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Histopathological and Immunohistochemical Studies on Endocrine Glands of Dogs with Special Emphasis on the Immunoexpression of β-cells of the Pancreas

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ABSTRACT

The aim of this study to evaluate the histopathological, histochemical and immunohistochemical changes of the endocrine glands namely, thyroid, pancreas and adrenal gland in dogs submitted to routine necropsy. A total number of 45 endocrine samples were collected from 15 carcasses of dogs received at Department of Veterinary Pathology, Madras Veterinary College, Chennai were chosen for the present study, namely thyroid, pancreas and adrenal gland. The tissue samples were collected in 10% neutral buffered formalin. Tissues were processed after 48 hours as per recommended procedure and 3 µm thickness sections were made and stained with haematoxylin and eosin for histopathological evaluation. The special stains like Masson's trichrome and Picrosirius red were used to assess the extent of replacement with collagen fibers. Immunohistochmical protocol was followed as per the manufactured recommendation (Pathnsitu, USA).The histopathological changes recorded in the pancreas included vacuolar changes (2/15), necrosis (2/15), periductular fibrosis (1/15), perivascular fibrosis (1/15), congestion (5/15), hemorrhages (3/15) and inflammatory changes (1/15) while in the adrenal glands included diffuse cortical hyperplasia (4/15) succeeded by nodular hyperplasia (3/15), adrenalitis (2/15), hyperaemia (2/15), hemorrhages (1/15) and atrophy (1/15). Histopathological examination of thyroid revealed atrophy of follicles suggesting thyroiditis (3/15), presence of follicular epithelial cells which were found separated from the basement membrane with cellular debris in the lumen indicating necrosis (2/15) and presence of large cystic gaps with eosinophilic proteinaceous material consistent with cystadenoma(2/15). The remaining samples showed hemorrhages (2/15), perivascular fibrosis (1/15) and congestion (2/15). However no histological alterations were noticed in 3 cases (3/15). Immunohistochemistry revealed that pancytokeratin was expressed by all epithelial cells including ducts. The expression of insulin was noticed in all the cases indicating that no reduction of β cells in all the above said pathological conditions.

Key words: Dog, Endocrine gland, Histopathology, Immunohistochemistry, β-cells (Insulin).

Endocrine system is a major system of the body that includes endocrine glands produce hormones directly into the circulation they are called as ductless gland. The primary glands are thyroid, parathyroid, adrenals, pineal, pituitary, pancreas, ovaries, testes and hypothalamus. Neuroendocrine gland includes the pituitary and hypothalamus glands (Clarke, 2015) and complex and tightly regulated by hormonal networks (Sturmhofel and Bartke, 1998). Endocrine system abnormalities appear in a broad spectrum in animal and human, often demonstrate hard to diagnosis Capen (2002). If endocrine glands are dysfunction be able to result failure by the desired cells to react to hormones, whether through faulty receptors or *via* adenyl cyclase (second messenger), systemic disease, metabolic disturbancesand administration of exogenous hormones (Miller, 2017). Companion animals serve an important part in human cultureand pet ownership is expanding dramatically around the world. These close human-pet connections mean that they share much of the same environment including exposure to similar levels of (Endocrine disrupting chemicals (EDCs) in daily life Pocar *et al.* (2023). However, it is currently understood that there has been a consistent rise in pet diseases which may be connected to the effects of pollutants, such as thyroid disorders and diabetes in dogs (Lea *et al.,* 2016).

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Few studies recorded the anatomical, histopathological, histochemical and ultrastructural changes of individual endocrine organs in animals Poonia *et al.* (2023); Vishen *et al.* (2021); Singh *et al.* (2023). Histopathological description of the endocrine glands of the dog still underexplored areas in Veterinary Pathology. Through this we can correlate the clinical sign and respective glands affection. However, this research initiated by the scarcity of existing literature on the multiple different endocrine glands histopathological alteration of the dog and also diabetes mellitus is one of the growing problems in the dog population. Immunohisto-chemistry is a one of the molecular technique to identify the loss of β cells within the pancreas. The study aims to investigate the histopathological alterations of certain endocrine organs and identification of the β cells within the pancreas in dogs for better diagnostic and prognostic approaches.

The endocrine gland samples, namely thyroid, pancreas and adrenal gland were collected from 45 samples in 15 cases received for post-mortem examinations in the department of Veterinary Pathology, Madras Veterinary College, Chennai during the period from 01.01.2019 to 31.12.2019. The representative tissue samples were collected in 10% neutral buffered formalin and processed. After 48 hours tissues were processed and embedded in paraffin and 3 µm thickness sections were made and stained with haematoxylin and eosin for histopathological evaluation. Tissues were also stained by Masson's trichrome and Picrosirius red Rieppo *et al.* (2019) to assess the extent of replacement with collagen fibers.

Immunohistochemistry

Immunohistochemical staining was performed as per recommendation of the manufacturer (Pathnsitu, USA) using super sensitive labeled poly HRP polymer method. Briefly, sections of 3-4 µm thick paraffin embedded tissue samples were collected on slides coated with polyL-lysine dried at 56°C for 3-5 hrs. Paraffin sections were dewaxed by xylene, rehydrated and finally antigen retrieval done in 1M Tris EDTA buffer (pH-9) in a pressure cooker for 20 min. Blocking was done with power block (casein) solution. The sections were incubated with the primary antibodies, pancytokeratin (Clone EPSIP, Pathnsitu), vimentin (Catalog No EP21, Pathnsitu) and insulin (Clone ICBTACLS, ThermoFischer Scientific) and in negative control only diluent was applied for 2 hrs in a humidified chamber. After rinsing twice with 1M Phosphate buffer saline+Tween 20 pH 7.4, the slides were sequentially treated with super enhancer and super sensitive horse radish Peroxidase (HRP) for 30 min. Diaminobenzidine (DAB) was used to develop a dark brown reaction product, counter stained with haematoxylin and washed with tap water air dried and cleaned with xylene and mount with DPX.

Pancreas

In our study total of 15 pancreas samples were collected from necropsy cases came for postmortem examination. Grossly, haemorrhages and necrotic changes diffusely were noticed. Histopathologically, congestion (5/15), haemorrghes (3/15), vacuolar changes (2/15), necrosis (2/15), periductular fibrosis (1/15), perivascular fibrosis (1/15) and inflammatory changes (1/15) were noticed. In congestion, the capillaries and veins were filled with blood and dilated (Plate 1 a). Among the various histopathological lesions vascular changes like congestion, haemorrhages were predominant. Pancreas is a major endocrine and exocrine organ. The islets of Langerhans of pancreas studded within the organs and size about 1.5 gm. It secrets the multifunctional hormone. The cells being alpha, beta, delta, gamma, endocromaffin which secrete glucagon, insulin, somatostatin, pancreatic polypeptide and serotonin, respectively. Thomson (1984) reported that massive haemorrhage in the pancreas mainly due to canine distemper and pneumonia and cardiovascular system derangement can result stasis of blood in internal organs. Vacuolated acinar cells, condensed cytoplasm and pyknotic nuclei of acinar cells were noticed focal to diffusely in vacuolar changes. Necrosis always accompanied with other vascular changes, the acinar cells were appeared in different stages of karyorrhexis and karyolysis (Plate 1 b). Jubb (2017) stated that necrosis in pancreas might be due to prolonged corticosteroid therapy, surgical manipulation of pancreas and feeding of high fat and low in protein. The clinical disease resulting from chronic pancreatitis might be related to the presence of pancreatic necrosis and pancreatic fat necrosis Bostrom *et al.* (2013). In fibrosis, varying degree of fibrous tissue extended into irregularly from interstitium into the adjacent lobules. In periductular fibrosis, fibro collagenous tissue around the ducts resulting lumen was reduced and lining epithelial cells were prominent (Plate 1c). There was periacinar and periductular fibrous tissue along with infiltration of mononuclear cells. Masson trichrome (Plate 1d) and picrosicirus red staining revealed presence of moderate collagen fibres in septa and around the blood vessels. Immunohistochemical expression of pancytokeratin taken all epithelial cells including ducts (Plate 1 e) and β cells of insulin revealed brown coloured cytoplasmic expression within the islets of Langerhans (Plate 1f). The expression of insulin noticed in all the cases indicated that no reduction of β cells in all the above said pathological conditions. Several studies reported that the strong and clear cytoplasmic expression of insulin β cells by immunohistochemistry in both normal and disease pancreas. Mcclaran *et al.* (2017) studied the β cells immunohistochemisry in insulinoma dog and described that the appeared strong, positive, cytoplasmic staining of islet cells within the sections of pancreas surrounding the mass. Harrington *et al.* (2017) described those insulin positive cells present with in the head of the pancreas with strong cytoplasmic staining pattern.

Adrenal gland

In the present investigation total of 15 adrenal samples were collected from necropsy. The main lesions occurred in adrenal glands were cortical hyperplasia (Diffuse)

(4/15), followed by nodular hyperplasia (3/15), degeneration (Plate 2b) (2/15), adrenalitis (2/15), hyperaemia (2/15), haemorrhages (1/15) and atrophy (1/15). Grossly, enlargement of gland, congestion and haemorrhages were noticed. Histologically, hyperplastic cortical cells were seen in diffuse and nodular hyperplasia, lymphocytic infiltration seen in adrenalitis, reduced the number of cortical cells of all three zone were seen in atrophy with increase fibrous tissue. Along with these, accessory nodules in capsule (Plate 2c) and cortex region were also recorded. The increasing pathological changes with age, hemosiderin accumulation, fatty changes, calcinosis, melanosis and amyloidosis can occur.

The various types of lesions encountered only at the time of image or post-mortem examination in humans (Fassnacht *et al.,* 2016) and domestic animals Myers (1997). The important clinical signs exhibited by dogs with adrenal alteration are Cushing syndrome, hypoadrenocorticism, anorexia, hyperadrenalin secretion (Silva *et al.,* 2018). Clinical data were not available to record clinical signs in the study. Among the lesion diffuse cortical hyperplasia was high followed by nodular hyperplasia, degeneration, adrenalitis, hyperaemia (Plate 2a), haemorrhages and atrophy. These findings were consistent with previous reports (Juodziukyniene *et al.,* 2014). They reported the highest prevalence of non neoplastic lesions was diffuse cortical hyperplasia. Both nodular hyperplasia and diffuse hyperplasia were observed in cortical areas, in which hyperplastic cortical cells were seen (Apple by *et al.,* 1980). Hyperplastic nodules further classified into micronodular (<0.5 cm) or macronodular (>0.5 cm) and distributed in a focal, multifocal or diffuse are found in zona glomerulosa or zona fasciculate DeLellis and Mangray (2004). Congenital adrenocortical hyperplasia

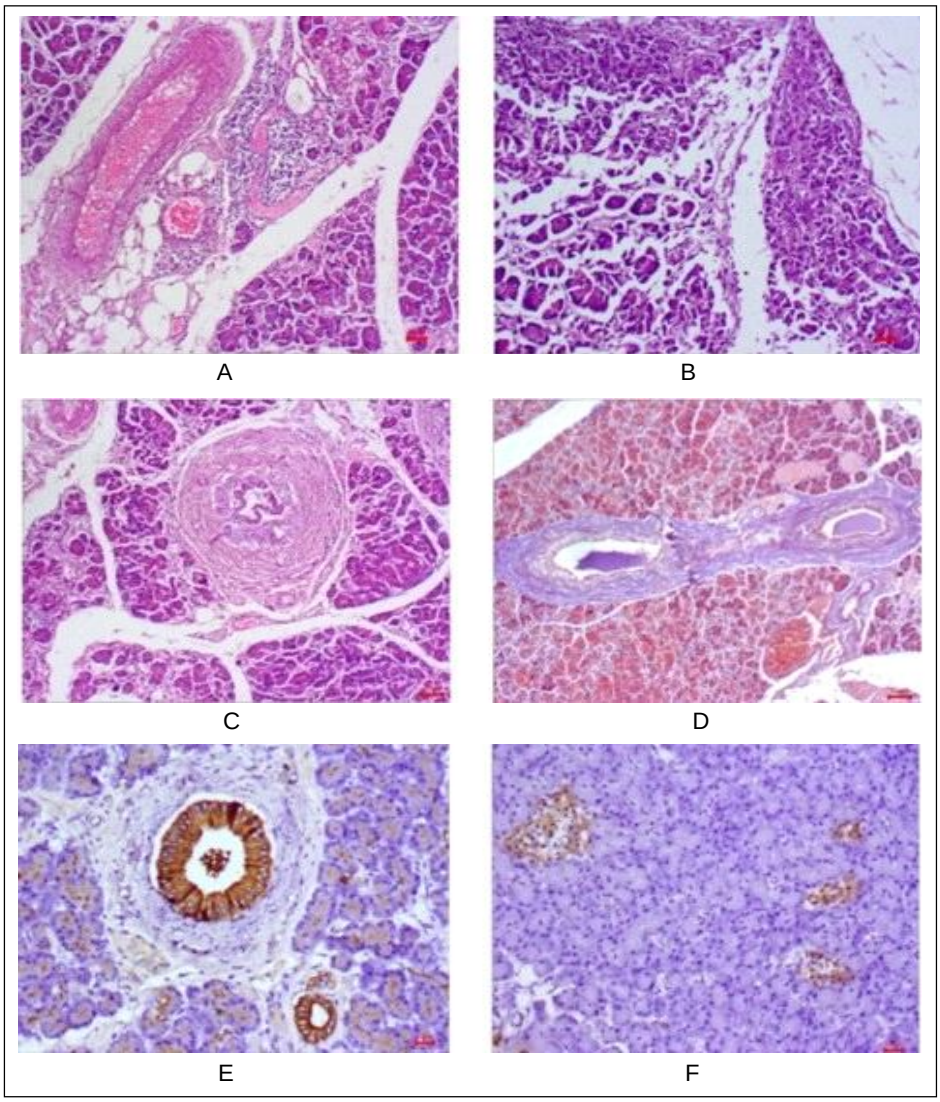


Plate 1: A.Congestion-Capillaries and veins filled with blood and dilated-H&E-20x, B. Necrosis-Karyorrhexis and karyolysis-H&E-20x., C. Periductular fibrosis-Fibro collagenous tissue around the ducts-H&E-20x., D. Periductular fibrosis-Masson trichrome blue coloured-20x, E. Pancytokeratin-Ducts-IHC-40x., F. Insulin-Islets of langerhans-β cells-IHC-20x.

and pituitary over secretion of ACTH hormone were responsible for diffuse hyperplasia Imaki *et al.* (2004). In the present study diffuse cortical hyperplasia characterized by hyperplastic and hypertropic cortical cells were seen in cortex. Masson's trichrome (Plate 2d) and picrosirius red special staining (Plate-2e) revealed the presence of extended collagen fibers deposition within the accessory nodules and capsular thickening cases. In case of nodular hyperplasia small micronodules (0.5 cm) seen in cortex with degenerative and fatty changes. Clinical signs like obesity, polyuria, polyphagic, pendulous abdomen, symmetric alopecia, hyperkeratosis, hyperpigmentation and cholesterolemia observed in adrenal hyperfunction (Zachry, 2017). Feldman and Nelson (1996) stated that atrophy of adrenal gland is mostly associated with decreased concentration of ACTH due to some drugs and pituitary adenoma which was produce hypoadrenocorticism. However the hormone level couldn't measured in this study. Immunohistochemical examination of pancytokeratin taken cells of zona glomerulosa, zona fasciculata and zona reticularis and vimentin taken fibroblast cells of zona glomerulosa (Plate 2f). The histopathological study of adrenal gland is an explicit diagnostic tool to uncover the adrenal disorder. Our results revealed that non functional pathological findings were more common when compared to functional (neoplastic) changes.

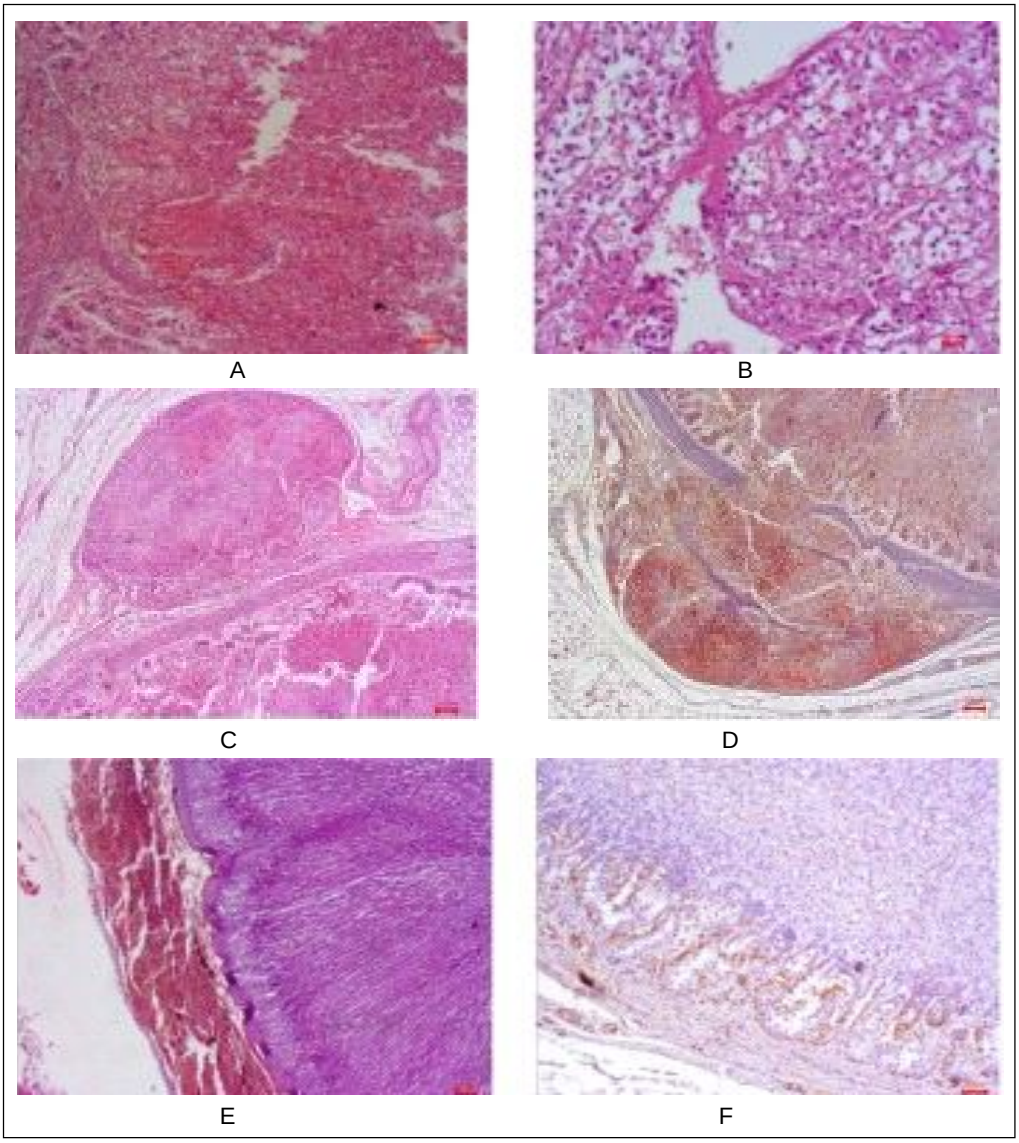


Plate 2: A. Congestion -Zona glomerulosa-H and E-20x, B. Degeneration-Zona glomerulosa-H&E-20x, C. Accessory nodules in capsule-H&E-10x, D. Red coloured-Collagen fiber-Accessory nodules-Masson's trichrome -10x, E. Capsular thickening-Picrosirius red-10x., F. Vimentin-Zona glomerulosa-IHC-10x.

Thyroid gland

Total of 15 thyroid glands collected from necropsy revealed enlargement of gland, congestion and haemorrhages noticed. Histopathological examination revealed diffuse infiltration of lymphocytes in the inter follicular area, varying sized follicles filled with pale stained colloid and atrophy of follicles deletion in three samples suggesting thyroiditis (3/15) Plate 3c). Follicular epithelial cells are detached from basement membrane and presence of cellular debris in the lumen suggestive of necrosis noticed in two thyroid tissue samples (2/15). Large cystic spaces (Plate 3d) with eosinophilic proteinaceous material consistent with cystadenoma (Plate 3e) were also noticed in two (2/15) of the samples. Remaining samples revealed congestion (2/15) (Plate 3a), haemorrhages (2/15) perivascular fibrosis (1/15) (Plate 3b) and no histopathological changes (3/15). Hypothyroidism usually occurs as a result of primary lesions in thyroid gland. Lymphocytic thyroiditis is responsible for clinical hypothyroidism in pet dogs (Gosselin *et al.,* 1981). Lymphocytic thyroiditis closely resembled Hashimoto's disease in humans. It is having hereditary predisposition (Fritz *et al.,* 1970). Chronic lymphocytic thyroiditis appears due to production of auto antibodies against thyroglobulin, thyroperoxidase or the TSH receptors. Thyroid gland may be slightly enlarged or reduced in size. It is characterized by multifocal to diffuse infiltration of lymphocytes, plasma cells and macrophages and remaining follicles may be atrophic and lined by columnar epithelium. Migration of lymphocytes and plasma

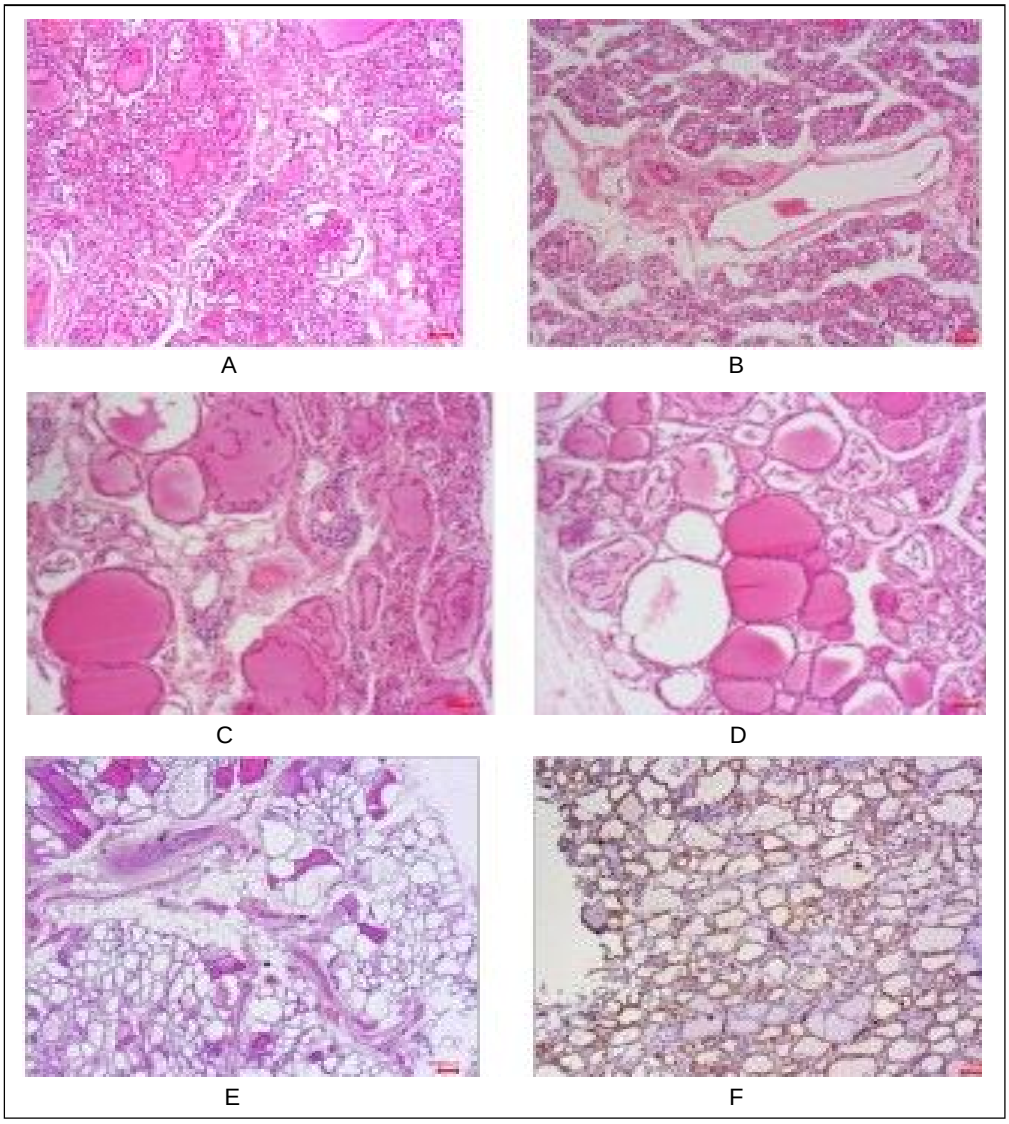


Plate 3: A. Congestion- Capillaries and veins filled with blood and dilated-H&E-10x, B. Perivascular fibrosis - Around the blood vessels-H&E-10x., C.T hyroiditis-MNC infiltration-H&E-10x., D. Large cavities filled with proteinaceous fluid-Cystic changes-H&E-10x., E. Cystadenoma-Varying size of follicles-H&E-10x., F. Pan cytokeratin-Epithelial cells of Follicles-IHC-4x.

cells cause detachment of the follicular cells from basement membrane and exfoliation along with lymphocytes in to the lumen, eventually causing destruction of follicles.

Out of fifteen cases two samples were showed cystadenoma lesions. Histologically, cystic adenoma consists of one or two large cavities filled with proteinaceous fluid, necrotic debris and erythrocytes. Cystic adenomas revealed one or two large cavities filled with proteinic fluid, necrotic debris and erythrocytes. Focal accumulations of tumor cells, forming either follicles or solid nests, are present in the capsule of dense fibrous connective tissue (Meuten, 2002). Cystadenoma with concurrent hyperthyroidism also reported in a German Shepard (Lawrence *et al.,* 1991). Boxers and Beagles have high predisposition to thyroid tumors. No sex predisposition noticed in animals. Most of the thyroid follicular tumors in dogs are malignant. Adenomas likely to occur as incidental finding on necropsy (Wucherer and Wilke, 2010). Non functional thyroid cystadenoma characterized by the presence of large cystic structures also reported in boxers (Maurin *et al.,* 2019). It was found that the strong association between progressive lymphocytic thyroiditis, hypothyroidism and thyroid neoplasia in a study conducted in Beagles. But in our study, there was no clinical data recording clinical signs. It is suggested that hypothyroidism may be a pre existing condition and chronic TSH stimulation promote neoplasia. Increased incidence of hypothyroidism reported above five years of age and neoplasia in dogs especially above 11 years. It implies that, prolonged untreated hypothyroidism could be contributing factor for thyroid tumors (Benjamin *et al.,* 1996). Immunohistochemical staining of pancytokeratin has taken all follicular epithelium (Plate 3f) in cystadenoma cases. Thyroiditis and necrosis of thyroid follicles are common pathological lesions encountered in this study, could be a secondary condition that might have developed due to prolonged hypothyroidism.

CONCLUSION

The histopathological diagnosis of endocrine glands alterations is a definite diagnosis technique for find out the endocrine disorder. Our outcome revealed that non functional pathological findings more common compared to functional (neoplastic) changes**.** The immune expression of β cells (insulin) revealed strong, cytoplasmic staining pattern in all the cases of canine pancreas. Histopathological studied revealed more haemodynamic changes like congestion, haemorrhages and vacuolar changes. Higher incidence of cortical hyperplasia recorded in adrenals. Thyroiditis followed by cystadenoma recorded of thyroid in this study.

Conflict of interest

All authors declare that they have no conflict of interest.

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