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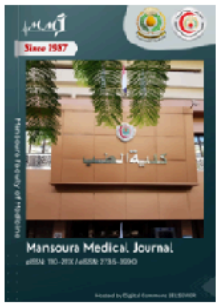

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MEDULLARY THYROID CARCINOMA: IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

MEDULLARY THYROID CARCINOMA : IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY

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ABSTRACT

Eleven cases of medullarly thyroid carcinomas (MTC) were evaluated in relation to the pattern, immunohistochemistry and electron microscopy. Six cases were classified as classical MTC with histochemical evidence of amyloid and 5 cases were atypical without histochemical evidence of amyloid . Immunostaining for thyrocalcitonin was positive in 4 out of 6 classical and 2 out of 5 atypical MTC cases. The neurosecretory granules were found in both the typical and the atypical MTC cases. The ultrastructural examination is essential in the diagnosis of MTC particularly where the histological and immunohistochemical evaluation is inconlusive. ABSTRACT

Eleven cases of medullarly thyroid carcinomas (MTC) were evaluated in relation to the pattern, immunohistochemistry and electron microscopy. Six cases were classified as classical MTC with histochemical evidence of amyloid and 5 cases were atypical without histochemical evidence of amyloid . Immunostaining for thyrocalcitonin was positive in 4 out of 6 classical and 2 out of 5 atypical MTC cases. The neurosecretory granules were found in both the typical and the atypical MTC cases. The ultrastructural examination is essential in the diagnosis of MTC particularly where the histological and immunohistochemical evaluation is inconlusive.

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a histogen tically distinct

type of thyroid neoplasms since they are thought to be derived from calcitonin-producing C (clear) cells. These C cells are currently felt to be derivation and a part of the complex APUD-cell system. These tumors can occur either sporadically or as a part of an autosomal dominant inherited syndrome 1,2 Though very uncommon, their possible imitation of papillary or follicular thyroid carcinoma specially on lacking amyloid stimulates the investigators on a trial to solve the diagnostic problem of amyloid negative medullary thyroid carcinoma [3,4].

The aim of the present work is to study immunohistochemically and ultrastrucurally the atypical (amyloid negative) MTC in comparison to the classical (amyloid positive) cases in a trial to find diagnostic criteria in atypical cases (amyloid negative) specially when thyrocalcitionin immunostaining is inconclusive.

MATERIAL AND METHODS

The present study included 11 cases of medullary thyroid carcinoma. Out of these, five cases were collected retrospectively from the files of department of Pathology, King Abdulaziz University Hospital in the period between 1983 to 1990. The other 6 cases were collected prospectively as fresh specimens from KAUH during the period of study from 1990 to 1993.

For the retrospective cases, slides and paraffin blocks were retrieved. More sections were cut for immunohistiochemistry on formalin fixed paraffin embedded sections. For routine work, the fresh specimens were fixed in 10% buffered formalin, dehydrated in ascending grades of alcohol and cleared in xylol and embedded in paraffin wax. Sections were cut from the prospective and retrospective cases and stained with hematoxylin and eosin stain and congo red stain for amyloid.

For the E/M study, the paraffin blocks were deparaffinized in xylol, rehydrated in descending grades of ethyl alcohol, washed in water and fixed in 3% gluteraldehyde. Both the fresh specimens already fixed in 3% gluteraldehyde and the rehydrated specimens were post fixed with 2% osmium tetraoxide and embedded in Epon LX-112 resin (Ladd, Burlington, VT). The ultrathin sections were stained with uranyl acetate and lead

citrate and examined with Phillipse elctron microscope.

Immunohistochemical staining was performed in the King Fahd Medical Research Center, King Abdulaziz University for thyrocalcitonin by using the avidin-biotin peroxidase complex (ABC) method with a Vectastain ABC kit . Following the incubation with 3% hydrogen peroxide in methanol to block the endogenous peroxidase activity, the deparaffinized tissue was successively treated and stained [5]. The monoclonal antibody for thyrocalcitonin was purchased from Immunotech Company (Marsellia, France).

RESULTS

Out of the eleven cases included in the present work, eight were females and 3 were males. The mean age was 48±8.5 years.

Histological findings :

Out of the eleven cases 6 cases were of typical histological pattern of MTC. They were characterized by solid proliferation of round (Fig. 1) to spindle cells (Fig. 2) having variable amount of granular amphophilic cytoplasm and medium sized nuclei separated by abundant hyalinized stroma showing typical amyloidosis by congo red stain (Fig. 3). The remaining 5 cases were considered atypical because they were negative for the amyloid stain.

In the five atypical cases, two cases showed adenoma like pattern with encapsulation and entrapped thyroid follicles retaining some colloid (Fig. 4). The third case showed masses of clear cells with relatively small nuclei separated by fibrous stroma (Fig. 5). The fibrous tissue at the border of the tumor was found to trap some clusters of these clear cells. The fourth case showed microfollicular and poorly differentiated follicular with fibrous septa rich in hyalinized collagen. The fifth case showed moderately differentiated papillary pattern (Fig. 6) but the nuclear features of papillary carcinoma were lacking . The cores of the papillae were rich in mature and hyalinized collagen.

Immunohistochemical staining :

Out of the eleven MTC, the six classical cases showed positive staining for thyrocalcitonin of variable degrees in five cases (83.3%) (Fig. 7)

while the atypical MTC showed positive staining in two out of five (Fig. 8). The immunostaining for thyrocalcitonin was in the form of cytoplasmic granular or diffuse staining with focal condensation on the cytoplasmic membrane(Fig. 9).

The E/M findings :

All the classical and the atypical MTC shared the presence of the neurosecretory granules. These granules consist of single delimiting membrane , an intermediate pale halo and a more or less electron dense core (Fig. 10). They were ranged between 200-300 nm in diameter and its size was nearly the same in all tumors. In three atypical MTC two types of neurosecretory granules were distinguished. A larger one was similar to the previously described and the other was small averaging 130 nm with a denser core and the characteristic peripheral electron-lucent halo(Fig.11).

In all the classical (6/6) and three of atypical MTC(3/5) cases showed that the malignant MTC cells have abundant rough endoplasmic reticulum (Fig 12) and conspicuous Golgi complexes with which the granules are often closely associated (Fig. 13). The mitochondria and lysozymes are readily found but in most cases (8/11) they were not prominent. In few cases (3/11) the mitochondria were abundant and swollen (Fig. 14). A well developed microtubular and microfilamentous system was seen in 7/11 of cases (Fig. 13). The nuclei were regular in shape with peripheral chromatin condensation (Fig. 13). In one classic and two atypical MTC cases, indented nuclear contours and cytoplasmic invagination was seen(Fig. 13&14). The stroma showed either coarse fibers of collagen and/or fine microfibrills of amyloid.

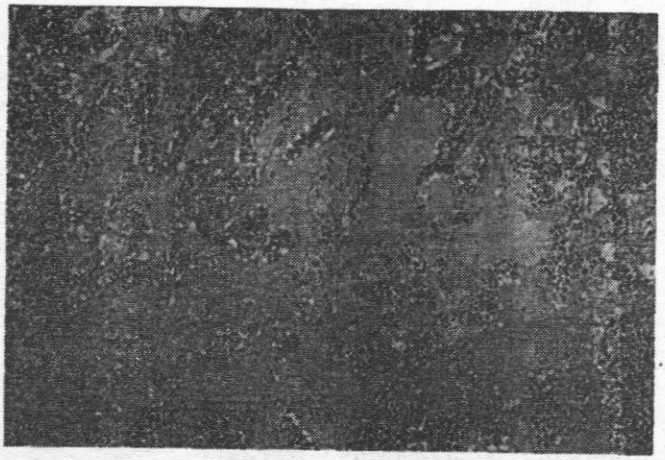

Figure 1. A classical pattern of MTC showing solid masses of small cells having rounded nuclei separated by abundant hyalinized stroma. (H&E, original magnification X 100)

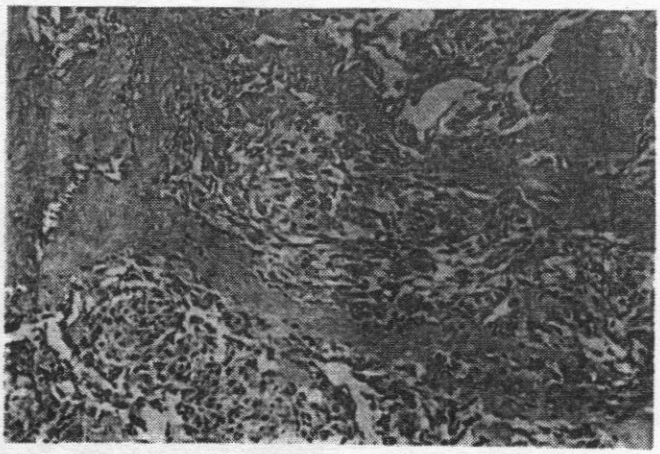

Figure 2. A classical medullary thyroid carcinoma showing abundant amyloid stroma separating masses of spindle cells having very thin rim of esinophilic cytoplasm. (H&E, original magnification X 200)

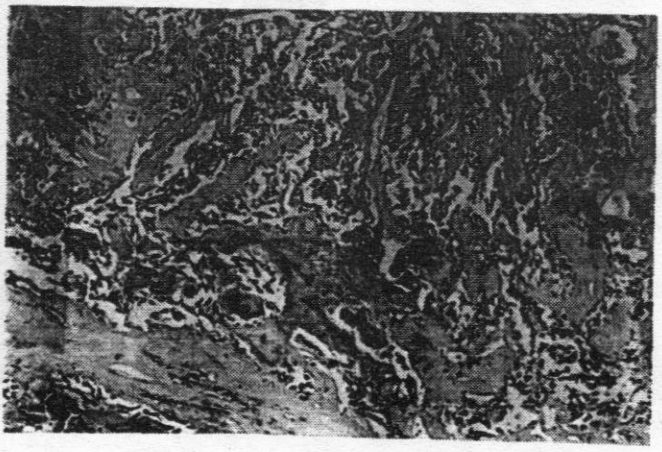

Figure 3. A classical medullary thyroid carcinoma showing congo red positivity of the stromal amyloid. (Congo red stain, original magnification X 100)

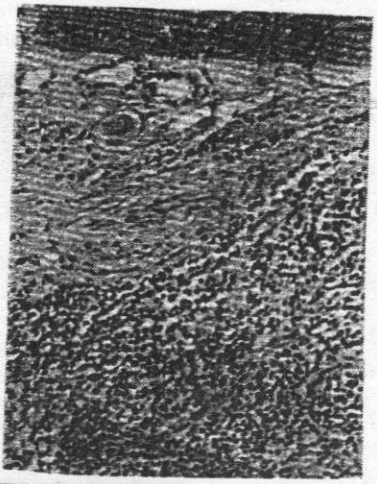

Figure 4. An atypical medullary thyroid carcinoma showing adenoma-like pattern with encapsulation. (H&E, original magnification X 200)

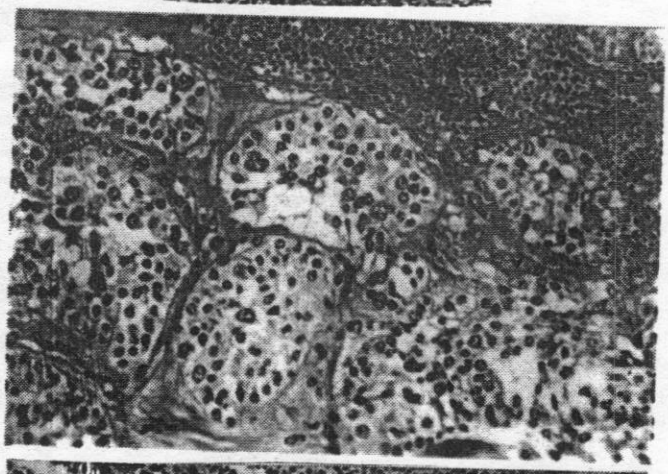

Figure 5. A clear cell variant of atypical medullary thyroid carcinoma showing masses of clear cells separated by fibrous stroma. (H&E, original magnification X 400)

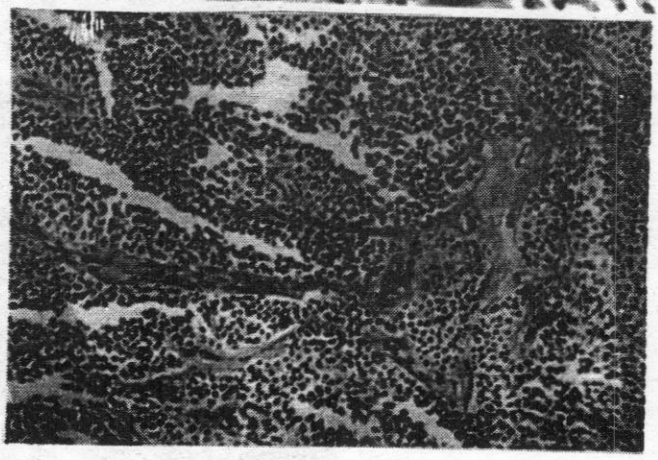

Figure 6. A papillary variant of atypical medullary thyroid carcinoma showing moderately differentiated papillae having fibrous hyalinized cores. (H&E, original magnification X 200)

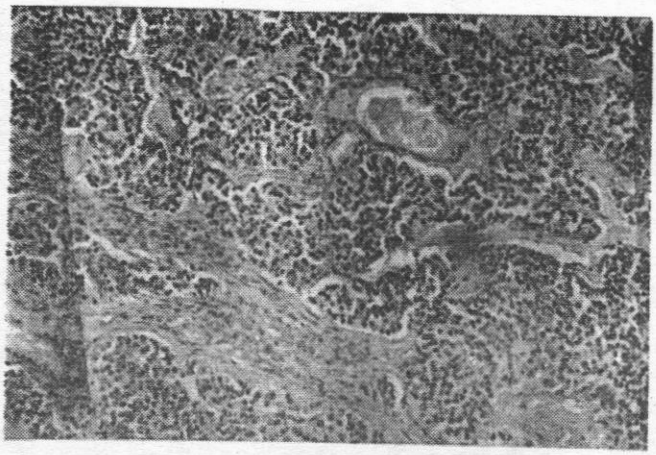

Figure 7. Patchy positivity of classical medullary thyroid carcinoma showing patchy diffuse cytoplasmic positivity. (Thyrocalcitonin immunstaining, original magnification X 200).

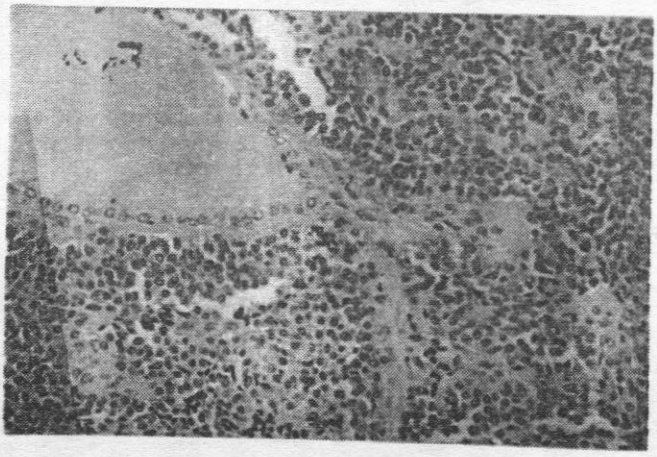

Figure. 8 Atypical medullary thyroid carcinoma of the adenoma-like pattern showing thyrocalcitonin patchy positivity with negative thyroid follicle as internal negative control. (Thyrocalcitonin immunstaining, original magnification X 200)

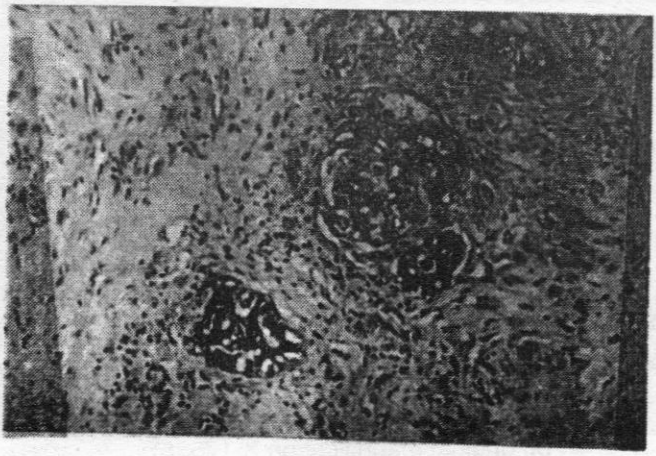

Figure 9. Thyrocalcitonin positivity of clear cell variant of medullary thyroid carcinoma entrapped in the hyalinized collagen. (Thyrocalcitonin immunstaining, original magnification X 200)

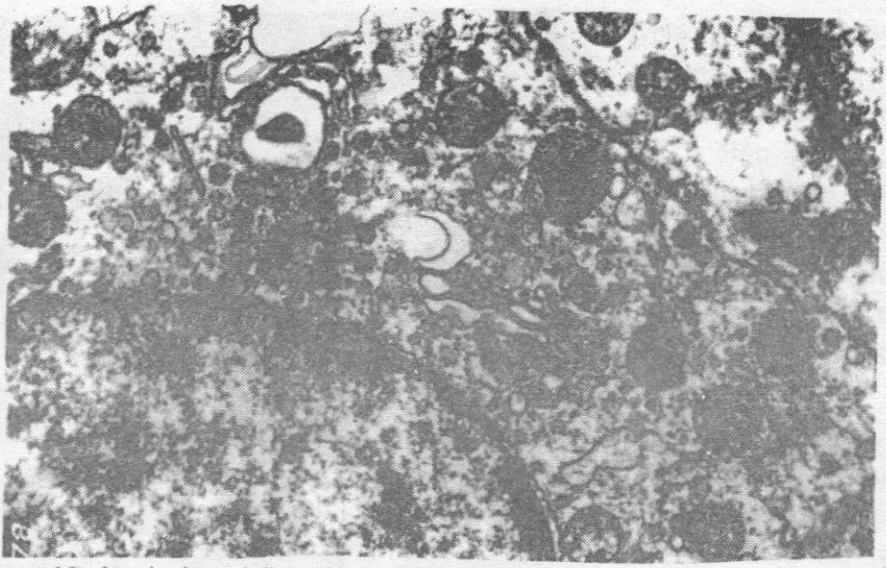

Figure 10. Atypical medullary thyr oid carcinoma cells showing both large(small arrow) and small (big arrow) neurosecretory granules consist of single delimiting membrane , an intermediate characteristic peripheral electron-lucent (pale) halo and an electron dense core . (E/M, original magnification X 17535).

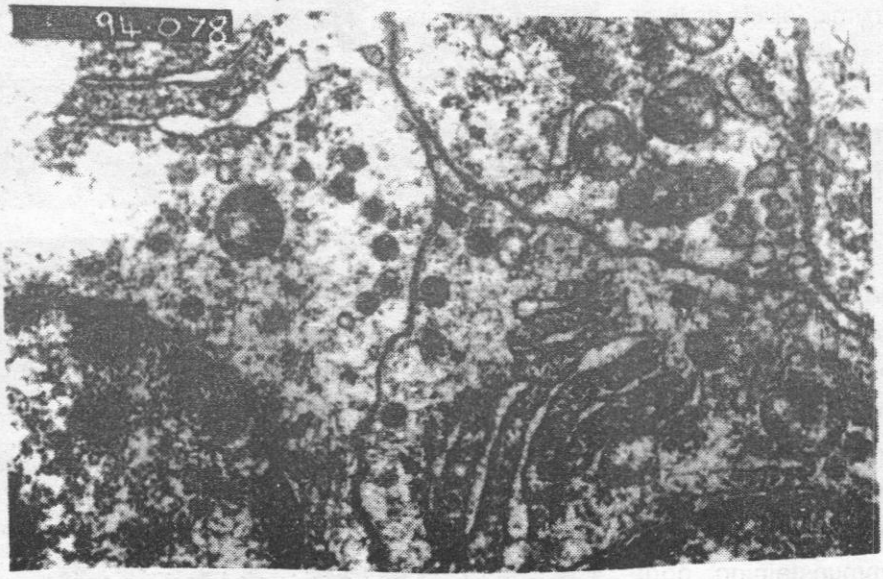

Figure 11. The lower right corner shows developed abundant rough endoplasmic reticulum in medullary carcinoma cell. Also, electron dense(small arrow) and electron less dense(big arrow) neurosecretory granules. (E/M, original magnification X 17535)

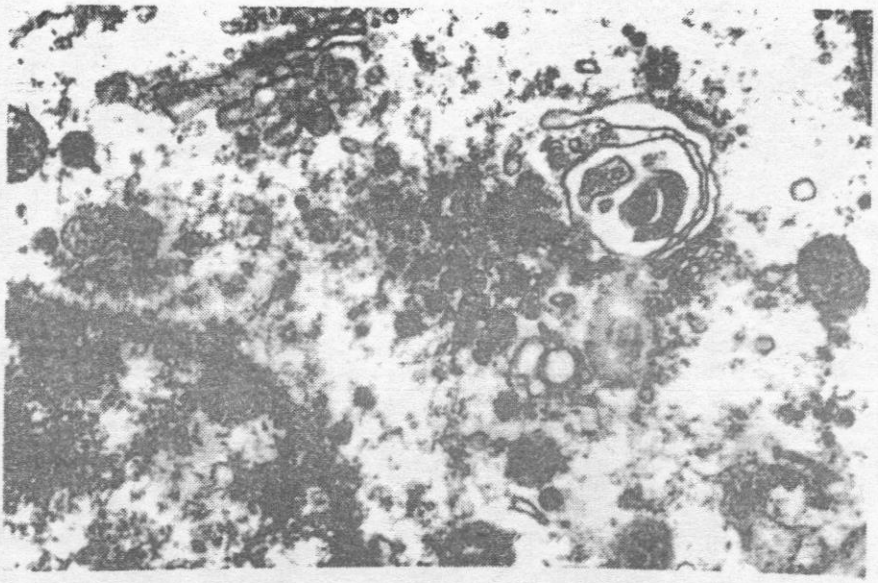

Figure 12. Both small and large neurosecretory granules closely related to a conspicuous Golgi complex . (E/M, original magnification X 17535)

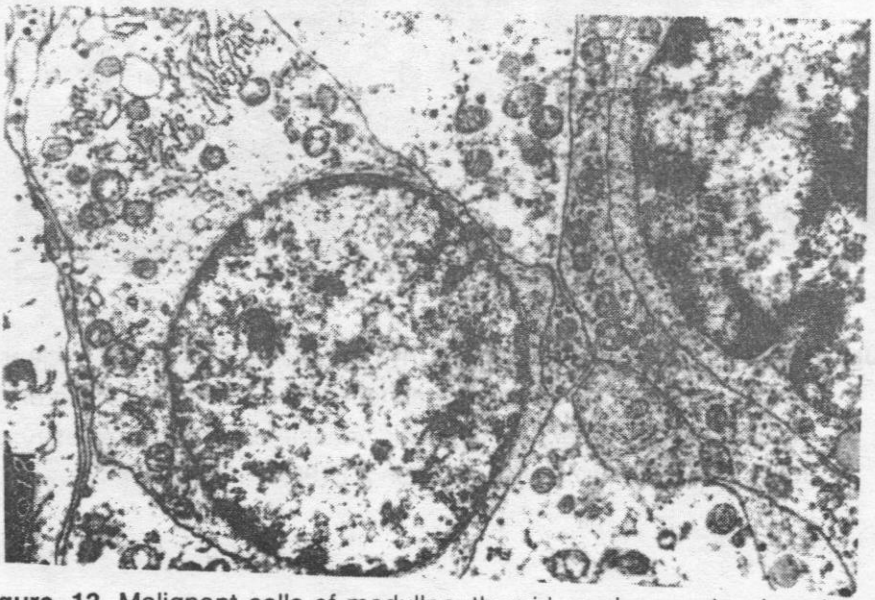

Figure. 13. Malignant cells of medullary thyroid carcinoma showing abundant mitochondria, few of them were swollen(arrow), with peripherally condensed nuclear chromatin. Well developed microtubules are seen in the upper left corner. (E/M, original magnification X 8190)

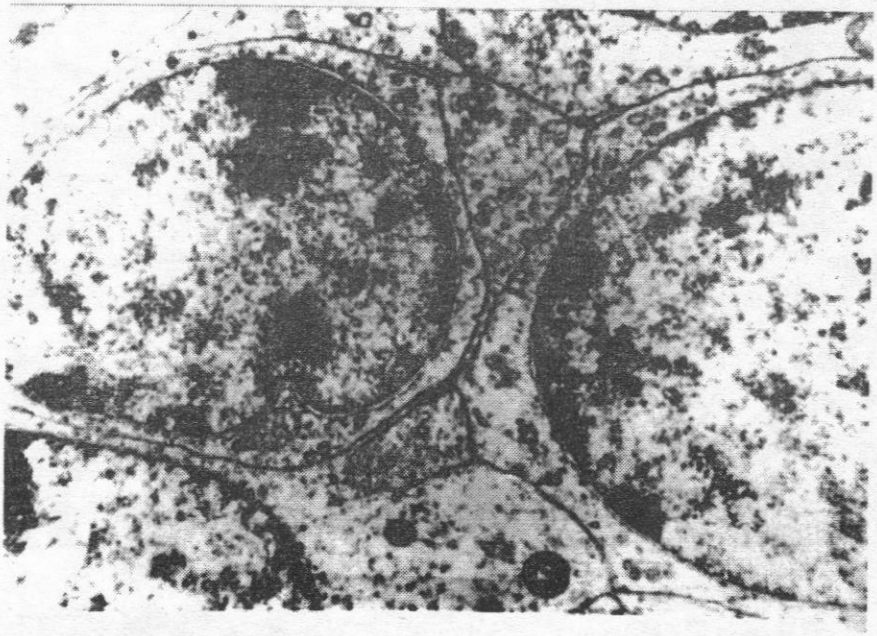

Figure 14. Malignant cells of medullary thyroid carcinoma showing nuclear indentation with focal peripheral condensation of the chromatin on the nuclear membrane and cytoplasmic variable-sized neurosecretory granules. (E/M, original magnification X 8190).

DISCUSSION

In the present work, eleven MTC cases were studied. Based on histological pattern, 6 cases (63.6%) were of classical pattern with amyloid in the stroma as described by others[2] and 5(36.4%) were atypical without amyloid. cases. This results is similar to that of others [5] who reported that 16 cases (71.4%) were classic and 5 cases (28.6%) were atypical in a study of 21 cases.

The histological atypical patterns in the 5 atypical cases were as follows: The atypical MTC could have the following histological variants: papillary variant [6]follicular variant [7,8], solid anaplastic [9]clear cell variant [10]giant cell variant [3]melanin producing pigmented variant [11], mucin producing variant [12,13]oncocytic variant[7] squamous variant[14]), paraganglioma-like variant (hyalinized trabecular adenoma like variant) 15and oat cell-like variant of MTC [16] Furthermore, MTC may occur as a component of other thyroid carcinomas as mixed papillary and medullary carcinoma and mixed follicular and medullary carcinoma [5,17,18]. The stroma may be scanty, ossified, contain floride foreign body-type giant cell reactions, psammomatoid concretions or typical psammoma bodies [6]. Out of these patterns in the present work, two cases were of adenoma like pattern with encapsulation, one case of clear cells with scanty stroma, one case with microfollicular and poorly differentiated follicular carcinoma like pattern and one case with the papillary pattern but lacking the nuclear features of papillary carcinoma. So based on the light microscopy, the atypical pattern as well as the absence of amyloid is not exclusive for MTC . It was the same conclusions of others [3,5,11,19]

Regarding the immunostaining for thyrocalcitonin, the reported figures vary from 50% to 61% [1,2,5] Some of the mixed MTC cases were thyrocalcitonin positive and some are negative [4,20,21] The overall percentage of

positive cases in the present work was 54.5%(6/11) which is in agreement with that of others [2] Out of the positive cases, 4 were of classic pattern and two of atypical MTC pattern. From the present work and the previous literatures, it is concluded that the presence of immunoreactivity for thyrocalcitonin is conclusive for the diagnosis of MTC while its absence is not exclusive for the possibility of MTC 8,16, 22,23,24,25.

Regarding the ultrastructural findings, both the fresh and the deparaffinized rehydrated tissue were suitable for the E/M study. Formalin fixed or even paraffin extraction did not prevent identification of the characteristic cytoplasmic granules as shown from the work of others [26].

The ultrastructural findings of both typical and atypical MTC were nearly similar. All the cases showed the characteristic neurosecretory granules. The type of neurosecretory granules were slightly different in the classical from that of the atypical MTC. In the classical MTC cases, they were mainly of the large type with a small percentage of the small types as described by others [27,28,29,30]. They added that the large neurosecretory granules predominate in quiescent-appearing cytoplasmic cells and the small neurosecretory granules are predominant in cell with rough endoplasmic reticulum. The presence of small and large neurosecretory granules in both the classical and the atypical MTC was the clue of others [31,32,33,34] for diagnosis of MTC secreting multiple polypeptide hormones (as ACTH, somatostatin, insulin, HCG and others) secreted by MTC cells due to the presence of different types of secretory granules.

It is concluded that the ultrastructural examination is essential for the diagnosis of MTC particularly when the histological, histochemical and immunohistochemical evaluation are inconclusive.

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