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Research Article

Microscopic and Histochemical Assessment of Structural Changes in the Human Thyroid Gland across Different Age Groups

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ABSTRACT
Background: Aging is associated with progressive structural and functional changes in the thyroid gland.
Objective: To assess microscopic and histochemical structural changes in the human thyroid gland across different age groups.
Methods: This comparative cross-sectional histomorphological study was conducted at Anatomy department in collaboration with Medicine department of DG Khan Medical College, DG Khan from January 2026 to June 2026, included 90 human thyroid specimens divided equally into three age groups: 18–35 years, 36–55 years, and 56–75 years. Routine hematoxylin and eosin staining was performed to assess general thyroid architecture, follicular size and shape, epithelial cell height, colloid content, stromal changes, vascularity, and the presence of age-related structural alterations.
Results: Mean follicular diameter increased significantly from 112.36±18.42 µm in the youngest group to 128.71±21.55 µm and 151.84±25.63 µm in the middle and older groups, respectively ($p<0.001$). Follicular epithelial height decreased from 8.42±1.31 µm to 7.36±1.24 µm and 6.18±1.17 µm ($p<0.001$). Colloid area increased from 54.63±7.85% to 61.72±8.41% and 69.58±9.12%, while stromal connective tissue increased from 11.48±3.62% to 16.37±4.18% and 23.76±5.43% ($p<0.001$). Irregular follicles, epithelial flattening, stromal fibrosis, vascular wall thickening, and lymphocytic infiltration were more frequent with advancing age.
Conclusion: Advancing age was associated with progressive follicular enlargement, epithelial flattening, colloid accumulation, reduced vascularity, increased fibrosis, and greater collagen deposition in the human thyroid gland.

Keywords: Thyroid Gland, Aging, Histology, Histochemistry, PAS Staining, Masson's Trichrome, Follicular Changes.

INTRODUCTION

Thyroid hormones are important hormones produced by thyroid gland which has a central role in the regulation of metabolism, growth and development as well as energy balance [1]. It is structurally made up of many small spherical follicles lined by follicular epithelial cells and filled with colloid with thyroglobulin in it. The delicate connective tissue framework, and rich capillary network surrounding the follicles, are important for hormone synthesis and secretion [2]. In addition, there are parafollicular cells in the gland which help to regulate calcium through the secretion of calcitonin [3]. The thyroid gland is different at different stages of life. In younger animals, the follicles tend to be more regular, the cuboidal epithelial cells are active and the distribution of the colloid is fairly uniform [4]. As age increases, there may be changes in the size of the follicles, height of the epithelium, colloid size, stromal tissue, and vascular tissue. Follicular enlargement, flattening of the epithelial cells, irregular follicular outline and an increase in connective tissue may also occur with age [5]. These changes may be due to a gradual change in the thyroid activity and also to the remodeling of tissues without actually the presence of thyroid disease. The follicular epithelium is, specifically, important when evaluating thyroid activity. A higher epithelial cell height is correlated with a higher functional activity while a flatter cell is correlated with a

relatively inactive cell [6]. As the age increases, the accumulation of colloid and the widening of the diameter of the follicles have also been linked together [7]. Older glands may show a greater degree of connective tissue, and some degree of fibrosis and stromal thickening may be more apparent [8]. Some of the other alterations reported in the ageing process are those of the blood vessels, such as thickening of vessel walls and decrease in microvascular organization [9].

Histochemical techniques can be utilized to provide information which cannot always be gained from routine hematoxylin and eosin staining. Carbohydrate-rich glycoproteins in thyroid colloid and basement membranes can be shown using periodic acid–Schiff staining [10]. The variation in staining intensity in PAS could be due to variations in the quantity and quality of thyroglobulin in the follicles [11]. Special stains for collagen may also be useful for the identification of age-related increases in connective tissue and fibrosis [12]. These methods enable evaluation of microscopic changes in structure along with the changes in tissue composition. Recent research has indicated that cellular metabolism, mitochondrial function, immune activity and gene expression also change as the thyroid ages [13]. Inflammatory and stromal changes that occur with age can be involved in changes to normal thyroid architecture [14]. Molecular analysis has also shown that there are variations in the follicular cell population and cellular stress pathways in older thyroid tissue [15]. The knowledge of these normal age-related changes is valuable because some of these changes can mimic early pathological changes.

Objective

To assess microscopic and histochemical structural changes in the human thyroid gland across different age groups.

METHODOLOGY

This comparative cross-sectional histomorphological study was conducted at Anatomy department in collaboration with Medicine department of DG Khan Medical College, DG Khan from January 2026 to June 2026. Specimens with known thyroid disease, gross nodules, malignancy, previous thyroid surgery, or marked autolytic changes were excluded. The included specimens were divided into predefined age groups, such as young adults, middle-aged adults, and older adults. Representative tissue samples were taken from both thyroid lobes, fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, and sectioned at approximately 4–5 µm thickness.

Data Collection

Routine hematoxylin and eosin staining was performed to assess general thyroid architecture, follicular size and shape, epithelial cell height, colloid content, stromal changes, vascularity, and the presence of age-related structural alterations. Periodic acid–Schiff staining was used to assess glycoprotein-rich colloid and basement membrane changes, while Masson's trichrome staining was used to evaluate connective tissue and fibrosis. Microscopic assessment was performed under standardized magnification. Quantitative measurements included mean follicular diameter, follicular epithelial height, proportion of colloid-filled follicles, and extent of stromal connective tissue. Histochemical staining intensity was graded using a predefined scoring system. Where available, digital image-analysis software was used to improve measurement accuracy. All observations were recorded on a structured data collection proforma.

Statistical Analysis

Data were entered and analyzed using SPSS version 26.0. Quantitative variables such as follicular diameter, epithelial height, and connective tissue proportion were expressed as mean±SD or median with interquartile range, depending on data distribution. Normality was assessed using the Shapiro–Wilk test. Differences among age groups were analyzed using one-way ANOVA for normally distributed variables or the Kruskal–Wallis test for non-normal data. Categorical variables and histochemical staining grades were compared using the Chi-square test or Fisher's exact test where appropriate. Post-hoc analysis was performed when significant differences were identified between age groups. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The study included 90 thyroid specimens, with 30 participants in each age group. Mean age was 27.43±5.12 years in the 18–35-year group, 45.67±5.48 years in the 36–55-year group, and 64.20±5.71 years in the 56–75-year group, with an overall mean age of 45.77±15.89 years ($p<0.001$). Males and females were equally

represented overall, 45 (50.0%) each, with no significant gender difference between groups (p=0.875). Right lobe specimens accounted for 48 (53.3%) and left lobe specimens for 42 (46.7%), with no significant difference across age groups (p=0.870).

Table I. Baseline Characteristics of Thyroid Specimens According To Age Group

Variable	18–35 years (n=30)	36–55 years (n=30)	56–75 years (n=30)	Total (n=90)	p-value
Age, years, Mean±SD	27.43±5.12	45.67±5.48	64.20±5.71	45.77±15.89	<0.001
Male, n (%)	16 (53.3)	15 (50.0)	14 (46.7)	45 (50.0)	0.875
Female, n (%)	14 (46.7)	15 (50.0)	16 (53.3)	45 (50.0)	
Right lobe specimen, n (%)	17 (56.7)	16 (53.3)	15 (50.0)	48 (53.3)	0.870
Left lobe specimen, n (%)	13 (43.3)	14 (46.7)	15 (50.0)	42 (46.7)	

With advancing age, mean follicular diameter increased from 112.36±18.42 µm to 128.71±21.55 µm and 151.84±25.63 µm, while epithelial height decreased from 8.42±1.31 µm to 7.36±1.24 µm and 6.18±1.17 µm. Colloid area increased from 54.63±7.85% to 61.72±8.41% and 69.58±9.12%, while stromal connective tissue increased from 11.48±3.62% to 16.37±4.18% and 23.76±5.43%. Vascular density decreased from 8.83±2.14 to 7.73±1.96 and 6.47±1.82 vessels/high-power field. All differences were statistically significant (p<0.001).

Table II. Quantitative Microscopic Changes in the Thyroid Gland across Age Groups

Histological Parameter	18–35 years	36–55 years	56–75 years	Test statistic	p-value
Follicular diameter, µm, Mean±SD	112.36±18.42	128.71±21.55	151.84±25.63	F=24.76	<0.001
Follicular epithelial height, µm, Mean±SD	8.42±1.31	7.36±1.24	6.18±1.17	F=24.91	<0.001
Colloid area, %, Mean±SD	54.63±7.85	61.72±8.41	69.58±9.12	F=22.80	<0.001
Stromal connective tissue, %, Mean±SD	11.48±3.62	16.37±4.18	23.76±5.43	F=55.12	<0.001
Vascular density, vessels/high-power field, Mean±SD	8.83±2.14	7.73±1.96	6.47±1.82	F=10.86	<0.001

Age-related microscopic changes became more frequent in older individuals. Irregular follicles increased from 4 (13.3%) to 11 (36.7%) and 22 (73.3%), flattened epithelium from 3 (10.0%) to 10 (33.3%) and 21 (70.0%), and colloid accumulation from 6 (20.0%) to 15 (50.0%) and 23 (76.7%). Stromal fibrosis increased from 2 (6.7%) to 9 (30.0%) and 20 (66.7%), while vascular wall thickening rose from 2 (6.7%) to 8 (26.7%) and 18 (60.0%). Focal lymphocytic infiltration was seen in 3 (10.0%), 7 (23.3%), and 12 (40.0%) patients. All changes were significant, with p<0.001 except lymphocytic infiltration (p=0.027).

Table III. Frequency of Microscopic Structural Changes across Different Age Groups

Microscopic Feature	18–35 years n (%)	36–55 years n (%)	56–75 years n (%)	χ^2	p-value
Irregularly shaped follicles	4 (13.3)	11 (36.7)	22 (73.3)	22.86	<0.001
Flattened follicular epithelium	3 (10.0)	10 (33.3)	21 (70.0)	23.44	<0.001
Colloid accumulation	6 (20.0)	15 (50.0)	23 (76.7)	19.34	<0.001
Stromal fibrosis	2 (6.7)	9 (30.0)	20 (66.7)	24.80	<0.001

Vascular wall thickening	2 (6.7)	8 (26.7)	18 (60.0)	20.53	<0.001
Focal lymphocytic infiltration	3 (10.0)	7 (23.3)	12 (40.0)	7.24	0.027

Histochemical changes also increased with age. Mean PAS staining score rose from 1.63±0.56 in younger adults to 2.07±0.64 in middle-aged adults and 2.47±0.57 in older adults (p<0.001). Strong PAS positivity increased from 5 (16.7%) to 12 (40.0%) and 20 (66.7%). The mean Masson's trichrome fibrosis score increased from 0.77±0.50 to 1.43±0.63 and 2.20±0.66, while moderate/severe collagen deposition increased from 3 (10.0%) to 11 (36.7%) and 22 (73.3%). These differences were statistically significant (p<0.001).

Table IV. Histochemical Findings According To Age Group

Histochemical Parameter	18–35 years	36–55 years	56–75 years	Test statistic	p-value
PAS staining score, Mean±SD	1.63±0.56	2.07±0.64	2.47±0.57	F=14.83	<0.001
Strong PAS positivity, n (%)	5 (16.7)	12 (40.0)	20 (66.7)	χ^2=15.43	<0.001
Masson's trichrome fibrosis score, Mean±SD	0.77±0.50	1.43±0.63	2.20±0.66	F=43.07	<0.001
Moderate/severe collagen deposition, n (%)	3 (10.0)	11 (36.7)	22 (73.3)	χ^2=25.24	<0.001

Histochemical scoring: 0=absent, 1=mild, 2=moderate, 3=strong/severe.

DISCUSSION

In the present study the age related changes in the human thyroid glands were clearly shown at microscopic and histochemical levels. Follicular diameter progressively increased from 112.36±18.42 µm in individuals aged 18–35 years to 128.71±21.55 µm in those aged 36–55 years and 151.84±25.63 µm in the 56–75-year group (p<0.001). In contrast, follicular epithelial height decreased from 8.42±1.31 µm to 7.36±1.24 µm and 6.18±1.17 µm, respectively (p<0.001). This is consistent with previous normal human thyroid tissue studies which showed that the mean follicular diameter increased with age, with younger age groups showing significantly smaller follicles than older age groups (p<0.001) [16][17]. The changes point to a progressive expansion of follicles with a decrease in the activity of the epithelium with age. These morphometric alterations were confirmed by the qualitative microscopic results. The percentage of irregularly shaped follicles grew from 13.3 per cent in the youngest group to 36.7 per cent in the middle age group and 73.3 per cent in the oldest group (p<0.001). The increase from 10.0% to 33.3% and 70.0% was also similar in flat epithelial cells of follicles (p<0.001). The histological features of thyroid gland changes which had been found in the previous studies and which were also seen in the older age group were follicular enlargement, significant difference in size of the follicles, increased irregularity, dilated follicles and age-related abnormalities in the epithelial cells of the follicles [18]. These findings are similar to each other, which helps validate the hypothesis that irregularity of the thyroid follicles and flattening of the epithelial cells is a hallmark of ageing of the thyroid. The age dependent variations in the colloid content were also significant. The mean colloid area was 54.63±7.85% in younger people, 61.72±8.41% in middle-aged people, and 69.58±9.12% in the older people (p<0.001). Similarly, visible accumulation of colloids was seen in 20.0%, 50.0% and 76.7% of the three age groups respectively. The light microscopy and histochemical studies of 83 human thyroid glands conducted earlier showed that there was an age-related rise in follicular colloid density and that progressive biochemical and physical changes in the follicular colloid occurred with age [19]. This is likely to reflect a relative decrease in activity of follicles with age, as the increase in colloid is coupled with a decrease in epithelial height. One thing that was especially striking is the progressive increase of stromal connective tissue. The mean values of stromal connective tissue were 23.76±5.43% in the oldest group and 16.37±4.18% in the middle aged group and 11.48±3.62% in the youngest group (p<0.001). Only 6.7% of younger specimens showed stromal fibrosis while 30.0% of middle aged and 66.7% of older specimens showed stromal fibrosis. Previous quantitative studies of aged human thyroids also showed that the connective tissue content became more and more prominent, becoming preponderant in old age [20]. In the elderly humans, thyroid fibrosis has also been

observed within the extracellular matrix by histological examination of old human thyroid tissue, which corroborates the present findings. Recent molecular studies confirm these structural observations. Single-cell RNA sequencing of pathologically normal human thyroid tissue showed fibrotic genes were more highly expressed in older age, there was a slight increase in the number of fibroblasts, and fibrotic signaling pathways were more activated by TGF-β [21]. The molecular changes that occurred may account for the higher stromal connective tissue percentage and fibrosis scores that were noted on the microscopic examination of the older age group in the present study. Another significant aspect of aging was the vascular changes. Mean vascular density decreased significantly from 8.83±2.14 vessels/high-power field in the 18–35-year group to 7.73±1.96 in the 36–55-year group and 6.47±1.82 in the 56–75-year group ($p<0.001$). Meanwhile, the vascular wall thickening rose from 6.7% to 26.7% and 60.0% respectively. We also compared young and aged human endocrine tissues to find a significant decrease in the number of microvessels and arterial numbers in aged human thyroid gland as was previously shown in other studies. Since the thyroid requires an extensive microvascular network for production and release of thyroid hormones, decreased vascularity can contribute to the changes in tissue maintenance and endocrine function seen with age. 10.0% of younger, 23.3% of middle aged and 40.0% of older specimens showed focal lymphocytic infiltration with a statistically significant difference between the groups ($p=0.027$). Previous studies on the thyroid glands of the elderly has showed focal lymphocytic infiltration in 17.2% of specimens and suggested that this finding may be an age-associated immunological process or an autoimmune process. This study had several limitations. Using a limited number of thyroid specimens in a relatively confined area can limit generalizability. The cross-sectional design does not allow the measurement of structural changes over time in the same individuals. Specimens from patients with known thyroid disease were excluded, but subclinical thyroid hormone and/or autoimmune disorders were not ruled out completely. Histochemical scoring was of a semi-quantitative nature, and may be subject to some observer variation. Moreover, other iodine, thyroid hormone, medication, smoking and metabolic disorders were not accounted for.

CONCLUSION

The study demonstrated clear age-related microscopic and histochemical changes in the human thyroid gland. Advancing age was associated with increased follicular diameter, greater colloid accumulation, more stromal connective tissue and fibrosis, stronger PAS positivity, and increased collagen deposition. In contrast, follicular epithelial height and vascular density progressively decreased with age. Irregular follicles, flattened epithelium, vascular wall thickening, and focal lymphocytic infiltration were also more common in older individuals. These findings suggest that normal aging produces progressive structural and tissue-compositional changes in the thyroid gland that should be considered during histopathological assessment.

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