ISSN 2563-7142

ORIGINAL ARTICLE

Immunohistochemical Study of C-cells Morphology, Distribution, and Localization in the Thyroid Gland: A Pilot Study in Cadaveric Tissue

Ghatak S[1], Potaliya P[2*], Bhattacharya S[3]

Ghatak S, Potaliya P, Bhattacharya S. Immunohistochemical Study of C-cells Morphology, Distribution, and Localization in the Thyroid Gland: A Pilot Study in Cadaveric Tissue. Int J Cadaver Stud Ant Var. 2020;1(1):11-19.

Abstract

Objective: Exact identification of C-cells in thyroid with defined morphological features, number of cells present in whole population, ratio of parafollicular to follicular cells and localization is a challenge with conventional analysis. A specific and sensitive calcitonin immunohistochemistry is necessary for the demonstration.

Method: Tissue obtained from 30 cadaveric specimens at AIIMS Jodhpur were processed, sectioned, and stained for immunohistochemical analysis using calcitonin. The results obtained were also compared with H&E staining for same section.

Results: C-cells were found distributed in both lateral lobes, concentrated around a vertical axis in cranio-caudal direction. It was a randomized distribution with hardly any symmetry. A complete homogeneous distribution of C-cells all over the thyroid was never demonstrated. C-cells were usually concentrated in the middle thirds of the thyroid, and more in the right lobe. Upper third predominantly had more C-cells as compared to lower third of the gland. Incidentally, no C-cells were found in the isthmus in any thyroid at all. When observed for the localization, most of the C-cells were in the interfollicular position.

Conclusion: The present study findings corroborate the use of IHC for C-cell analysis in thyroid gland. Calcitonin proved to be an important diagnostic marker in C-cell identification. Quantification was done reliably, proving calcitonin used in the procedure justified due to its high specificity and affinity.

Key Words: *C-cells; Thyroid; Cadaveric; Staining; Calcitonin; Immunohistochemistry*

Introduction

Difficulties arise from the subjective nature of conventional histological analysis for C-cells in thyroid. Exact identification with defined morphological features, number of cells present in whole population, ratio of parafollicular to follicular cells and localization as C-cell hyperplasia with its classification are then a challenge. To overcome these challenges, it is required to have better diagnosis in not only C-cell Hyperplasia (CCH) but carcinoma of C-cell i.e. Medullary Carcinoma. Thyroid malignancy constitutes 1% of solid organ malignancies, out of which the medullary sub-type cancer, forms around 3-5% of all thyroid cancers. A multidisciplinary approach is required for developing better diagnostic criteria of C-cells in thyroid to have a check on this emerging carcinoma which can be a threat in future.

Calcitonin, the main product of C-cells, has a high stake in pathophysiology of individuals. It lowers the concentration of calcium in the blood by suppressing bone resorption achieved by osteoclasts. Massive degranulation occurs through experimentally induced

[1]Professor and Head, Department of Anatomy, All India Institute of Medical Sciences, Jodhpur, Rajasthan -342005, India
[2]Assistant Professor, Department of Anatomy, All India Institute of Medical Sciences, Jodhpur, Rajasthan -342005, India
[3]Ex-Professor and Head, Department of Pathology, All India Institute of Medical Sciences, Jodhpur, Rajasthan -342005, India

[*]Corresponding author: Pushpa Potaliya, Assistant Professor, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan-342005, India, Tel: +91 9460481123; E-mail: ppotaliyaa@gmail.com

Received: June 12, 2020, Accepted: August 30, 2020, Published: September 30, 2020

hypercalcemia. The calcium level in the blood, which in turn depends upon the rate of demineralisation of the bone by osteoclasts, controls the calcitonin secretion. The release of the calcitonin results in diminishing the mineral resorption and returning calcium level to normal. Calcitonin receptors are also found in the kidney where they regulate the calcium excretion in the urine. Calcitonin also enhances the production of vitamin D1 by the kidney. Calcitonin secretion is also stimulated by gastrin level and increase rapidly in response to pentagastrin stimulation.

The exact number of C-cell is unknown and for this reason it is difficult to define a true hyperplasia [1]. Our focus in the present study was on the delineation between what one must consider normal or C-cells hyperplasia (CCH). CCH is also related to other conditions than thyroid pathology as prolonged hypercalcemia, hyperparathyroidism and hypergastrinemia. Besides this it is also considered as the precursor of genetically determined medullary thyroid carcinoma (MTC). More recently, CCH associated to sporadic, non-genetically determined MTCs was described in several studies [2,3]. Therefore, keeping in mind the details and significance of C-cells, this study was carried out to develop a strategy for observing and analysing the morphology, localization and distribution of C-cells in cadaveric thyroid tissue in small group of Indian origin. It was initially done by using conventional method of H&E staining. In addition, focus was on using immunohistochemistry technique and the results were compared. This differentiation is important for therapy and for prognosis to come with an ensemble portraying normal and C-cell hyperplasia (with accurate differentiation of its sub-types). The exact number of C-cells is important to establish a true hyperplasia. Range of C-cells in normal thyroid is established as of more than 50 immunostained C-cells, per one low-power field [4]. It is crucial therefore to delineate between what we must contemplate normal or C-cells hyperplasia. So, this study was taken to establish the count in normal thyroids to rule out hyperplasia. This study also accounts for an accurate use of calcitonin as a diagnostic marker in C-Cell hyperplasia and medullary carcinoma diagnosis in our setup at A.I.I.M.S, Jodhpur. Another matter of interest to take the study was the criteria for neoplastic CCH is when intrafollicular or nodular C cells with nuclear pleomorphism are seen on H&E and are morphologically distinct from follicular cells [5]. So careful examination in H&E stain was done to confirm this fact. Therefore, the aim of the study was

i. To study the morphology, localization, and distribution of C-cells in thyroid in a small group of Indian origin.

ii. To study growth pattern of C-cells for delineation between normal and C-cell hyperplasia.

iii. Role of calcitonin in C-cell analysis by immunohistochemistry and its use as diagnostic marker in C-cell hyperplasia.

Materials and Methods

1. Collection of tissue specimen: The tissue samples were collected from 30 cadavers voluntarily donated to be used for gross anatomy dissection in the Department of Anatomy, AIIMS Jodhpur. Permission for the research work was obtained from the institutional ethics committee. Out of thirty cadavers, 19 were males and 11 were females. The age of cadavers ranged between 28- 52 years with 21 cadavers in the age group 35-45 years.

Inclusion criteria:

a. Fresh cadavers of age 18 years and older with intact thyroid gland without any skin incision.

Exclusion criteria:

a. Any clinical condition or previous surgery of neck region

b. Thyroid enlargement and/or cadavers with a history of thyroidectomy

The thyroid carefully dissected from the neck of each cadaver and fixed in 10% formaldehyde solution.

2. Whole thyroid were then bisected for two lateral lobes and an isthmus.

3. Sampling method: Four sections were taken:

a. Three horizontal sections in each lateral lobe were done and three regions were considered (upper one third: upper region, middle one third: middle region and lower one third: lower region)

b. Vertical sections in the complete isthmus, at 3 mm intervals.

Thus, for each thyroid gland obtained from a single cadaver, six blocks (from above and below each plane) were made for each lateral lobe and one complete vertical section from the isthmus.

4. The collected tissue were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax (melting point 60 degrees). Serial transverse sections, 5 μ thick were cut.

5. Staining: Out of two sections from each region (one from each lateral lobe), one section of each region was stained in conventional H&E staining and the other one for immunohistochemistry staining. Subsequently

the H&E stained slides were examined under a light microscope to confirm tissue architecture.

For immunohistochemistry, the sections were first deparaffinised in xylene and dehydrated through graded alcohols. Deparaffinised sections were incubated in citrate buffer and the endogenous peroxidase activity was blocked with 0.03% hydrogen peroxide (H_2O_2) in 50 mL methanol for 45 mints. After washing with working solution of phosphate buffer with 0.1% Triton X, slides were treated with normal horse serum for 2 h for blocking the non-specific antigen. The sections were then incubated with calcitonin antibody at a dilution 1:200, at 4ºC overnight. Slides were then treated with biotinylated secondary antibody. The chromogenic visualization reaction was done by using 3,3-diaminobenzidine hydrochloride (DAB).

6. All H&E stained sections of each region were assessed qualitatively under the light microscope, available in department of anatomy at All India Institute of Medical Sciences.

7. For the analysis of immunohistochemistry stained slides a close visualization and observation of C-cells were done for its morphology. Quantification of C-cells and their localization and distribution were done. For visual inspection, Photographic image analysis Technique was utilized by using Nikon research motorized microscope model no. Cx-43 which was available in department of pathology, AIIMS (Jodhpur). By this, number of cells were counted (follicular and parafollicular). Population variation of cells was also determined.

8. Criteria for CCH (true or neoplastic) and its classification were marked during observation depending upon maximum number of cells in a region of lobe.

Statistical analysis

The collected data were screened and entered excel spreadsheet and then analysed using Statistical Package for the Social Sciences (SPSS) for Windows Version 21.0 (SPSS Inc; Chicago, IL, USA). Paired sample 2 tailed t-test was used to observe the difference between different variables; an alpha error of <0.05 was considered as statistically significant.

Results

Gross anatomy results: On gross examination of 30 thyroid glands cadaveric specimen, 27 specimens were characterized by presence of right and left lobes joined by isthmus. In one specimen, the isthmus was found to be absent. Also, two specimens were observed with presence of additional pyramidal lobe attached to the isthmus**.** The thyroid gland was measured in-situ for its dimensions (lobes and isthmus) prior to fixation. In 19 cadaveric specimens, right lobe was found to be longer than left one. While in 10 specimen left lobe was found to be longer than the right lobe. In one specimen it showed an equal length of both right and left lobes. The arterial blood is supplied by a pair of superior and inferior thyroid arteries. One cadaver was also found to have thyroid ima artery**.**

The total weights of thyroid gland from different cadavers were observed. Also, each lobe and isthmus were weighed separately for each of the gland**.** The peak weight of thyroid gland was 65.52 gm, which exceeded much more when compared to mean weight i.e. 30.85 gm (Table 1).

TABLE 1
Mean ± standard deviation of total weight and of various parts of the thyroid gland.

Weight (in gm)	Minimum	Maximum	Mean	Standard error	Standard deviation
Total	8.26	65.52	30.85	4.57	16.4828
Right lobe	3.61	37.06	13.82	2.38	8.58
Left lobe	3.54	28.46	13.33	2.01	7.26
Isthmus	0.74	14.22	4.31	4.32	4.05

Also, each lobe of the thyroid gland was measured for its dimensions and Maximum length i.e. 7.9 cm was found for right lobes (Table 2).

Microscopic study results: The variability in total C-cell numbers was observed in the thyroid tissues from randomly selected thirty cadavers of the varied age and of both the sexes. The number of C-cells in

TABLE 2
Mean ± standard deviation of morphometric parameters/dimensions of various parts of the thyroid gland.

	Parameters (in cm)	Minimum	Maximum	Mean	Standard error	Standard deviation
Right lobe	Length	1.9	7.9	4.86	0.522	1.8843
	Breadth	2.4	5.2	3.93	0.306	1.1034
	Thickness	1.2	4.1	2.29	0.277	0.9995
Left lobe	Length	1.5	7	4.8	0.551	1.9887
	Breadth	1.3	6.8	3.62	0.422	1.5221
	Thickness	0.6	3.7	2.08	0.312	1.1283
Isthmus	Length	1.4	2.4	1.83	0.342	0.3585
	Breadth	1.1	4.6	2.17	0.411	1.0247
	Thickness	0.2	0.9	0.5	0.214	0.253

n= (P>0.05), *= (P<0.05)

the different sections of each collected specimen was also examined. In routine Haematoxylin and Eosin staining, the gland showed presence of spherical follicles. Each follicle was seen lined with a single layer of follicular cells, which was all cuboidal due to inactive state of gland in cadavers. These follicles were seen to be surrounded by reticular fibres. The lumen of principal follicle had colloid filled in it. In addition, we looked for another type of cells called parafollicular cells or C-cells which are also present in thyroid gland. Either they should be looked peripherally in between the follicles as interfollicular C-cell or within the follicles as intrafollicular or para-follicular C-cells (Figure 1). In the later, they are generally separated from the lumen by the follicular cells in vicinity.

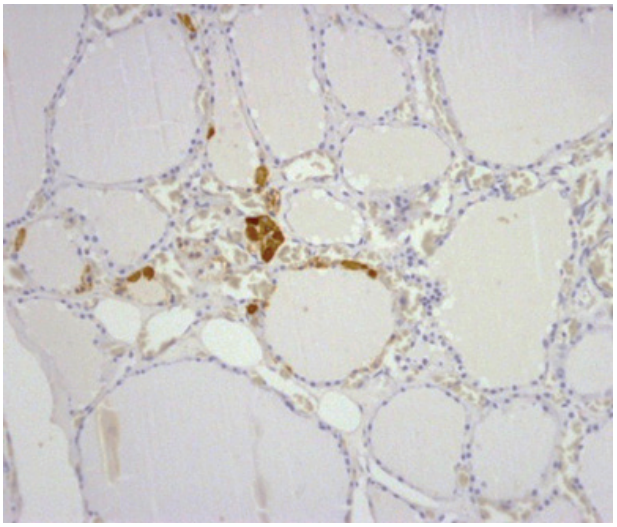

Figure 1) *Normal number of C-cells in at follicular (blue) and interfollicular (red) positions (Calcitonin immunostaining x100).*

It was observed that it was not possible to discriminate with full conviction the thyroid C-cells from follicle cells in routine H&E staining. Only a few places larger cells different from follicle cells were seen. But still affirmation was yet not there. Localization, exact identification, and quantification were also not possible in H&E staining. Thus, for the accurate C-cell identification which was a prerequisite for the quantification we relied on immunohistochemistry (IHC).

In IHC, C-cells were observed positive for the most specific marker i.e. calcitonin which is the main hormone product with its characteristic brown color. The C-cells with their nuclei were conspicuously visualized at all stages of the sections. They were found to be present singly, in groups, or in linear display in relation to follicles. The C-cells were unevenly distributed throughout the glands, being more numerous in the central than in the peripheral regions in most of the sections. Out of 30 thyroid glands, all showed presence of C-cells except one lobe on the right side. C-cells were found to be absent in all the isthmus (Figure 2a and Figure 2b) (Table 3). In the middle part of both lateral lobes it had diffuse pattern of the C-cells (Figure 3).

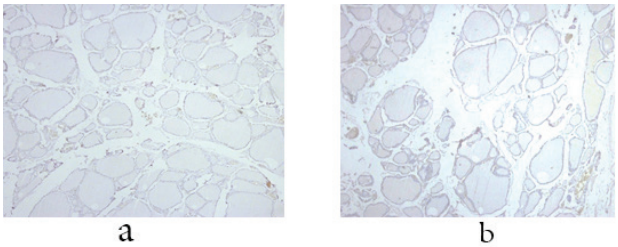

Figure 2) *a) Showing upper part of a lateral lobe with sparsely present C-cells; b) Showing thyroid gland with colloid filled follicles and total absence of C-cell in the region of isthmus.*

TABLE 3
Number of lobes showing presence of C-cells in various thyroid glands.

Parts of thyroid gland	Total no. of lobes observed	No. of lobes in which C-cells found
Right lobe	30	14
Left lobe	30	15
Isthmus	29	nil

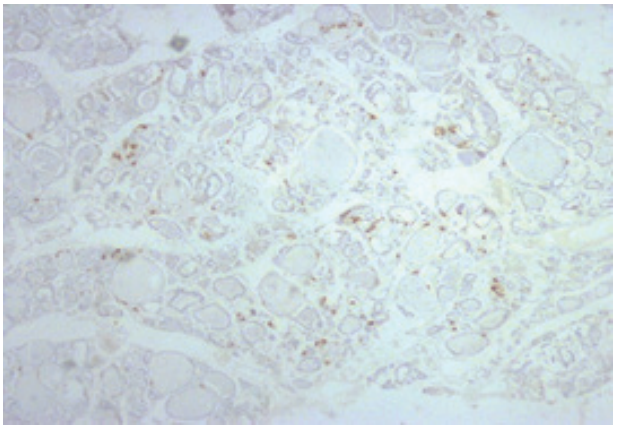

Figure 3) *Showing low magnification to demonstrate the diffuse pattern of C-cells throughout thyroid gland as found in middle part of lateral lobe. (calcitonin immunostaining x40).*

Localization of the C-cells: To observe the localization, the low power fields were selected which showed the presence of C-cells in it. In the stepwise displacement throughout the section the fields were counted. Within the positive field for C-cell, it was easy to recognize the individual cells by moving the plane of focus perpendicularly. The thyroid lobules were easily identifiable as they were surrounded by partial fibrovascular septa. The C-cell localization was observed inside these lobules as well as in neighboring ones. In both the right and the left lobes maximum fields with positive C-cells were found in the middle region. Right lobe showed comparatively more i.e. 41 fields positive whereas on the left little less around 33 fields was observed positive. Besides it, upper region of both the lobes also showed presence of positive fields for C-cells. It is important to note that the isthmus was found completely devoid of any field with C-cell. In the lower region, though fields with C-cells were observed in the left lobe, we could not find any positive field for C-cells in the lower region of right lobe (Table 4).

TABLE 4
Distribution of C-cells in the thyroid gland in low power fields (X 100 magnification).

Parts of thyroid gland	Region	Number of lobes in which C-cells found	Number of low power (X 100 magnification) fields showing C-cells
Right lobe	Upper (R_a)	4	8
	Middle (R_b)	10	41
	Lower (R_c)	Nil	nil
Isthmus	complete	nil	nil
Left Lobe	Upper (L_a)	4	7
	Middle (L_b)	9	33
	Lower (L_c)	2	5

Quantification of the C-cells: After the identification of positive fields, quantification was done. The number of C-cells was predicted by a systematic precise observation. Again, a stepwise displacement in the x and y axis, was done. The first field of vision in each thyroid section was selected marked from a fixed corner. Thereafter, the fields were sampled systematically and independently of their content stepwise. Predetermined movements of the stage in the x and y directions were done. To avoid any bias, the quantification was done by two observers. In total, 1726 C-cells were observed in 30 thyroid tissue samples. Isthmus and lower region of right lobe showed complete absence. Maximum of 1405 were observed in middle region of left lobe. Invariably in both the lobes maximum c-cells were found in middle region only but statistically significant difference was there between right and left lobes. When observed for the localization, more of the C-cells were in the interfollicular position. A maximum of 1231 interfollicular position was in the middle region of left lobes of various samples. Interesting, least number of interfollicular C-cells were also found in the lower region of same lobe. In follicular position, 261 C-cells were observed. It was also observed that when C-cells are in confines of a follicle, they are always separated

from the follicular lumen by neighboring follicular cells (they may also be termed as parafollicular). The minimum number of follicular positions were found in upper region of left while middle region showed maximum of i.e. 174 C-cells (Table 5).

Out of the total observed fields region wise, C-cells were found most of the time in a diffuse pattern. When seen for number of C-cells per low power field, they ranged from 04-43 C-cells per field. So approximately on an average 19 C-cells per field were observed in the present study (Table 6).

TABLE 5
Localization of C-cells in various regions of different lobes according to their position.

	Region	Total number of C-cells	Follicular	Interfollicular
Right lobe	Upper (R_a)	40	10	30
	Middle (R_b)	219	69	150
	Lower (R_c)	nil	nil	Nil
Isthmus	complete	nil	Nil	Nil
Left Lobe	Upper (L_a)	41	4	37
	Middle (L_b)	1405	174	1231
	Lower (L_c)	21	4	17
	Total no. of C-cells	**1726**	**261**	**1465**

TABLE 6
Showing average number of C-cell found per field.

	Region	Fields positive for C-cells	Total number of C-cells	Average of C-cell in each field
Right lobe	Upper (R_a)	8	40	5
	Middle (R_b)	41	219	6
	Lower (R_c)	nil	nil	nil
Isthmus	complete	nil	nil	nil
Left Lobe	Upper (L_a)	7	41	6
	Middle (L_b)	33	1405	43
	Lower (L_c)	5	21	4
	Total	**94**	**1726**	**19**

Discussion

Thyroid gland showed a number of morphological and functional alterations with advancing age. The extent to which such alterations are dependent on the aging or age coupled thyroidal diseases is still uncertain [6].

The most established early account of C-cells can be credited to E. Cresswell Baber for his work on a canine's thyroid gland in 1876. He assumed that stratum of cells flanked by the vesicles which is commonly now called follicles, symbolize the renaissance collection of the vesicles [7]. Later on another investigating group termed it as 'interfollicular epithelium', and confused it with oncocytic cells which are follicular derived [8]. Nonidez et al., in nineteenth century are credited for coining the term parafollicular cells, which were identified as interfollicular cells present in thyroid of dogs as a binary module of epithelium only [9]. On the other hand, in humans it was reported as typical C-cells which primarily situated inside the thyroid follicles i.e. interfollicular cells with meagre in parafollicular or intrafollicular site. The present study findings are much in agreement with this study of DeLellis et al., revealing that human C-cells are intrafollicular and basement membrane shields it from interstitium of thyroid [10].

Bilobed structure of thyroid gland is derived from the caudal end of thyroglossal duct embryologically.

The major constituent here is follicular cells derived from median anlagen. Simultaneously the paired lateral anlagen crop up as ultimo-branchial bodies from the IVth - Vth branchial pouch complex. At the time of the descent, these masses get separated from the parathyroid IV and fuses to form 'solid nest cells' in postnatal life, at the junction of the upper third with the medium third of its lateral lobes. These solid nest cells exhibit secluded or grouped tangentially situated calcitonin-immunoreactive specialised C-cells in abundant number [11,12]. Ultimobranchial bodies here served as vectors for the neural crest cells to be there and get incorporated to form an integrated composite together [13]. Specific quantification is lacking for precisely marking C-cell hyperplasia (CCH). Still the most widely accepted delineation in literature is presence in at least one area of bare minimum 50 C-cells per one low power field in both thyroid lobe [1,3].

C-cells in the present study was found in various regions (upper, middle and lower) in both the lobes except lower region in right lobe. Maximum number of C-cells were in the middle region and that too in the medial subareas, in concord to the preferential location of C-cells referred by workers [12-16]. The embryological origin clearly states that the C-cells are derived from neural crest cells which only reach lateral lobe and not the isthmus. In present study also it is found that there were no C-cells present in the isthmus. The proximity of the C-cells to the remnants of the ultimobranchial body and the solid nest cells were reported by many workers [14,15]. This was an invariable observation in our study that in spite of localised variation, C-cells were intimately present in proximity of thyroid follicles. Literature also reported the lack of C-cells in the lingual thyroid and in the thyroglossal duct remnants [14,15]. In the present study no such observation was done, as analysing presence of C-cells in various accessory thyroid tissue was beyond the scope of study. Also, in our limited sample size we did not find the remnants of thyroglossal duct [14-16].

In our study the numbers of C-cells were found to be of significant difference between the two lobes which was in concurrence with the findings of several authors [17-25]. It was also said that the incidence of more number in the left lobe is might be due to the smaller sample size [18].

The precise quantification of C-cells is unknown and based on this rationale it is easier said than done to define a true hyperplasia [13]. This is accredited to the substantial variation according to age, sex, sampling method and the allied thyroid pathology. Various explanations are given by several researchers concerning it. Most accepted is that morphologically, three types of C-cell hyperplasia are illustrious based on their pattern of growth. That includes focal, diffuse, and nodular C-cell hyperplasia [19,20]. In the present study the distribution of C-cells was diffused throughout but no hyperplasia was observed.

In C-cell hyperplasia, morphologically distinct C-cells were observed which could be recognised in routine stains. Guyetant et al., in 2003 stated that the morphology of the exceeding number of the C-cells also defines a form of C-cell hyperplasia, the neoplastic C-cell hyperplasia [2]. They are morphologically distinct from the follicular cells and also recognizable in routine stains (H&E). On microscopic examination, neoplastic C-cells are outsized than normal and mildly to moderately atypical, similar to those of invasive medullary thyroid carcinoma [2,3]. In the present study each section was also observed for routine H&E staining where absolute lack of decipherable C-cells was there. It was also observed that the cuboidal cell lined colloid filled follicles were seen with the interstitial area with no atypical large sized C-cells. Therefore, the present study ruled out presence of any neoplastic C-cell.

The key product of C-cells i.e. calcitonin has its role in lowering the calcium concentration in the blood by limiting the bone resorption done by osteoclasts. C-cells are the chief source of calcitonin during routine physiological conditions. For this reason, they can be considered a valuable tumour marker for medullary thyroid carcinoma. Medullary Thyroid Carcinoma (MTC) can be microscopically diverse ranging from papillary, follicular, tubular, and varied histological prototype has been depicted. Even the reliability of distinctive feature of MTC, as existence of amyloid is not always certain. It may be devoid of it or also found supplemented with thyroid tumors. Therefore, for the diagnosis of MTC, the calcitonin immunohistochemistry can be considered as a reliable mean [21-24].

Pertaining to the weight of thyroid gland, findings of various authors implied to a considerable negative correlation amid age and the thyroid weight. It was generally found that in elderly individuals i.e. in 65-70 years' age group, the weight of thyroid gland was about 20-30% less than the individuals in their second decade of life. The major cause of it was atrophy of follicles and the loss of major portion out of total number of follicles [25]. The maximum weight of thyroid gland was at the early age around twenties and thirties. Subsequently it declines at the age of 40-50 years. Afterward a considerable turn down was evident

in elderly faction above 60 years [26,27]. A negative correlation was also seen between age and length of right and left lobes of thyroid gland. It was documented that the utmost length of thyroid gland lobes is found in the third decade and then it illustrated decline slowly with each successive decade [28]. In present study the average weight of thyroid gland was found to be 30.85 gm whereas the maximum weight found for a thyroid was 65.52 gm. The average length of two lobes was 4.83 cm whereas breadth was approximately 3.77 cm. The average weight and other morphometric dimensions were found to be slightly on lower side, can be accredited to the cadavers from which tissue were obtained as most of them fall in advancing age group.

Clinical implication: Neuroendocrine tumours are indifferent malignancies erupting from cells generating hormones, dispersed all over the body. Medullary thyroid carcinoma commences from the C cells of thyroid gland. It in particular has an elevated mortality ratio with escalating prevalence. Noteworthy is these tumors are responsible for mere 3-5% of entire thyroid carcinoma, but it exemplifies even fourteen and greater percentage of thyroid-cancer associated deceases and incidence is gender unbiased.

Conclusion

The present study findings corroborate the use of IHC for C-cell analysis in thyroid gland in a small group of Indian origin. In analyzing the usefulness of methods used for C-cell identification, use of calcitonin proved to be an important diagnostic marker. Quantification was done reliably, proving calcitonin used in the procedure justified due to its high specificity and affinity. The conspicuously visualized C-cells in IHC being more numerous in the central than in the peripheral regions in most of the sections. The position in abundance was interfollicular and more on left lobe. Invariably middle region of both lobes showed maximum C-cells with significant difference between two lobes. The isthmus was found to be completely devoid of any C-cells.

Limitations of the study: In this pilot study a small sample size was selected. All the samples selected were from normal cadaveric tissue without any thyroid pathology.

Conflict of interest: The authors have NO conflicts of interest to declare.

Financial Assistance: This work was carried out with the financial support from AIIMS Jodhpur under the cluster of Intramural funding.

References

1. Albores Saavedra J, Monforte H, Nadji M, et al. C-cell hyperplasia in thyroid tissue adjacent to follicular cell tumors. Hum Pathol.1988;19:795-9.
2. Guyetant S, Josselin N, Savagner F, et al. C-cell hyperplasia, and medullary thyroid carcinoma: Clinicopathological and genetic correlations in 66 consecutive patients. Mod Pathol. 2003;16:756-63.
3. Yadav M, Agrawal V, Pani KC, et al. C-cell hyperplasia in sporadic and familial medullary thyroid carcinoma. Indian J Pathol Microbiol. 2018;61:485-8.
4. Machens A, Hoffmann F, Sekulla C, Dralle H. Importance of gender-specific calcitonin thresholds in screening for occult sporadic medullary thyroid cancer. Endocr Relat Cancer. 2009;16:1291-8.
5. Sakorafas GH, Nasikas D, Thanos D, et al. Incidental thyroid C cell hyperplasia: clinical significance and implications in practice. Oncol Res Treat. 2015;38:249-52.
6. van de Ven AC, Netea Maier RT, de Vegt F, et al. Associations between thyroid function and mortality: the influence of age. Eur J Endocrinol. 2014;171:183-91.
7. Baber EC. Contributions to the minute anatomy of the thyroid gland of the dog. Phil Trans R Soc. 1876;166:557-68.
8. Hurthle K. Beitrage zur Kenntniss des Secretionsvorgangs in der Schilddruse. Pflugers Arch. 1894; 56:1-44.
9. Nonidez JF. The origin of the 'parafollicular' cells: a second epithelial component of the thyroid gland of the dog. Am J Anat. 1932;49:479-505.
10. DeLellis RA, May L, Tashjian AH Jr, et al. C-cell granule heterogeneity in man. An ultrastructural immunocytochemical study. Lab Invest. 1978;38:263-9.
11. Rios Moreno MJ, Galera Ruiz H, De Miguel M, et al. Inmunohistochemical profile of solid cell nest of thyroid gland. Endocr Pathol. 2011;22:35-9.

12. Martin V, Martin L, Viennet G, et al. "Solid cell nests" et pathologies thyroidiennes. Etude retrospective de 1390 thyroïdes. Ann Pathol. 2000;20:196-201.

13. Tiedje V, Schmid KW, Weber F, et al. Differentiated thyroid cancer. Internist. 2015;56:153-66.

14. LiVolsi VA. Surgical pathology of the thyroid. WB Saunders Company, Philadelphia. 1990.

15. Katoh H, Yamashita K, Enomoto T, et al. Classification and general considerations of thyroid cancer. Ann Clin Pathol. 2015;3:1045.

16. Vandernoot I, Sartelet H, Abu Khudir R, et al. Evidence for calcitonin-producing cells in human lingual thyroids. J Clin Endocrinol Metab. 2012;97:951-6.

17. Sherman SI. Targeted therapies for thyroid tumors. Modern Pathology. 2011;24:S44-S52.

18. Hadoux J, Pacini F, Tuttle RM, et al. Management of advanced medullary thyroid cancer. Lancet Diabetes Endocrinol. 2016;4:64-71.

19. Baloch Z, LiVolsi VA, Tondon R. Aggressive variants of follicular cell derived thyroid carcinoma; the so called 'real thyroid carcinomas' J Clin Pathol. 2013;66:733-43.

20. Perry A, Moldberg K, Albores Saavedra JA. Physiologic versus neoplastic C-cell hyperplasia of the thyroid: Separation of distinct histologic and biologic entities. Cancer.1966;77:750-6.

21. Kushchayev SV, Kushchayeva YS, Tella SH, et al. Medullary Thyroid Carcinoma: An Update on Imaging. J Thyroid Res. 2019; 2019:1-17.

22. Hu MI, Cote GJ. Medullary thyroid carcinoma: who's on first? Thyroid. 2012;22:451-3.

23. Nilsson M, Williams D. On the origin of cells and derivation of thyroid cancer: C cell story revisited. Eur Thyroid J. 2016;5:79-93.

24. Roy M, Chen H, Sippel RS. Current understanding and management of medullary thyroid cancer. Oncologist. 2013;18:1093-100.

25. Suzuki S, Nishio S, Takeda T, et al. Gender specific regulation of response to thyroid hormone in aging. Thyroid Res. 2012;5:1.

26. Trimboli P, Guidobaldi L, Bongiovanni M, et al. Use of fine-needle aspirate calcitonin to detect medullary thyroid carcinoma: a systematic review. Diagn Cytopathol. 2016; 44:45-51.

27. Avdeenko L, Khmel O. Morphological characteristics of the thyroid of adults from St-Petersburg. J Arkh Pathol. 2001;63:22-6.

28. Keith LM, Arthur FD, Anne MR. Clinically Oriented Anatomy. (5thedn), Lippincott Williams and Wilkins, Philadelphia. 2006.