Results

RESULTS

In this work 65 cases representing various thyroid neoplastic lesions were examined. The benign thyroid lesions were observed to occur in younger people, in contrary to malignant lesions which commonly occur in the fifth decade. However, the malignant lesions can occur in any age. Anaplastic thyroid tumors and lymphomas were found only in people over the age of 40 years. The preponderance of female incidence was obvious in all groups of thyroid neoplastic lesions (Table 1).

Table (1): **Age and sex incidence in various thyroid tumors.**

Tumor type	No of cases	Age		Sex	
		Average	*Mean*	*Female*	*Male*
Benign:					
Follicular adenoma	9	15-38	25	7	2
Malignant:					
Follicular carcinoma	10	12-62	42	8	2
Papillary carcinoma	18	18-65	41.9	14	4
Medullary carcinoma	12	22-62	45.5	7	5
Anaplastic carcinoma	14	42-70	65	10	4
Lymphoma	2	60, 65	62.5	1	1

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF VARIOUS GROUPS OF THYROID NEOPLASIA

1. ***Follicular adenomas:*** H & E stained sections of 9 cases were reviewed. All examined cases covered most of the histological patterns of follicular adenoma as following:
   a) Microfollicular adenoma (2 cases).
   b) Macrofollicular adenoma (2 cases).
   c) Trabecular adenoma (2 cases), one of them showed Hurthle cell differentiation.
   d)Mixed pattern, showed variable proportions of microfollicular, colloid, normofollicular and trabecular adenoma (3 cases).

Immunohistochemistry: 7 cases (77.8%) of follicular adenoma were positively stained for Tg, however, all cases were absolutely negative for calcitonin antibody (Table 2).

Table (2): **Immunohistochemical staining results of follicular thyroid adenoma for various antibodies used.**

Antibody	No of cases	+ve	%	-ve	%
TG	9	7	77.8%	2	22.2%
CK	9	8	88.9%	1	11.1%
Calcitonin	9	-	-	9	100%

The staining pattern for Tg antibody was usually diffuse and strong over the majority of follicles in most cases, in contrary, cytokeratin immunoreactivity showed patchy distribution and moderate degree of intensity (Table 3).

Table (3): Immunohistochemical staining pattern of epithelial follicular adenoma for various antibodies used.

Antibody	No of cases	Distribution				Intensity		
		-	+	++	+++	+	++	+++
TG	9	2	1	2	4	1	2	4
CK	9	1	1	6	1	2	5	1

There was an obvious discrepancy in Tg immunoreactivity between different patterns of follicular adenoma. The tumors or areas of tumors formed of small follicles showed strongest stainability in the form of cytoplasmic staining which was more obvious over the luminal surface of the cells (Fig. 1). In contrast the stainability became weaker and patchy in tumors or areas formed of large follicles distended with abundantly formed colloid. Single case of pure trabecular adenoma showed lesser degree of the stainability that was usually focal in distribution, however in mixed adenoma, the trabecular area showed more strong staining over that of pure type. The remaining cases that failed to be stained; one was macrofollicular and other was trabecular adenoma with Hurthle cell differentiation (Table 4).

Immunohistochemical staining for low molecular weight CK was positive in 8 out of 9 cases (88.9%), most of them showed patchy and moderate degree of reactivity regardless of cellular or morphologic pattern of the tumor (Table 5, Fig. 2).

Table (4): **Immunohistochemical stainign pattern of follicular thyroid adenoma for Tg with reference to their morphologic subtypes**

Histologic type	No. of cases	Distribution								Intensity					
		Epithelial				*Colloidal*				*Epithelial*			*Colloidal*		
		-	+	++	+++	-	+	++	++++	+	++	+++	+	++	+++
Microfollicular Adenoma	2	-	-	-	2	-	-	-	-	-	-	2	-	-	-
Macrofollicular adenoma	2	1	-	1	-	1	-	1	-	-	1	-	-	1	-
Mixed adenoma	3	-	-	1	2	-	-	1	2	-	1	2	-	2	1
Trabecular adenoma															
1. Normal cell	1	-	1	-	-	-	-	-	-	1	-	-	-	-	-
2. Hurthle cell	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-

Table (5): **Immunohistochemical staining pattern of follicular adenoma for CK with reference to their morphologic subtypes.**

Histologic type	No of cases	Distribution				Intensity		
		-	+	++	+++	+	++	+++
Microfollicular adenoma	2	-	-	1	1	1	-	1
Macrofollicular adenoma	2	-	-	2	-	1	1	-
Mixed follicular adenoma	3	1	1	1	-	-	2	-
Trabecular adenoma	2	-	-	2	-	-	2	-

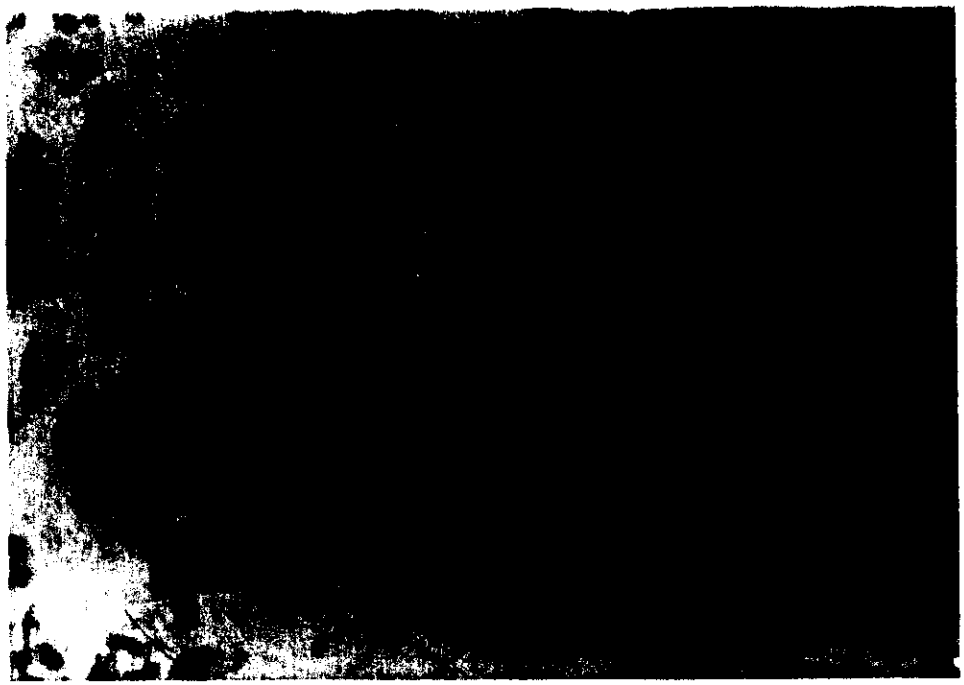


Figure (1): Follicular adenoma showing diffuse immunostaining of both follicular cells and colloid. (Tg immunohisto. x 10).

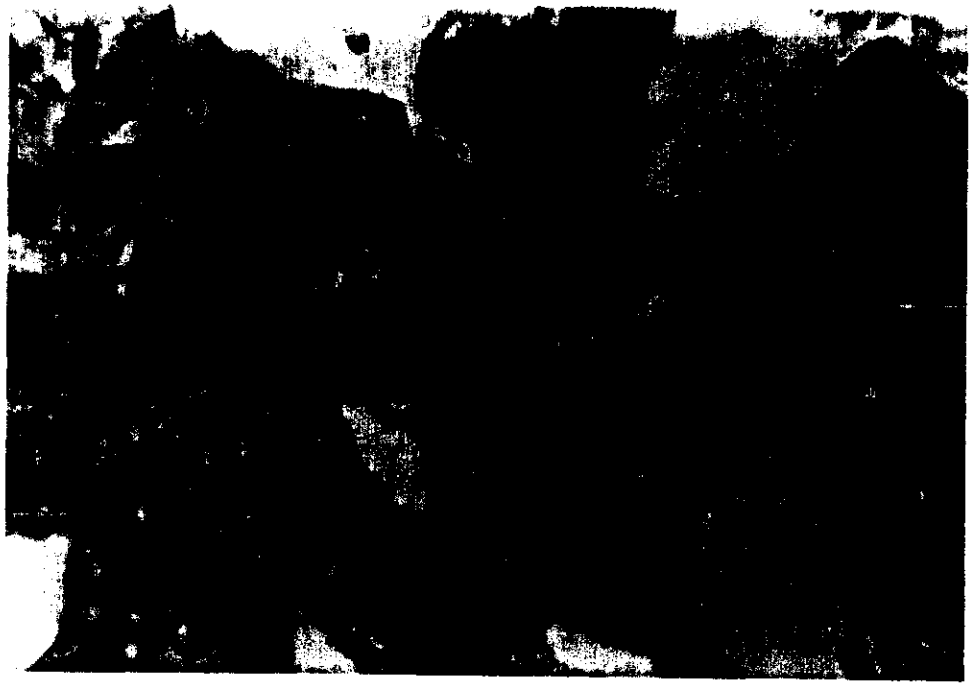


Figure (2): Follicular adenoma showing positive cytoplasmic staining of follicular cells. (CK. immunohisto. x 20).

PAPILLARY CARCINOMA

Histopathology

Eighteen cases of papillary carcinomas stained with conventional stain were re-examined.

The cases were subcategorized into 4 groups according to the proportional amounts of both follicular and papillary structures observed in each case.

1. ***Predominantly papillary:*** 11 cases were observed to be formed of predominantly or exclusively papillary structures. Regarding the cell type, 10 tumors were formed of ordinary cells of papillary carcinoma and one case formed of Hurthle cell type. There was one case composed of a small papillary area in addition to a solid area. The solid area was formed of whorled spindle-shaped cells and groups of cells with squamous differentiation abutting about and in between the papillary fronds. This case was diagnosed as moderately differentiated carcinoma.

2. ***Mixed papillary and follicular:*** 4 cases were observed to be formed of relatively equivocal amount of both follicular and papillary structures. There was one case showed Hurthle-cell differentiation. There was a case showed differentiated papillary and follicular structure; sharply demarcated from solid (insular) portion forming the predominant component of the tumor. The insular part was composed of varying sized well-defined nests of neoplastic cells, often surrounded by hyalanized collagen. Small rounded follicles were haphazardly distributed within the solid nests. The neoplastic cells were relatively smaller and monomorphous with focal pleomorphism than that of the associated differentiated area. Many of the nuclei were

vesicular and some of them were optically clear. Although these nuclei were closer to each other due to scanties of the cytoplasm, nuclear overlapping was not a prominent feature. This case was diagnosed as poorly differentiated carcinoma.

3. ***Predominantly follicular:*** 2 cases were found to be formed of more than 50% of examined section of follicular structures. There was one case showed small area of insulae admixed within the differentiated part of tumor diagnosed as poorly differentiated carcinoma.

4. ***Follicular variant:*** one case was formed of exclusively well formed follicular structures.

All cases (100%) showed the characteristic ground glass and overlapping nuclei of papillary thyroid carcinoma in both follicular and papillary structures.

Immunohistochemistry

Fourteen cases (77.7%) of papillary carcinoma were positively stained for Tg antibody, 16 cases (88.9%) were positively stained for LMW cytokeratin. There was absolute negativity for calcitonin (Table 6).

Table (6): Immunohistochemical staining results of papillary carcinoma for various antibodies used.

Antibody	No of cases	+ve	%	-ve	%
TG	18	14	77.8%	4	22.2%
CK	18	16	88.9%	2	11.1%
Calcitonin	18	-	-	18	100%

The immunostaining pattern for Tg was considerably varied between different morphological pattern of papillary carcinoma. The stainability of tumor got more diffuse and stronger of both epithelial and colloidal material with shift of tumor pattern from predominantly papillary to follicular pattern. In the follicular variant the staining pattern showed the strongest intensity while the predominantly papillary pattern showed the lowerest degree of reactivity and only 7 out of 11 cases of predominantly papillary pattern showed positive immunostaining (Table 7).

Table (7): Immunohistochemical staining pattern of various groups of papillary carcinomas forTg.

Histologic pattern	No. of cases	Distribution								Intensity					
		Epithelial				*Colloidal*				*Epithelial*			*Colloidal*		
		-	+	++	+++	-	+	++	++++	+	++	+++	+	++	+++
Predominantly papillary	11	4	2	4	1	-	3	-	-	4	2	1	-	3	-
Mixed papillary and follicular	4	-	2	1	1	1	-	2	1	1	2	1	1	2	-
Predominantly follicular	2	-	-	1	1	2	-	-	-	1	-	1	-	-	-
Follicular variant	1	-	-	-	1	-	-	1	-	-	-	1	-	-	1

The staining pattern of predominantly papillary type showed weak to moderate focal cytoplasmic staining which became more intense in the apical part of the cells lining the papillae (Fig. 3). The positivity was usually patch in distribution except one case showed negative staining of all cells lining the papillae where the follicles embedded in core of papillary stalk showed obvious positive staining (Fig. 4).

The 4 negatively stained cases, two were morphologically well differentiated, one was moderately differentiated; where both solid and papillary areas were negatively stained while the fourth case showed Hurthle cell differentiation.

All cases of mixed papillary and follicular pattern (100%) were positively stained for Tg. The positivity in the papillary areas showed moderate diffuse cytoplasmic staining which become more stronger in the apical part of cells and over the luminal surface. The positivity still patchy in distribution except one case showed diffuse stainability (Fig. 5). In contrast, in the follicular areas, staining pattern become more diffuse involving the majority of the follicles and the intensity of the staining was strong and diffusely cytoplasmic involving most of the cells lining the follicles (Fig. 6).

One case with Hurthle cell differentiation showed focal staining of follicular area while the papillary portion was absolutely negative. There was moderate diffuse cytoplasmic staining which became more intense over the luminal border of cell lining the follicles. The immunostaining of poorly differentiated tumor showed moderate cytoplasmic immunoreactivity in few clusters of cells haphazardly distributed in the solid (insular) portion of tumor, however, the staining was particularly strong in cells forming microfollicles embedded within the insular part (Fig. 7). The associated differentiated papillary or follicular structures showed staining pattern similar to that described as above (Fig. 8). There was one case showing small area with tendency to squamoid differentiation was positively stained of Tg (Fig. 9). The two cases of predominantly follicular pattern were positively stained for Tg. The follicles showed strong cytoplasmic staining of cells lining the follicles while colloidal material was obviously negatively stained. However, the positivity still

patchy in distribution in one case, while involving the great majority of follicles in the second one (Fig. 10). The small associated papillary area showed focal, weak staining only over the luminal surface. There was poorly differentiated area in one case showed clear negativity.

The follicular variant case was stained positively for Tg. There was strong diffuse cytoplasmic staining of all cells lining the follicles. The colloid was always positively stained showing the same degree of intensity as follicular cells (Fig. 11).

From the above results there was no absolute correlation between the degree of differentiation and immunostaining pattern however strong intensity was exclusively observed in well differentiated tumors (Table 8).

Table (8): Immunohistochemical staining pattern of epithelial Tg in various groups of papillary carcinomas regarding the degree of differentiation.

Histologic pattern	No. of cases	Distribution				Intensity		
		-	+	++	+++	+	++	+++
Predominant papillary								
• Well differentiated	10	3	2	4	1	4	2	1
• Moderately differentiated	1	1	-	-	-			
• Poorly differentiated								
Mixed papillary and follicular								
• Well differentiated	3	-	1	1	1	-	2	1
• Moderately differentiated								
• Poorly differentiated	1	-	1	-	-	1	-	-
Predominant follicular								
• Well differentiated	1	-	-	-	1	-	-	1
• Moderately differentiated								
• Poorly differentiated	1	-	-	1	-	1	-	-
Follicular variant								
• Well differentiated	1	-	-	-	1	-	-	1
• Moderately differentiated								
• Poorly differentiated								

Immunostaining pattern for low molecular weight CK; showed a mild variation to some extent in intensity and distribution of the staining between the papillary, follicular structures, where the papillary areas showed stronger and diffuse stainability rather than follicular structures, however, no systemic difference could be observed except between predominantly papillary and follicular variant (Table 9). Immunostaining for CK was positive in 10 out of 11 cases showed predominantly papillary pattern. The positively stained cases showed homogeneous, strong, diffuse cytoplasmic staining of almost all cell lining the papillae (Fig. 12, 13). The follicular structure showed somewhat lesser degree of stainability. Three out of 4 cases of mixed papillary and follicular pattern were positively stained. In general the staining pattern still diffuse showing moderate to strong staining intensity (Fig. 14). The cases of predominantly follicular pattern were positively stained involving most cells of follicles, but the degree of reactivity was still less pronounced than that of the associated papillary area. The case of follicular variant was positively stained but with weaker intensity (Fig. 15).

Regarding the degree of differentiation, tumors contained the solid or insular regions showed positive staining did, not differ considerably from that of papillary or follicular areas, however areas of the squamous differentiation got stronger intensity (Fig. 16).

Table (9): Immunohistochemical staining pattern of various groups of papillary carcinoma for ck antibody.

Histologic pattern	No of cases	Distribution -	+	++	+++	Intensity +	++	+++
Predominantly papillary	11	1	-	3	7	-	2	8
Mixed papillary & follicular	4	1	-	1	2	-	2	1
Predominantly follicular	2	-	-	1	1	-	2	-
Follicular variant	1	-	-	1	-	1	-	-

Figure (3): Papillary carcinoma with predominantly papillary pattern showing cytoplasmic staining of scattered group of cells lining the papillae, which become more intense in the apical part and over the luminal surface of the papillae (Tg immunohisto. x 40).

Figure (4): Papillary carcinoma with predominantly papillary pattern showing obvious negativity of all cells lining papillae with positive staining of follicular structure embedded with papillary stalk. (Tg immunohisto. x 20).

Figure (5): Papillary carcinoma of mixed pattern showing diffuse positive staining involving most of the papillae (Tg immunohisto x 10).

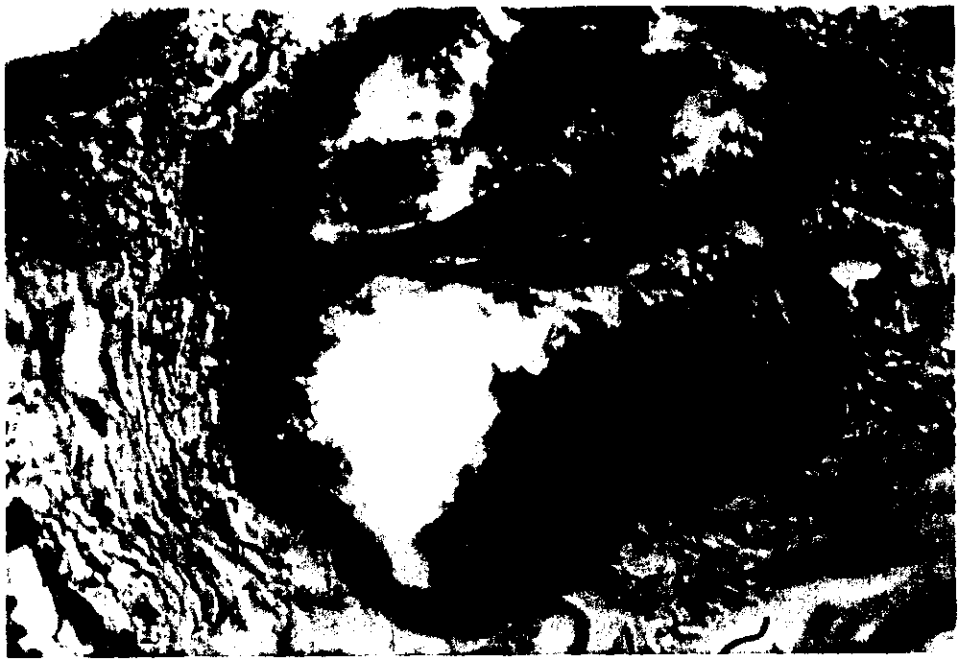


Figure (6): Papillary carcinoma of mixed pattern showing strong staining involving almost all cells lining the follicle (Tg immunohisto x 40)

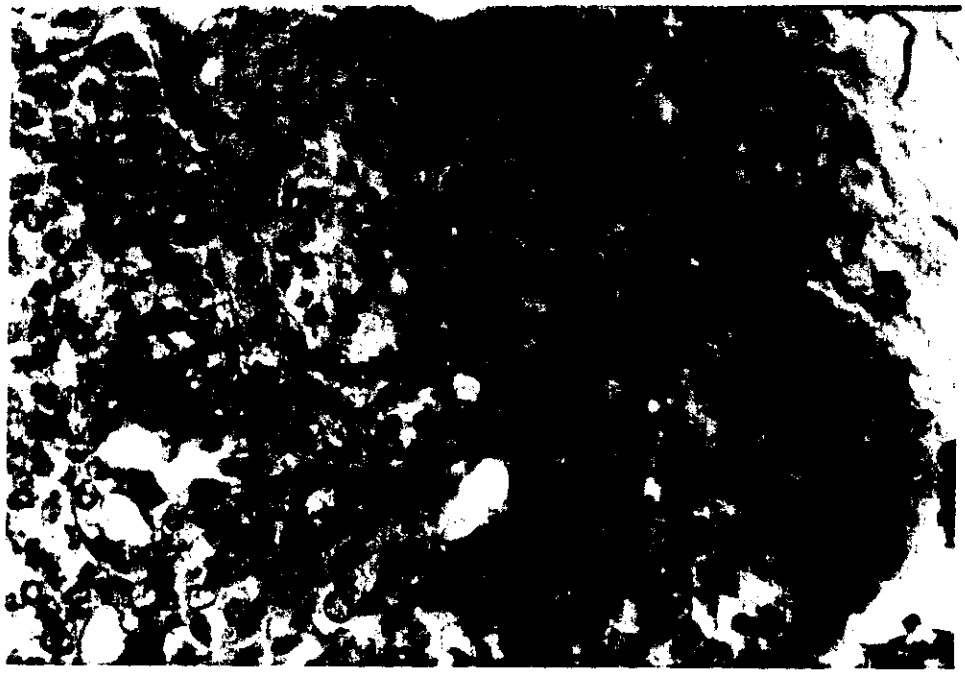


Figure (7): Poorly differentiated area of papillary carcinoma (mixed pattern), showing strong staining in isolated group of cells and cells lining microfollicles (Tg immunohisto x 20).

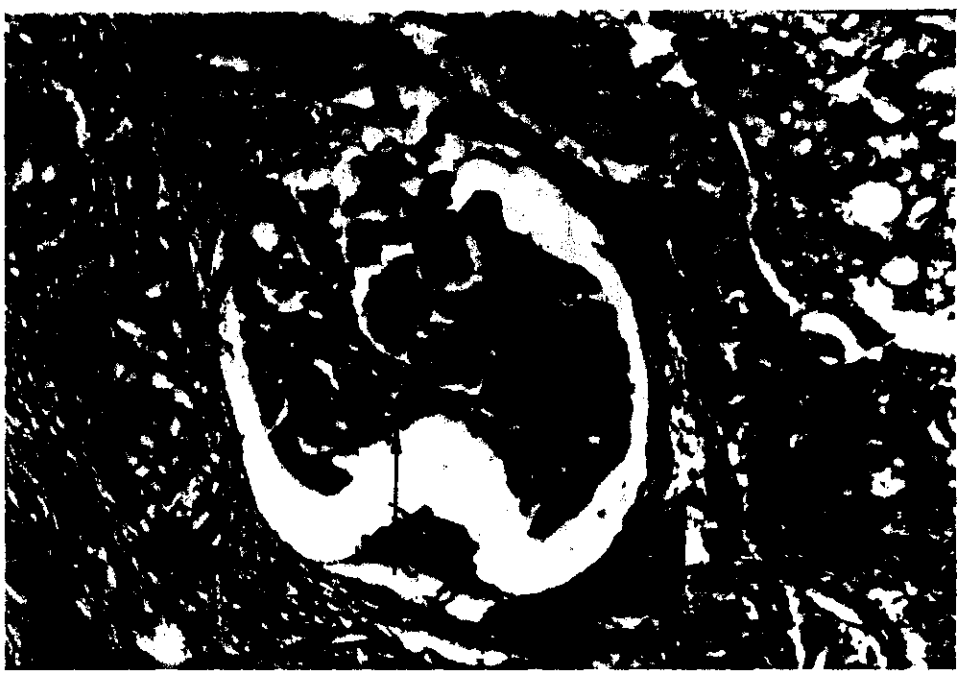


Figure (8): The same case above showing papillary area with focal staining over luminal surface of cells lining papillae *(arrow)*, while there was a diffuse staining of follicular structure beside it *(arrow head)*. (Tg immunohisto x 10).

Figure (9): Papillary carcinoma (mixed pattern), showing moderate cytoplasmic staining of small group of cells *(arrow head)* intervening between group of follicles some of them showed cytoplasmic staining of lining cells (Tg immunohisto x 40).

Figure (10): Papillary carcinoma (predominantly follicular pattern showing strong positive staining of cells lining most follicles while the colloid was negatively stained (Tg immunohisto x 40).

Figure (11): Papillary carcinoma (Follicular variant) showing strong staining of both follicular cells and colloid. (Tg immunohisto x 20).

Figure (12): Papillary carcinoma (predominantly papillary pattern) showing diffuse strong cytoplasmic staining in almost all cells lining papillae. (ck immunohisto x 10 "A" x 20 "B")

Figure (13): Another case of papillary carcinoma (predominantly papillary pattern) showing strong diffuse cytoplasmic staining, in virtually all cells lining the papillae (CK immunohisto x 20).

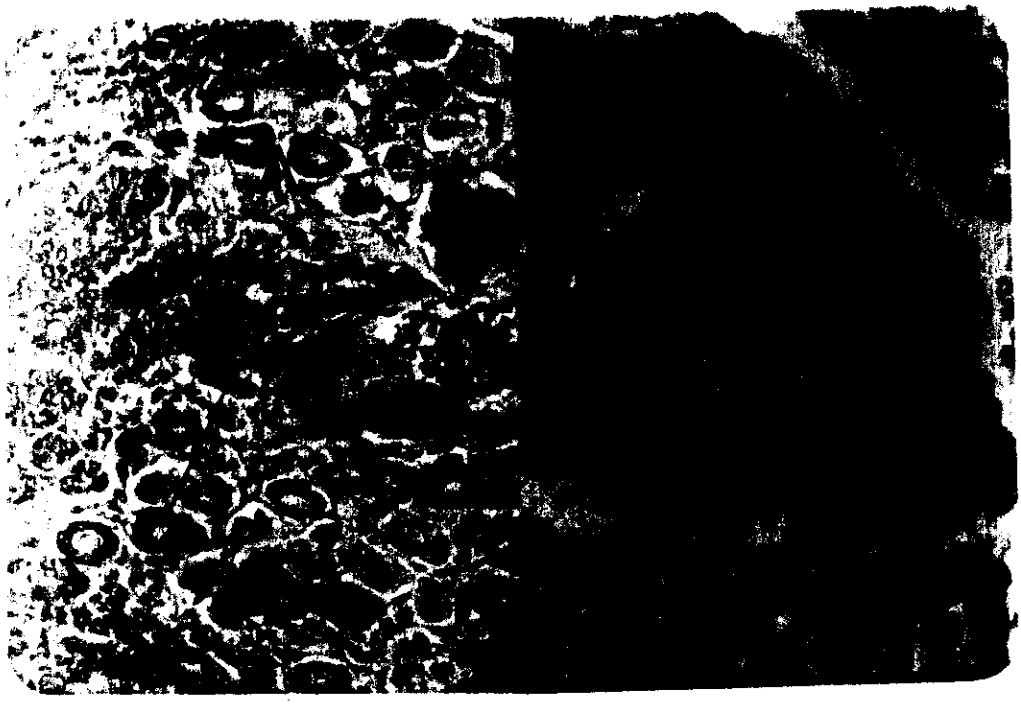


Figure (14): Papillary carcinoma (mixed pattern) showing diffuse staining of follicular structures (arrow) but with moderate intensity. (CK immunohisto x 10 "CA" x 20 "B").

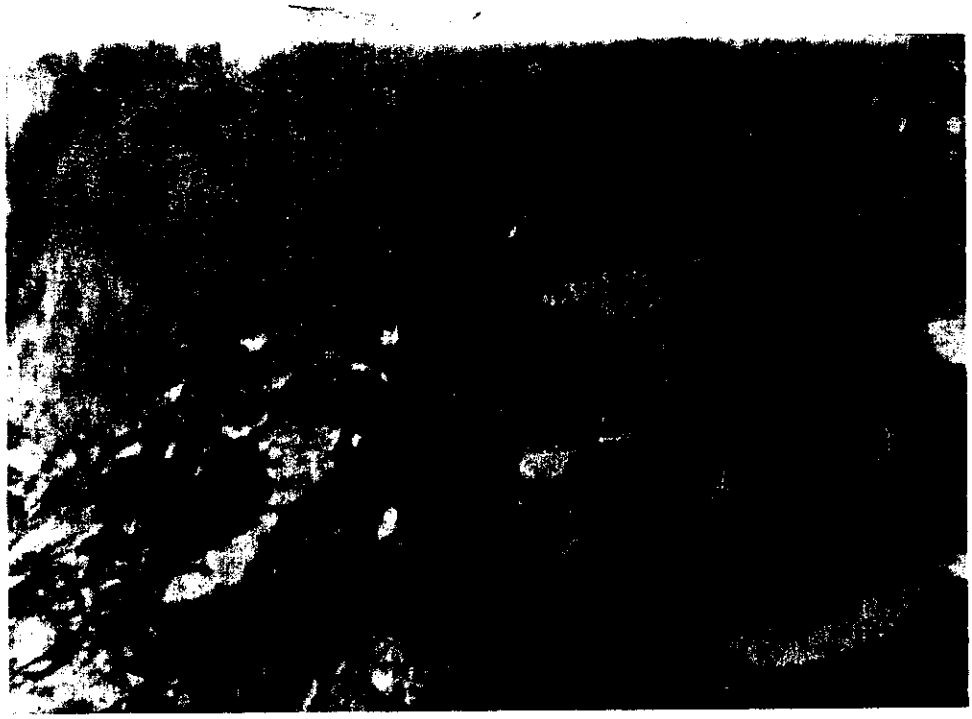


Figure (15): Papillary carcinoma (follicular variant) showing diffuse positive staining of all follicles but with a weak degree of intensity. (CK immunohisto. x 20).

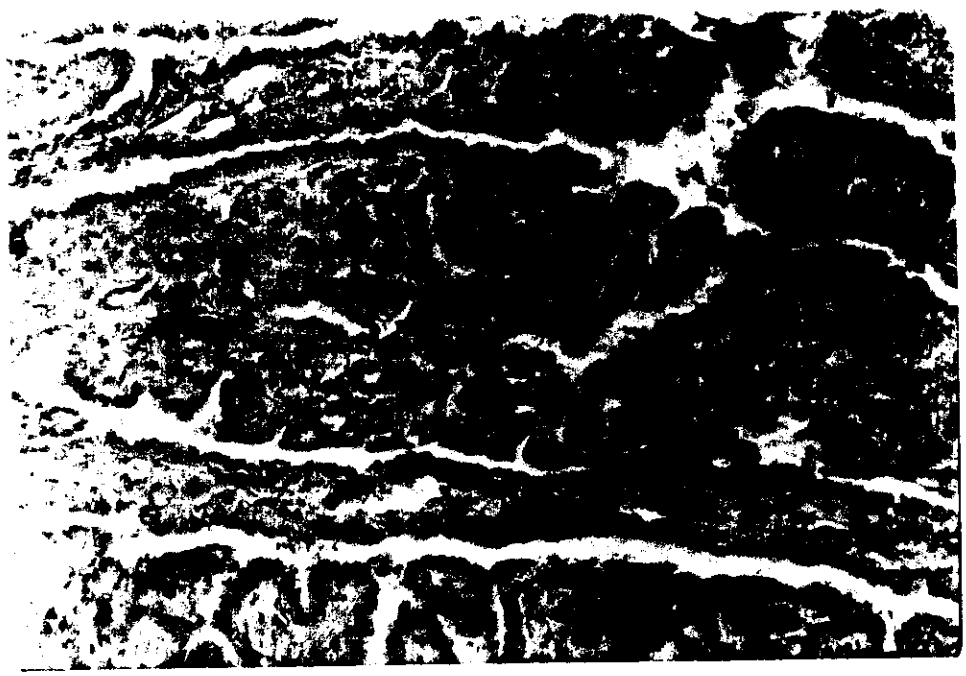


Figure (16): Solid area with squamous differentiation in a papillary tumor showing diffuse and moderate degree of reactivity (CK immunohisto. x 10).

FOLLICULAR CARCINOMA (FC)

Histopathology

H & E stained sections of follicular carcinomas were reviewed an reclassified according to the degree of histopathologic differentiation of the tumors into the following groups:

1. ***Well differentiation F.C. (5 cases):*** the tumors were composed solely or almost solely of well formed follicular structures.

2. ***Moderately differentiated F.C. (2 cases):*** the tumors were composed of trabecular or solid structures combined with follicles as well. The trabeculae referred to the cords two to several cell thick and separated from each other by thin fibrous connective tissue septa. A solid arrangements referred to large solid cell area usually containing microfollicles to normal size one. According to the proportional amount of both follicular or trabecular and solid structures; there was one tumor of predominantly follicular structures with focal trabecular area while the other tumor was formed of predominantly a solid structures.

3. ***Poorly differentiated F.C. (3 cases):*** The tumors were characterized by the formation of well defined nests (insulae) of tumor cells, often had geographic configuration and occasionally surrounded by hyalanized collagen and often separated from these framework by artificial clefts. Variation in this basic appearance were represented by an area with diffuse trabecular or festoon like configuration. Many insulae displayed small rounded haphazardly distributed small follicles, when these formations were numerous a cribriform or sieve like appearance resulted. Some of these follicles were empty, the other were occupied by a dense acidophilic colloid.

The insulae had a monotonous appearance which was largely due to relatively uniform nature of the tumor cells which were characteristically small both in term of the nuclear and cytoplasmic size. The cells had pale eosinophilic cytoplasm, nuclei with smooth or slightly irregular nuclear membrane, clumped chromatin and inconspicuous nucleoli. The first case in addition to the above mentioned criteria was characterized by the presence of follicular pattern which was actually predominate over the solid (insular) portion. There was no sharp demarcation between the two component, instead, they were blended together. In addition to small microfollicles hidden in solid nests, there were large and better developed follicles, simulating that of follicular variant of papillary carcinoma however, well formed papillae were not seen. The second case was composed entirely of solid (insular) carcinoma, the nests were rounded to oval generally smoothed, and the artificially created clefts were pronounced. The nests were entirely solid without follicular formation. There was a central punctate foci of necrosis within some tumor nests. The cells had abundant eosinophilic cytoplasm and nuclei with clumped chromatin. This case was diagnosed primarily as medullary carcinoma, however it was having features suggestive of poorly differentiated follicular carcinoma also. So this case was considered as diagnostic problem grouped with other poorly differentiated follicular carcinoma for immunohistochemical study for differential diagnosis. The third case had a small area of solid nest intermingling within differentiated follicular component.

There was one case diagnosed primarily as follicular carcinoma, however, the differentiated carcinoma forming, relatively, most of the tumor part admixed with undifferentiated area showing giant, bizzar or even multinucleated cells

with other spindle form cells. This case was grouped to other undifferentiated or anaplastic tumor of the thyroid.

Immunohistochemistry

Generally immunohistochemical staining showed that most of cases (90%) were positively stained for Tg, however, all cases showed clear negativity for calcitonin antibody. Cytokeratin immunoreactivity was observed in 80% of cases (Table 10).

Table (10): **Immunohistochemical staining results of epithelial thyroid follicular carcinoma for various antibodies used.**

Antibody	No of cases	+ve	%	-ve	%
TG	10	9	90%	1	10%
CK	10	8	80%	2	20%
Calcitonin	10	-	-	10	100%

Immunohistochemical staining showed great variation and discrepancy between different groups of follicular carcinoma included in this work as following:

1. *Well differentiated F.C.:* Immunohistochemical staining using polyclonal thyroglobulin antibody showed that all 5 cases (100%) were clearly positive. In general the staining pattern was diffuse, involving mostly all follicles. The lining cells of small follicles showed diffuse moderate cytoplasmic staining which gets stronger in the apical part and over the luminal border of the cells usually; the small follicles were devoid of colloid (Fig. 17, 18).

   In the large follicles there was diffuse moderate to weak cytoplasmic staining of all cells lining the follicles (Fig. 19). The colloid if present

showed diffuse homogeneous staining with moderate staining intensity but sometimes got intense especially at the interface of follicular lumen with follicular cells. Sometimes the lining cells of large follicles showed weak or negative staining in comparison to strong staining of cells lining small follicles (Fig. 20).

The discrepancy in the size of follicular structures of different tumors examined, created a systemic difference of Tg staining intensity, whenever the tumor composed almost entirely of small follicles it showed stronger intensity of the staining.

Immunohistochemical staining for low molecular weight cytokeratin showed positivity in 4 out of 5 cases. The staining pattern was observed involving mostly all follicles whether of large or small size. The lining cells showed commonly diffuse weak to moderate cytoplasmic staining (Fig. 21).

All the above results were included in table (11).

Table (11): Immunohistochemical stainign pattern of well diferetniated follicular carcinoma for various antibodies used.

Antibody	No. of cases	Distribution								Intensity					
		Epithelial				*Colloidal*				*Epithelial*			*Colloidal*		
		-	+	++	+++	-	+	++	++++	+	++	+++	+	++	+++
TG	5	-	-	-	5	2	-	2	1	-	1	4	-	1	2
CK	5	1	1	2	1	-	-	-	_	1	2	1	-	-	-
Cal.	5	--	-	-	-	-	-	-	-	-	-	-	-	-	-

2. *Moderately differentiated F.C.:* Immunohistochemical staining showed that both cases were positively stained for Tg and CK antibodies (Table 12).

Table (12): **Immunohistochemical staining results of moderately differentiated F.C. for various antibodies used.**

Antibody	No of *cases*	+ve staining *Epithelial*	*colloidal*	-ve staining *epithelial*	*colloidal*
Tg	2	2	2	-	-
ck	2	2	-	-	-
Calcitonin	2	-	-	2	-

However, the staining pattern for the Tg was quite different between the two cases. The case of predominantly follicular showed positive immunoreactivity for Tg in both trabecular and follicular structures. The staining pattern was patchy in distribution in both follicular and trabecular areas, however, the intensity of the staining was moderate in trabecular one (Fig. 22) which got strong and obvious in follicular part. The colloid was weakly positive. The other case showed absolute negativity of solid portion together with its small microfollicular structure in contrast to positive moderate staining of the follicular part similar to that of well differentiated follicular carcinoma. The colloid showed weak to moderate staining intensity (Table 13).

The immunostaining for monoclonal low molecular weight cytokeratin was positive in both cases. The positivity was patchy in distribution showing moderate to weak degree of positivity of either solid, trabecular or associated differentiated follicular structures (Table 14).

Table (13): Relationship between Tg immunostaining pattern and various histologic patterns of moderately differentiated follicular carcinoma.

Histologic pattern	Follicular part				Solid/trabecular part	
	Epithelial		*Colloidal*		*Epithelial*	
	Dist.	Int.	Dist.	Int.	Dist.	Int.
Predominantly solid	+	++	++	+	-ve	-
Predominantly follicular	++	+++	+	+	++	++

Table (14): **Relationship between ck immunostaining pattern and various histologic patterns of moderately differentiated follicular carcinoma.**

Histologic pattern	Follicular		Trabecular/solid	
	Distribution	*Intensity*	*Distribution*	*Intensity*
Predominantly solid	+	+ +	+ +	+ +
Predominantly follicular	+ +	+	+ +	+ +

3. ***Poorly differentiated F.C.:*** 2 out of 3 cases showed positive epithelial immunoreactivity for both polyclonal Tg and monoclonal L.M.W. ck. The colloidal positivity for Tg antibody was observed in two cases (Table 15). Immunohistochemical staining for Tg showed in general weak positive reaction in occasional scattered cells or group of cells in poorly differentiated area. The immunostaining of the first case showed scattered

follicle with positive colloidal staining, otherwise the rest of examined section showed almost negative staining in epithelium of both follicular and solid portions (Fig. 23). The staining of the second case showed weak cytoplasmic reactivity in focal areas with few scattered cells showed moderate intensity of immunostaining (Fig. 24). The third case showed more diffuse and intense staining for both colloid and cells lining follicles in differentiated follicular area in contrast to sparsely weakly stained cells in solid portion (Table 16). Immunostaining for L.M.W. ck was usually focal and of moderate intensity to strong involving both forms, follicular and insular pattern (Table 17).

Table (15): **Immunohistochemical staining results of poorly differentiated FC for various antibodies with reference to epithelial and colloidal reactivity.**

Antibody	No of *cases*	+ve staining *Epithelial*	*colloidal*	-ve staining *epithelial*	*colloidal*
Tg	3	2	2	1	1
ck	3	2	-	1	-
Calcitonin	3	-	-	-	-

Table (16): Relationship between Tg immunostaining pattern and various histologic pattern of poorly differentiated follicular carcinoma.

Histologic pattern	No of *cases*	Follicular part Epith. *Dist.*	Follicular part Epith. *Int.*	Follicular part Coll. *Dist.*	Follicular part Coll. *Int.*	Insular part Epith. *Dist.*	Insular part Epith. *Int.*
Insular/ Follicular	1	-ve	-	+ +	+	-ve	-
Insular	1	-	-	-	-	+	+
Follicular/ insular	1	+ +	+ +	+ +	+	+	+

Epith. *Epithelial*
Coll. *Colloid*
Dist. *Distribution*
Int. *Intensity*

Table (17): Relationship between ck immunostaining pattern and various histologic patterns of poorly differentiated follicular carcinoma.

Histologic pattern	Follicular *Dist.*	Follicular *Int.*	Insular *Dist.*	Insular *Int.*
Insular/follicular	+ +	+	+ +	+
Insular	-	-	+	+ + +
Follicular/insular	-ve	-	-ve	-

Dist. *Distribution*
React. *Reactivity*
Int. *Intensity.*

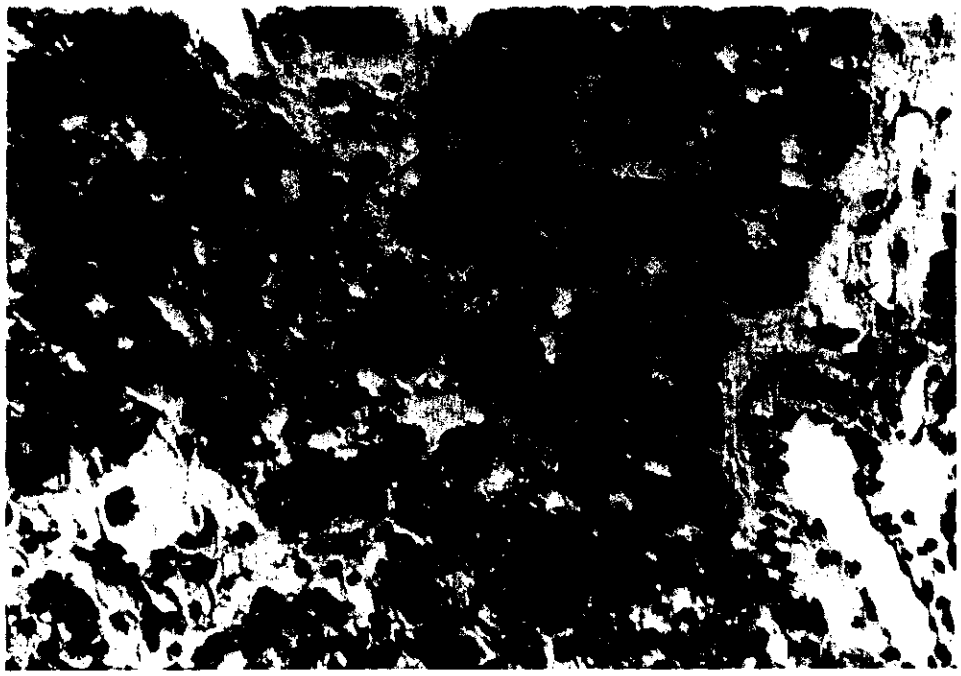


Figure (17): Well differentiated follicular carcinoma showing diffuse immunostaining which get stronger over the luminal surfaces of follicular cells (arrow). (Tg immunohisto x 10).

Figure (18): High power view of the above case.

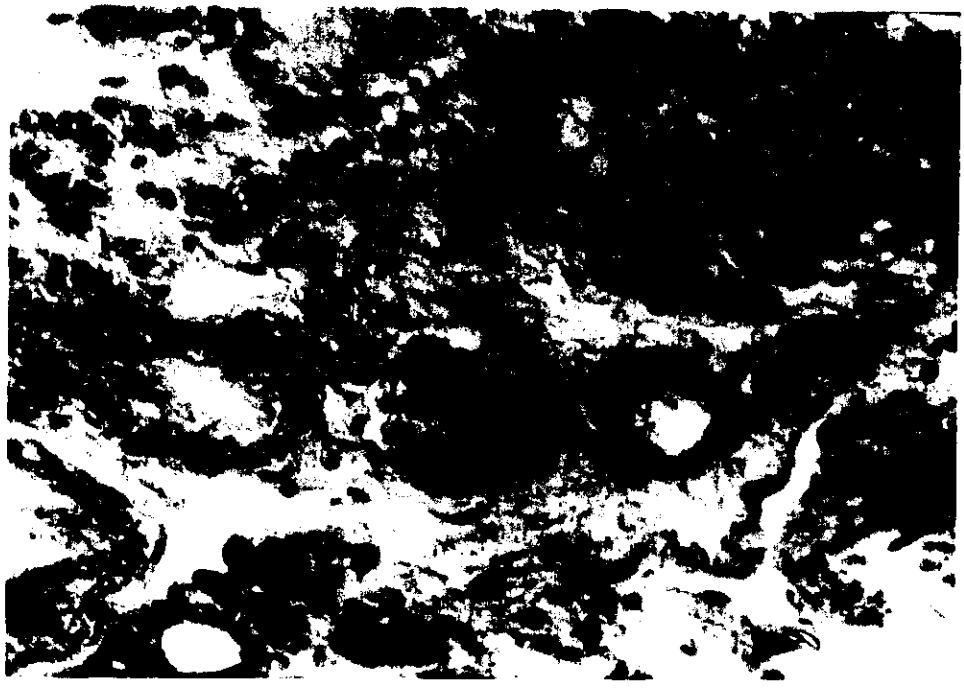


Figure (19): Well differentiated follicular carcinoma show diffuse immunostaining of large follicles. Colloidal positivity is noticed also in all follicles (Tg immunohisto. x 20).

Figure (20): Well differentiated follicular carcinoma showing strong immunostaining in cells lining small follicles, whereas cells of large follicles showed weak or negative staining. The colloidal reactivity is strong at the interface of follicular lumen (Tg immunohisto x 40).

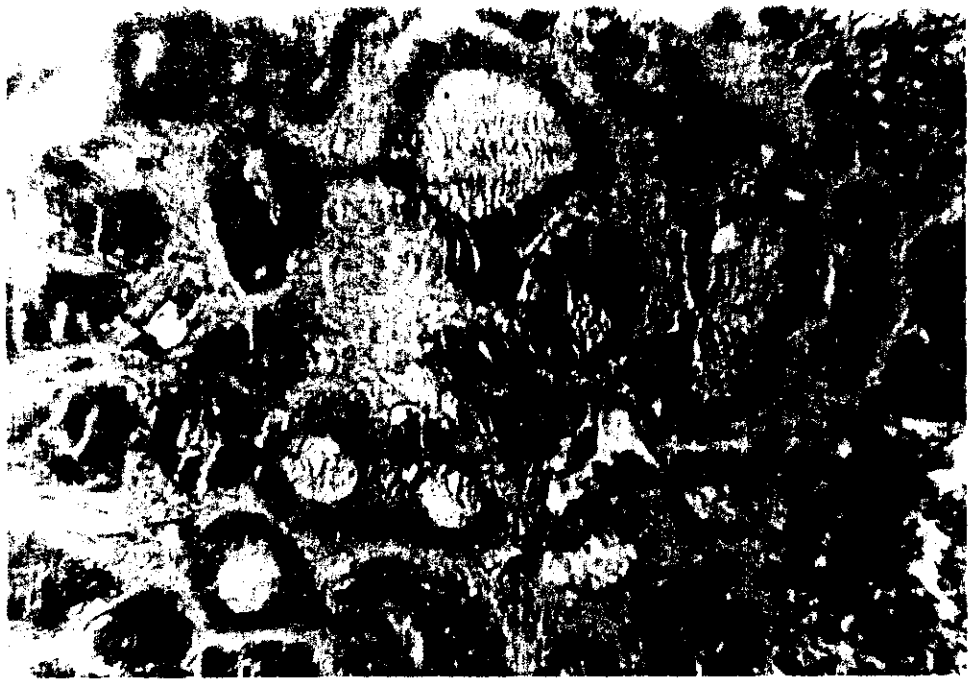


Figure (21): Well differentiated follicular carcinoma showing positive immunostaining in the follicular cells whether of the small or large thyroid follicles (CK immunohisto. x 10).

Figure (22): Moderately differentiated follicular carcinoma showing positively stained trabecular area with moderate intensity which get stronger in area with follicular differentiation *(arrow)*. The neighbouring part is negatively stained. (Tg immunohisto x 20).

Figure (23): Poorly differentiated follicular carcinoma (case 1) showing negative staining of epithelium of both, follicular and insular *(arrow)* structures. The colloidal positivity is observed in few follicles (Tg immunohisto x 10).

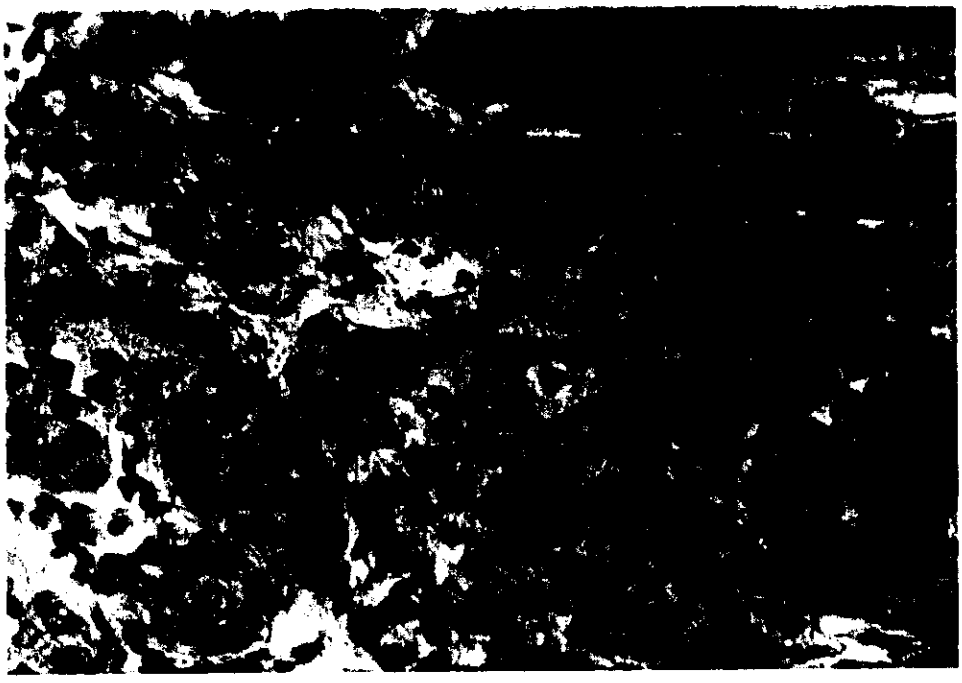


Figure (24): Poorly differentiated follicular carcinoma (case 2) showing weak cytoplasmic staining in few scattered cells *(arrow head)*. The rest of the tumor was negative (Tg immunohisto. x 20).

MEDULLARY CARCINOMA

Examination of stained sections of medullary tumors with conventional stain showed that all tumors were composed of varying proportion of polygonal and spindle cells arranged in solid nests or sheets or masses. The amyloid material was present in all cases with varying proportion differed from one case to another and differed even in same tumor areas. Mitotic activity was observed rarely in most cases, however, the cases diagnosed clinically as recurrent medullary carcinoma showed small areas of cellular anaplasia formed of giant cells, necrosis and high mitotic index. All cases included in this work could be interpreted morphologically as classical medullary carcinoma. The cases were subcategorized in three groups according to the location of the tumor, 4 cases; the tumors were confined to thyroid gland. Five cases; the primary thyroid tumor showed metastasis to cervical lymph nodes, 3 cases were diagnosed clinically as recurrent medullary carcinoma with lymph node and distant metastasis.

Immunohistochemistry

Ten cases out of 12 cases showed positive cytoplasmic staining for polyclonal calcitonin antibody. However, all tumors were negatively stained for polyclonal Tg. The cytokeratin positivity could be observed in 10 cases also (Table 18).

Table (18): Immunohistochemical staining of medullary thyroid carcinoma.

Antibody	No of cases	+ve	%	-ve	%
TG	12	-		12	100%
CK	12	10	83.3%	2	16.7%
Calcitonin	12	10	83.3%	3	16.7%

The characteristic fine granular cytoplasmic immunoreactivity for calcitonin could be observed sometimes in associated normal orlyperplastic areas of C-cells of thyroid tissue (Fig. 25). The immunoreactivity for calcitonin observed in stained cases showed differences in the staining pattern between different groups included in this work. The medullary tumors localized in the cervical region without obvious distant metastasis; the immunostaining was moderate to strong involved most tumor area. In contrast the staining pattern get weaker and focal or even negatively stained in those tumors showed distant metastasis (Table 19).

Table (19): **Relationship between calcitonin immunostaining and the virulence of medullary thyroid carcinoma.**

Tumor group	No of cases	Distribution					Intensity		
		-	+1	+2	+3	+4	+	++	+++
Localized	4	-	-	1	1	2	-	1	3
Metastatic	5	-	1	1	2	1	1	2	2
Recurrent	3	2	1	-	-	-	1	-	-

Metastatic: cases showed lymph node metastasis only.

So localized medullary tumors showed homogeneous distribution of calcitonin immunostaining (Fig. 26). However, there was slight variation in the staining intensity in some tumors where some areas or few isolated cells showed stronger stainability than the other associated areas (Fig. 27, 28). In metastatic or recurrent medullary tumors a principle observation was heterogeneous cellular distribution for calcitonin immunostaining. In some tumors with lymph node metastasis, only small focal areas showed weak to moderate staining (Fig. 29), other tumors showed groups of isolated cells distributed throughout the field (Fig. 30). However only sparsely isolated cell showing positive reactivity

where the rest of the lesion showing virtual lack of hormone reactivity; could be observed only in the tumor with distant metastasis (Fig. 31).

The staining pattern for low molecular weight cytokeratin showed generally moderate degree of stainability regardless of tumor cell type or the tumor category (Table 20, Fig. 32).

Table (20): **Relationship between ck immunostaining and the virulence of medullary carcinoma.**

Tumor group	No of cases	Distribution				Intensity		
		-	+	++	+++	+	++	+++
Localized	4	1	1	2	-	-	2	1
Metastatic	5	-	1	3	1	2	2	1
Recurrent	3	1	1	1	-	1	1	-

Figure (25): Normal thyroid tissue showing fine granular cytoplasmic immunostaining of isolated group of C-cells in between thyroid follicles (Cal. immunohisto x 20).

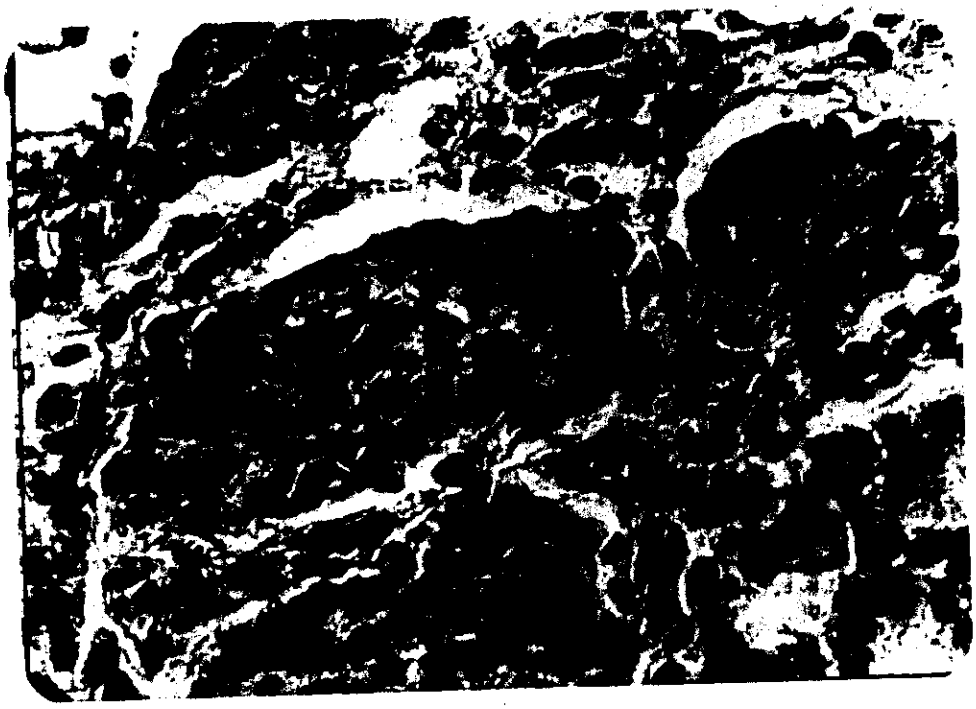


Figure (26): Localized medullary thyroid carcinoma showing homogeneous, diffuse immunostaining involving almost, all cells (Cal immunohisto x 20).

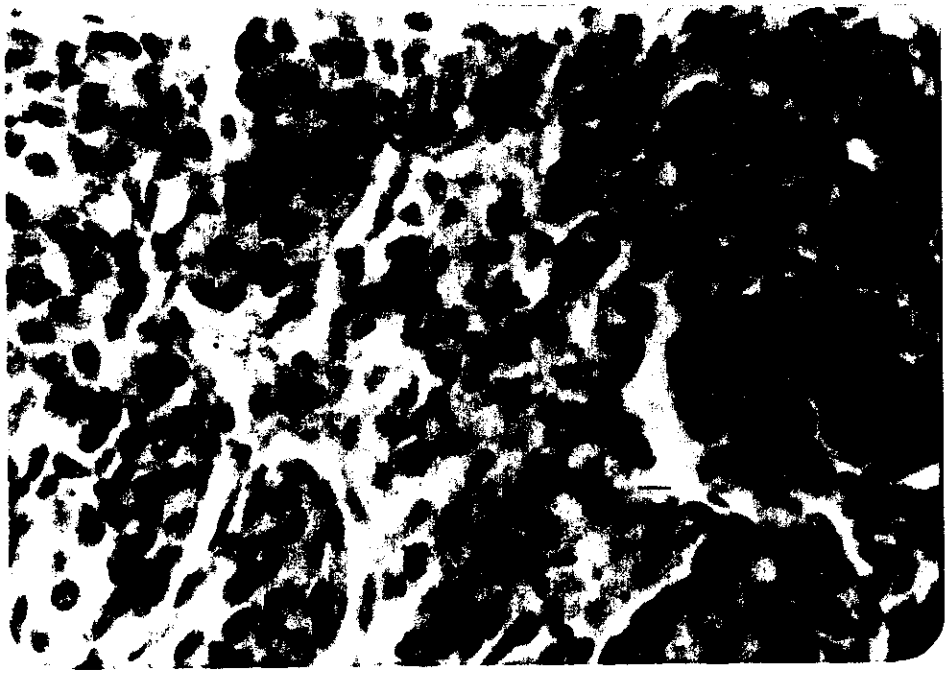


Figure (27): Localized medullary thyroid carcinoma showing diffuse staining. Small area (c) showing strong reactivity, while the rest of the tumor showed moderate reactivity. The staining is observed in both polygonal and spindle shaped cells (Cal immunohisto. x 20).

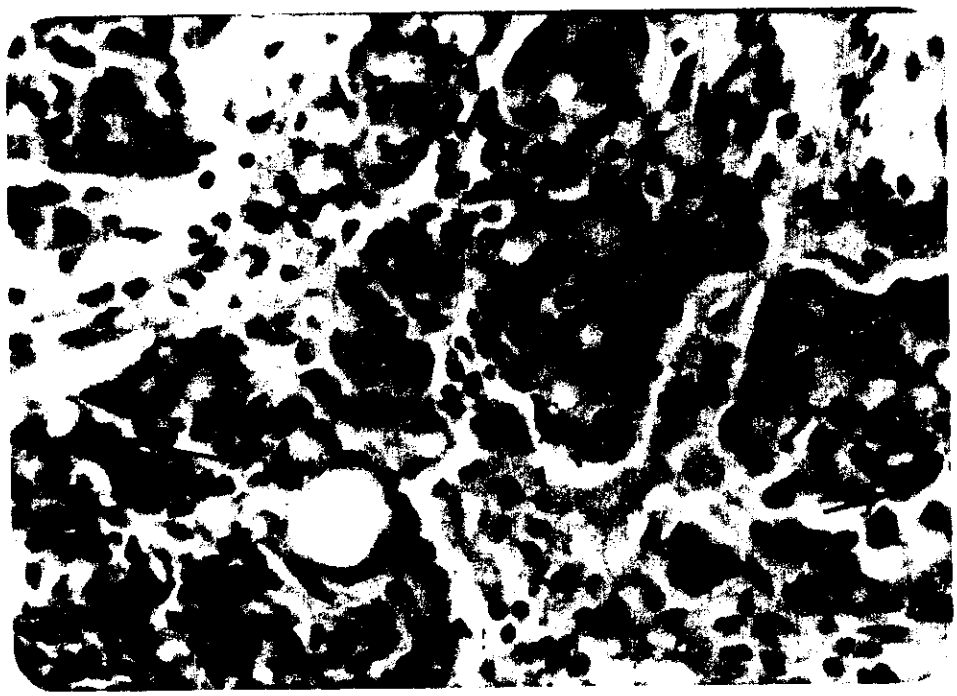


Figure (28): Localized medullary thyroid carcinoma showing homogeneous diffuse moderate staining with few isolated cells showing strong reactivity *(arrow)*. The amyloid material *(arrow head)*, showing also positive staining (Cal immunohisto x 20).

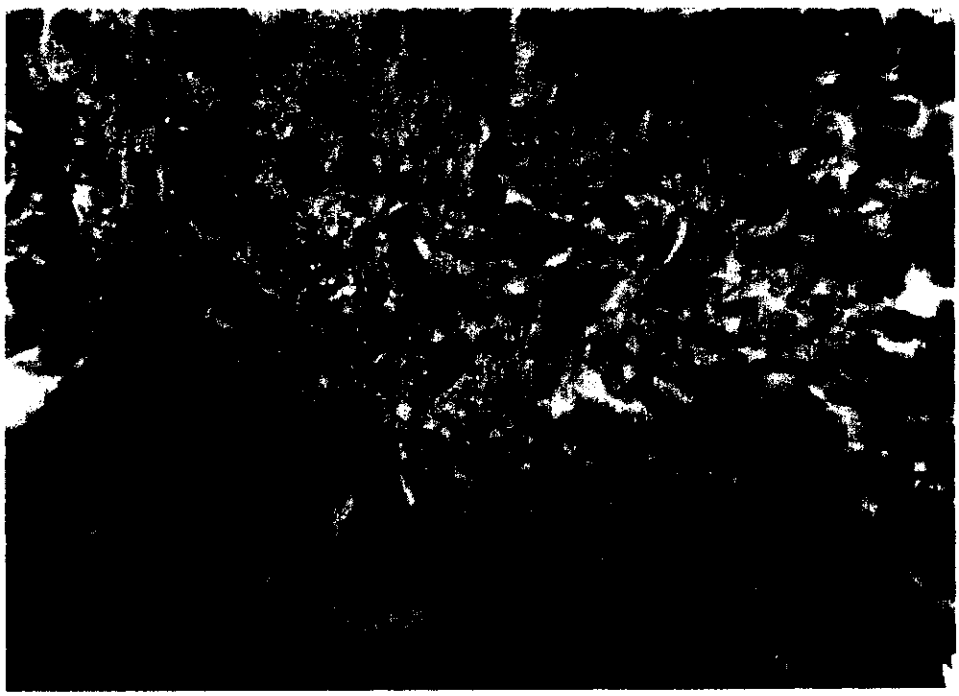


Figure (29): Metastatic medullary thyroid carcinoma showing small focal area with moderate to weak staining intensity (Cal immunohisto x 20).

Figure (30): Metastatic medullary thyroid carcinoma showing groups of isolated positively stained cells (Cal. immunohisto. x 20).

Figure (31): Recurrent medullary thyroid carcinoma, showing positively stained isolated cells, while the rest of the tumor is negatively stained (Cal. immunohisto. x 20).

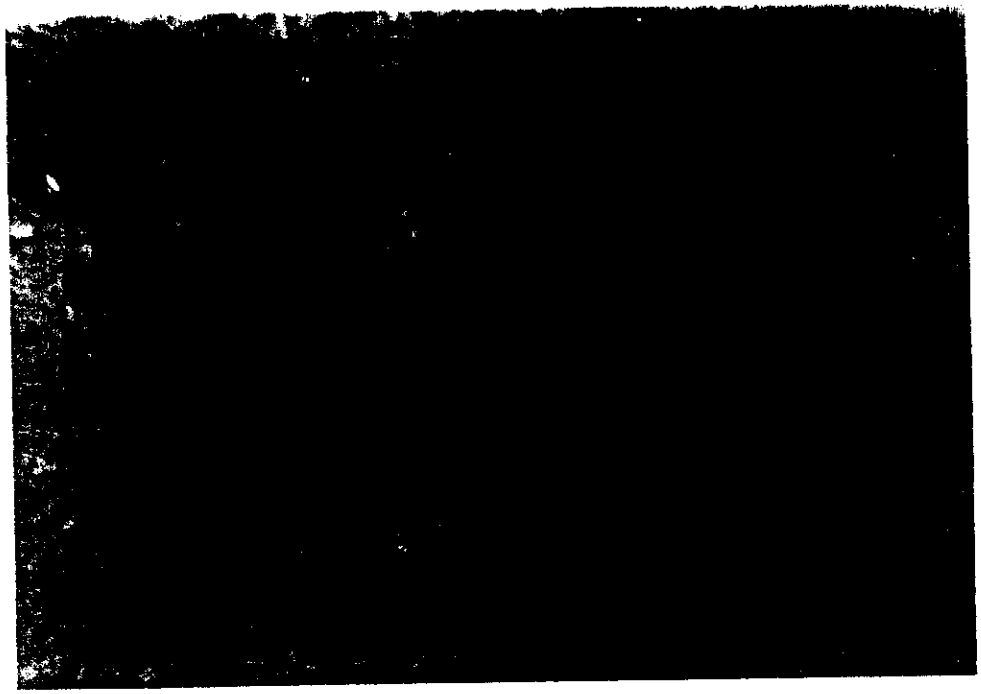


Figure (32): Medullary thyroid carcinoma showing positively stained area formed mainly of spindled shape cells (CK immunohisto. x 20).

ANAPLASTIC CARCINOMA

Histopathology

In the anaplastic (undifferentiated) thyroid carcinoma 14 cases could be found after excluding cases with features of squamous cell carcinoma. Three cases were obtained by needle biopsies while the rest cases were surgical biopsies. H & E stained sections of various cases were reviewed and evaluated regarding the type and form of cells. 12 cases showed only one cell type; 5 cases (35.7%) were of spindle cell type, 4 cases (28.6%) were of giant cell type, 2 cases (14.3%) were of small cell type and one case (7.1%) was of squamoid cell type. The other 2 cases (14.3%) of the tumors showed admixture of spindle and pleomorphic giant cells.

Spindle Cell Pattern

The tumors were predominantly composed of spindle cells. These tumors usually had sarcomatoid appearance where the spindle cells are often arranged in fascicules resembling fibrosarcoma or in a storiform pattern similar to that of malignant fibrous histiocytotomas (Fig. 33). Sometimes the tumor cells were arranged in palisading manner around necrotic areas, or arranged in a manner similar to that of nerve cell tumors.

Giant Cell Pattern

The tumors were composed predominantly of large anaplastic cells characterized by striking degree of pleomorphism and the presence of numerous tumor giant cells having bizarre; sometimes multiple hyperchromatin nuclei and eosinophilic cytoplasm, that on occasion presented as Rhabdoid-like appearance (Fig. 34).

The pattern of growth was usually solid but sometimes artifactual separation of the cells lead to the formation of pseudo-alveolar pattern. Cell

with clear cytoplasm was observed in one case. The cells sometimes were simulating the ganglion tumor cells or myosarcomatous tumor. There was tumor composed almost exclusively of cells simulating either epithelioid sarcoma or simulating differentiated liposarcoma (Fig. 35).

Squamoid Cell Pattern

This case was characterized by the presence of tumor nests which showed area simulating squamous differentiation but keratin pearl formation was not seen (Fig. 36, 37). Numerous multinucleated benign appearing osteoclast-like cells were observed frequently (Fig. 38).

Small Cell Pattern

The tumor was found to be composed of diffuse non-cohesive round cells with little or no cytoplasm. The cytoplasm when present was slightly eosinophilic, the nuclei, though small, were larger than those of mature lymphocytes and varied somewhat in size and shape. Some cells had prominent nuclear membrane and nucleoli, while the other had dark compact nuclei (Fig. 39). Vague alveolar patterns were present in some areas of the neoplasms that also showed diffuse infiltration by neoplastic cells.

Generally hemorrhage, necrosis and inflammatory cells were common findings in all tumors. The vascular and nerve trunk invasion was a peculiar pattern that had been observed. In some tumors there was endothelial proliferation account to occlude the vascular lumen. There was an obvious intra and extrathyroidal invasion in all the cases.

There was only 21.4% of the tumors had residual foci of well differentiated carcinoma. One of them had differentiate area of medullary carcinoma, the other two had differentiated area of follicular carcinoma. An

obvious zone of transition between the differentiated follicular carcinoma and the anaplastic area; composed of spindle cells in one case and giant cells in the other case, was seen.

Immunohistochemistry

Immunohistochemical study showed that only two cases (14.3%) showed positive reaction for Tg antibody however, there was no reactivity at all for calcitonin antibody, the cytokeratin reactivity was observed in 9 cases (64.3%) (Table 21).

Table (21): **Immunohistochemical staining results of anaplastic thyroid carcinoma for various antibodies used.**

Antibody	No of cases	+ve	%	-ve	%
Tg	14	2	14.3%	12	85.7%
CK	14	9	64.3%	5	35.7%
Cal.	14	-	-	14	100%

The only 2 cases showed positive reactivity for Tg were, a case of small cell carcinoma and the other one showed features of giant cell pattern. As regard the case of the small cell pattern the positivity was observed in clusters of neoplastic cells nearby ruptured thyroid follicles which also stained positively with other normal entrapped thyroid follicles. However, the rest and vicinity of the tumor away from thyroid follicles was absolutely negative (Fig. 40, 41).

The other case of giant cell pattern was associated with well differentiated follicular carcinoma and a transitional zone in between. The positivity was observed in the transitional zone in few clusters of cells near by follicles of well-differentiated follicular carcinoma which showed strong

immunostaining (Fig. 42). However, the rest of the tumor in the anaplastic area was negatively stained (Fig. 43).

The well differentiated follicular carcinoma associated in the above case and other case of spindle cell pattern was strongly stained, however the anaplastic spindle cells were absolutely negative in the vicinity of the tumor or even in cells admixed or nearby the differentiate carcinoma in the transitional zone. The anaplastic area of this case was completely devoid of any traces of normal or neoplastic thyroid follicles even when serial sections were taken, (Table 22).

Table (22): **Immunohistochemical staining pattern of various types of anaplastic thyroid carcinoma for thyroglobulin antibody.**

Histologic pattern	No of cases	Distribution					Intensity		
		0	+1	+2	+3	+4	+	++	+++
Spindle cell carcinoma	5	5	-	-	-	-	-	-	-
Giant cell carcinoma	4	3	1	-	-	-	1	-	-
Giant-spindle cell carcinoma	2	2	-	-	-	-	-	-	-
Squamoid cell Carcinoma	1	1	-	-	-	-	-	-	-
Small cell carcinoma	2	1	1	-	-	-	-	1	-

Immunohistochemical staining for low molecular weight cytokeratin: showed that the cases of anaplastic small cell pattern were negatively stained. The other cell patterns of anaplastic carcinoma showed variable immunostaining results.

The squamoid cell pattern (one case) showed the strongest and diffuse stainability where the reactivity was observed in over 75% of cells. Three out of 5 cases (60%) of anaplastic tumors consisted predominantly of spindle cell were positively stained. The positively stained cases showed the weakest intensity of reaction in few isolated cells (less than 25% of cells). Five out of 6 cases (83.3%) of tumors diagnosed as giant cell anaplastic carcinoma, either composed predominantly of giant cells or mixed with spindle cells were positively stained, over 50% of cells in 3 cases (60%) showed strong staining intensity. The giant cell anaplastic tumor formed of epithelioid cells was negatively stained (Table 23).

Table (23): **Immunohistochemical staining pattern of various types of anaplastic thyroid carcinoma for monoclonal cytokeratin.**

Histologic pattern	No of cases	Distribution					Intensity		
		0	+1	+2	+3	+4	+	++	+++
Spindle cell carcinoma	5	2	3	-	-	-	2	1	-
Giant cell carcinoma	4	1	-	1	1	1	-	1	2
Giant-spindle cell carcinoma	2	-	-	1	-	1	-	1	1
Squamoid cell Carcinoma	1	-	-	-	-	1	-	-	1
Small cell carcinoma	2	2	-	-	-	-	-	-	-

Immunostaining of spindle cell showed great discrepancy in staining pattern between different tumors containing it, since these cells showed diffuse and strong degree of reactivity where the cells intermixed with giant cells showed histologic epithelial differentiation (Fig. 44). However, in the tumors formed solely or predominantly of spindle cells with obvious histologic sarcomatous appearance, the positivity was only observed in isolated sparsely distributed cells with very weak intensity of the staining (Fig. 45). In some areas the positivity observed in cells which could be represent either remnant part of destructed thyroid follicle or anaplastic cells with tendency toward follicular differentiation (Fig. 46). The associated follicular carcinoma or trapped thyroid follicles within the tumor tissue were focally and weakly stained (Fig. 47). Immunostaining of the giant cell tumors usually showed diffuse cytoplasmic staining whether in mononuclear or bizzar anaplastic multinucleated giant cells with the same degree of staining intensity in both types of cells (Fig. 48). However, the paranuclear globular staining pattern was observed in one case (Fig. 49). The light microscopic analysis showed that this tumor was composed of giant cells having rhabdoid-like features and characterized by eosinophilic cytoplasm and eccentric nuclei. Another case characterized by the presence of membranous immunostaining rather than cytoplasmic staining (Fig. 50, 51). The light microscopic analysis showed that all or most of these cells had vacuolated clear cytoplasm. The tumor formed histologically from cells simulating ganglionic cells showed diffuse homogeneous immunostaining (Fig. 52).

The immunoreactivity observed in a case of giant cell carcinoma associated with differentiated follicular carcinoma and transitional zone showed positive staining in both differentiated follicular area together with diffuse staining of undifferentiated area. However, the intensity of the staining of well

differentiated area was less stronger and homogeneous than that observed in the anaplastic and transitional zone (Fig. 53).

The squamoid carcinoma showed homogeneous strong diffuse positivity observed in most cells. (Fig. 54). however, in some area the central part of the nests formed from more differentiated squamous like cell, got less degree of stainability rather than that of the peripheral spindled shaped cells (Fig. 55). The associated multinucleated osteoclast-like cells showed strong homogeneous cytoplasmic staining (Fig. 56).

Immunohistochemical staining for polyclonal calcitonin: showed universal negativity in all cases except the differentiated medullary area associated with spindle cell anaplastic case. The positivity was observed in focal areas and isolated groups of cells less than 25% of tumor mass.

Immunohistochemical staining for monoclonal leucocytic common antigen: showed almost negativity in tumor diagnosed as large (giant) anaplastic cell carcinoma. In contrary the two cases of small cell carcinomas showed moderate patchy cytoplasmic staining in many cells with localization of the stain in the cell membrane in few group of cells (Table 24; Fig.57)

Table (24): Immunohistochemical staining pattern of various types of anaplastic thyroid carcinoma for monoclonal LCA.

Histologic pattern	No of cases	Distribution					Intensity		
		0	+1	+2	+3	+4	+	++	+++
Spindle cell carcinoma	5	5	-	-	-	-	-	-	-
Giant cell carcinoma	4	4	-	-	-	-	-	-	-
Giant-spindle cell carcinoma	2	2	-	-	-	-	-	-	-
Squamoid cell Carcinoma	1	1	-	-	-	-	-	-	-
Small cell carcinoma	2	-	-	1	1	-	-	2	-

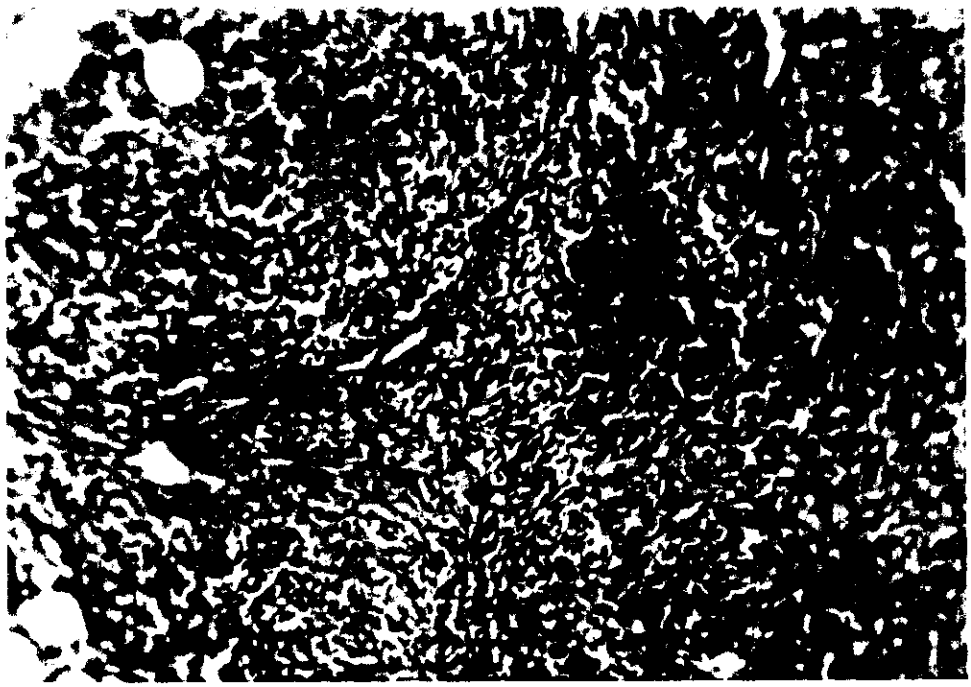


Figure (33): Anaplastic thyroid tumor formed of spindle cells arranged in a manner simulating malignant fibrous histiocytoma (H & E x 10).

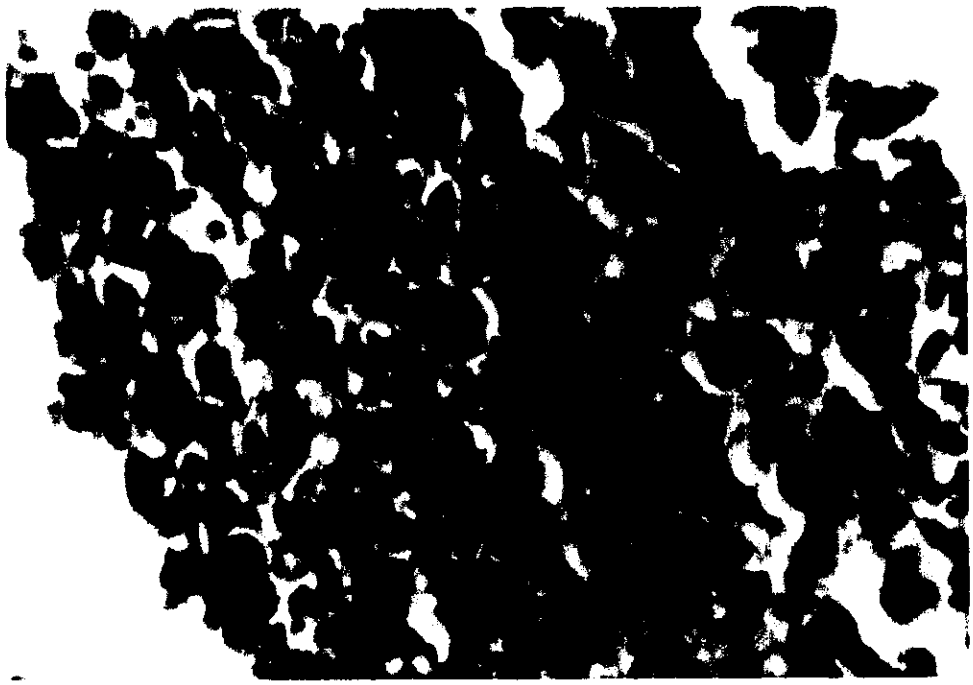


Figure (34): Anaplastic thyroid tumor formed of giant cells with abundant acidophilic cytoplasm simulating rhabdoid sarcoma (H & E x 20).

Figure (35): Anaplastic thyroid tumor composed of cells simulating either epithelioid sarcoma or simulating differentiated liposarcoma (H & E x 20).

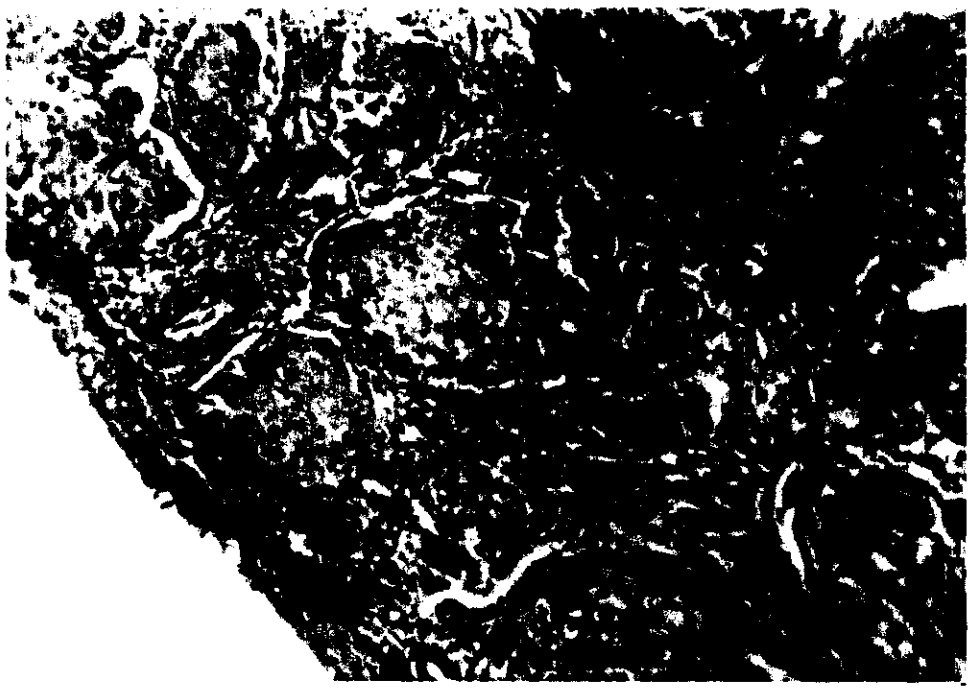


Figure (36): Anaplastic thyroid carcinoma composed of masses of cells bearing squamous differentiations, but there was no keratin formation (H & E x 10).

Figure (37): High power view of squamoid pattern anaplastic carcinoma (H & E x 40).

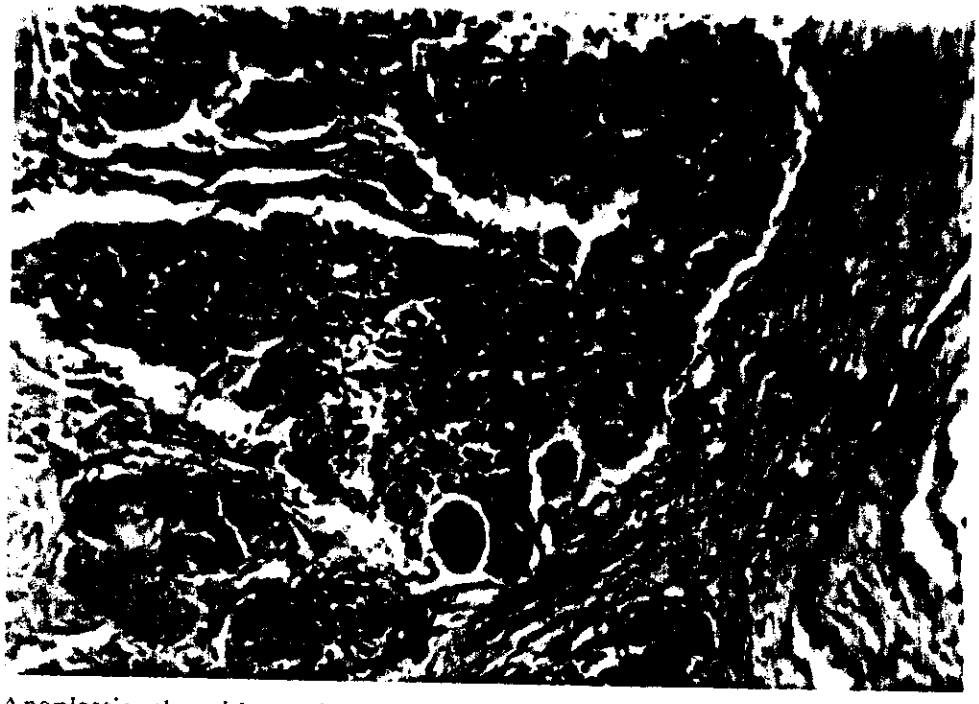


Figure (38): Anaplastic thyroid carcinoma "squamoid pattern" contain scattered multinucleated benign appearing osteoclast-like cells (H & E x 10).

Figure (39): Anaplastic thyroid tumor composed of diffuse sheets of small cells. Many of these cells with eosinophilic cytoplasm, while other with clear cytoplasm (H & E x 20).

Figure (40): Anaplastic thyroid tumor of small cell pattern showing strong immunostaining of entrapped thyroid follicle (both epithelium and colloid). The nearby areas *(arrow)* showing also positive immunoreactivity of tumor cells (Tg immunohisto. x 10).

Figure (41): High power of above case, showing positive immunostaining of ruptured thyroid follicle (area A) and small cells of anaplastic tumor (area b) (Tg immunohisto x 20).

Figure (42): Giant cell anaplastic carcinoma (transitional zone) showing strong immunostaining of differentiated follicular carcinoma. A cluster of anaplastic cells *(arrow)* showing strong staining while the rest of tumor tissue is negatively stained (Tg immunohisto. x 20).

Figure (43): The same case above showing positive immunostaining of thyroid follicles, while the anaplastic cells in between are negatively stained (Tg immunohisto. x 10).

Figure (44): Anaplastic thyroid carcinoma of mixed cell type showing diffuse and strong immunostaining of an area formed of spindle-shaped cells (CK immunohis x 20).

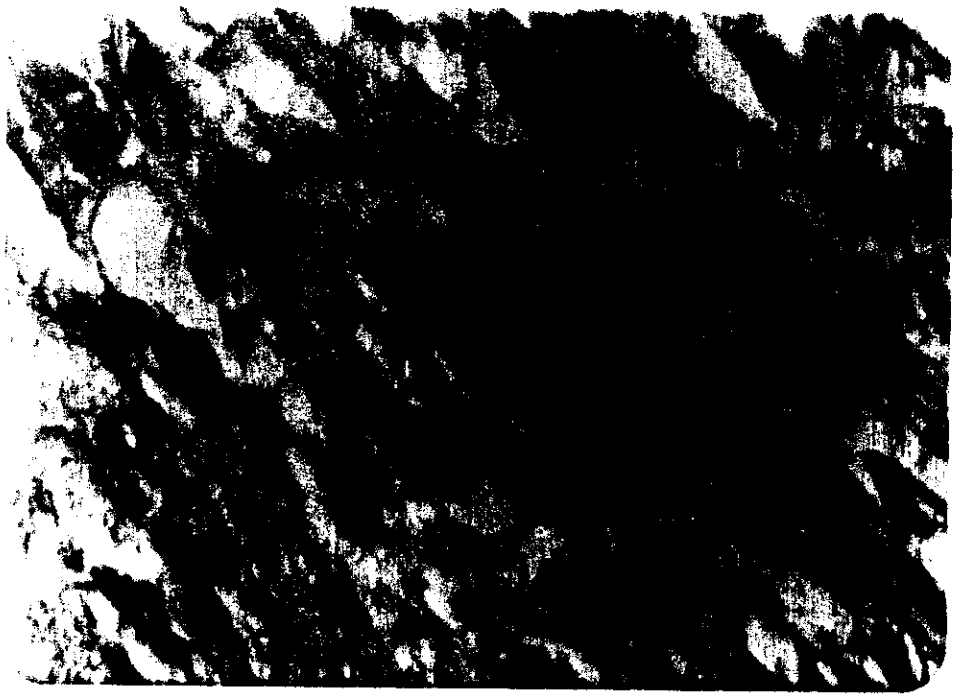


Figure (45): Anaplastic thyroid tumor formed predominantly of spindle shaped cells showing weak, positive immunostaining in single isolated cells, while the rest of the tumor showed clear negativity (CK immunohisto. x 40).

Figure (46): Anaplastic spindle-cell thyroid tumor showing positive immunostaining in cluster of cells with tendency toward follicular differentiation, the rest of the tumor is negatively stained (CK immunohisto x 40).

Figure (47): Anaplastic spindle-cell thyroid tumor showing weak positive immunostaining in two entrapped thyroid follicles. The surrounding anaplastic cells are negatively stained (CK immunohisto x 40).

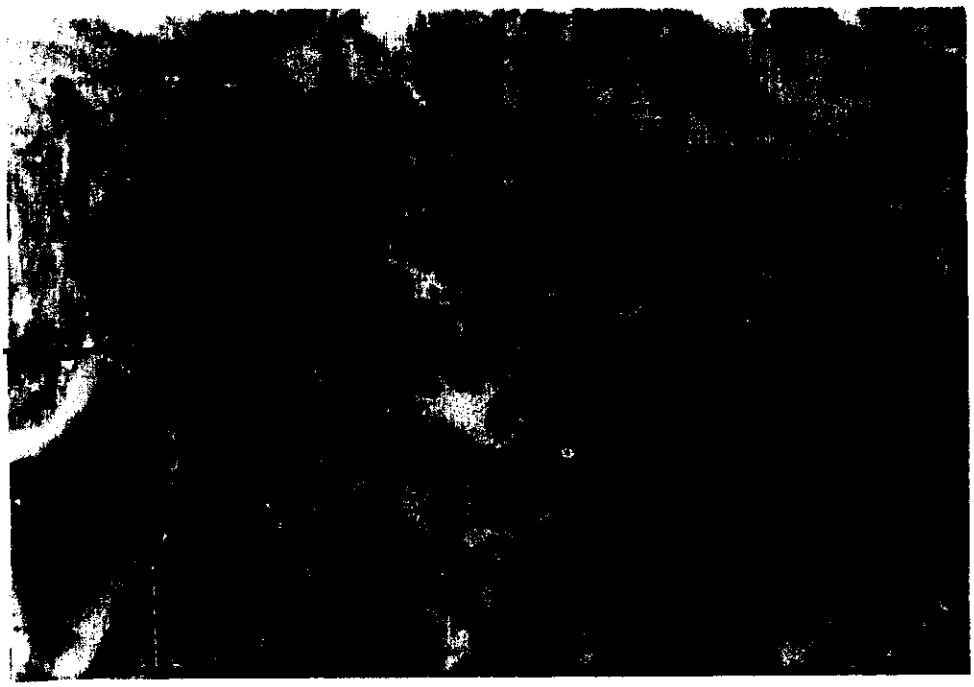


Figure (48): Anaplastic giant cell tumor showing strong, diffuse cytoplasmic staining, whether mononuclear or malignant bizzar-shaped, multinucleated giant cells *(arrow)* (CK immunohisto. x 20) (insert x 40).

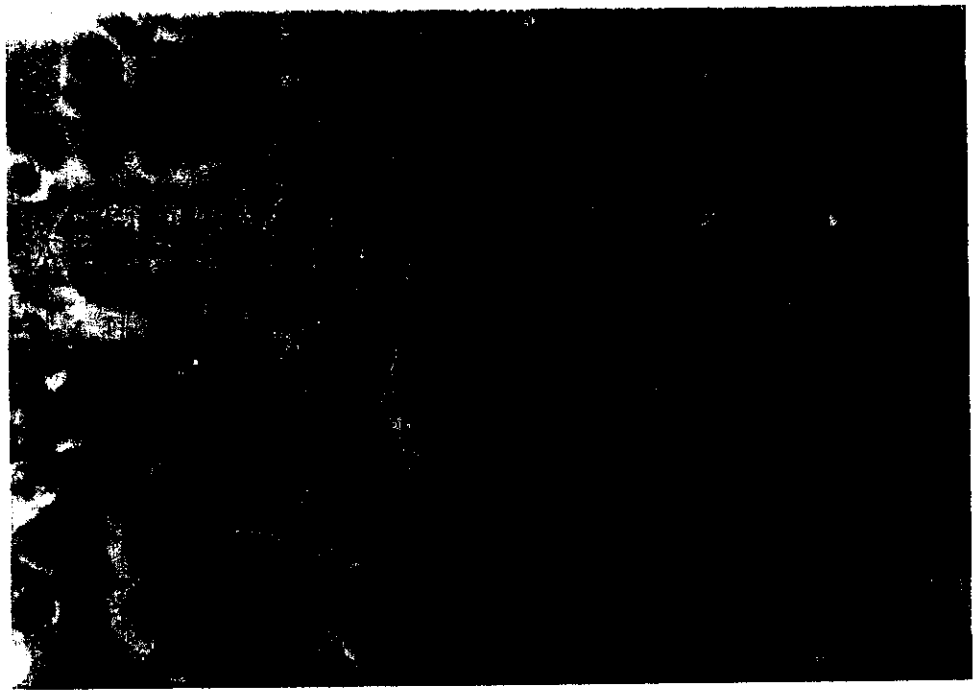


Figure (49): Anaplastic giant cell tumor showing paranuclear cytoplasmic positivity in cells having histologic feature of rhabdoid like sarcoma (CK immunohisto. x 20).

Figure (50): Anaplastic giant cell tumor showing diffuse, strong immunostaining localized mainly in the cell membrane (CK immunohisto. x 10 "A" x "20" B).

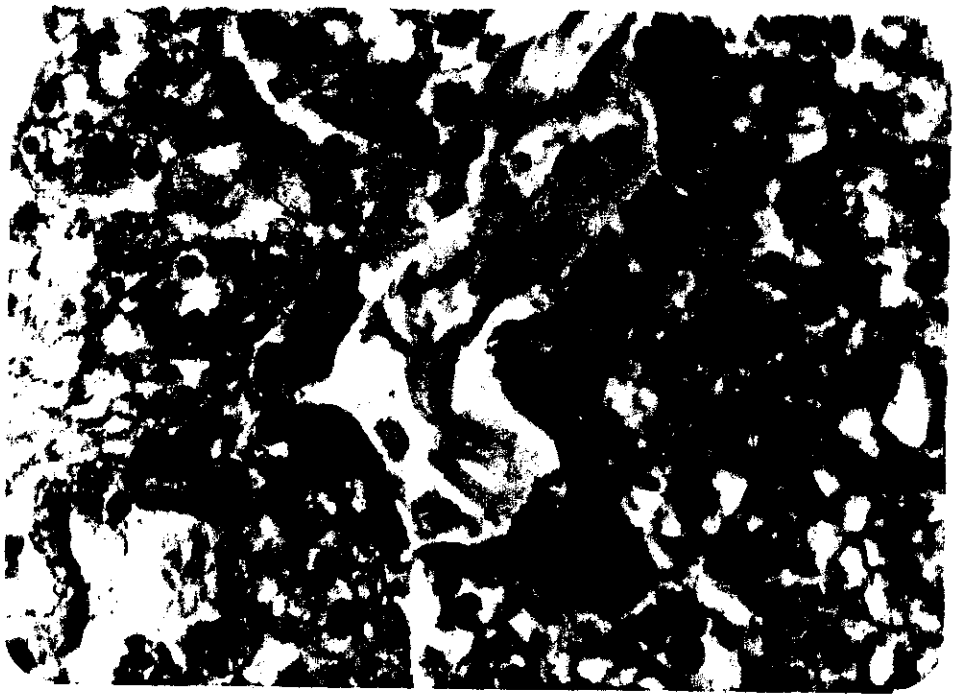


Figure (51): The same case as above showing strong diffuse cytoplasmic positivity in cells showing bizzar nuclear shape or multinucleated form *(arrow)* (CK immunohisto. x 20).

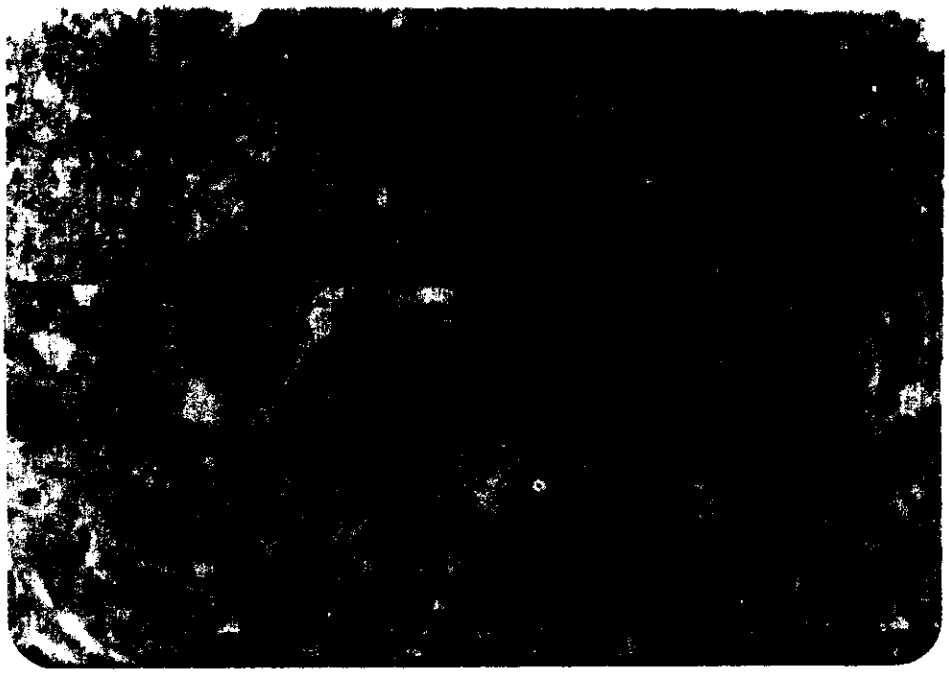


Figure (52): Anaplastic giant cell tumor, showing diffuse, moderate immunostaining of giant (large) cells *(arrow)*, simulating histologically, ganglionic cells. Small cells in between large one showing weak positivity *(arrow head)* (CK immunohisto x 10 "A" x 20 "B").

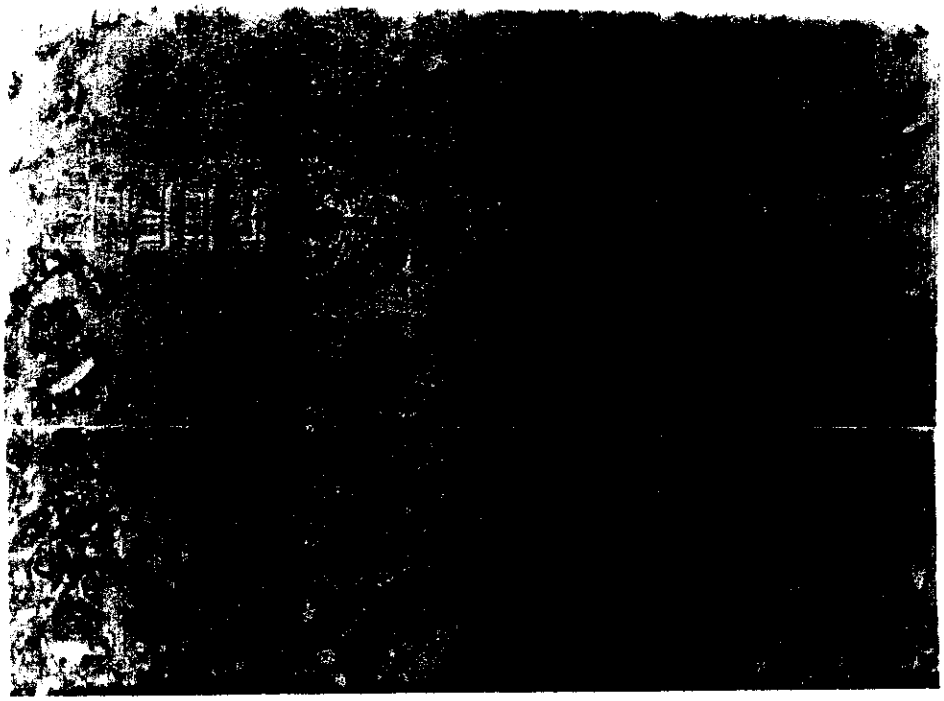


Figure (53): Anaplastic thyroid tumor showing diffuse moderate immunostaining in area "D" of well differentiated follicular carcinoma, which get stronger in transitional zone "T" and anaplastic area "A" (CK immunohisto/ x 10 "D and T" x 20 "A").

Figure (54): Anaplastic thyroid tumor of squamoid cell pattern, showing diffuse, homogeneous, and strong immunostaining (CK immunohisto. x 10).

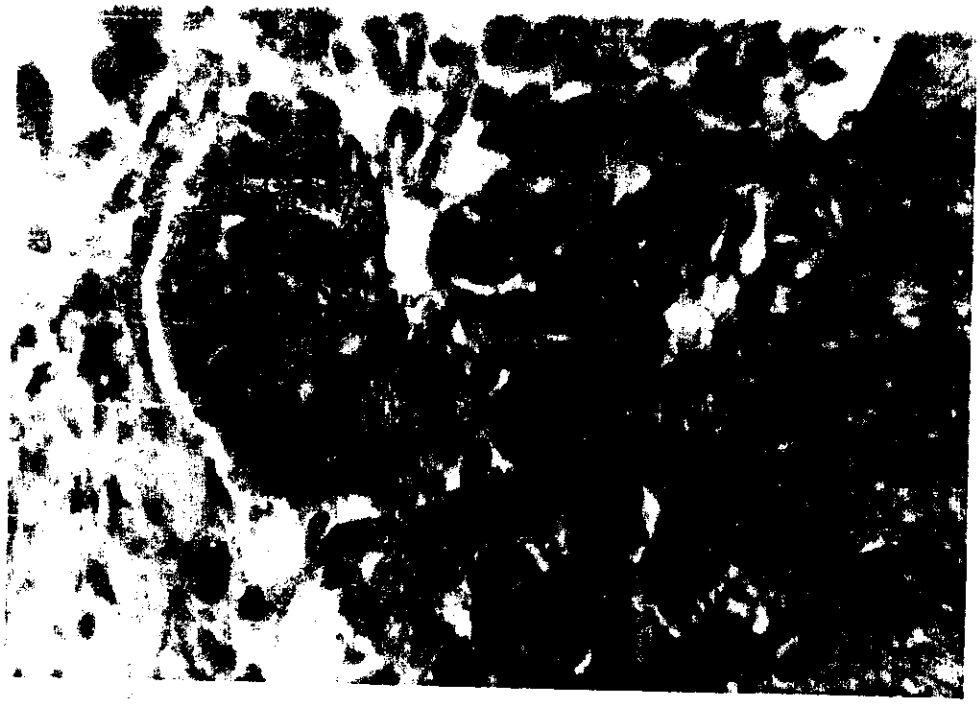


Figure (55): High power view of above case showing diffuse immunostaining of both central area "C" with more squamous differentiation and surrounding peripheral zone "G" formed of spindle cells (CK immunohisto. x 40).

Figure (56): The same case as above showing strong immunostaining in multi-nucleated osteoclast-like cells (CK immunohisto x 20 "insert x 40"

Figure (57): Anaplastic, small cell thyroid tumor showing diffuse moderate cytoplasmic staining. Few cells showed membranous staining also *(arrow)* (LCA immunohisto x 20).

LYMPHOMA

Examination of both cases of thyroid lymphoma with H & E stain showed that one of them was associated with chronic lymphocytic thyroiditis. Packing of thyroid follicles with malignant lymphoid cells which considered as conclusive histologic features for thyroid lymphoma was observed in both cases (Fig. 58).

Immunohistochemical staining for LCA showed that the thyroid lymphomas were positively stained. The staining pattern was usually diffuse in distribution and commonly presented as membranous stain (Fig. 59). However, there was a case in which the cytoplasmic stain was usually observed. The cases were negatively stained for low molecular weight CK, Tg and calcitonin antibodies.

Study of Tg in benign and malignant thyroid tumors

Most of benign thyroid tumors (77.8%) showed positive Tg immunostaining in both epithelium and colloid while only 44.6% of malignant thyroid tumors; including medullary; anaplastic and lymphoid tumors, showed positive immunostaining (Table 25).

Table (25): The incidence of thyroglobulin immunostaining in thyroid neoplasms.

Tumor type	No. of cases	+ve	%	-ve	%
Benign	9	7	77.8%	2	22.2%
Malignant	56	25	44.6%	31	55.4%

The thyroglobulin immunopositivity was significantly higher in follicular carcinoma (90% of cases positive) rather than papillary or anaplastic carcinomas. The medullary carcinomas were absolutely negative for Tg immunostaining (Table 26), Histogram (I).

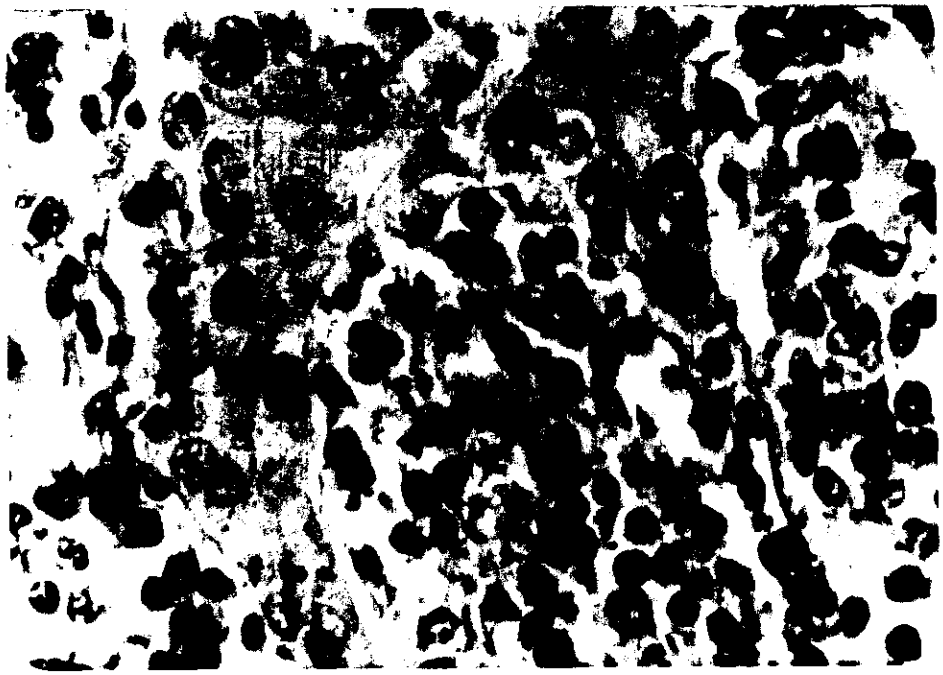


Figure (58): Thyroid lymphoma showing packing of thyroid follicle (F) with partial replacement of follicular cells with malignant lymphoid cells (L) (L & E x 40).

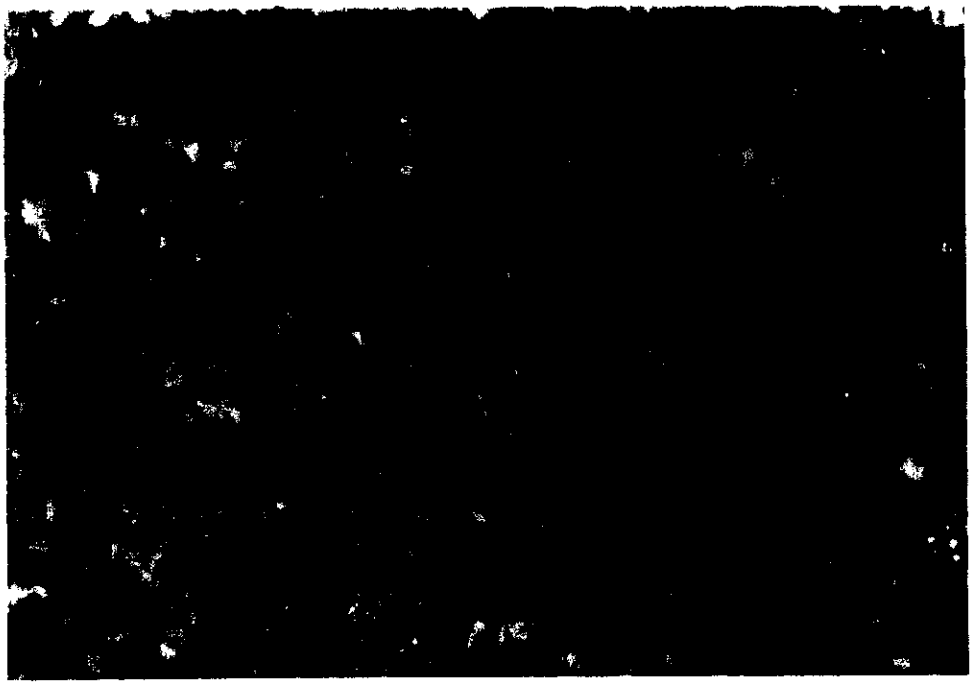


Figure (59): Thyroid lymphoma showing diffuse weak membrane staining of lymphoid cells (LCA immunohisto. x 20).

Table (26): **Immunohistochemical staining results for thyroglobulin in various types of malignant thyroid tumors.**

Histologic type	No of cases	+ve	%	-ve	%
Follicular carcinoma	10	9	90%	1	10%
Papillary carcinoma	18	14	77.8%	4	22.2%
Medullary carcinoma	12	--	--	12	100%
Anaplastic carcinoma	14	2	14.3%	12	85.7%
Lymphoma	2	-	-	2	100%

The immunoreactivity was obviously strong in (44.4%) of follicular carcinoma. In contrast 28.5% of papillary carcinoma cases showed strong positivity, while the anaplastic carcinoma only showed weak positivity in two cases (Table 27), Histogram (II).

Table (27): **Relationship between thyroglobulin immunoreactivity and various types of thyroid follicular cell carcinomas.**

Histologic type	No of cases	Reactivity degree +	%	++	%	+++	%
Follicular carcinoma	9	2	22.2%	3	33.3%	4	44.4%
Papillary carcinoma	14	6	42.9%	4	28.5%	4	28.5%
Anaplastic carcinoma	2	2	100%	--	--	--	--

Reactivity degree: Intensity

With regard the degree of differentiation there was clear increase in the number of positively stained cases with more differentiated tumor where 85% of well differentiated tumors were positively stained in contrast to 14.3% of anaplastic tumors. No significant differences between moderately differentiated and poorly differentiated group could be detected (Table 28), Histogram (III). However, the degree of intensity of the staining was obviously strong in well

differentiated tumor which got weaker in less differentiated tumors, where moderately differentiated tumors showed moderate stainability and poorly differentiated or anaplastic tumors, showed only weak staining intensity (Table 29), Histogram (IV).

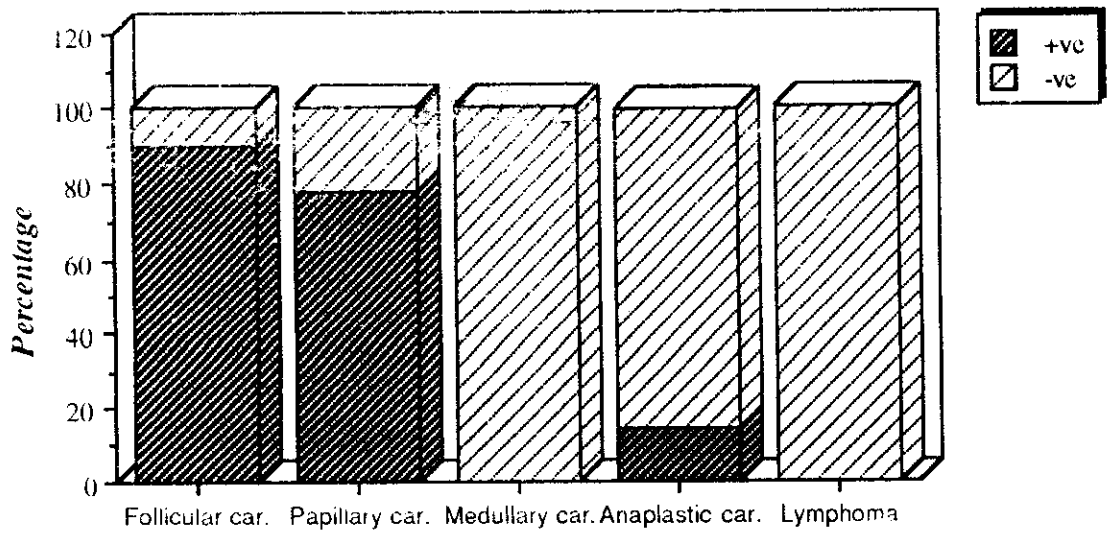
Table (28): The relationship between epithelial thyroglobulin immunostaining and degree of differentiation of various types of thyroid follicular cell carcinomas.

Histologic diagnosis	No of cases	+ve	%	-ve	%
Well differentiated	20	17	85%	3	15%
Moderately differentiated	3	2	66.7%	1	33.3%
Poorly differentiated	5	4	80%	1	20%
Undifferentiated	14	2	14.3%	12	85.7%

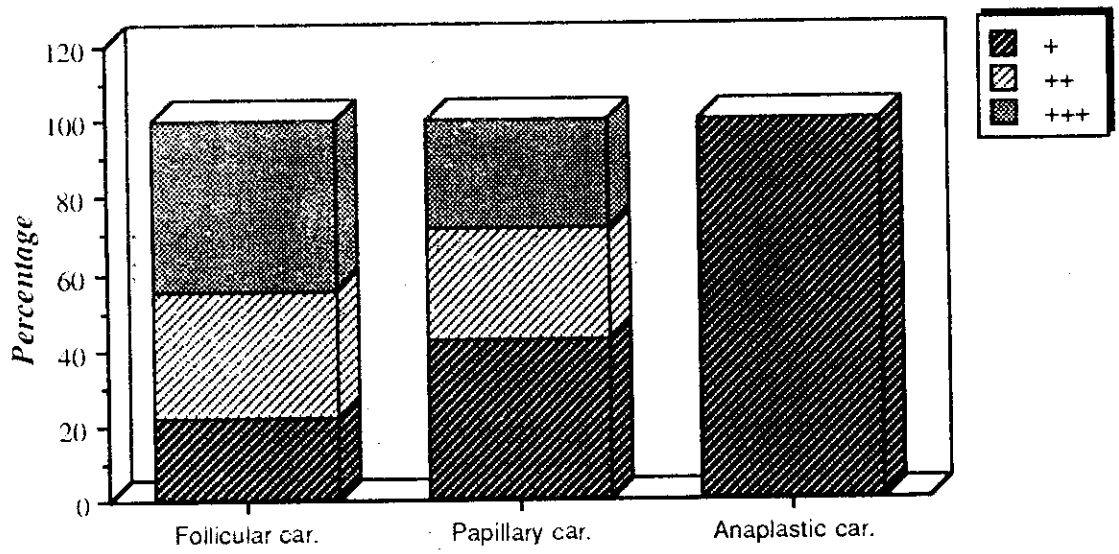
Table (29): Relation between epithelial thyroglobulin immunoreactivity and degree of differentiation of various follicular cell carcinomas.

Histologic diagnosis	No of cases	Reactivity degree +	%	++	%	+++	%
Well differentiated	17	4	23.5%	5	29.4%	8	47.1%
Moderately differentiated	2	-	-	2	100%	-	-
Poorly differentiated	4	4	100%	-	-	-	-
Undifferrentiated	2	2	100%	-	-	-	-

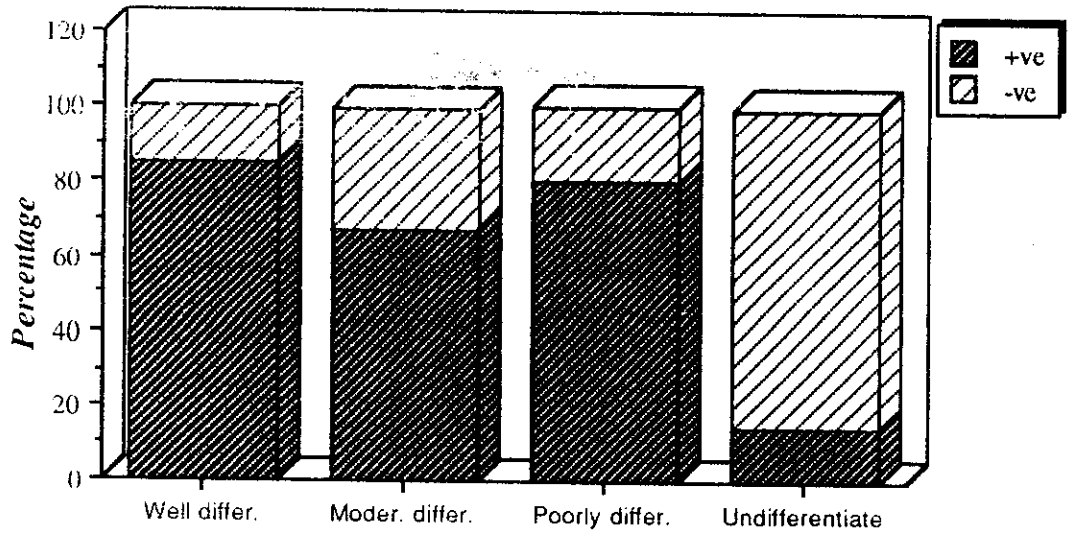
Reactivity degree: Intensity



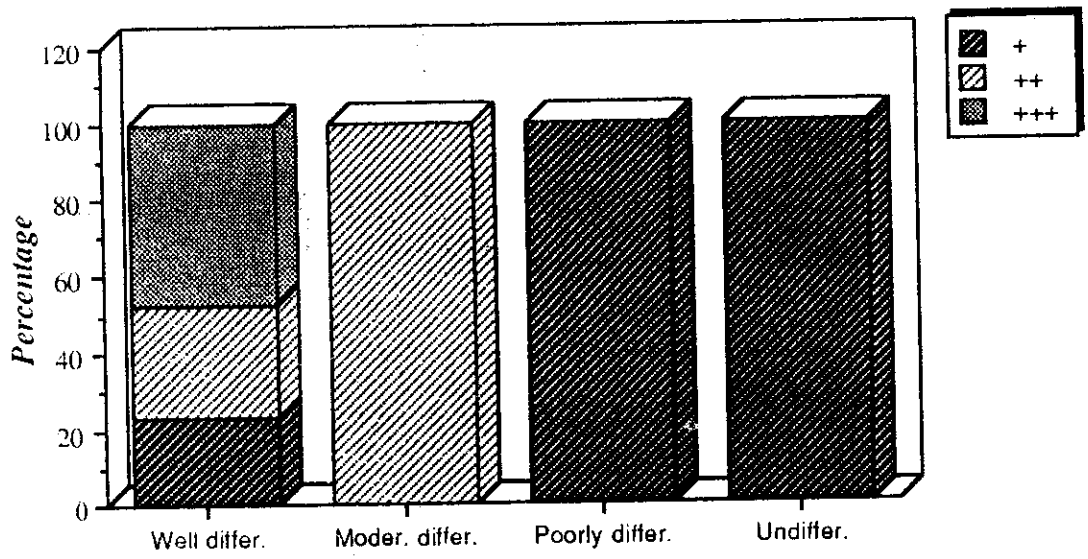
Histogram (I): ***Immunohistochemical staining result for thyroglobulin in various types of malignant thyroid tumor.***



Histogram (II): ***Relationship between thyroglobulin immunoreactivity and various types of thyroid folicular cell carcinomas.***



Histogram (III): *The relationship between epithelial thyroglobulin immunostaining and degree of differentiation of various types of thyroid follicular cell carcinomas.*



Histogram (VI): *Relationship between epithelial thyroglobulin immuno-reactvity and degree of differentiation of various thyroid follicular cell carcinomas.*

Study of CK Immunostaining in Benign and Malignant Thyroid Tumors

Monoclonal cytokeratin immunostaining showed that almost all thyroid tumors benign or malignant contain low molecular weight ck (Table 30). The anaplastic tumors were representing the lowest group showing positivity (64.3%), while the papillary carcinoma was the highest group showing positivity (88.9%) (Table 31), Histogram (V).

Table (30): Incidence of ck immunostaining in thyroid neoplasms

Tumor type	No of cases	+ve	%	-ve	%
Benign neoplasm	9	8	88.9%	1	11.1%
Malignant neoplasm	56	43	76.8%	13	23.2%

Table (31): Immunohistochemical staining results for ck in various types of malignant thyroid tumors.

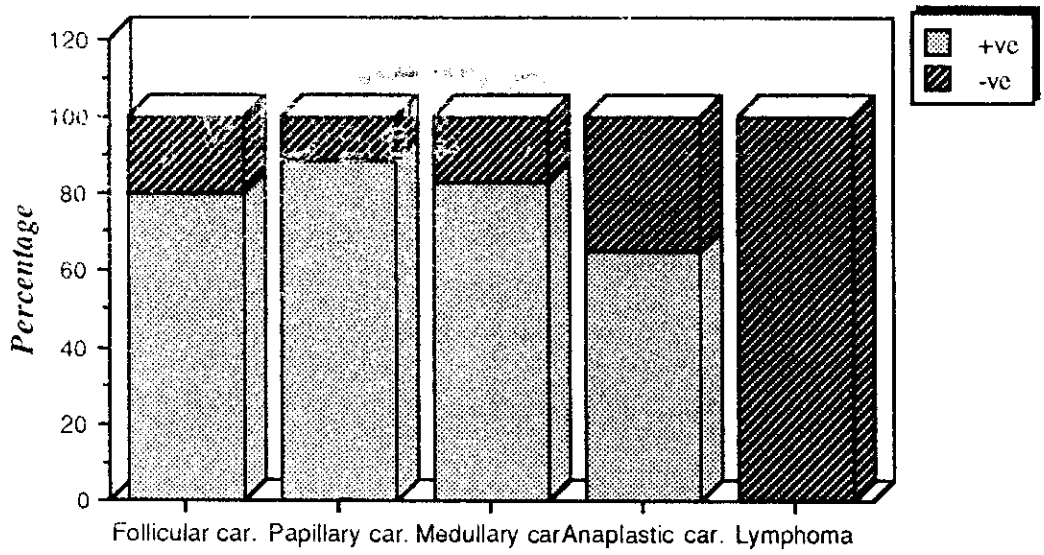
Histologic diagnosis	No of cases	+ve	%	-ve	%
Follicular carcinoma	10	8	80%	2	20%
Papillary carcinoma	18	16	88.9%	2	11.1%
Medullary carcinoma	12	10	83.3%	2	16.7%
Anaplastic carcinoma	14	9	64.3%	5	35.7%
Lymphoma	2	-	-	2	100%

Regarding the degree of reactivity, strong staining intensity was observed commonly in papillary carcinoma where 56.3% of cases was strongly stained while anaplastic tumor showed strong reactivity in 44.4% of cases. The follicular and medullary carcinomas were usually observing moderate degree of reactivity (Table 32), Histogram (VI).

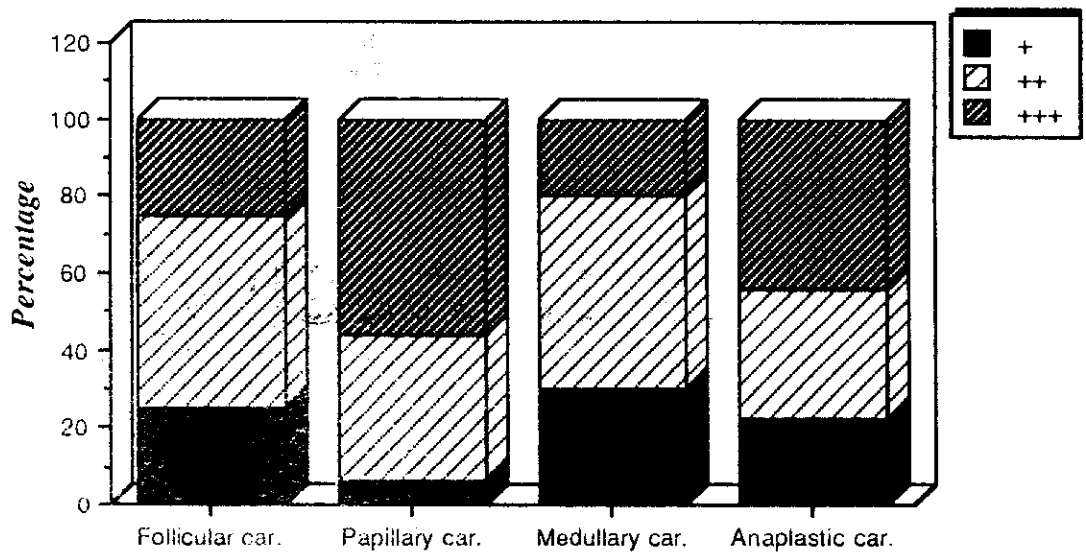
Table (32): **Relationship between cytokeratin immunoreactivity and various types of thyroid carcinoma.**

Histologic type	No of cases	Reactivity degree +	%	++	%	+++	%
Follicular carcinoma	8	2	25%	4	50%	2	25%
Papillary carcinoma	16	1	6.25%	6	37.5%	9	56.3%
Medullary carcinoma	10	3	30%	5	50%	2	20 %
Anaplastic carcinoma	9	2	22.2%	3	33.3%	4.	44.4%

Reactivity degree: Intensity



Histogram (V): ***Immunohistochemical staining results for CK in various types of malignant thyroid tumors.***



Histogram (VI): ***Relationship between cytokeratin immunoreactivity and various types of thyroid carcinoma.***

HISTOCHEMISTRY

Alcian blue

Thyroid tumors including benign or malignant lesions stained for alcian blue stain (pH 2.5) showed that all benign lesions were devoid of acid mucosubstances, while 35.2% of malignant lesions displayed a variable amount of mucin (Table 33).

Table (33): **Relationship between the presence of acid mucosubstances and type of thyroid neoplasms.**

Type of tumor	No of cases	+ve	%	-ve	%
Benign	5	-	-	5	100
Malignant	54	19	35.2%	35	64.8%

Mucin was found, most frequently, with papillary, medullary carcinomas (about 45% of the cases), it occurred somewhat less frequently with follicular carcinoma and rarely with anaplastic tumors (Table 34).

Table (34): **Mucin study in various types of malignant thyroid tumors.**

Tumor type	No of cases	+ve	%	-ve	%
Papillary carcinoma	18	8	44.4%	10	55.6%
Follicular carcinoma	10	3	30%	7	70%
Medullary carcinoma	12	7	58.3%	5	41.7%
Anaplastic carcinoma	14	1	7.1%	13	92.9%

The amount of mucinous substances varied considerably from one case to the another. Eight cases were weakly positive and 8 were moderately positive and only 3 tumors were strongly positive, one of them showed follicular differentiation and 2 were papillary carcinoma, the last one was medullary carcinoma (Table 35). In papillary and follicular carcinomas the mucin was often extracellular either in the colloid or along the luminal surfaces of the papillae and follicles, but it may be cytoplasmic (Fig. 60, 61). The distribution of mucin in medullary carcinoma was observed also commonly in extracellular spaces and less often intracellulary. The case of anaplastic tumor; showed positive staining was consisted of giant cells, many of them showed clear vascuolated cytoplasm.

Table (35): **Relationship between degree of Mucin positivity and the type of malignant tumor.**

Tumor type	No of cases	Strong +ve		Moderate +ve		Weak +ve	
		No.	%	No.	%	No.	%
Papillary carcinoma	8	1	12.5%	3	37.5%	4	50%
Follicular carcinoma	3	1	33.3%	1	33.3%	1	33.3%
Medullary carcinoma	7	1	14.3%	4	57.1%	2	28.6%
Anaplastic carcinoma	1	-	-	-	-	1	100%

Congo Red

Congo red reaction in the form of organ red colour was demonstrated in all cases of medullary thyroid carcinoma and observed in 4 cases of anaplastic tumors. The distribution of amyloid positive material in medullary carcinomas varied considerably from one case to another. In few cases the amyloid was demonstrated within the cytoplasm as well as extracellulary within the stroma, some patches of the stroma stained positively for amyloid also showed positive calcitonin immunoreactivity.

Grimelius, Silver Stain

Silver stain for argyrophilia was demonstrated in 10 out of 12 cases of medullary carcinoma. The distribution of positively stained cells had nearly a similar pattern of calcitonin immunostaining (Table 36).

Table (36): **Argyrophilic staining pattern of medullary carcinoma in accordance to the virulence of tumor type.**

Tumor type	No of cases	Distribution 0	+	++	+++
Localized	4	-	-	2	2
Metastatic	5	-	1	1	3
Recurrent	3	2	1	-	-

Argyrophilic silver stain showed positive tumor cells in 2 cases showed histologically a squamoid and giant cell pattern (Table 37). The staining was always focal and confined to a few tumor cells and appeared in the form of coarse cytoplasmic granules. The pattern was very different from the fine diffuse homogeneous staining seen in the medullary thyroid carcinoma used as control. Curiously a pattern of fine cytoplasmic granularity was seen only in areas of obvious necrosis.

Table (37): **Study of argyrophilic reactivity in medullary and anaplastic carcinomas.**

Tumor type	No of cases	+ve	%	-ve	%
Medullary	12	10	83.3%	2	16.7%
Anaplastic	14	2*	14.3%	12	85.7%

* ***Positivity was observed as focal coarse cytoplasmic granules.***

Figure (60): Papillary carcinoma showing positive staining over luminal surface of the papillae *(arrow)* (Alcian blue x 20).

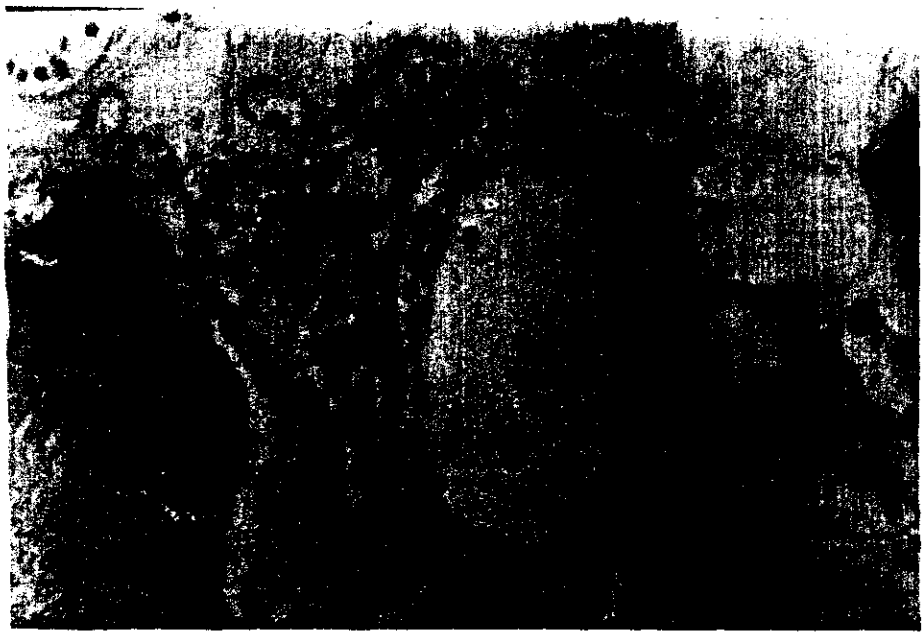


Figure (61): Follicular carcinoma showing positive staining of colloid and over the luminal surface of follicular cells *(arrow head)* (Alcian blue x 20).

ELECTRON MICROSCOPY

Two anaplastic cases were selected for DEM study. The first case composed histologically of purely spindle cells simulating sarcomatous tumors. It was associated with well differentiated follicular carcinoma with transitional zone. Immunohistochemical staining showed positive staining for ck in few isolated cells which may not be sufficient to conclude that the tumor was of epithelial origin. The semithin sections stained with toluidine blue showed clusters of bizarre-shaped cells with features of cellular and nuclear anaplasia with intervening collagenous and osteoid like matrix (Fig. 62). Some of these cells were in close contact to each other (Fig. 63), which gave suggestion of cellular junction between them. The examination of ultra-thin sections showed large nuclei with irregular contour, moderately rich clumped chromatin, deep indentation of nuclear membrane and very bizarre in shape and prominent nucleoli. There was intranuclear filamentous structure which has been also observed intracytoplasmic, which could be representing intermediate filaments (Fig. 64).

Generally the cytoplasm was relatively abundant in organelles where the free ribosomes, polyribosomes and flat profile of rER were numerous and scattered randomly in the cytoplasm of most of the tumor cells (Fig. 65). However, in few cells, the cytoplasm was abundantly filled with mitrochondria and well developed rough endoplasmic reticulum (Fig. 66). The specialized cell junctions like desmosomes and hemidesmosomes and short stunted microvilli projecting in irregularly shaped intercellular spaces were observed rarely (Fig. 67). These features were considered conclusive for epithelial differentiation of tumor cell. A collagen stroma with frequent electron dense calcium deposit or intervening electron dense osteoid-like matrix were observed infrequently (Fig. 68).

The second case composed histologically of diffuse small cells and diagnosed primarily as small cell anaplastic carcinoma. Immunohistochemical staining for LCA gave patchy cytoplasmic staining with membranous localization in few cells. Although the tumor was negatively stained for ck, which indicate that the tumor was not epithelial but the staining pattern for LCA was not absolutely conclusive that the tumor was of lymphoid origin. The semithin sections stained with toluidine blue showed malignant cells with hyperchromatic nuclei, scanty cytoplasm, ill-defined cell border (Fig. 69). These histologic features were suggestive for lymphoid neoplasm. These cells inducing partial destruction and replacement of lining cells of normal thyroid follicles (Fig. 70). Examination of ultra-thin sections showed that the nucleus take the characteristic prominent pattern of heterochromatin (clumped chromatin) which commonly subjacent to the nuclear envelop, deep cleavage and nuclear pocketing commonly seen in lymphoid tumor. There was a relatively small cytoplasmic volume and paucity of organelles which was consisted mostly of free ribosomes and polyribosomes and few scattered mitochondria in some cells (Fig. 71, 72). No specialized cellular junction could be detected even between cells closely packed together (Fig, 73).

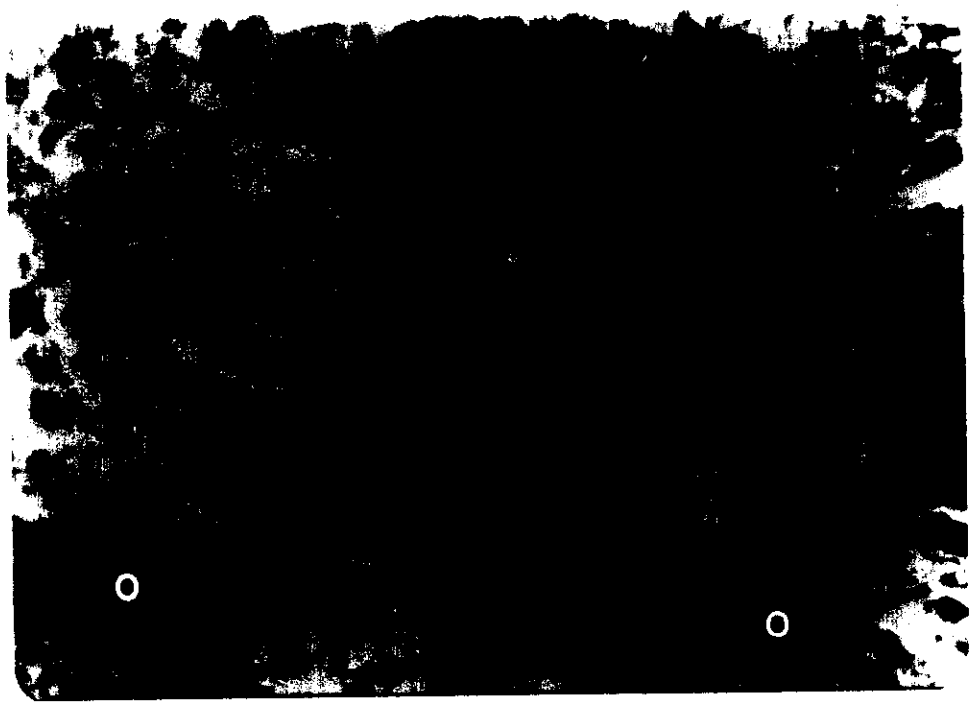


Figure (62): Semithin Epon section showing population of anaplastic spindled-shaped cells with intervening collagenous, and dense basophilic osteoid like matrix (0) (Toluidin blue x 40).

Figure (63): Semithin Epon section (closer view) showing aggregate of anaplastic cells in close contact to each other (Toluidin blue x 1000).

Figure (64): *Two malignant cells with bizarre shape large nuclei (N) showing irregular contour, deep identations and prominent nucleolus (nl). The cytoplasm showing abundant free ribosomes and polysomes (r) intermixed with profile of rough endoplasmic reticulum (rER). Apparent intranuclear filamentous structure as well as cytoplasmic condensation are present (arrow). (Uranyl-acetate lead x 4500).*

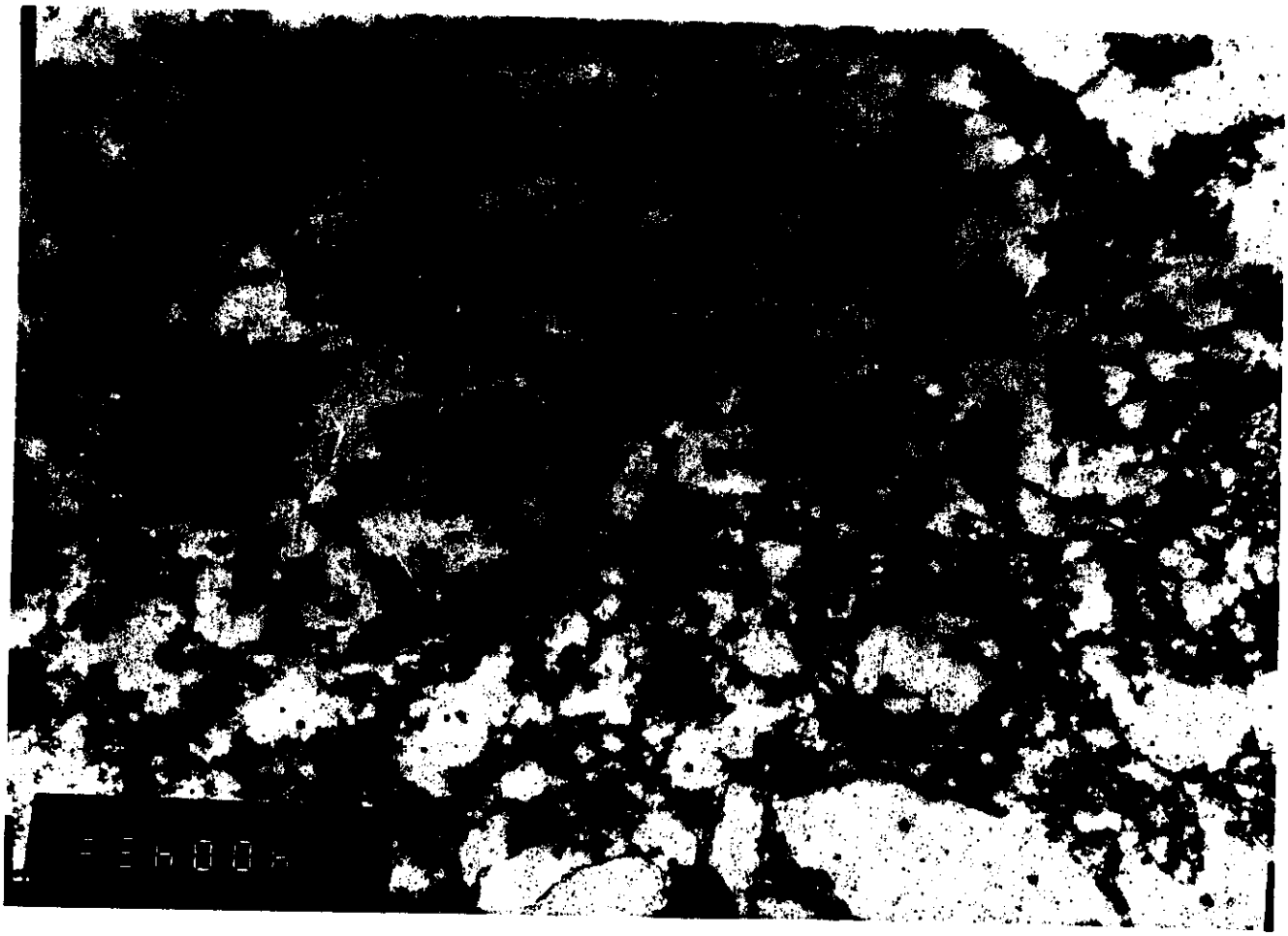


Figure (65): Higher power showing rudimentary rough endoplasmic reticulum (rER); many are inclose contact to nuclear membrane (arrow head) indicating higher rate of cellular proliferate-cytoplasm is filled with free ribosomes and polysomes (r). Small filamentous structures (arrow) could be tonofilament (Uranyl - acetate lead, x 6000). N = nucleus.

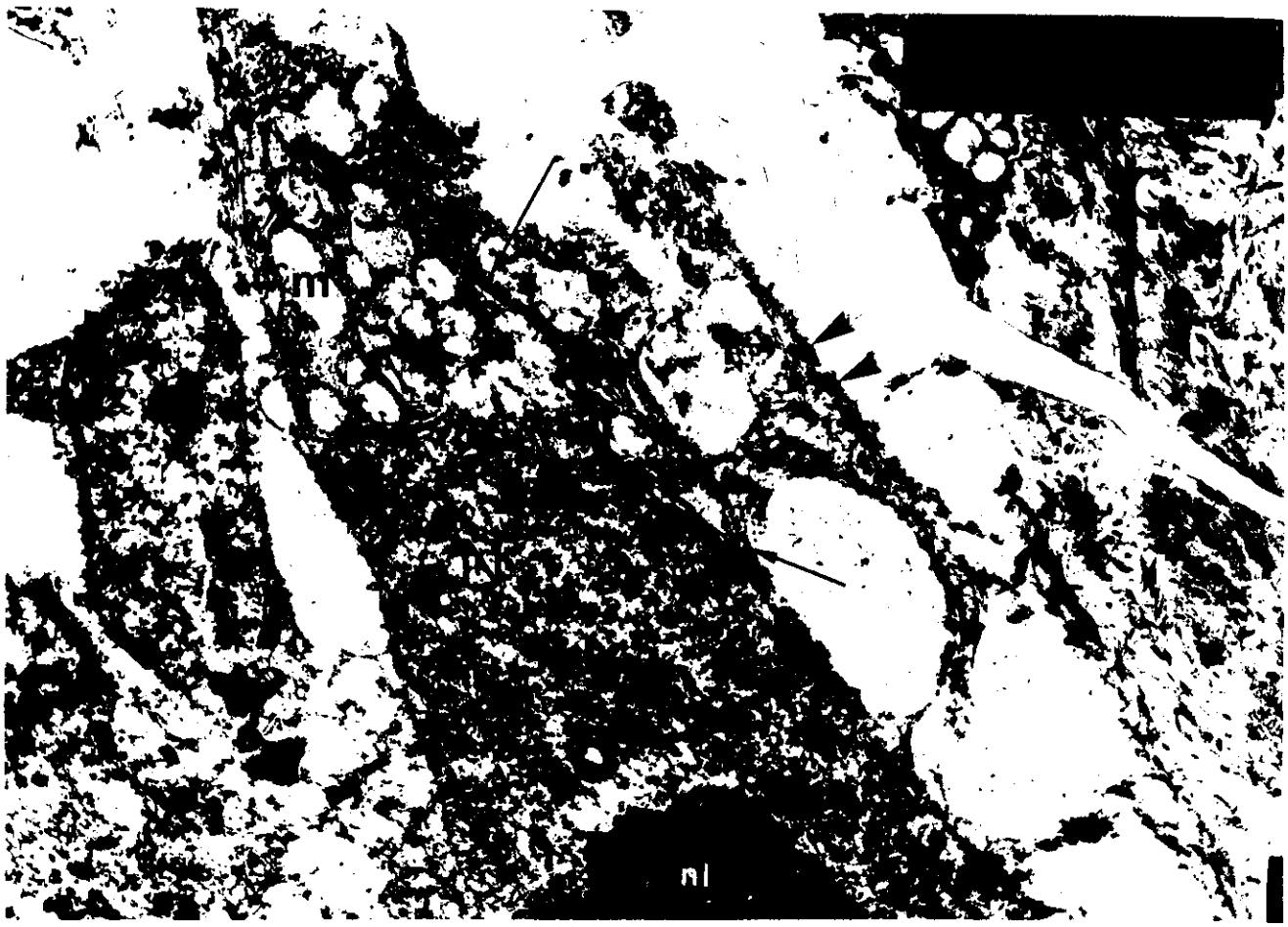


Figure (66): Malignant cells with large bizarre nucleus (N) and prominent nucleolus (nl). Cytoplasm filled with mitochondria (m) and rough endoplasmic reticulum (arrow). hemidesmosomes are observed (arrow head) between the cells and surrounding collagenous matrix (C) (Uranyl - acetate lead x 4000).

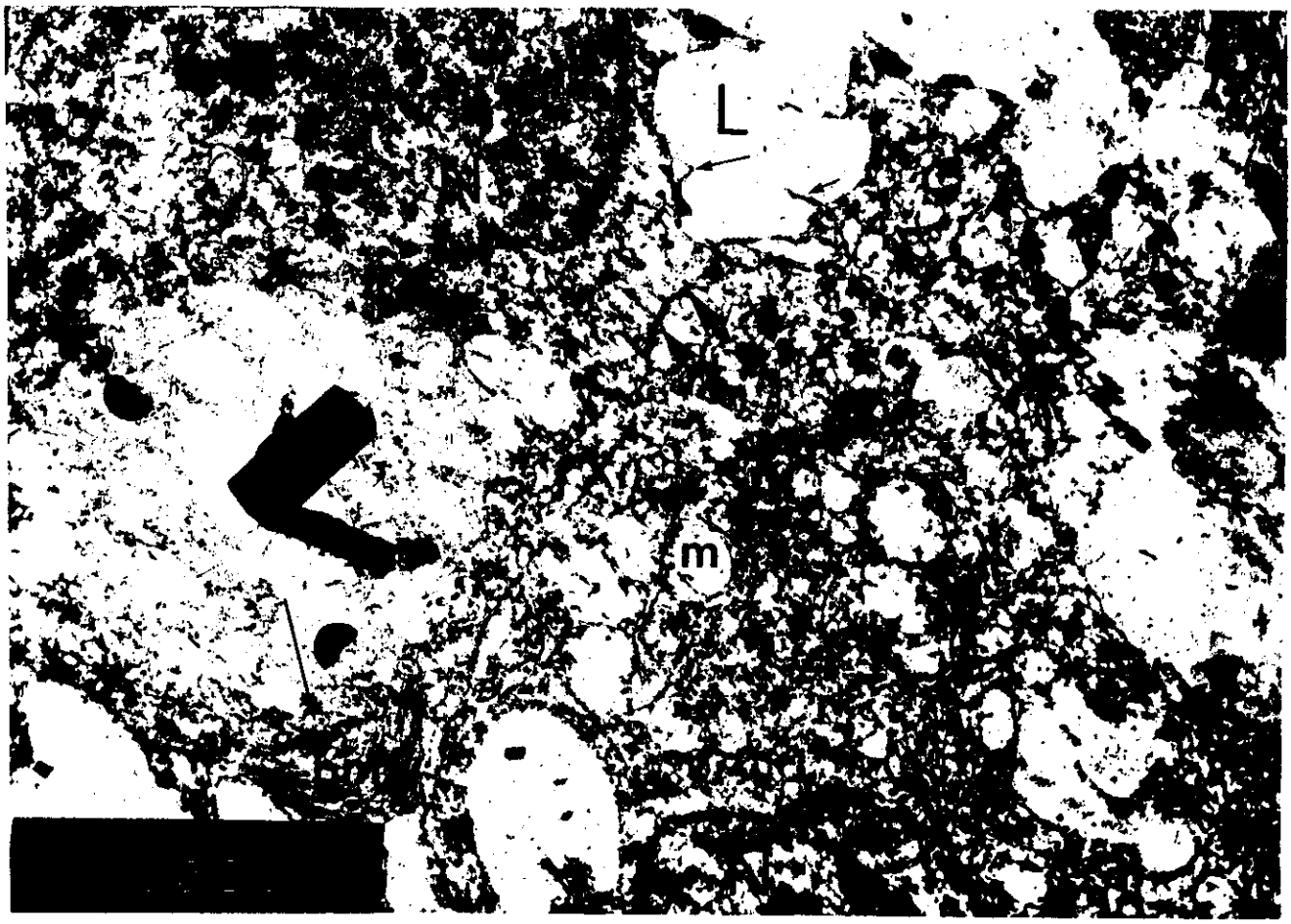


Figure (67): *Two tumor cells displaying short rudimentary, microvilli (arrow) projecting within luminal space (L). Specialized cellular function, desmosomes (arrow head) between the two cells and hemidesmosomes (arrow) between the cell and thickened basement membrane (bm). N: nucleus, m: mitochondria (Uranyl-acetate lead x 4000).*

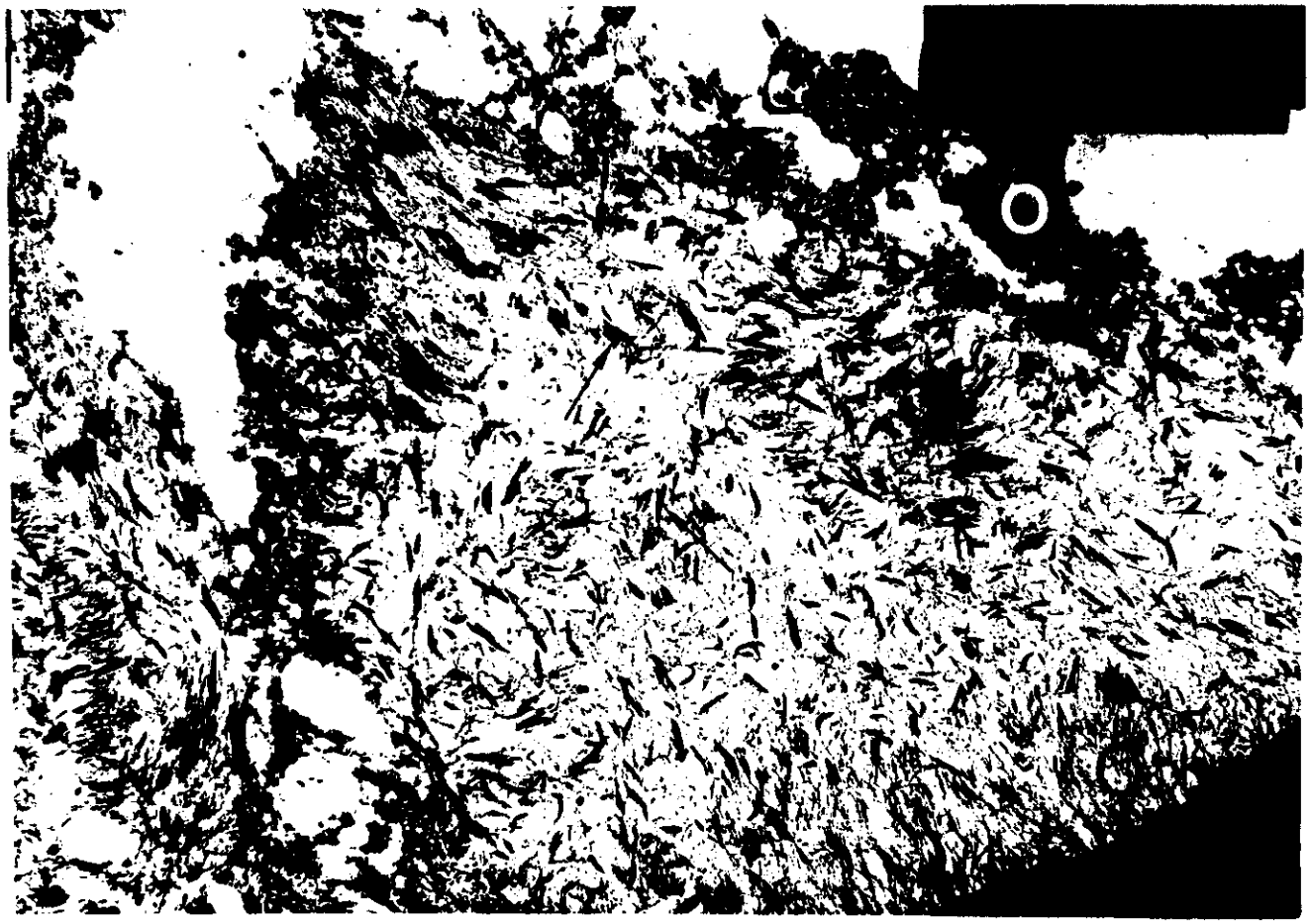


Figure (68): Stroma between tumour cells showing collagen (C) matrix with diffuse electron dense calcium (hydroxy apetite crystals) deposits (arrow). Intervening of electron dense osteoid like matrix (0) is seen. (Uranyl acetate lead x 6000).

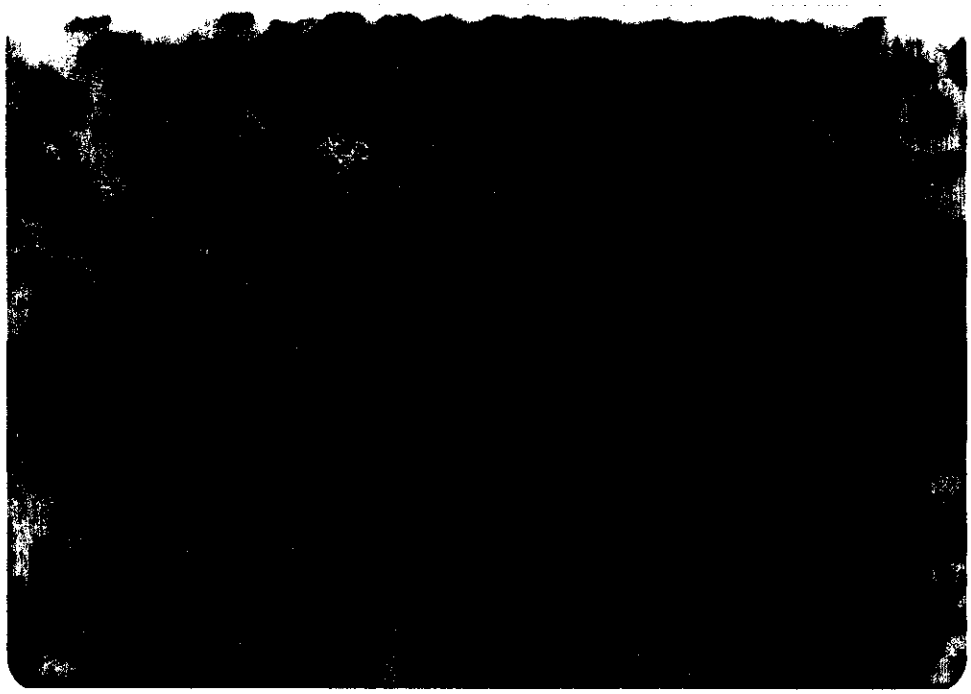


Figure (69): *Semithin Epon section showing normal thyroid follicle surrounded by cells with hyperchromatic nuclei, ill defined cell border and scanty cytoplasm (Toluidin blue x 40).*

Figure (70): *Semithin Epon section showing clusters of neoplastic cells (arrow) which represent either neoplastic transformation or replacement of follicular cells of normal thyroid follicle (CF) (Toluidin blue x 40 "A" x 1000 "B").*

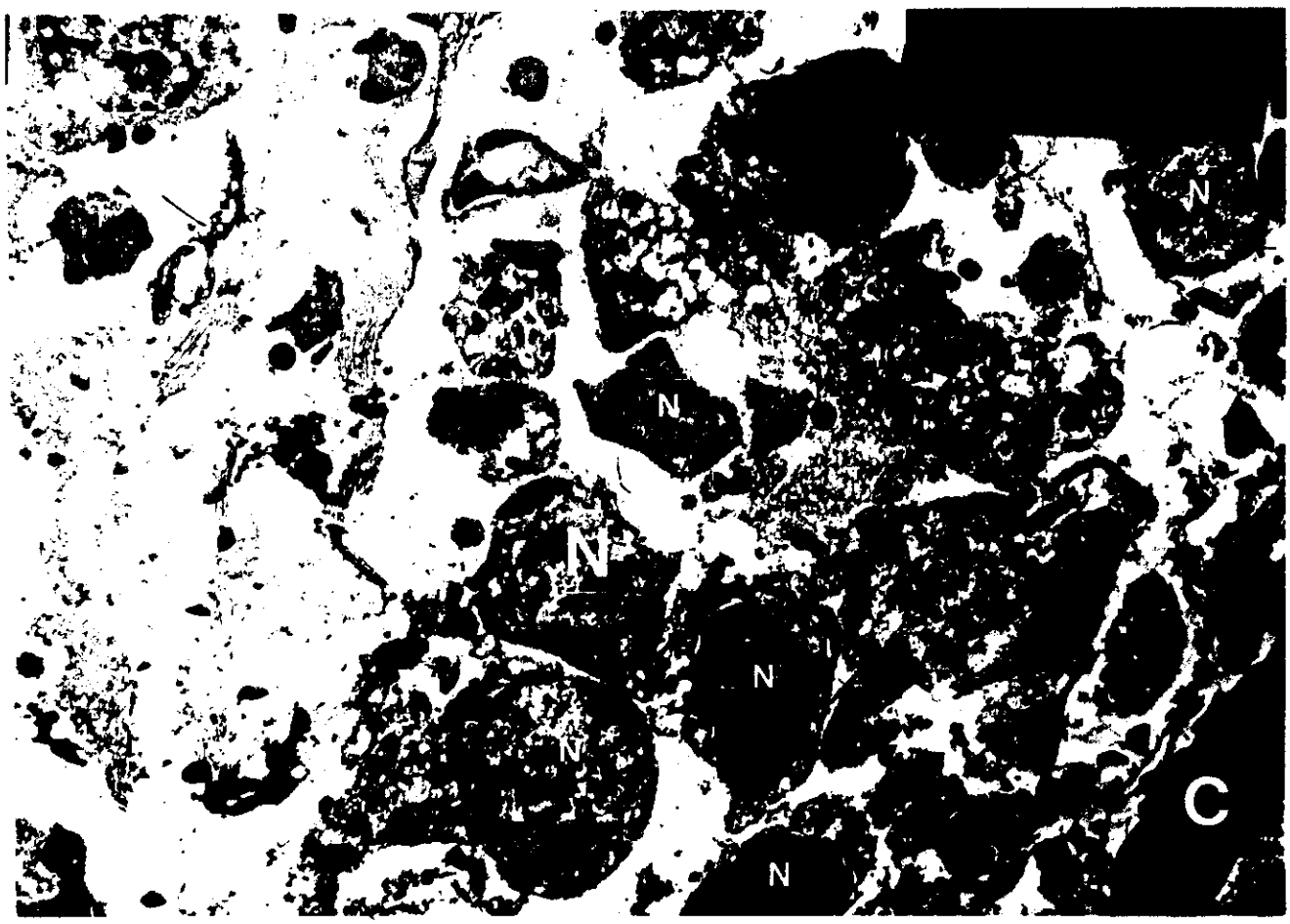


Figure (71): *Group of small and intermediate sized lymphocytes representing the clusters of neoplastic cells in previous figure. They are closely packed together, however, no apparent cellular junction, microvilli or interluminal space. N: nucleus, C: colloid (Uranyl acetate lead x 3500).*

Figure (72): *Group of lymphoid cells, with large nuclei (N) showing nuclear cleavage (arrow) and nuclear pocketing (arrow head) inconspicuous, nucleoli, heterochromatin is subjacent to the nuclear membrane. Cells showing minimal amount of cytoplasm filled with numerous ribosomes and polyribosomes (r) however other cytoplasmic organelles are absent or sparsely present, m: mitrochondria (Uranyl acetate lead x 5000).*

Figure (73): High power view, showing absence of intercellular junction, instead the two cells are in close contact to each other. N: nucleus, r: ribosomes, m: mitochondria (Uranyl-acetate lead x 6000).