whom reported in medulla of adenal in guinea pig contains irregular cords of chromaffin cells in bwteen them many blood sinusoids. Measuring of the medulla in present study detected significant deference between the immature and adult cat, which were in left and right gland $23.63 \pm 0.36 \mu\text{m}$, $22.57 \pm 0.26 \mu\text{m}$ in immature, but were $29.39 \pm 0.48 \mu\text{m}$, $27.74 \pm 0.35 \mu\text{m}$ in adult cat respectively. This variation may concerning to age difference.

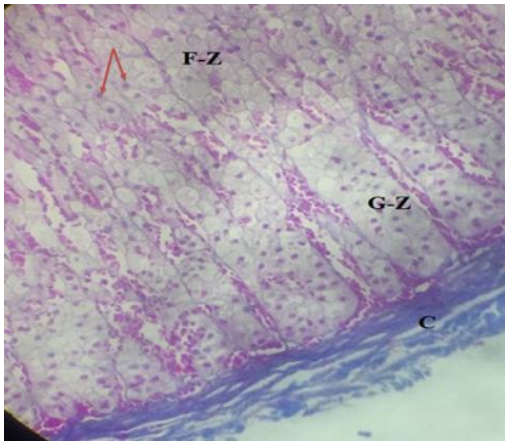


Figure 1. Microscopic photograph in the adult cat illustrate the gland of adrenal: capsule (C), and zones of cortex, glomerulosa: G-Z, fasciculata: F-Z which involve polygonal cells (brown arrow), (X100 Masson trichrom stain)

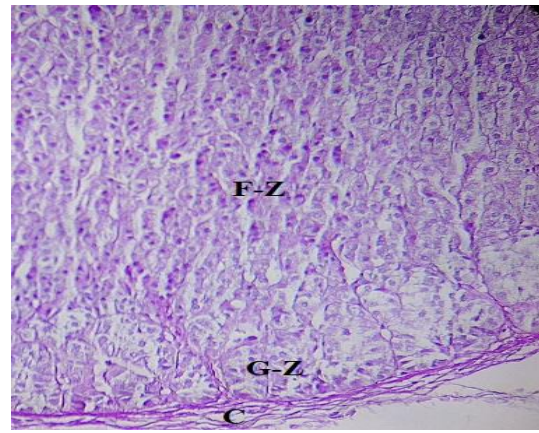


Figure 2. Microscopic photograph in the immature cat illustrate the gland of adrenal: fasciculata (F-Z) and glomerulosa (G-Z) zones surrounded with capsule (C), (X100 H&E stain).

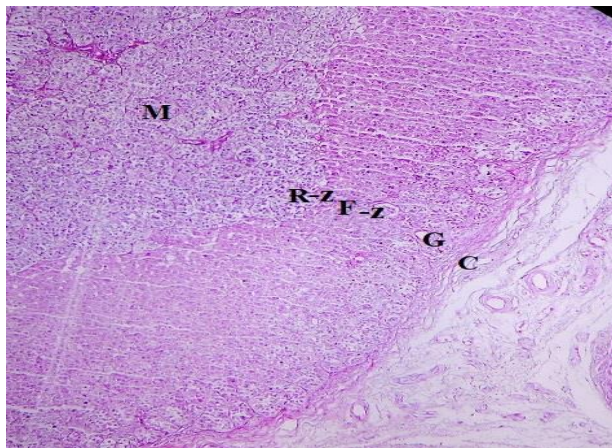


Figure 3. Microscopic photograph in the immature cat illustrate the gland of adrenal: capsule (C), and the layer of glomerulosa (G), fasciculata (F-Z), reticularis (R-Z) in the cortex, in addition to medulla (M), (X40 H&E stain).

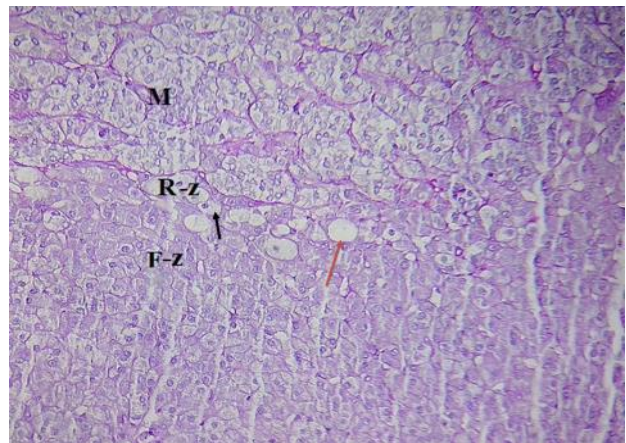


Figure 4. Microscopic photograph of adrenal in the immature cat illustrate: medulla (M), reticularis (R-Z) and fasciculata (F-Z) zones, and presence of venule (brown arrow), vacuoles cytoplasm (X200 PAS stain).

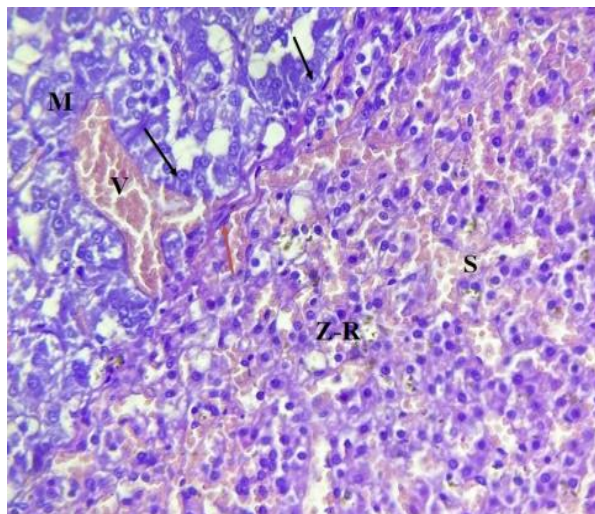


Figure 5. Microscopic photograph of adrenal in the adult cat illustrate: zone reticularis(Z-R), medulla (M), endothelial cell (brown arrow), blood vessel (V), sinusoid (S), and chromaffin cells (black arrow). (X200 PAS stain).

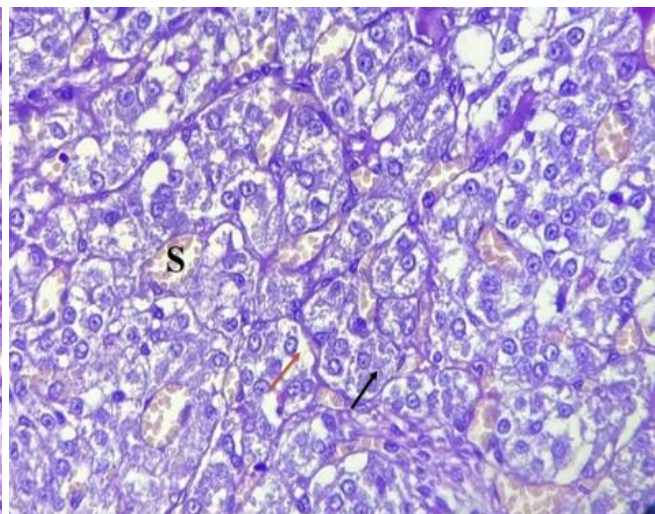


Figure 6. Microscopic photograph in the adult cat illustrate the gland of adrenal: the medulla contains cells of Epinephrine (black arrow), Norepinephrine (brown arrow) as well as sinusoid (S). (X400 PAS stain).

Table 1: Microscopic measurements of adrenal in adult and immature domestic cats (Mean ± SE, in μm)

Kind of cat	structure	Left gland	Right gland
Adult	Capsule thickness	4.93± 0.32	5.61± 0.21
	Glomerulosa thickness	30.16±0.60	32.26± 0.36*
	Fasiculata thickness	58.57±0.93	63.89± 0.72*
	Reticularis thickness	18.36±0.24	20.18± 0.41*
	Medulla thickness	29.39±0.48	27.74± 0.35*
	Capsule thickness	3.76 ± 0.17	4.12 ± 0.13
Immature	Glomerulosa thickness	25.10±0.15	28.43± 0.28*
	Fasiculata thickness	51.31±0.27	54.62 ± 0.43*
	Reticularis thickness	15.92±0.53	17.50 ± 0.22*
	Medulla thickness	23.63±0.36	22.57 ± 0.26*

(*) represent to significant difference at level (P< 0.05).

CONCLUSION

The histological examination of adrenal in present experiment displayed that the structures in immature domestic cats were distinct for most of the components like those observed in adult cats. Histological measurements determined the presence of significant differences in the thickness of the structures that make up the gland, as there were significant

differences between those in the right gland and in the left gland. In addition, it was detected significant differences in histological measurements of adrenal gland structures between immature and adult ages in local domestic cats. The present findings were proven that the adrenal glands were well developed in immature domestic cats.

CONFLICT OF INTEREST

With the publication of current study, the authors reported no quarrel of interest.

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