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Original Article

Immunohistochemical Assessment of Neuroendocrine Differentiation in Colorectal Carcinomas and Its Relation with Age, Sex and Grade Plus Stage

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ABSTRACT

Background and Objective: **Neuroendocrine differentiation has not been proved to have effects in behavior of colorectal carcinomas. The aim of this study was Immunohistochemical evaluation of neuroendocrine differentiation in colorectal cancer.**

Patients and Methods: **In this cross-sectional study, 83 paraffin blocks from patients admitted in Rasoul-e-akram Hospital, Tehran, Iran, during 2003 to 2008, were evaluated in Pathology Department. All sections were stained with immunohistochemistry method for neuron specific enolase (NSE) and Chromogranin A(CgA). Data were analyzed using SPSS 12.0.**

Results: **Median age of patients was 56 yr. Forty four cases (53%) were female. According to TNM staging system, 11% of cases were in stage I, 29% in IIa, 7% in IIb, 2% in IIIa, 23% in IIIb, 24% IIIc and 2% were in stage IV. Thirteen cases (16%) were NSE positive, 15 cases (18.1%) were CgA positive. Two, 8 and 5 percent of the patients in grade I, II and III were CgA positive, respectively. Two, 6 and 5 percent of the patients in grade I, II and III were NSE positive. In grades II and III, NSE and CgA were significantly higher than grade I (P<0.001). CgA incidence was higher significantly in mucinous carcinomas (P<0.05).**

Conclusion: **Less than 20% of colorectal cancers showed neuroendocrine differentiation. There was no significant relationship between NSE and CgA incidence with stage or tumor site. There was a relationship between histologic grade and above-mentioned markers; this finding may help us in our knowledge about tumor behavior.**

Keywords: Colorectal Cancer, Cell Differentiation, Neuroendocrine Cell, Neuron Specific Enolase, Chromogranin A

Introduction

Colorectal carcinoma is the most common and most treatable carcinoma of gastrointestinal (GI) tract. In Iran, colorectal carcinomas were the most common malignancy of the GI tracts after gastric carcinoma and a younger age distribution compared to Western reports is suggested (1).

There are available evidences that colorectal carcinoma with neuroendocrine differentiation has worsen prognosis comparing the ones without them. However, clinical value and prevalence of

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neuroendocrine differentiation markers have not been determined yet (2).

In colorectal carcinomas with neuroendocrine differentiation, expression of Chromogranin A (CgA) and neuron specific enolase (NSE) studied by immunohistochemistry methods are considered as factors for tumor differentiation (3).

There are discrepant data in literature as to whether the presence of a neuroendocrine cell component as determined immunohistochemically carries prognostic implications(2) but Atosy *et al*. found relation with grade and stage(4). Hamada and Famulski did not find any association with grade, stage, age or sex in different studies (5, 6). Syversen *et al*. only found relation with embryologic origin of the tumor (7).

The aim of this study was the immunohistochemical assessment of neuroendocrine differentiation in colorectal carcinomas and its association with age, sex, grade as well as stage of the tumor. The significance of the study was that if we could find the association of neuroendocrine differentiation of tumor cells with tumor grade and stage, it might play a role in tumor behavior.

Material and Methods

Eighty-three paraffin wax embedded blocks of colorectal tumors from 83 patients admitted in Rasoul-e-akram Hospital, Tehran, Iran during 2003 to 2008 undergone surgical resection were obtained in Pathology Department. Age of the patients, staging and grading of the tumor and histopathologic findings were recorded for each case. Four µm-thick sections were stained with Hematoxyline & Eosin method. Well-differentiated carcinomas were regarded as grade I, moderately differentiated carcinomas as grade II and poorly differentiated carcinomas as grade III. Pathologic staging was performed according to American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) TNM staging system (8).

For immunohistochemistry studies, a labeled-streptavidin-biotin method (Dako, Denmark) was performed. Immunohistochemical markers included Neuron Specific Enolase(NSE) and Chromogranin A(CgA). Each paraffin-embedded tissue section (4 µm in thickness) was deparaffinized, hydrated, incubated in 3% H_2O_2 and microwaved to block endogenous peroxidase activity. The tissue sections were subjected to antigen retrieval by microwaving in 10 mM citrate buffer. The sections were incubated with serum blocking solution (Reagent A) to block nonspecific binding and then with the primary antibodies in moist chamber. After rinsed with PBS, the sections were incubated with the biotinylated secondary antibody (Reagent B) and rinsed with PBS. The sections were followed by incubation with enzyme conjugate (Reagent C). Subsequently, the sections were stained with DAB and counterstained with Hematoxylin. Serum blocking solution (Reagent A) in place of the primary antibody was used as a negative control. Appendiceal carcinoid tumor was used as a positive control for both of the markers. Immunostaining labeling intensities were defined as: 0, no positive tumor cell or less than 2% of the positive tumor cells; +, 2%–9% of the positive tumor cells; ++ more than 10% of the positive tumor cells; - negative labeling (5-7).

Statistical analysis was performed using chi-square test and Student's *t*-test for evaluation of association/difference between clinical parameters including gender, age, tumor location, stage and immunohistochemical markers using software of SPSS 12.0 (SPSS Inc., Chicago, IL). The statistically significant difference was set $P < 0.05$ (two-sided probability).

Results

The median age of the patients at time of diagnosis was 56 yr, ranging from 14 to 101 yr. Thirty-nine patients were male (47%), with a male-to-female ratio of 0.88:1. The tumors were located in the cecum in 10 cases (12%), the ascending colon in 10 cases (12%), the descending colon in 4 cases (4.8%), the sigmoid colon in 29 cases (34.9%) and the rectum in 30 cases (36.1%).

Tumors differentiation (histological grade) were as follows: grade I for 37 cases (44.6%), grade II for 37 cases (44.6%) and grade III for 9 cases (10.8%). Fifteen cases were mucinous adenocarcinomas. The pTNM staging system for tumors were stage I for 11%, stage IIa for 29%, IIb for 7%, IIIa for 2%, IIIb for 23%, IIIc for 24% and IV for 2% of cases.

Immunohistochemical reactivity of the samples was positive for NSE in 16% of cases (8 sample + and 5 sample ++).15 cases (18.1%) were CgA positive (7 cases + and 8 cases++).Fig. 1 and 2 show positive reactions for NSE and CgA, respectively.

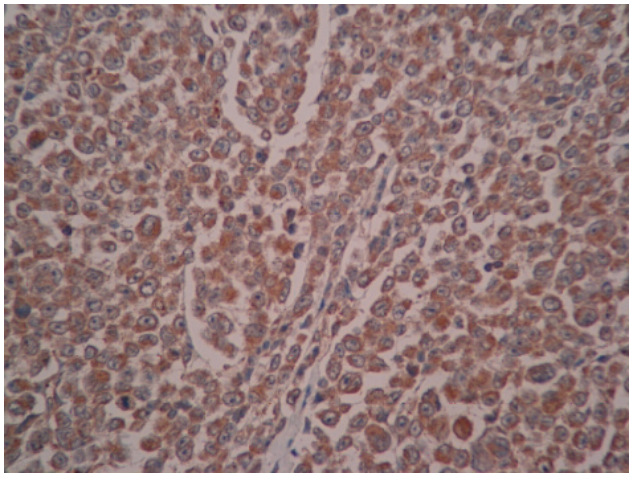

Fig. 1. 2^+ Positive Chromogranin A immunohistochemical marker

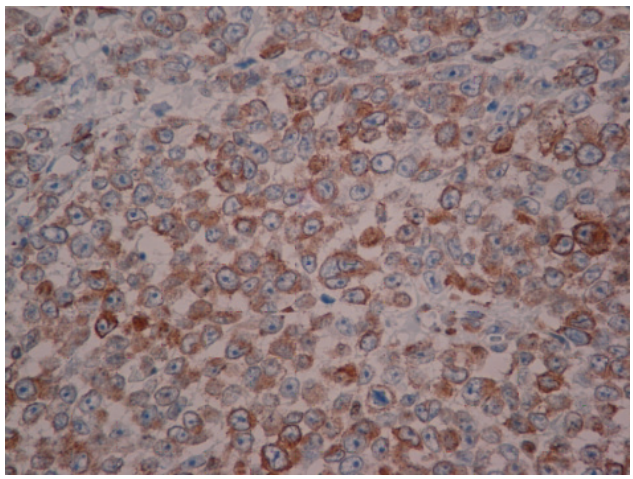

Fig.2. 2^+ Positive NSE immunohistochemical marker

Both NSE and CgA were significantly more prevalent in grade II and III (chi-square $P<0.001$ for each one). CgA was more prevalent in mucinous tumors (chi-square $P<0.05$).

No significant association was present between presence of CgA and NSE with embryologic origin of primary tumor (mid gut or hindgut).

We could not find any relation between immunoreactivity of CgA and NSE with pathologic tumor stage. In addition, we compared patients in stage III and IV with stage I and II but any statistical difference revealed.

According to *t* student's test findings, there was no relation between reactivity of the above immunohistochemical markers and age of the patients. In addition, no correlation found with patients' sex.

The relation of grade, stage, type and embryologic origin of the tumor with positivity or negativity of CgA and NSE immunohistochemical markers are compared in Table 1.

Table 1: Relation of chromogranin a and NSE IHC markers with grade, stage, tumor type and tumor embryologic origin

Variable \ Marker		Chromogranin A		*P* value	NSE		*P* value
		Neg	Pos		Neg	Pos	
Grade	I	35	2	0.002	34	2	Less than 0.001
	II	29	8		31	6	
	III	4	5		3	5	
Stage	I	8	1	0.07	9	0	0.052
	IIa	21	3		23	2	
	IIb	2	4		3	3	
	IIIa	2	0		2	0	
	IIIb	15	4		12	6	
	IIIc	17	3		17	2	
	IV	2	0		2	0	
Tumor type	Non mucinous	59	9	0.025	57	9	0.21
	Mucinous	9	6		11	4	
Tumor Origin	Mid gut	18	4	0.98	17	5	0.31
	Hind gut	50	11		51	8	

NSE; Neuron specific enolose; Neg: Negatitve ; Pos:Positive

Discussion

In our study, the prevalence of immunohistochemical neuroendocrine differentiation was significantly higher in grades II and III. We also found immunoreactivity for CgA in 6 out of 9 mucinous adenocarcinomas.

In 50 cases of colorectal carcinoma, thirty-eight and 26 percent of cases had positive reaction for CgA and NSE, respectively (4). CgA positivity was associated with grade and stage of the tumors and was associated inversely with survival, moreover, CgA was the most sensitive and specific neuroendocrine marker and in case of its presence, appeared to bear a poor prognosis in patients with colorectal cancers (4).

Hamada *et al.* found no correlation between Chromogranin immunoreactivity and tumor location, grade, or stage in 212 patients. However, they concluded that the neuroendocrine differentiation was an independent prognostic factor among patients with colorectal cancer (5).

Famulski *et al*. did not find any statistically significant relationships between CgA, NSE and/or Synaptophisin(Syn) expression with tumor site, histopathological type, grading, lymph node metastases, age and sex of patients in 48 cases of colorectal cancer (6).

Syversen *et al*. reported CgA and NSE immunostaining in 15 and 36% of tumors, respectively. Furthermore, they found that the expression of NSE was significantly higher in colorectal carcinomas derived from the mid gut than in those of hind gut origin (7).

Neuroendocrine differentiation is often seen in small cell undifferentiated colorectal cancers and correlates with a more aggressive course of the disease (9).

Indinnimeo *et al*. found significant association between CgA-positivity and lymph node metastasis. They concluded that CgA over expression could reflect a more aggressive tumor and adjuvant chemotherapeutic protocol should be considered in the CgA positive colon cancer patients (10).

Schwander *et al*. used 94 rectal carcinomas for immunehistochemical analysis of CgA, Syn and NSE. Neither for Syn nor for NSE significant association with clinical or histopathological variables could be found. Expression of CgA significantly correlated with age and differentiation. They found no significant association between neuroendocrine markers and tumor stage. However, incidence of metachronous distant metastases was significantly associated with CgA expression. The writers concluded that the expression of the neuroendocrine marker CgA seemed to have a prognostic impact in primary rectal cancer for the incidence of metachronous distant metastases (11).

Secco *et al*. found no significant association between CgA expression and location of primary tumor, bowel wall infiltration, stage of disease or tumor grade. To conclude, they did not recommend CgA expression as a marker to identify prognostic subgroups in colorectal cancer patients (12).

In another study, Grabowski *et al*. studied the correlation of survival of 116 patients with colorectal cancer of stages III (n = 59) and IV (n = 57) with the extent of neuroendocrine differentiation. They found a significant difference in survival of the 96 patients of groups 0 and 1(< 2% cells staining positive for neuroendocrine markers), comparing the 20 patients of group 2 (mean survival were 48.9 months and 18.6 months, respectively). The difference was most striking in stage III disease with 79.4 months' survival for combined groups 0 and 1, and 38.9 months' survival for group 2. Using the multivariate Cox regression model, the presence of more than 2% of cells with neuroendocrine differentiation was found to be an independent prognostic parameter for stage III and IV disease and no correlation was observed between neuroendocrine differentiation and tumor location, grade, depth of invasion or stage (13).

The aim of the present study was to determine the amount of neuroendocrine differentiation in 83 samples of colorectal tumors. Median age and male to female ratio of our patients were much like to other mentioned studies. Furthermore, the location of primary tumors as expected according to findings of other studies was in right hemi colon and rectum in 74% of cases (2, 3).

Most of our samples had the histological grade of I and II and less than 10% of the tumors had grade III. Moreover, considering TNM system, about half of our cases were in stage III.

Sixteen percent of samples were NSE-positive,

18.1% were CgA positive and 9.9% were both NSE and CgA-positive samples. These findings are similar to Schwander and Secco studies but incidence of NSE and CgA-positive samples were less in our study comparing other mentioned studies (4-13).

We also found that prevalence of neuroendocrine differentiation is significantly higher in grade II and III, which is consistent with the finding of Atosy and Schwander and in contrast with the findings of other mentioned studies. Another point to be mentioned is that in our study no association between neuroendocrine markers and tumor stage was present. This finding is along with those of all mentioned studies except Atosy *et al*.(4). Likewise, study of Grabowski *et al*. is supporting this fact by defining neuroendocrine markers as independent factors affecting survival, but without any association with tumor stage (13).

Presentation of CgA in mucinous adenocarcinomas was noticeable in 6 out of 9 tumors. In current study, we found no association between NSE, CgA presentation and embryonic origin of tumor; in contrast, Syversen *et al*. described such association in their study (7).

One of our cases showed histological pattern of neuroendocrine differentiation and it was the only case in which CgA and NSE markers were both positive.

Conclusion

Although less than 20% of our cases expressed neuroendocrine differentiation markers, NSE and CgA prevalence were not associated with tumor stage and/or location, also not with age and sex of the patients. Consequently, these markers may be use to predict invasive behavior according to positivity in higher grades. Furthermore, presence of histological pattern of neuroendocrine differentiation can predict expression of NSE and CgA.

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