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ORIGINAL ARTICLE

CHROMGRANIN-A EXPRESSION IN COLORECTAL CARCINOMAS; ASSESSING ITS PROGNOSIC SIGNIFICANCE

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Aim: *The frequency and clinical significance of neuroendocrine features in conventional carcinomas has not been settled since few studies have been performed with conflicting results. The aim of this prospective study is to investigate neuroendocrine differentiation in colorectal carcinomas in relation to its prognostic significance.*
Methods: *The resected specimens of sixty-two colorectal cancer patients were examined grossly and microscopically for histopathology, grades and stages. For neuroendocrine cell detection, immunohistochemical staining with anti-chromogranin A monoclonal antibody (Dako A/S, Denmark, cat. No MO869) was done. The distribution of positively stained cells was evaluated and divided into focal and diffuse patterns. The pattern of chromogranin a staining was correlated with the histologic type, grade, stage, disease free survival and overall survival.*
Results: *Focal chromogranin a expression was detected in 71% of cases and diffuse staining in 29% of cases. Diffuse staining pattern of chromogranin A indicated more neuroendocrine differentiation of tumor tissue and was significantly correlated with histologic type, high grade and advanced stage of the tumors. Also, diffuse staining significantly lowered disease-free survival and overall survival; however, staging was the main predictor of survival*
Conclusion: *Chromogranin A is a sensitive and specific neuroendocrine marker. Diffuse chromogranin A positivity appears to bear a poor prognosis in patients with colorectal cancers.*

Keywords: *Neuroendocrine tumors, Immunohistochemistry, Colorectal cancer.*

INTRODUCTION

Neuroendocrine cell (NEC) carcinomas are occasionally accompanied by adenocarcinomas in the gastrointestinal tract but the relationship between these two distinct tumors is unclear.(1) Morphologically, such lesions are classified into two subgroups: composite-type tumors, in which both components appear to be mixed haphazardly(2,3) and collision-type tumors, which are considered as double tumors with a "side by side" or "one upon another" pattern.(4,5) Two hypotheses have arisen regarding the mechanism for the association of adenocarcinoma and NEC carcinoma. One is that both are derived from a common multipotential epithelial stem cell, the NEC carcinoma component resulting from differentiation from the adenocarcinoma to the NEC phenotype during tumour progression.(6) The second hypothesis is that adenocarcinoma and NEC carcinoma arise from a multipotential epithelial stem cell and a primitive NEC, respectively, and that they exist next to each other coincidentally.(7) Chromogranin A (cga), a marker for tissues with neuroendocrine features, was originally isolated as the major secretory protein of adrenal medulla chromaffin granules.(8) While the precise function of cga is unknown, recent data suggest that it may play a critical role in the maturation of secretory granules.(9) Immunoreactivity of this 71–kD acidic protein has now been extensively characterized and shown to be specifically present in normal and malignant cells of the diffuse neuroendocrine system, so that, chromogranin stain has become the most widely used pan-endocrine marker.(10)

In this study, we have used immunohistochemistry to characterize the expression of cga in a variety of of adenocarcinomas of the colon that unexpectedly express cga and to determine the prognostic relevance of neuroendocrine differentiation in these patients.

PATIENTS AND METHODS

This study included 62 colorectal cancer patients who were treated in Mansoura University Hospital (Colorectal Surgery Unit) during January 2002 to December 2004. Staging of the disease was according to modified Dukes' classification.[11] After operations, the resected specimens were examined grossly and sent for histopathology. The resected specimen was opened along its longitudinal dimension and fixed in 10% formalin. The central tissue slice taken from each tumor contained the largest longitudinal dimension. Hematoxylin and eosin stained sections of each tumor were examined by light microscopy for the original histological diagnosis and grading of the tumors. The tumors were graded according to Broders system.[12]

Immunohistochemical staining: For immunohistochemical staining, 4-µm sections were cut from the paraffin blocks prepared from the resected specimens and stained with anti Chromogranin-A monoclonal antibody (Dako A/S, Denmark, cat. No MO869) using peroxidase-antiperoxidase (PAP) technique described by Sternberger et al (13). Detection kits used in this study were Histostain- SP (peroxidase) broad spectrum-DAB (diamino-benzidine) kits (Zymed, USA, cat No 95-9643). Chromogranin-A positivity appeared as fine intracytoplasmic granules. The percentage of positively stained cells counted in 100 tumor cells in randomly selected high power fields (x400) was calculated. According to WHO classification,[14] when at least 30% of tumor cells show positive staining, the tumor will be "neuroendocrine tumor". For interpretation, staining will be considered focal when percent of stained cells is less than 30% and diffuse if it is more than 30%. Slides prepared from human adrenal gland and stained with anti Chromogranin-A monoclonal antibody were used as positive control. In negative control slides, the primary antibody was replaced by normal mouse serum.

All patients were followed up for two years for incidence of recurrence (local or peritoneal) or metastasis (hepatic). Disease free survival and over-all survival were reported.

Statistical methods: The Findings assessed were calculated as numbers, simple percentages and mean ± standard deviation. Statistical analysis was done using Chi-square test, log rank test and Cox regression analysis.

RESULTS

The study included 62 colorectal cancer patients (40 colonic and 22 rectal). They were 35 males (56.5 %) and 27 females (43.5 %). The age of the patients ranged from 24 – 76 years with mean age of 49.32 ± 14.98 years. Gross examination of the resected specimens revealed ulcerative form in 29 patients (46.8 %), annular in 15 patients (24.2 %) and cauliflower lesions in 18 patients (29.0 %). Microscopic examination diagnosed adenocarcinoma in 55 patients (88.7%) and graded it into grade 1 (10 patients) (16.1 %), grade 2 (35 patients) (56.5%) and grade 3 (10 patients) (16.1%), on the other hand, 7 patients showed undifferentiated carcinoma (grade 4) (11.3 %). Forty-six patients had Dukes' stage B (74.2%) and 16 patients had Dukes' stage C (25.8%). Table 1.

Table 1. Clinico-pathological Features of the studied cases (n=62).

		No	%
Age	≤ 45	25	40.3%
	> 45	37	59.7%
Sex	male	35	56.5%
	female	27	43.5%
Site	Colon	40	64.5 %
	Rectum	22	35.5 %
Gross Appearance	Ulcer	29	46.8 %
	Annular	15	24.2 %
	Cauliflower	18	29.0 %
Pathology	Adenocarcinoma	55	88.7 %
	Undifferentiated	7	11.3 %
Grade	1	10	16.1 %
	2	35	56.5 %
	3	10	16.1 %
	4	7	11.3 %
Stage	B1	25	40.3 %
	B2	21	33.9 %
	C1	11	17.7 %
	C2	5	8.1 %

On immunohistochemical staining with chromogranin-A monoclonal antibody, 44 patients (71%) showed focal staining (Figs. 2,3) and 18 patients (29%) showed diffuse staining (Figs. 4 & 5).

Thirty-six patients had no recurrence (58%), local recurrence occurred in 8 patients (12.9%), peritoneal recurrence in 5 patients (8.1%) and hepatic metastasis in 13 patients (21%).

There was significant association between pattern of staining (focal or diffuse) and location (P=0.001), gross appearance (P=0.009), histological type (P=0.001), grade (P=0.001), stage (P=0.001) and incidence of recurrence (P=0.001). Table 2.

Table 2. Correlation between clinico-pathological features and chromogranin staining of studied patients (n = 62).

		Focal (n=44)	Diffuse (n=18)	Chi-square test	
				X2	P
Site	Colon	34 (54.8%)	6 (9.7%)	10.773	0.001(S)
	Rectum	10 (16.1%)	12 (19.4%)		
Gross Appearance	Ulcer	25 (40.3%)	4 (6.5%)	9.455	0.009(S)
	Annular	11 (17.7%)	4 (6.5%)		
	Cauliflower	8 (12.9%)	10 (16.1%)		
Pathology	Adenocarcinoma	44 (71%)	11 (17.7%)	19.289	0.0001(S)
	Undifferentiated	-	7 (11.3%)		
Grade	1	10 (16.1%)	-	34.612	0.0001(S)
	2	31 (50%)	4 (6.5%)		
	3	3 (4.8%)	7 (11.3%)		
	4	-	7 (11.3%)		
Stage	B1	22 (35.5%)	3 (4.8%)	36.269	0.0001(S)
	B2	20 (32.3%)	1 (1.6%)		
	C1	1(1.6%)	10 (16.1%)		
	C2	1 (1.6%)	4 (6.5%)		
Recurrence	None	36 (58%)	-	41.989	0.0001(S)
	Local	4 (6.5%)	4 (6.5%)		
	Hepatic	1 (1.6%)	12 (19.4%)		
	Peritoneal	3 (4.8%)	2 (3.2%)		

Mean survival time between studied patients was 17.1 ± 1.0 months. There was significant association between pattern of chromogranin A staining (focal or diffuse) and patient's overall survival (P= 0.0505) (Fig. 1).

Tumor grade, stage and chromogranin pattern of staining were entered in Cox regression using enter method without stratification where the main predictor of survival was staging of the disease followed by diffuse chromogranin-A staining, and lastly, tumor grading. Table 3.

Table 3. Survival predictors (Cox regression analysis).

	P
Grade	0.763
Stage	0.018
Chromogranin	0.661

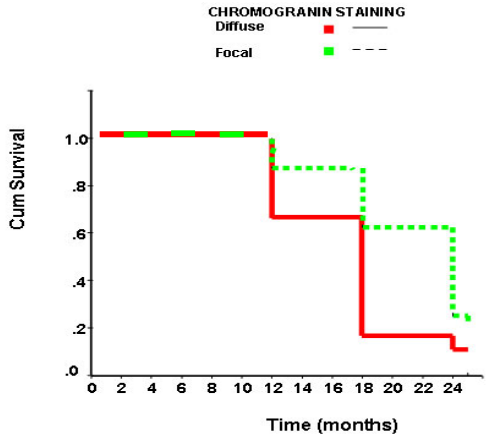

Fig 1. Overall survival in focal and diffuse chromogranin staining (log rank test {P= 0.0505}).

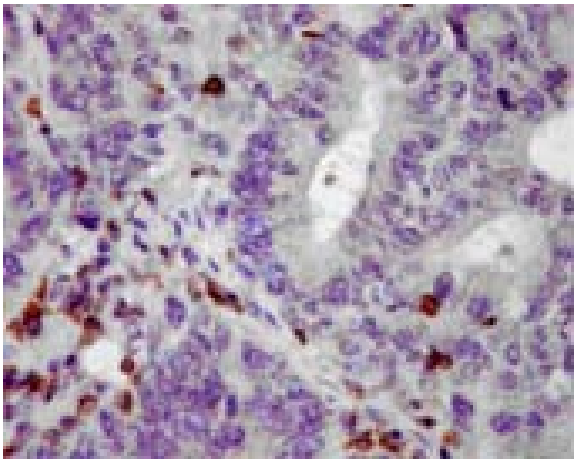

Fig 2. Grade I adenocarcinoma with focal cga staining (10%) (Immunoperoxidase, DAB x 400)

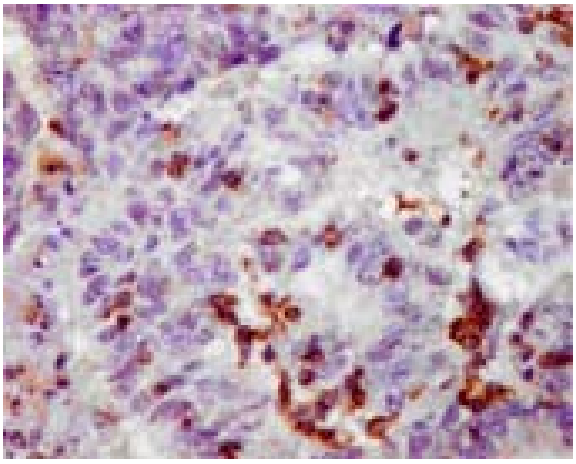

***Fig 3. Grade II adenocarcinoma with focal cga staining (20%)** (Immunoperoxidase, DAB x 400).*

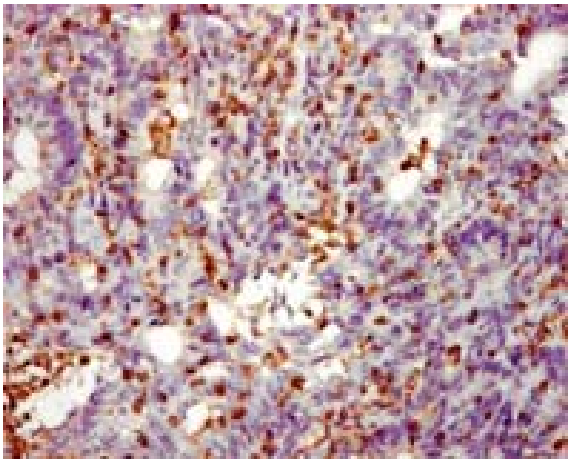

***Fig 4. Grade III adenocarcinoma with diffuse cga staining (70%)** (Immunoperoxidase, DAB x 100).*

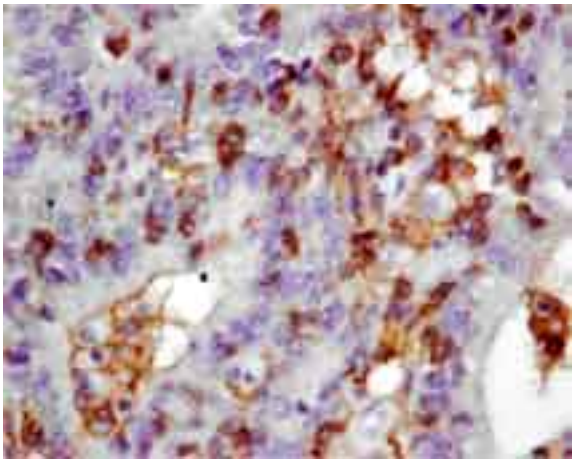

***Fig 5. Grade II adenocarcinoma with diffuse cga staining (40%)** (Immunoperoxidase, DAB x 400).*

DISCUSSION

Neoplastic proliferations of neuroendocrine cells (NEC) may occur throughout the entire GI tract. It encompasses a heterogeneous gross and microscopic structural spectrum, ranging from inconspicuous microproliferations ("mucous membrane nevi") to bulky tumor masses.[15]

In this prospective study, we searched for neuroendocrine cell differentiation within colorectal cancer specimens which was diagnosed as adenocarcinomas and undifferentiated carcinomas by using Chromogranin A (cga) monoclonal antibody (Carcinoid tumors were excluded). Both light and electron microscope studies have shown a possible relationship between cga immunoreactivity and Grimeliu's argyrophilia. In a literature, the presence of argyrophil cells, which are detected by argyrophil stain, did not influence the prognosis of patient with colorectal carcinomas.[16] On the other hand, other authors reported that colorectal carcinomas with positive enterochromaffin cells showed more aggressive behavior than tumors without these cells.[17] In fact, positive cells containing neurosecretory granules were more easily detected by cga than by Grimueliu's staining.

The mean age of the patients was lower by two decades than reported worldwide.[18] Also, undifferentiated carcinoma represented 11.3% which is much higher than reported in literature but moderately differentiated adenocarcinoma was the same as reported in the same study.[19]

Lee et al[20] suggested that the measurement of cga may prove to be a useful tool by which biologically distinct subsets of tumors that may arise during normal colonic mucosal maturation may be recognized.

Based on the degree of immunoreactivity, tumors were divided into group 0 (<2% cells stained positive) and group 1 (>2% cells stained positive).[21] The recent WHO classification recommends to use the term of neuroendocrine tumor when the tumors containing at least 30% of obviously neuroendocrine cells; some authors recommend to use higher thresholds, of at least 50%, in order to avoid overdiagnosis.[14] In our series, pattern of staining was divided into diffuse (29%) where more than 30% of cells gave positive immunoreactivity and focal staining (71%).

Diffuse staining was encountered in much little manner in colon carcinomas but more in rectal carcinoma, also it was encountered infrequently with annular and ulcerative lesions but frequently observed in cauliflower lesions. We noticed that there is significant correlation between poorly and undifferentiated tumors and diffuse pattern of staining. The statement "cauliflower lesions are proliferative in nature, well differentiated with less lymphatic and local spread and of good prognosis"[22] does not go in hand with our results.

Diffuse staining had also a significant association with advanced stage of tumors and incidence of metastasis and recurrence. These results go in hand with results obtained by many authors.[23-25] One of the authors[23] stated that neuroendocrine cell carcinoma is a rare malignant tumor similar in histology, behavior and histochemistry to oat cell carcinoma of the lung. It is an extremely aggressive tumor and shows wide dissemination at the time of its discovery. In another study, patients with numerous endocrine cells had a significantly worse prognosis than patients without endocrine cells[24].

However, some literatures reported conflicting results; one of these publications[26] showed that the presence or absence of chromagranin positive cells did not influence the prognosis. The other one[27] did not find a significant correlation between cga expression and any of the clinicopathological parameters (tumor type, tumor grade, Dukes stage, and survival time). However, a significant positive correlation was observed between cga and BCL2 expression. These findings indicate that BCL2 may be involved in neuroendocrine differentiation in addition to its role in protecting cells from apoptosis.

In our study, diffuse pattern of chromogranin staining significantly affected patient's survival ($P = 0.05$).

Our results are similar to many literatures,[21,24,28] one of these publications[21] correlated the survival with the extent of neuroendocrine differentiation and concluded that neuroendocrine differentiation is often seen in small cell undifferentiated colorectal cancer and is correlated with a more aggressive course of the disease. Another author[28] reported a case of a midgut neuroendocrine tumor metastatic to the breast. Immunohistochemical analysis showed strongly positive cga staining.

In one of the recent studies,[29] the median survival for neuroendocrine carcinomas were 16.4 months. The study concluded that colorectal neuroendocrine tumors are extremely rare showing biologically aggressive behavior. Nevertheless, improved survival may be achieved by aggressive multimodality therapy.

Another study stated that poorly differentiated (PD) adenocarcinoma often retains the capacity for neuroendocrine (NE) cell differentiation. It is important to detect the presence of NE cell differentiation in advanced colorectal carcinomas because these carcinomas have been shown to produce distant metastasis at the time of diagnosis and to have a particularly poor prognosis.[30] In

our study, patients with numerous endocrine cells had a significantly worse prognosis than patients without.

By immunohistochemical staining for chromogranin A, NE cell differentiation was detected in 16.7% of patients with PD adenocarcinoma.[30]

Indinnimeo et al[31] found a significant association between cga-positivity and lymph-node metastasis and concluded that cga over expression could reflect a more aggressive tumor and stated that cga + colon cancer patients are at risk for lymph-node disease and therefore include them in adjuvant chemotherapeutic protocol. Our results confirmed these and those patients should be included for adjuvant therapy.

In Conclusion Chromogranin A is a sensitive and specific neuroendocrine marker. Neuroendocrine cell differentiation is significantly related to grade and stage of the tumor. Diffuse chromogranin A positivity bear a poor prognosis. Patients with neuroendocrine tumors had a significantly worse prognosis than patients without, so it is recommended to give them aggressive multimodality therapy where survival may be improved.

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