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Original Research Article

Age-Associated Histological Changes in the Thyroid Gland of Guinea Pigs *(Cavia porcellus)*

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Abstract: Thyroid glands demonstrate structural alterations throughout their life; however, there is little information on age-related histology features of guinea pigs. The aim of this research was to analyze the histological features of the thyroid glands of adult guinea pigs of different ages. Thirty-six adult guinea pigs were analyzed and assigned to four age categories (9 specimens in each category), which were 6, 12, 24 and 36 months old. Masson's trichrome and PAS staining of thyroid tissues fixed in formaldehyde solution and processed routinely were carried out. Follicle size and distribution, colloid appearance, follicular epithelium morphology, distribution of parafollicular cells, interstitium structure and blood vessel features were diverse for different age groups among the investigated tissue samples. In 6-month-old animals, mostly relatively small follicles, located in loose connective tissue, were seen. The variability of follicles sizes and colloid distribution increased with age. Large follicle, foamy-appearing colloid, low colloid areas, flat follicular epithelium, follicular epithelium degeneration and vascular congestion occurred much more often in 36-month-old animals. Such results present a descriptive study of the histological characteristics of the thyroid gland of the guinea pig depending on its age. As the level of thyroid hormones and histomorphometrical measurements have not been examined in the study, the described changes in the histological structure cannot be considered as an indirect indication of thyroid dysfunction.

Keywords: Guinea Pig, Cavia Porcellus, Thyroid Gland, Aging, Histology, Thyroid Follicles, Colloid, Parafollicular cells.

1. INTRODUCTION

The Thyroid gland is one of the important endocrine glands that play a very important role in controlling metabolism, growth, development, thermogenesis, and other physiological functions. The thyroid hormones T3 and T4 control cellular metabolism and are more significant during growth and development (Dellmann *et al*., 1981; Quesenberry *et al*., 2004).

Histologically, the thyroid gland is characterized by a unique organization among endocrine glands due to the presence of an extracellular accumulation of its secretory substance (colloid) in the lumen of thyroid follicles. The structure of the gland mainly includes thyroid follicles, lined with follicular epithelium cells, and colloid. The other essential cells of the gland are para-follicular or C cells which are located between follicular cells and in inter-follicular tissue. C cells are involved in the synthesis of calcitonin and thus play a role in calcium balance (Dellmann *et al*., 1981). The guinea pig thyroid gland was studied both anatomically and histologically. Comparative histological studies showed that the thyroid gland of guinea pigs contains follicles of various sizes, lined with follicular epithelium cells and containing colloid, and also parafollicular cells were found in the glandular tissue (Batah *et al.,* 2019). This structure serves as an important ground for the evaluation of age-related changes in the thyroid gland. Depending on species, physiological state, sex, and age, thyroid structure and its functioning can differ. Regarding guinea pigs, there is not much information about the functioning of the thyroid gland and age-related structural changes. The research of Müller *et al.,* (2009) analyzed serum thyroxine levels in healthy guinea pigs and found out the reference values for this species. This research showed that serum thyroxine levels differed from one animal to another, but there was no correlation between thyroxine levels and age. Aging influences the morphology and physiological activity of thyroid gland and the changes in the structure of thyroid gland caused by aging do not consist only in hormonal changes. Morphological changes may relate to the size and form of thyroid follicles, follicular epithelium, colloid and stroma. However, it must be noted that the interpretation of morphological changes should be done carefully because structural changes in themselves do not indicate any change in the synthesis of thyroid hormones (Franceschi *et al*., 2019; Mammen *et al*., 2023). Another aspect of the clinical significance of the thyroid gland in guinea pigs is related to the development of thyroid diseases and spontaneously occurring neoplasias of the gland. Mayer *et al*., (2010) reviewed thyroid disorders in guinea pigs and provided descriptions of clinical cases of hyperthyroidism and rarer hypothyroidism. Spontaneously occurring thyroid gland neoplasms in guinea pigs and different morphological and immunohistological forms of the tumors of the thyroid gland were later described (Gibbons *et al*., 2013). Spontaneously occurring thyroid carcinoma has also been found in guinea pigs. Zarrin, (1974) describes spontaneously developing thyroid tumor nodules consisting of carcinoma with an ossifying spindle cell component, showing that spontaneous thyroid neoplasia may develop in this animal. Attention was paid especially to age-related alterations in thyroid parafollicular cells. Yamasaki studied C-cell follicles in guinea pigs of 1 to 29 months old and found a significant increase in number of C-cell follicles in animals of 20–29 months. It was supposed that an increase is caused by proliferation of C-cells in older animals (Kameda, 1986). The structure of the vascular system of the thyroid gland is very rich and specialized. Yamasaki examined the thyroid and thymic arteries in guinea pigs and revealed anatomical peculiarities of the arterial supply of the thyroid gland (Yamasaki, 1995). Clinically, guinea pigs are important because of thyroid diseases which have been found in this animal, mainly hyperthyroidism. On the other hand, there is insufficient information about the normal age-related morphological features of the thyroid gland in guinea pigs when compared to that in humans and other animal models (12). The vascular structure of the thyroid gland is important in understanding any interstitial vascular alterations noted upon histological observation. Previous research works that have focused on the general morphology and pathology of the thyroid gland in guinea pigs have not given detailed observations on the age-related histological features in various adult age groups. It was in this context that the current study sought to assess the histological characteristics of the thyroid gland in guinea pigs at four different age levels.

2. MATERIALS AND METHODS

2.1. Ethical Approval

All animal experiments were performed following the guidelines for animal care and use and were approved by [College of Veterinary Medicine / Tikrit University], approval number [Tu.VET.122].

2.2. Experimental Animals

In this experiment, 36 guinea pigs (Cavia porcellus) were used. The animals were acquired from the animal house of College of Veterinary Medicine, Tikrit University, Iraq. They were subdivided into four age categories, each containing nine animals: 6, 12, 24 and 36 months. The animals were maintained under laboratory conditions and were observed prior to tissue sampling.

The animals were equally subdivided into four age groups, each containing nine animals:

- Group I: 6 months old (n = 9)
- Group II: 12 months old (n = 9)
- Group III: 24 months old (n = 9)
- Group IV: 36 months old (n = 9)

The animals were maintained under proper the laboratory conditions and periodically examined for their general health condition prior to collecting tissues.

2.3. Thyroid Gland Tissue Specimen Collection

The animals were first anesthetized prior to dissection. The cervical area was prepared, and incision of the skin was made at the anterior side of the neck. The surrounding tissues were dissected to reveal the trachea and the thyroid gland.

The thyroid gland and trachea were excised with care. The excised samples were then washed thoroughly to clean off any blood residue and placed in labeled containers filled with formalin fixative solution to preserve the integrity of the tissues.

Each sample was individually identified based on the type of animal used and specimen number.

Methodological caution: The chloroform was used for the anesthesia process in the original manuscript. This may only be used if true in the actual experimental procedure and animal use process.

2.4 Histological Procedures

[Thyroid glands] were fixed in [10% neutral buffered formalin] for [24 hrs/days]. Tissues were fixed, dehydrated in successive concentrations of alcohol, cleared in xylene and embedded in paraffin wax. The sections of [10 µm] thickness were made and attached on glass slides. Sections were stained with hematoxylin and eosin. Collagen fibres and connective tissue elements were highlighted using Masson’s trichrome staining (Kang *et al*., 2021) and Periodic acid-Schiff (PAS) staining was performed following a previously published method (Takase *et al*., 2025). Stained sections were observed under the light microscope) Suvarna *et al*., 2018).

2.5 Histological Study

The thyroid sections were observed with special emphasis on the size and distribution of thyroid follicles, morphological structure of follicular epithelial cells, colloid content and morphology, distribution and morphology of parafollicular cells, interstitial connective tissue, inflammatory cell infiltration, blood vessels and their congestion, degenerative changes in follicular cells, and lipid droplets presence (Mahmoudi, 2024).

3. RESULTS

3.1. Thyroid Gland at 6 Months Old

The parenchyma of the thyroid gland of the 6-month-old guinea pigs consisted of small thyroid follicles that were arranged in lobulated form. Follicles were divided by loose connective tissue space. In most follicles, there was colloid present, while only a few of them were relatively empty or had no colloid. Cells in the follicle and parafollicular regions appeared relatively immature in comparison with those found in the older animals (Fig. 1 and 2).

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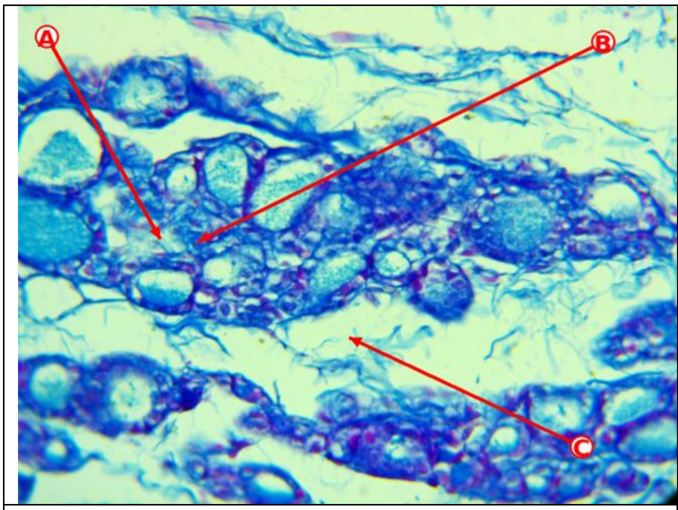
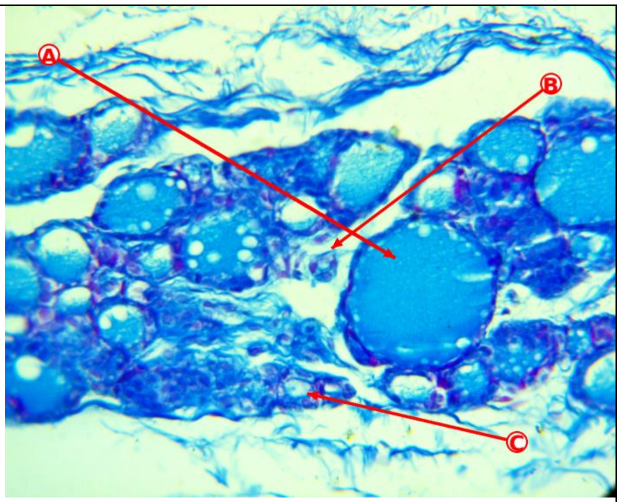
<tr><td>
</td><td>
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<tr><td>Figure (1): Show the thyroid lobules had small thyroid follicles with colloid (A) the thyroid follicular cells and parafollicular cells were un well developed (B), the loose C.T between the thyroid lobules was wide (C) (40 X mason trichrome) 6- months age (A).</td><td>Figure (2): Show the thyroid lobules had small thyroid follicles with colloid (A) the thyroid follicular cells and parafollicular cells were un well developed (B), the loose C.T between the thyroid lobules was wide (C) (40 X mason trichrome) 6- months age B.</td></tr>
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3.2. Thyroid Gland at 12 Months of Age

In the 12-month-old thyroid gland, there was considerable variation in the size of the follicles, which were mainly lined by simple cuboidal epithelium of follicular cells. There was colloid in the majority of follicular spaces, but in some follicles there was a lack of colloid. Parafollicular cells were seen among the follicular epithelial cells and the interstitial connective tissues. They formed small groups of cells, which were relatively pale in their cytoplasm in some places. Interlobular connective tissue was relatively loose. There was mild infiltration by inflammatory cells in some regions. Some follicles had shrinking of colloid, but others had relatively abundant colloid.

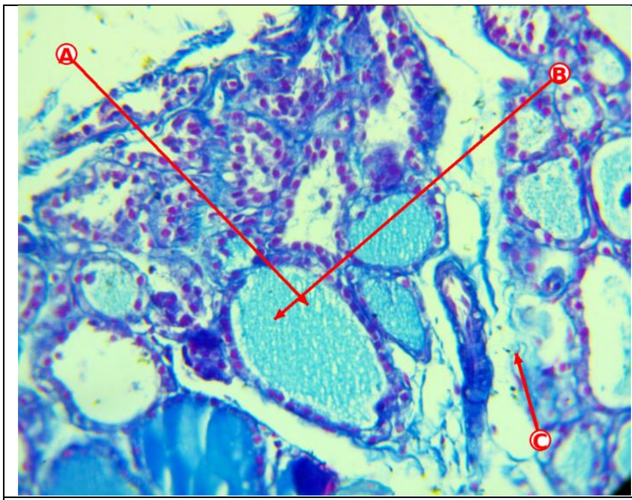

Figure (3): Show The thyroid follicles were variable in size and these are filled with colloid lined by simple cuboidal cells which are the colloid follicular cells (A) parafollicular cells were present in between the follicular cells and in the interstitial CT in the form of groups (B) the loose CT in between the thyroid lobules (C) (x 40 MTC) 12- months age A.

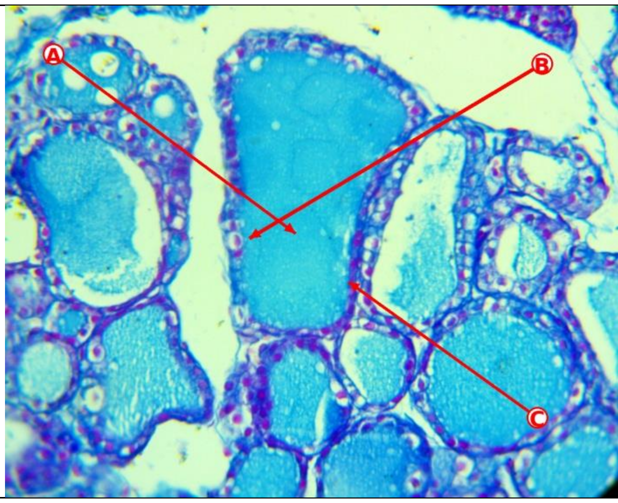

Figure (4): The follicles were well developed occupied its lumens by colloid (A)the follicle cells were simple cuboidal cells (B) surrounded by many parafollicular cells (C) (40 x MTC) 12- months age B.

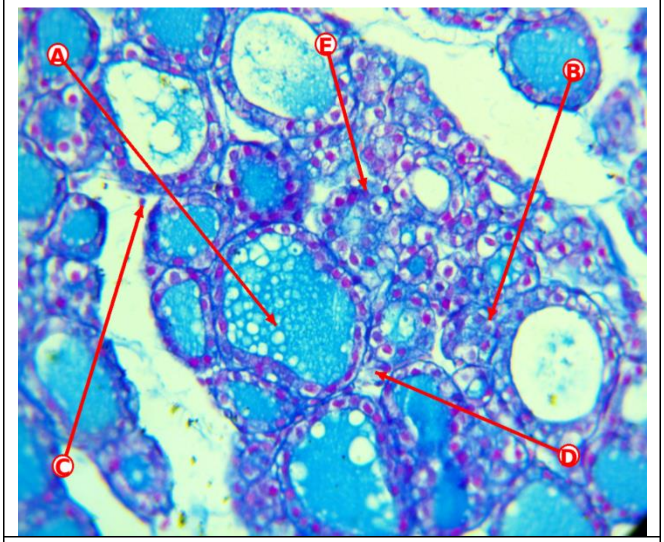

Figure (5): Show the large follicles and thyroid were contained foam colloid (A) or empty (B) the smaller follicles were containing homogenized colloid (C) the para follicular cells were many which were present between the follicle cells (D) and in the interstitial CT (E)(x 40 MTC) 12- month age C.

3.3. Thyroid Gland at 24 Months of Age

Thyroid parenchymal tissue of the 24-month-old rodents exhibited follicles with varying sizes. The follicles had predominantly been lined with simple cuboidal epithelial cells and varied in the amount of colloid within. Parafollicular cells could be easily distinguished between the follicular epithelial cells and in the interfollicular connective tissue. They looked relatively large when compared to the follicular cells and displayed pale cytoplasm. Connective tissue surrounding the thyroid gland contained collagen and elastic fibers. Lymphocytes could be found in the interfollicular connective tissue on occasion. Most of the follicles contained colloid, even though some follicles appeared to be shrunken or colloid unevenly distributed (Figs. 6,7 and 8).

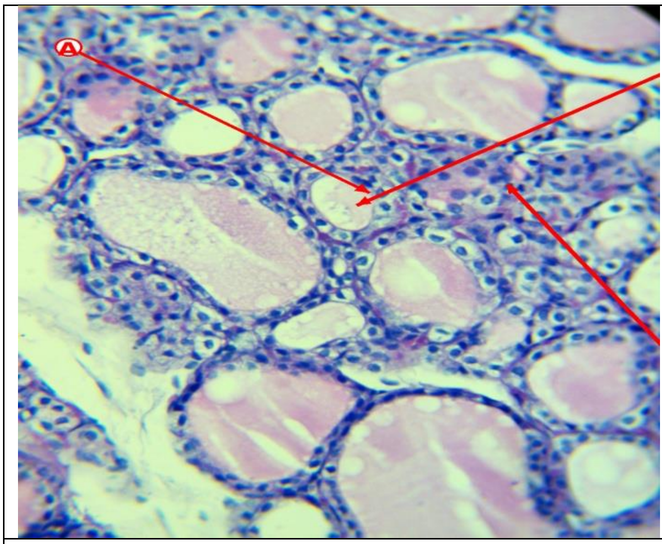

Figure (6): Show the parenchyma of gland had thyroid follicles of different size filed with colloid (A) surrounded by follicular cells (B) parafollicular cells (C) 40 x PAS 24-month age A).

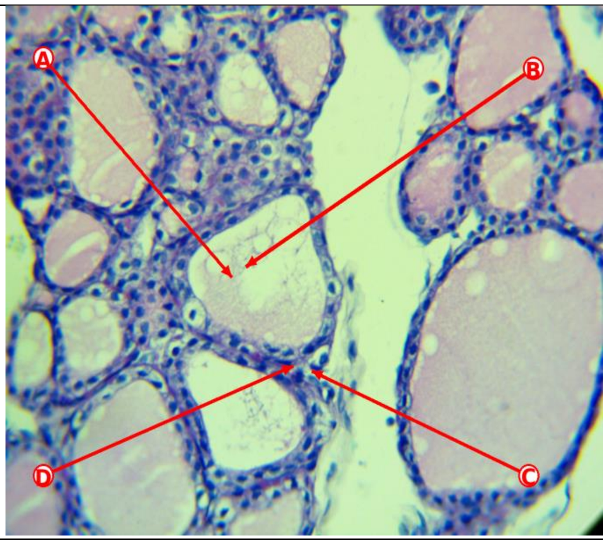

Figure (7): Show thyroid gland follicular cells of simple cuboidal epithelium (A) colloid in lumen of follicle (B) shrinkage of colloid (C) parafollicular cells (D) 40 x PAS) 24-month age B.

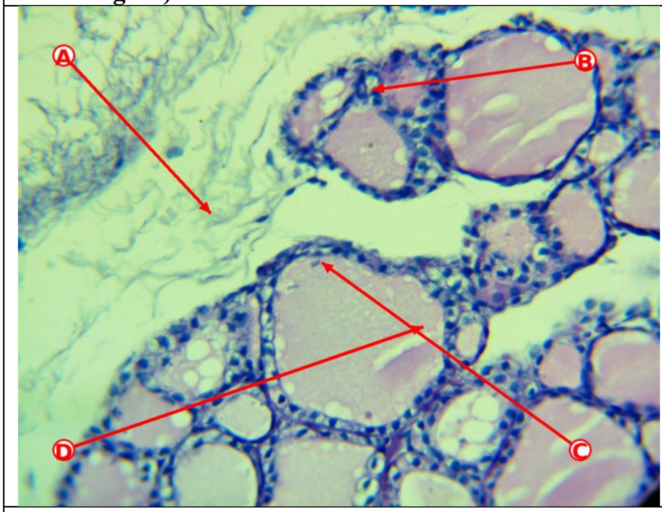

Figure (8): Show fascia of loose CT (A) around the thyroid follicles, collagen fiber with elastic fibers (B) macrophage (C) thyroid follicles with colloid (D) 40 x PAS 24-month age C.

3.4. Thyroid Glands in 36-Month-Old Guinea Pigs

Thyroid glands in 36-month-old guinea pigs were characterized by high variability in follicle size, and there were many large follicles in the thyroid gland tissue. Follicles were lined by follicular epithelium cells having various sizes – mostly cuboidal and relatively flattened in some places. All follicular lumen contained colloid, and in some cases, its foamy appearance could be observed. There were lipid droplets in follicular lumen in some follicles. In some cases, there was shrinking or depletion of colloid in follicles. There were degenerative changes in follicular epithelium in some regions. Interstitium of thyroid glands was rich in blood vessels, some of which were congested. There were small follicles without visible colloid. Parafollicular cells were mainly found in interfollicular connective tissue (Figs. 9, 10 and 11).

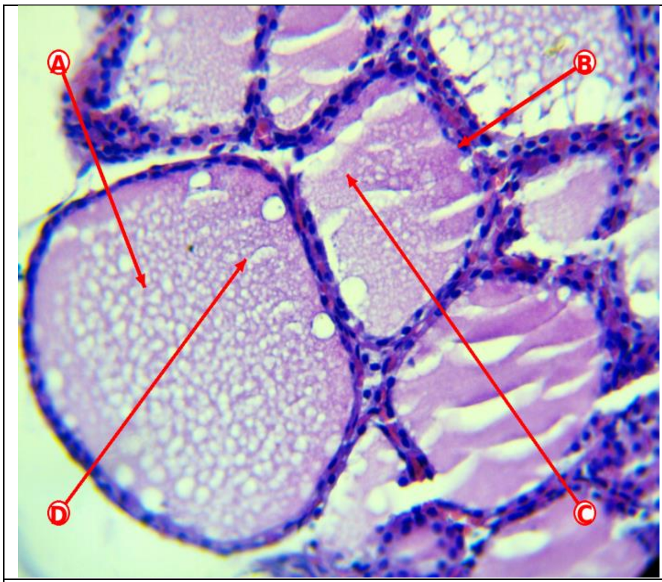

Figure (9): Show the thyroid follicle of great size have colloid with bubbles (A) fat droplets (B) follicle cells with are simple squamous epithelium (C) shrinkage of colloid (D) x 40 PAS 36 -month A.

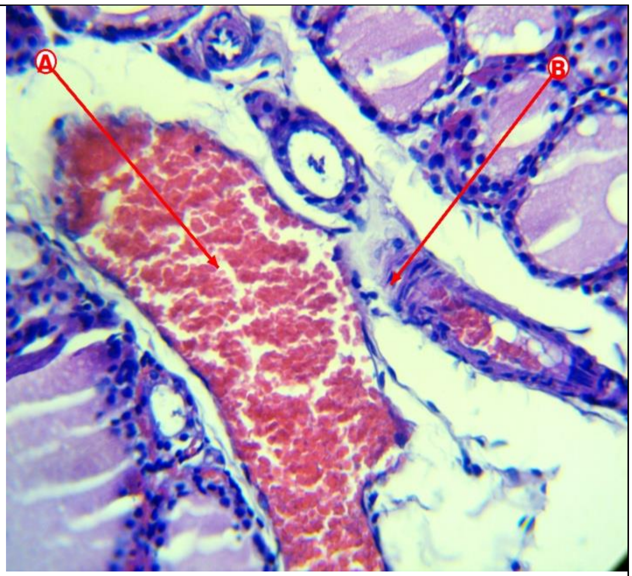

Figure (10): Show the interstitial CT of thyroid stroma had congested blood vessels (A) degeneration of follicular cells (B) (X40 PAS) 36- months age B.

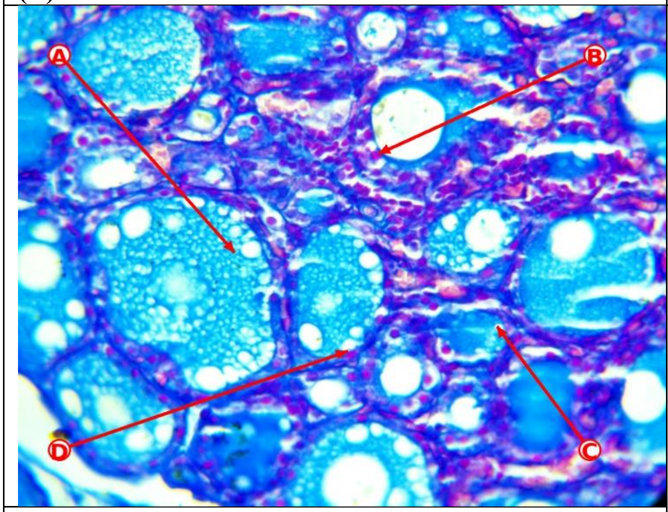

Figure (11): Show the whole lumens of thyroid follicles had colloid in the form of foamy appearance (A) and degenerated most of thyroid follicular cells (B) empty of follicles from colloid (C) blood capillaries in the interstitial CT (D) (40 x MTCH) 36- months age C.

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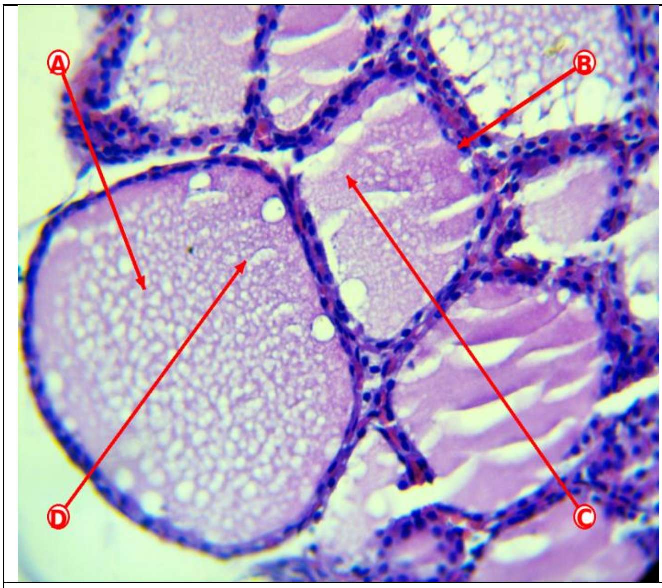

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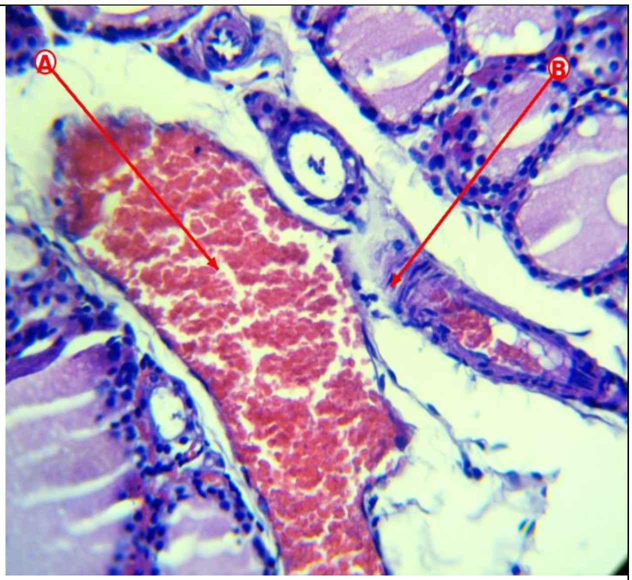

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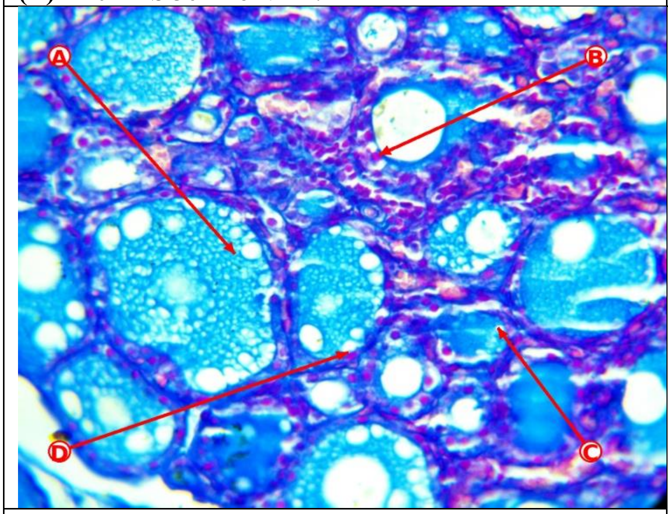

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4. DISCUSSION

The current study proved the presence of significant histological differences between studied age groups of guinea pigs with regard to the structure of the thyroid gland. The main ones were follicle size and localization, quantity and appearance of colloid, the shape of follicular epithelium, parafollicular cell localization, connective tissue and vascular system localization. It means that there is a certain structural change of the thyroid gland at aging. Some mammalian species have also undergone similar changes related to aging of the thyroid gland, however, the pattern and quantity of these changes might be different for different species (Lee *et al*., 2016; Soleimani *et al*., 2021).

At 6 months, the thyroid gland of the guinea pig consisted mostly of small follicles with loose connective tissue around them. Most follicles contained colloid, however, some had none or only small quantity of it. The follicle epithelium was cuboid. This description correlates with the general organization of the thyroid gland as a structure with follicles, which consist of follicular epithelium and colloid. The size of thyroid follicles and the height of the follicular epithelium It is dependent on the physiological status of the gland (Hussein *et al*., 2023).

Follicles displayed more diversity in size at 12 months. The follicles consisted of cuboidal epithelial cells while parafollicular cells were seen among the follicular cells as well as the inter-follicular connective tissue. In some follicles, there was an abundance of colloid material, but in others, the amount of colloid was reduced or seemed to be reduced in size. There are other mammalian species which show variations in the sizes of follicles and their contents in a single thyroid gland and this can be due to the variations in the structure of individual follicles (Lee *et al*., 2016).

The 24-month results were particularly intriguing given the easy detection of parafollicular cells, along with great variability in the size of the follicles and the location of colloid. It has been shown in previous studies on guinea pigs that there is an increased number of C-cell follicles with age, more specifically in animals aged 20 to 29 months (Kameda, 1986). These findings serve as an easy reference point for the current results because the parafollicular cells were clearly identifiable in the older animals. Nevertheless, the present experiment was carried out through only morphological identification without any staining for calcitonin or quantification of cells.

The most prominent structural modifications were identified in animals aged 36 months. The presence of numerous rather big follicles, with cuboidal or flattened follicular epithelium in some regions, could be noted. Some follicles contained less or no colloid, while others possessed colloid that was foamy. Degenerative changes of follicular epithelial cells and vascular congestion were also identified. Similar modifications were reported for aged mice and humans: differences in follicular sizes, irregular follicular structure, flatting of follicular epithelium, and follicles containing no or decreased amounts of colloid (Lee *et al*., 2016). In another animal study, it was demonstrated that aging led to alterations of thyroid volume, follicular structure, colloid, and the interstitium; thus, it was proven that aging affects multiple structural elements of the thyroid gland (Soleimani *et al*., 2021). On the molecular level, the process of thyroid aging is characterized by alterations in immune and inflammatory mechanisms, mitochondrial functioning, cytoskeleton structure, and metabolism (Liu *et al*., 2022). They might contribute to structural alterations in aged thyroid.

The large size of follicles and the flattened nature of epithelial cells in the oldest animals could possibly indicate an age-related change in follicular structure. However, such a conclusion should be made with care. Epithelial follicle height is dependent on the activity of the thyroid gland. However, the current experiment does not contain any measures of circulating T3, T4 and TSH. Hence, no statement can be made about the relationship between age-related structural changes and age-related changes in hormone levels. The reason is that age-related structural changes are not always associated with age-related changes in thyroid hormone levels (Franceschi *et al*., 2019; Van Heemst, 2024).

The variations in the colloid content detected in the current experiment are also worth noting. The colloid contains thyroglobulin that is the main protein stored in thyroid follicles. The presence of the colloid changes depending on follicular activity and colloid absorption (Hussein *et al.,* 2023). According to previous studies, the distribution of colloid is changed and colloid-deficient follicles are seen in the aged thyroid gland (Lee *et al*., 2016). Therefore, the presence of colloid-rich and colloid-reduced follicles in the old guinea pigs may reflect individual differences in the state of structures of follicles. However, the presence or absence of colloid in itself was not a sufficient criterion for judging if the thyroid gland produced more or fewer hormones.

Degenerations of the follicular epithelium were more pronounced in 36-month-old animals. Aging is associated with changes in cell maintenance, oxidative stress, mitochondria, inflammation and extracellular matrix, which may impact tissue structure (Franceschi *et al*., 2019). Therefore, such mechanisms may provide an alleged explanation of degenerative changes observed in older animals. However, apoptosis, oxidative stress, cellular senescence or biomarkers were not assessed in the current study, and thus, degeneration of the follicular epithelium should be considered a morphological rather than a cellular phenomenon.

Vascular congestion observed in older animals may also be related to changes in the thyroid microenvironment because of aging. The thyroid gland is highly vascularized, and such features of the organ are closely associated with its endocrine and metabolic functions (Hussein *et al.,* 2023).

Nevertheless, in the current study, none of the vascular density, vessel diameter, and blood flow were measured. Thus, vascular congestion represents a microscopic finding and a description, which does not mean that there is any impairment in thyroid circulatory system.

The interstitial connective tissue differed in the two groups of animals as well. There were large areas of loose connective tissue in younger animals and sometimes there was more collagen containing connective tissue in the older animals' samples. Ageing of the tissues results in changes in the extracellular matrix and stroma, which can affect the structural organization of the endocrine tissues (Franceschi *et al*., 2019). Nevertheless, in the current study, Masson's trichrome staining was done mainly for morphological observations and no quantitative analysis of the collagen area was done. Therefore, it would be too early to consider the results as unambiguous signs of age-related fibrosis. Recent studies have demonstrated that ageing of the thyroid gland is characterized by cellular senescence and changes in the phenotype of the thyroid epithelial cells. Another element of the thyroid is para-follicular cells that secrete calcitonin and whose distribution and organization vary from one animal species to another (Kameda, 1986; Comparative histology of C thyrocytes, 2022). According to the results obtained during the current research, para-follicular cells were identified in the inter-follicular connective tissue adjacent to the follicular cells, mainly in the older groups. This finding agrees with the previous research conducted on guinea pigs showing age-related alterations in C-cell follicles (Kameda, 1986, Yassin *et al.,* 2024). It should be noted, however, that calcitonin immunohistochemistry was not done and hence the current results can be considered only as the results of morphology.

According to our results, aging in guinea pigs affects multiple elements of the thyroid structure. Changes were more evident in the oldest age group, including larger and more diverse follicles, modifications in the colloid appearance, flattening and degeneration of some follicular epithelial cells, vascular congestion and interfollicular connective tissue alterations. Similar modifications have been reported in other experimental animals, mostly aged rodents, taking into account species differences in the structure of thyroid glands when comparing them with findings obtained in guinea pigs (Matsumoto, 2016; Soleimani *et al.,* 2021).

All these findings are functionally significant and should be taken into account. Modern studies on the aging process of the thyroid gland note that structural and functional modifications are not always related to each other and several biological and environmental factors influence aging of the thyroid gland (Franceschi *et al.,* 2019; van Heemst, 2024). The present study does not provide any data on serum T3, T4 and TSH levels and quantitative histomorphometric evaluation. Therefore, it is necessary to interpret these findings as the age-related structural modification of the thyroid gland but not thyroid dysfunction.

Additional studies with histomorphometric evaluation, immunohistochemical localization of calcitonin and thyroglobulin and determination of serum T3, T4 and TSH will provide strong evidence concerning their correlation with each other.

5. CONCLUSION

A number of histological alterations specific to age have been observed in the thyroid glands of guinea pigs: changes in follicle sizes, colloid structure, follicular epithelial structure, the localization of parafollicular cells, connective and vascular tissues. The most pronounced changes have been observed in the oldest group of animals, which include enlarged follicles, flattened follicular epithelium, colloid alteration in some areas, degeneration of the epithelium, and congestion of vessels. Such findings offer an adequate description of thyroid gland morphology in different ages. However, histology does not help assess the functional importance of the above alterations. Further studies are required in histomorphometry and immunohistochemistry.

6. Recommendations

Future studies should involve quantification of the follicular diameter, follicular area, colloid area, and follicular epithelial thickness. Staining for thyroglobulin and calcitonin using immunohistochemistry technique would provide further insight into characterization of follicular and parafollicular cells. Studies involving a greater sample size and including both genders would be more informative in understanding the correlation between thyroid morphology and function in old age in respect to T3, T4, and TSH concentrations.

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AUTHOR'S DECLARATIONS

Publication Consent: The author has demonstrated their consent for the publication of the current manuscript.

Data and Material Availability: All data is available on request

Conflict of Interests: The authors did not declare any conflicts of interest.

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Contributions by Author

Conceptualization, methodology, investigation, histological analysis, data acquisition, data analysis, interpretation and manuscript preparation was done by Saif Mohammed Fanar. The author has seen and approved the final manuscript and takes complete responsibility for the entirety of the work.

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