e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(6); 233-240

Original Research Article

Age-Related Changes of Thyroid Gland: A Histological Study

Margret William[1], Cessy Job[2]

[1]Assistant Professor, Department of Anatomy, Government T.D. Medical College, Alappuzha
[2]Professor, Department of Anatomy, Government Medical College, Konni

Received: 25-03-2024 / Revised: 23-04-2024 / Accepted: 26-05-2024
Corresponding Author: Dr. Margret William
Conflict of interest: Nil

Abstract:

Background: The thyroid gland is an important endocrine gland that has a role in growth and development, rate of metabolism, and blood calcium level maintenance. In the course of aging anatomical features and functions of the thyroid gland undergo age-related changes. Literature is giving controversial results on age-related changes. This study aims to identify the age-related changes that occur in the thyroid gland.

Methods: A descriptive study on age-related changes of thyroid gland conducted in Department of Anatomy, Govt Medical College Kottayam. 100 specimens of the thyroid gland were collected from the Department of Forensic Medicine during autopsy. A histological study was conducted on the follicular cells and mean follicular diameter after staining the slide with Haematoxylin & Eosin stain. Data was entered in MS Excel and analysed using SPSS software.

Results: Specimens were grouped into 0-20, 21-40, 41-60, and >60 years. Histological studies showed a significant negative correlation between age and mean follicular diameter (p-value .006, <0.05). Mean follicular diameter was maximum in the less than 20 years group (135.07±20.17µm) and gradually decreased and became 122.67±68µm in 20-40 years of age and 113.59±55µm in 40-60 years of age. Mean follicular diameter was minimum in >60 years (81.6±42.37µm). In individuals ≤20 years and >60 years, 74% of specimens were having squamous lining for thyroid follicles. In 21 to 60 years of age, 82% of follicular epithelium was either cuboidal or columnar, and only 18% had a squamous lining. Squamous epithelium implies that the gland is inactive in 74% of people at extremes of age. This observation was significant statistically.

Conclusion: This study shows that there are significant changes in the histology of the thyroid gland as age advances. As the age of the person increases there is a significant reduction in mean follicular diameter. In the majority of specimens (74%) follicular cells showed inactive squamous epithelium in the <20 and >60 years age group.

Keywords: Thyroid Glands, Follicular Cells, Mean Follicular Diameter, Squamous Epithelium.

Introduction

The thyroid gland is an important endocrine gland situated in the anterior aspect of the lower part of the neck. It is a butterfly-shaped organ composed of two lobes, left and right, connected by a narrow isthmus. The functional unit of the thyroid gland is the thyroid follicle lined by follicular cells, filled with colloids composed of iodinated thyroglobulin. The follicular cells vary in shape depending on the level of their activity.

Normally (at an average level of activity) cells are cuboidal, and the colloid in the follicles is moderate in amount. When inactive (or resting) the cells are flat (squamous) and follicles are distended with abundant colloid. Lastly, when the cells are highly active, they become columnar, and colloid is scanty. By assessing the follicular cells, we can identify the level of activity of the gland. Thyroid gland diseases cause multiple systemic manifestations. Symptoms of thyroid gland disease in the elderly may mimic manifestations of normal aging. To rule out pathology it is important to know about normal appearance and changes related to the aging of the thyroid gland. Previous studies have given controversial data regarding age-related changes in the thyroid gland. Some histological studies show that the thyroid gland in an elderly person is characterised by increased fibrosis and a reduction in the number and size of thyroid follicles whereas some show an increase in the size of follicles.

The thyroid gland is the only endocrine gland that depends on the external environment for the raw materials of its hormones. Dietary intake of iodine has an important role in thyroid hormone secretion. So, there is a possibility of structural and functional variation of the gland in different populations. No

previous studies on the histological structure of the thyroid gland are available in the South Indian population which increases the relevance of this study.

Materials & Methods

A hundred specimens of the thyroid gland were collected from the Department of Forensic Medicine, Govt. Medical College Kottayam, during autopsy.

The following procedures were employed in autopsy specimens. The thyroid gland was dissected en-mass from autopsied bodies. Tissue washed in running tap water. Specimen number, age, and sex were labelled. Bits of thyroid tissue were collected from both the lobes. These tissues were fixed and preserved in 10% formalin.

Tissue processing and staining: After fixation of thyroid tissue, bits were kept overnight in 70% alcohol, dehydration was done with ascending grades of alcohol, cleared with xylene, and embedded in paraffin wax using Leuckhart's L blocks. Paraffin blocks were labelled and cut at 6 micron thickness using LEICA 2125 rotary microtome with disposable blades. The individual sections were gently lowered onto the surface of the water at 60-70^0 C to remove the folds. The sections were taken on egg albumin-coated slides. Slides were kept in an incubator for 45 min and stained with Haematoxylin and Eosin stain. A special stain like PAS was also used for selected slides. The slides were observed under the light microscope.

The size of follicles was measured using an ocular micrometer. Follicles were grouped into large (>2.5 ocular unit = 240µm), medium (0.5 to 2.5 ocular unit = 48 to 240 µm), and small-sized (<0.5 ocular unit = 48µm) according to the diameter. The number of follicles in each group per low power field, the size of follicles, and the type of follicular epithelium were studied and compared in different age groups. The mean follicular diameter was calculated by measuring the diameter of large, medium-sized, and small-sized follicles in a randomly selected low-power field using an ocular micrometer and counting the number of large (>240µm), medium (48- 240µm), small-sized (<48µm) and total number of follicles in that low power field and calculating the average of their diameter. Photographs of the histological slides were taken using photomicrographic equipment from the Department of Pathology. After collecting the data from all the samples, the data was numerically coded and entered into MS EXCEL Spreadsheet. The analysis was done using the SPSS software package.

Inclusion Criteria: Specimens collected from autopsied bodies from the Department of Forensic Medicine within 24 hours of death.

Exclusion Criteria: Decomposed bodies, hanging cases, any crush or cut injury of the neck, the histological finding of thyroid disease, and death following chronic illness were excluded from the study

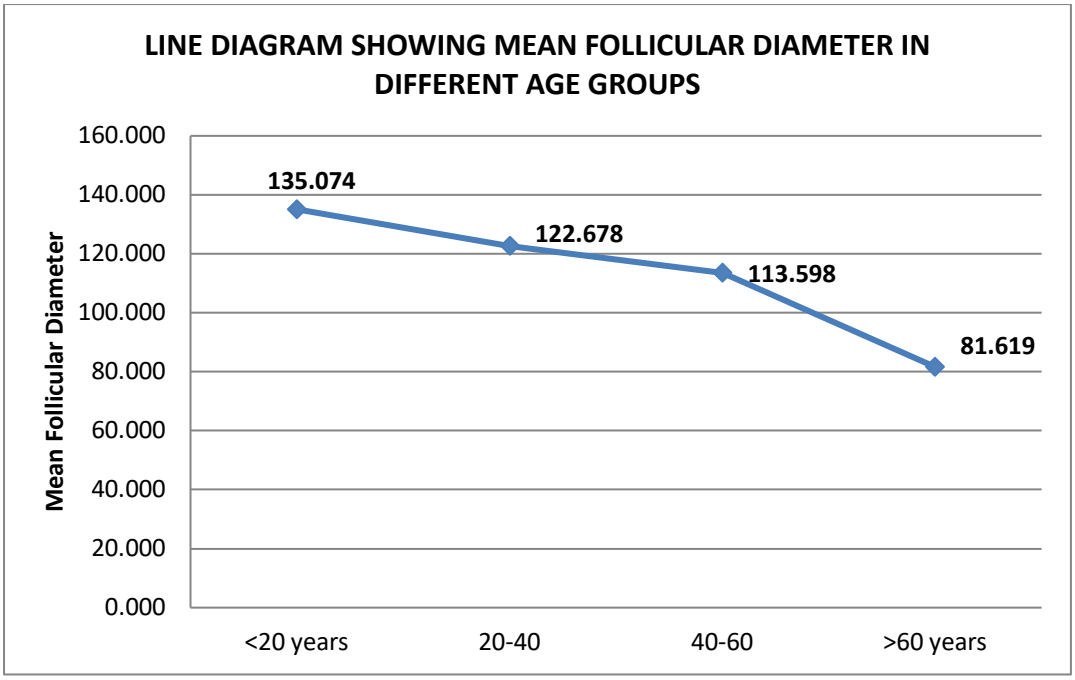
Results

Out of 100 specimens, 87 were from males and 13 were from females. Specimens were grouped according to the age of the individual into 0-20 years, 21-40 years, 41-60 years and >60 years, and the age-related changes were assessed. The majority of the study population was in the 41-60-year group (40%) followed by the 21-40 year group (33%). Only 5% of the study population was in the 0-20 year group. The age of the population ranged from 5 to 94 years

Mean follicular diameter

This study showed significant age-related changes in the thyroid gland. Mean follicular diameter reduces as the age of the person increases. One-way ANOVA test showed a significant difference in mean follicular diameter in these four age groups (p-value 0.042, <0.05). The mean follicular diameter in the age group less than 20 years was 135±20.17µ and that of the age group >60 years was 81.6±42.37µ. As the age of the person increases there is a significant reduction in mean follicular diameter

Pearson correlation showed a significant negative correlation between age and mean follicular diameter (correlation coefficient -0.274, p-value .006, <0.05). Fig 1-3 shows the thyroid gland section of younger individuals with more large and medium-sized follicles and Fig 4-7 represents the thyroid gland section of older individuals with more small sized follicles. Mean follicular diameter significantly reduces as the age of the person increases.



Graph 1: Line Diagram Showing Mean Follicular Diameter in Different Age Groups

The mean follicular diameter in males of the study population was 112.76 ± 59.47µ and that of females was 84.98 ± 45.73 µ. However, this difference in mean follicular diameter with sex was not statistically significant.

Follicular cells of thyroid follicle

A study on follicular cells lining the follicle showed a higher incidence of squamous epithelium at extremes of age. In individuals ≤20 years and >60 years, 74% of specimens (ie, 20 out of 27) had shown squamous lining. At 21 to 60 years of age, 82% of follicular epithelium was either cuboidal or columnar, and only 18% had a squamous lining. Squamous epithelium implies that the gland is inactive in 74% of people at extremes of age. The chi-square test showed that this difference in follicular cells is significant (p-value 0.000).

Table 1: Frequency of different types of follicular epithelium in different age groups

Age groups	Squamous epithelium	Cuboidal/ columnar epithelium	Total no of specimens
≤20 and >60 years	20	7	27
21 – 60 years	13	60	73

Fig 2 shows the high-power view of the thyroid gland section of a 16-year-old male (≤20 year group) stained with PAS. The squamous lining of thyroid follicles can be visualized. Fig 3 shows the high-power view of the thyroid gland section of a 22-year-old male, PAS staining, it belongs to the 21-40 year group and the follicular cells are cuboidal.

Fig 4 represents the thyroid gland section of a 34-year-old male (21- 40 group) stained with H&E showing scalloping of colloid. Columnar epithelium shows that the gland is highly active, scalloping of the colloid is due to active reabsorption of the colloid for hormone synthesis by the follicular cell.

Studies on the follicular epithelium showed that the gland is more active in 21 to 60 years of age and at extremes of age majority of the population had inactive thyroid glands.

Parenchyma and stroma of thyroid gland

The number of follicles per low-power field also showed significant age-related changes. The total number of large follicles showed a significant negative correlation with age (p-value 0.026, <0.05 and correlation coefficient -0.223), and the total number of small follicles per low power field showed a significant positive correlation with age (p-value 0.008 and correlation coefficient 0.263). This result shows that the total number of large follicles decreases and small follicles increase as age advances. However, the total number of follicles per low-power field and the number of medium sized follicles did not show any significant change.

The amount of connective tissue in between the follicles was less in younger age groups, where the follicles appeared to be closely packed (fig 1,2,3,4). As the age advanced there was an increase in the stroma of the thyroid gland (fig 5,6,7). As the mean

follicular diameter decreases with age and the total number of follicles per low power field did not change significantly this can be inferred that the stroma of the thyroid gland increases and parenchyma decreases as age advances. Since the colloid within the follicle is PAS positive, the periodic acid stain was also done in some selected slides. The colloid will appear magenta and the nuclei of the cells appear blue by PAS with Haematoxylin stain. Peripheral scalloping of colloid due to active reabsorption of colloid by the follicular cells was demonstrable by the PAS staining (fig 6). Vacuoles were observed in histological sections 41% of study specimens. This was thought to be the first post-mortem change in the thyroid gland (fig 3,7).

Figure 1: Thyroid gland section of a 5-year-old male, H&E staining, low power view, showing follicles lined by cuboidal epithelium, filled with colloid, lobules separated by connective tissue septae

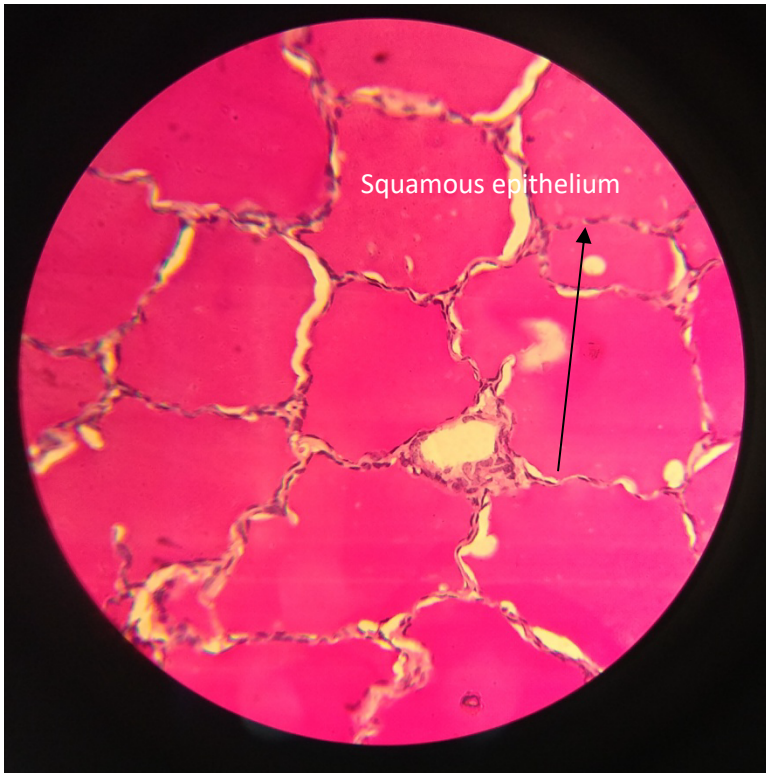


Figure 2: Thyroid gland section of a 16-year-old male, PAS staining, high power view, showing follicles lined by squamous epithelium

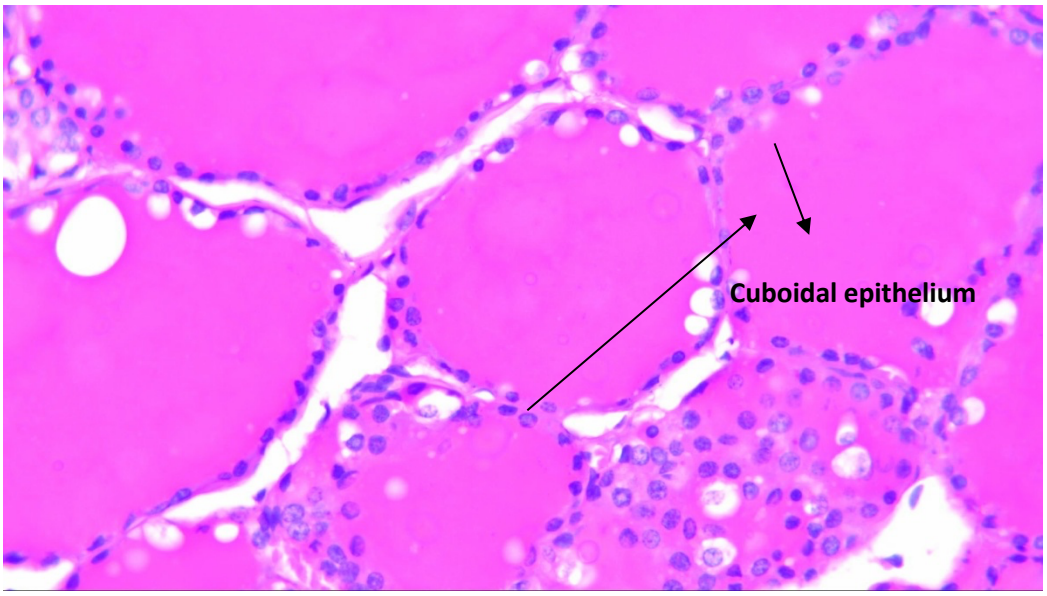


Figure 3: Thyroid gland section of a 22-year-old male, PAS staining, high power view showing cuboidal lining of follicles

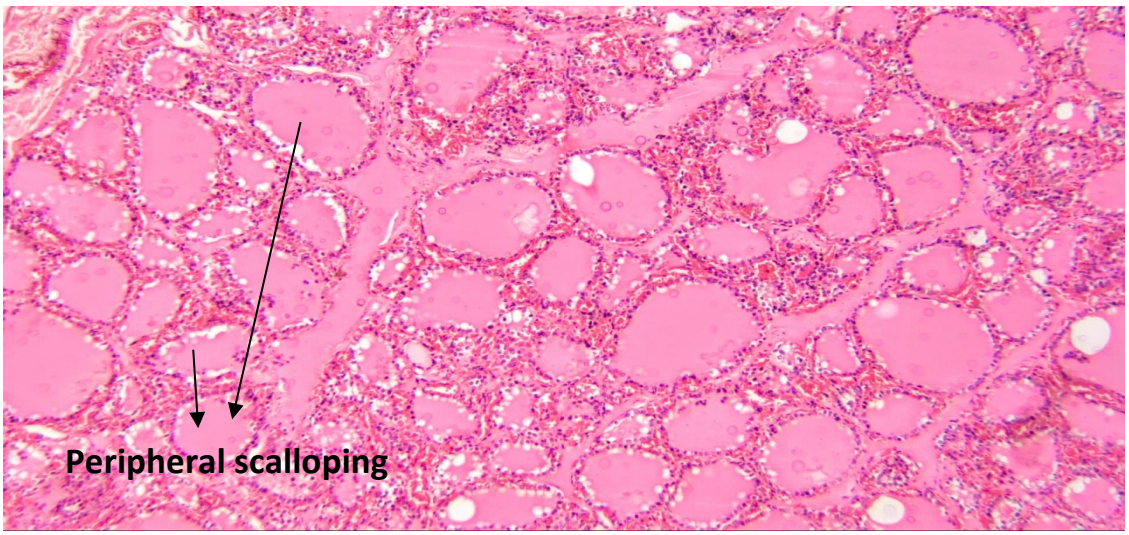


Figure 4: Thyroid gland section of a 34-year-old male, H&E staining, low power view showing more of medium-sized follicles and peripheral scalloping of colloid

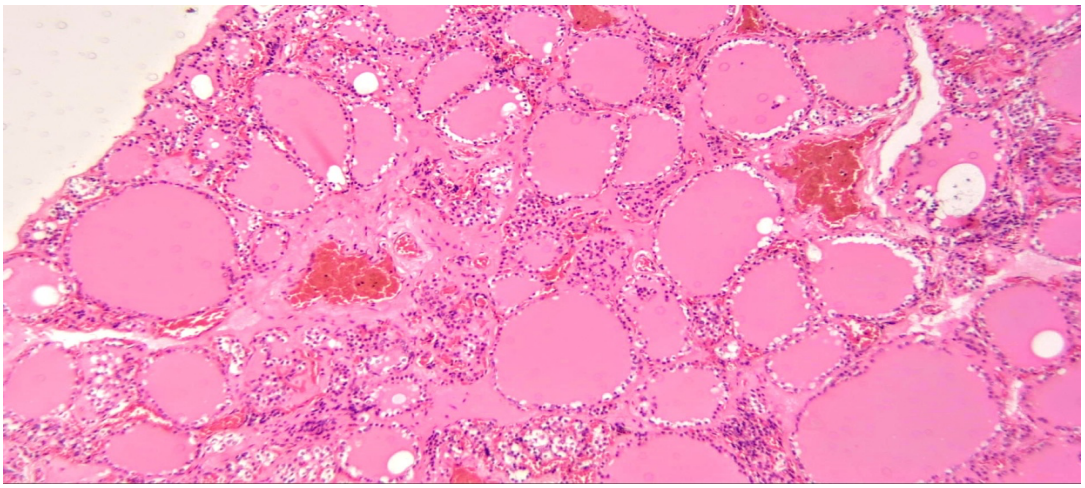


Figure 5: Thyroid gland section of a 54 year old male, H&E staining, low power view showing more amount of connective tissue between the follicles compared to younger age group

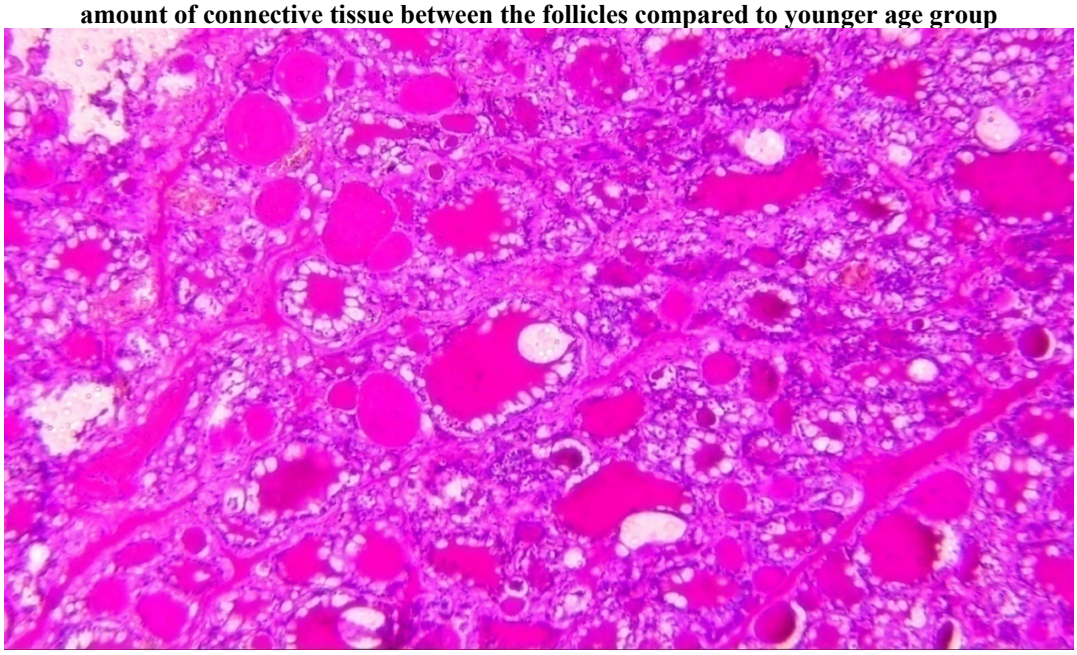


Figure 6: Thyroid gland section of a 56 year old female, PAS staining, low power view

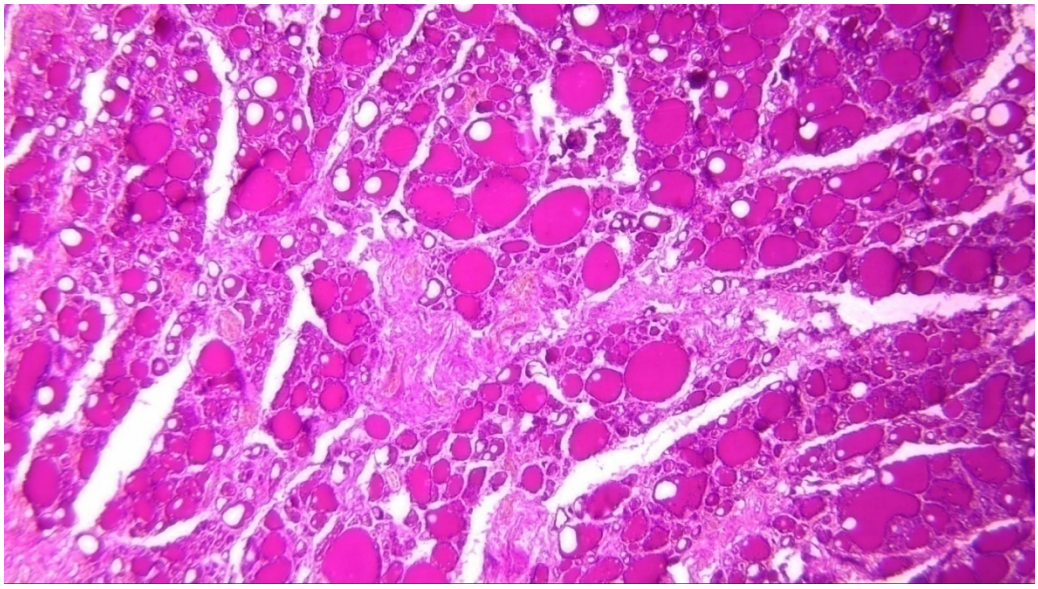


Figure 7: Thyroid gland section of a 85 year old male, PAS staining, low power view, showing more small sized follicles with vacuoles within colloid and more amount of stroma

Discussion

Mean follicular diameter: Mean follicular diameter in different age groups was calculated in this study. That showed a significant decrease in mean follicular diameter as age increased. Mean follicular diameter was maximum in the <20 years age group (135.07±20.17µm). In 20-40 years of age, it became 122.67±68.31µm. Further reduction was noticed in 40-60 years (113.59±55.88µm). The mean diameter of follicles was minimal in the elderly, >60 years group (81.61±42.37µm). There was a significant negative correlation between age and mean follicular diameter in the present study. The average size of the thyroid follicle studied by Nurunnabi et al [1] (2009) obtained values slightly higher than the diameter obtained by the present study.

He reported the size of thyroid follicle in age group 10-20 years is 171.29±3.95µm, age group 21-50 years is 210±20.02µm and >50 years is 170.55±4.31µm. This shows an increase in the size of follicles till middle age and a decline thereafter.

The present study showed the maximum mean follicular diameter in the <20 years age group. In contrast to this observation, a study conducted by Malik P et al [2] (2016) revealed that the mean size of thyroid follicles in age group ≤20 years is

56.38±14.12µm age group 21- 50 years is 130.08±30.67µm and age group >50years is 96.05±12.86µm. It shows that the mean size of thyroid follicles is higher in the 21 -50 years age group, followed by >50 years, followed by ≤ 20 years. Here the minimum diameter was obtained in the ≤20 years group.

This study showed an increase in the number of small-sized follicles (<48µm) and a decline in large-sized follicles (>240µm) as age increases. Older individuals had more small-sized follicles and younger individuals had more large-sized follicles. The number of medium-sized follicles per low-power field (48-240µm) and the total number of follicles per low-power field did not show significant age-related changes. In contrast to this finding in 2004 Schlumberger et al [3] observed that in children there is a higher proportion of small follicles (30-100µm) and intermediate follicles (100-200µm).

Sulthana et al [4] (2007) measured the mean diameter of follicles and the number of follicles per square millimeter area. The highest mean diameter of follicles was found in the 19-45 years age group which had fewer follicles per unit area of the section. The total number of follicles per low-power field counted in the present study did not show age-related change. Similar to the present study Brown RA et al [5] (1986) observed that the total number of acini in the gland did not show any great age changes.

However, studies conducted in other animals show observations different from those observed in the human thyroid gland. Studies conducted by Huntingdon Research Centre (1981-1984) on Sprague-Dawley rats showed an increase in internal follicular diameter and total number of follicles with an increase in age of the rats [6]. A similar observation was made by Sawicki B et al [7] (1992) in a study conducted on European bison. The thyroid of calves showed an increase in the number and size of follicles in old age.

Follicular cells

The present study showed a significant difference in the lining epithelium of thyroid follicles in different age groups. 74% of specimens at extremes of age ie, ≤ 20 years and >60 years were lined by squamous epithelium (fig 2) whereas the majority, 84%, between 21-60 years of age showed either cuboidal (fig 3) or columnar (fig 6) lining. Squamous lining was observed only in 18% of cases in 21 - 60 years of age.

Squamous lining shows inactive follicles, follicular cells are cuboidal in moderately active glands and columnar in highly active glands. Follicles with differing levels of activity may co-exist in a section, the lining of the majority of follicles noted to compare the activity in different age groups in the present study. In contrast to this observation, Stoffer et al [8] (1961) noted that the average height of the follicular epithelium decreased between the ages 20 and 50, but thereafter showed little change. Studer et al [9] (1978) studied the age-related changes in mice and observed that 80% of all thyroid follicles in 13-month-old mice. Morphologically, the "Cold" follicles are characterized by a larger than-normal colloid volume and a comparatively flat epithelium for any given follicle size. The present study also showed more specimens with squamous lining in the elderly.

Similar to the present study histological study conducted by Abdullah et al [10] (2010) also showed a strong positive correlation between age and the number of squamous cells of the follicular epithelial lining.

Firdous Dar et al [11] (2013) conducted a study on female Kuttanad ducks from day old to 24 weeks of age and found that the mean epithelial height of active follicles increases from day-old duckling up to the sixth week. From eight weeks onwards there was a gradual decrease in the mean epithelial height up to 24 weeks. This observation is similar to that of the present study. Snehangsu Singu et al [12] (2015) conducted a study on 42 Assam Pati ducks from day old to 40 weeks of age. The epithelial height of the active follicles increased up to the eighth week and the epithelial height of inactive follicles showed a decreasing trend.

Similar observation was made by Igbokwe CO et al [13] (2017), who studied the morphological changes in the thyroid gland of West African Dwarf goats during the foetal and postnatal development stages. The follicular cells were of low cuboidal shape in foetuses, assumed high cuboidal or columnar form in the prepubertal group, and squamous in older pubertal age.

Parenchyma and Stroma

The number of follicles per low-power field showed significant age-related changes in the present study. The number of large-sized follicles (>240µm) showed a significant negative correlation and the number of small-sized follicles (<48µm) showed a significant positive correlation with age. But the total number of follicles per low-power field and the number of medium-sized follicles (48-240µm) did not show significant age-related change. But Abdullah et al [15] (2010) observed a significant negative correlation between age and number of thyroid follicles.

Nurunnabi et al (2009) [1] observed that the number and average diameter of thyroid follicles and percentage of parenchyma increase during the first 50 years of life and decrease thereafter. Similar to this present study also observed a decrease in mean follicular diameter. There was a

reduction in the number of large-sized follicles significantly but as a result of an increase in small-sized follicles, the total number of follicles did not show a decrease with age. The present study also noticed an increase in stroma along with age. Younger age groups had closely packed thyroid follicles with less amount of connective tissue in between. As age progressed there was an increase in the amount of connective tissue in between the follicles. Similar to the present study Mustacchi et al [14] (1950) compared 40 glands from old people with those from younger subjects. In two-thirds of thyroid glands from elderly subjects, he found an increase in connective tissue that is similar to this study.

Conclusion

Histological studies showed a significant negative correlation between age and mean follicular diameter (p-value .006, <0.05). Mean follicular diameter was maximum in the less than 20-year group (135.07±20.17µm) and gradually decreased and became 122.67±68µm in 20-40 years of age and 113.59±55µm in 40-60 years of age. Mean follicular diameter was minimum in >60 years (81.6±42.37µm). As the age of the person increases there is a significant reduction in mean follicular diameter. Mean follicular diameter did not show a significant difference with the gender of the person.

The number of thyroid follicles per low-power field showed significant age-related changes. The number of larger follicles significantly reduced and the number of smaller follicles significantly increased as age increased. Follicular cells showed a higher incidence of squamous epithelium at extremes of age.

In individuals ≤20 years and >60 years, 74% of specimens were having squamous lining. At 21 to 60 years of age, 82% of follicular epithelium was either cuboidal or columnar, and only 18% had a squamous lining. Squamous epithelium implies that the gland is inactive in 74% of people at extremes of age. This observation was significant statistically.

References

1. Nurunnabi ASM, Mahbub S, Shahriah S, et al. Thyroid Follicles and Parenchyma are found to increase with advancing age during the first 50 years of life in Bangladeshi People. Bangla J Soc Physiol. 2009; 4(2): 88-92.
2. Malik P, Srivastava SK, Sen R, Singh K, Grover S, Bansal S. Age related changes in size of thyroid follicle in north Indian population: A histologic study. Int J Med Res Health Sci. 2016; 5(5):96–100.
3. Schlumberger M, Faggiano A, Coulot J, Bellon N, Talbot M, Caillou B, et al. Age-Dependent Variation of Follicular Size and Expression of Iodine Transporters in Human Thyroid Tissue. J Nucl Med. 2004; 45:232-237.
4. Sultana SZ, Khan MK, Sultana S, Rahman MH, Khalil M, Khalil M. Histomorphometric study of thyroid gland of Bangladeshi people. Mymensingh Med J MMJ. 2007 Jan; 16(1):36–42.
5. Brown RA, Al-Moussa M, Beck J. Histometry of normal thyroid in man. J Clin Pathol. 1986; 39(5):475–482.
6. Rao-Rupanagudi S, Heywood R, Gopinath C. Age-related changes in thyroid structure and function in Sprague-Dawley rats. Vet Pathol. 1992; 29(4):278–287.
7. Sawicki B, Siuda S, Kasacka I. Bisoniana 107. Microscopic structure of the thyroid gland in the European bison. Acta Theriol (Warsz). 1992 Oct 8; 37:171–9.
8. Stoffer R P, Helwig C A, Welch J W, McCusker E N. The thyroid gland after the age 50. In: Geriatrics. 1961;16: 435–43.
9. Studer H, Forster R, Conti A, Kohler H, Haeberli A, Engler H. Transformation of Normal Follicles into Thyrotropin-Refractory "Cold" Follicles in the Aging Mouse Thyroid Gland. Endocrinology. 1978 May 1; 102(5):1576–86.
10. Abdullah SI, Al-Samarrae A-JJ, Mahood A-KS. The Effect of Aging on Human Thyroid Gland: (Anatomical and Histological Study). Iraqui J Coom Med. 2010 Jul; (3):158–64.
11. Firdous Dar, Karalathukaran Lucy. Epithelial Diversification of Thyroid Gland in Kuttanad Duck (Anas platyrhynchos domesticus) - A Postnatal Study. Br J Poult Sci. 2013; 2(1):7–10.
12. Snehangsu Sinha, Munmun Sarma, K B Dev Choudhury. Postnatal Changes in Epithelial Lining of Follicles in Thyroid Gland of Pati Ducks (Anas Platyrhynchos Domesticus) Of Assam at Different Age Groups. J Agreculture Vet Sci. 2015 Jan; 7:2319–72.
13. Igbokwe CO, Ezeasor DN. Age-related Microscopic Changes in the Thyroid Gland of West African Dwarf Goat During Foetal and Postnatal Periods of Development. Not Sci Biol. 2017 Mar 30; 9(1):59.
14. Mustacchi P O, Lowenhaupt E. Senile changes in histologic structure of thyroid gland. In: Geriatrics. 1950; 268–73. (5).
15. Sami I Abdullah, Abdul Jabbar J, Abdul Karim S. The effect of aging on Thyroid gland: (Anatomical and histological study). Iraqi J Comm. Med. July2010;(3):158-164.