

Neuroendocrine Differentiation in Colorectal Carcinomas – An Immunohistochemical Study

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ABSTRACT

Purpose: Neuroendocrine differentiation (NED) and its role in behavior of colorectal carcinomas (CRCs) is still debatable. The clinico-pathological pattern and the immunohistochemical expression of NE markers, chromogranin A (CgA) and neuron specific enolase (NSE) and their comparison with the histidine decarboxylase (HDC) immunohistochemistry in various subtypes of CRCs was carried out.

Methods: Tissue biopsies from 100 patients presenting with variable morphology, histological grades and clinical stage of the CRCs were included. Clinical history was recorded and tissue sections were stained with CgA, NSE and HDC antibodies. Data were analyzed using SPSS 18.0.

Results: Median age of patients was 57.5 years and 78% were males and 22% were females. Mucinous (74%), non mucinous (22%) and signet ring cell carcinomas (4%) were diagnosed. Well, moderate and poorly differentiate tumours formed 13%, 24% and 63% of all biopsies. Ten percent of cases were in stage I, 35% in II, 53% in III and 2% were in stage IV. Immunohistochemical staining of 21%, 15% and 11% of colorectal adenocarcinomas demonstrated HDC, CgA and NSE positivity respectively. HDC expression in detecting the NE foci in colorectal adenocarcinomas was stronger and more sensitive than CgA and NSE ($P=0.006$) and that too even in poorly differentiated tumours ($P<0.001$).

Conclusions: A total of 20% of CRCs showed NED that related significantly with the tumour grade. HDC may reliably be employed in a panel of markers to assess NED in malignancies.

Keywords: Colorectal carcinoma, tumour, malignancy, adenocarcinoma

INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer deaths in Western countries¹ however, a rising incidence has been observed in developing countries and Pakistan, where it ranks among the top ten cancers in both genders; the more so in younger age group compared to the Western figures² and more frequently presenting with late stage disease and thus poorer prognosis³.

NED in a tumour, means that some or most of the cells constituting that tumour mass have resemblance with the cells of nervous and endocrine (hormonal releasing) tissues. NED has frequently been reported in CRC^{4,5}. It is difficult to distinguish the NE cell by routine hematoxylin and eosin staining. Previously, investigators applied argentaffin and argyrophilic techniques to demonstrate NED but immunohistochemistry has replaced those methods for last a few decades. By far, the most commonly used immunostains for the demonstration of NED in CRC include Chromogranin^{6,7} and Synaptophysin⁸. However, the role of NED as prognostic marker is debatable because the previous reports show

conflicting results^{5,9} conforming association with poor prognosis particularly in stage III-IV CRC⁵. It is important to detect this NED in advanced CRCs because these carcinomas have been shown to produce distant metastasis at the time of diagnosis⁴. Keeping in view the predictable significance of NED as a prognostic as well as potential therapeutic target in various carcinomas, this study has been carried out to determine immunohistochemical expression of NED in CRCs with respect to the different histological subtypes, grades and clinical features in our population presenting with CRC.

MATERIALS AND METHODS

This cross-sectional descriptive study comprised 100 consecutive patients, both males (M) and females (F), from Sheikh Zaid Medical Institute Lahore and Services Hospital Lahore (67, 33 cases respectively) who underwent resection of their primary colorectal malignancy from January 2010 to January 2015. Cases with tumour recurrence or taking therapy for their malignancy (follow-up cases) were excluded. All selected patients gave written informed consent in the post-op period. Relevant clinical and laboratory data of these patients including age, gender, presenting symptoms, tumor location and type of surgical procedure were recorded in separate proformas. After confirmation of the microscopic

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diagnosis, the histological grading of these tumours was undertaken following the criterion by American Joint Committee on Cancer (AJCC) and the World Health Organization (WHO).¹⁰Immunohistochemistry was performed using the standard Avidin Biotin Peroxidase method. The epitope retrieval was carried out in citrate buffer at pH 6. The primary antibodies used were mouse monoclonal antibodies to Chromogranin A (CgA), Neuron specific enolase (NSE) and Histidine decarboxylase (HDC) employed with a specified protocol (DAKO, Carpinteria, CA). The expression of each immune-marker was assessed and assigned a score based on the degree of immunoreactivity. The tumours were classified as 0 (no expression of neuroendocrine markers), 1 or low expression (< 2% tumour cell cytoplasm staining positive for neuroendocrine markers) and 2 or high expression (>2% tumour cell cytoplasm staining positive for neuroendocrine markers)¹¹.The data was entered and analysed using SPSS, version 20.0.

RESULTS

As regards the gender, 78% patients were males and 22% were females with a male to female ratio being 3.5:1. The mean age of these patients was 57.5± 12.31 years (age range: 35–80 years) with 6% cases below the age of 40 years, 74% between the age of 40–60 years and 20% between 61 and 80 years of age.

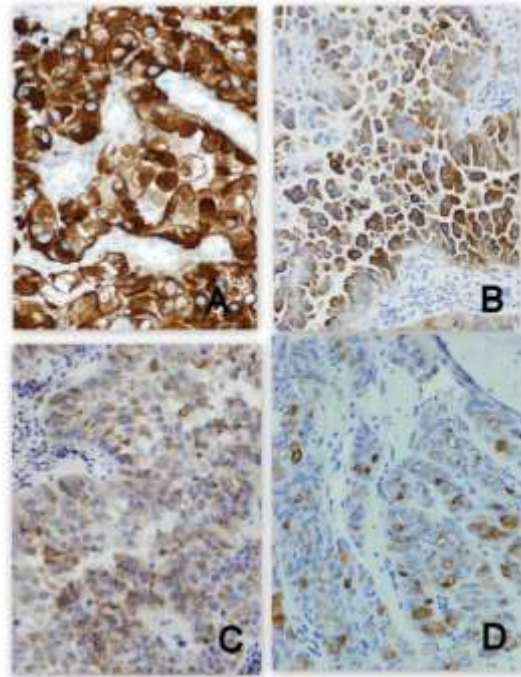
Clinical history was more or less common in both genders with the maximum number of patients, (62%), presenting with bleeding per rectum with or without altered bowel habits. Other complaints were pain in abdomen (56%), palpable abdominal mass (22%), tenesmus(28%), mucus discharge (10%) and intestinal obstruction (34%). Rectum was involved in 44%, sigmoid in 26%, caecum in 12%, descending colon and anal canal in 10%, transverse and ascending colon was involved in 4% cases each. Non-mucinous (74%), mucinous (22%) and signet ring cell (4%) variants of adenocarcinoma were diagnosed.

A total of 17(22.9%) and 57(77%) of the non-mucinous, 4(18.1%) and 18(81.8%) of mucinous and 1(25%) and 3(75%) of signet ring cell cases were females and males respectively. 13% well differentiated (WD), 24% moderately differentiated (MD) and 63% poorly differentiated (PD) adenocarcinomas were diagnosed. All signet ring cell adenocarcinomas were also classified as grade III, owing to their poor differentiation characteristics in terms of histology and prognosis. Gender distribution of CRC is shown in Table 1.

According to TNM staging system, 10% of cases were in stage I, 31% in IIa, 4% in IIb, 5% in IIIa, 27% in IIIb, 21% IIIc and 2% were in stage IV.

Table: 1: Gender distribution with respect to histological grades and subtypes of CRC in n=100 cases

Fig. 1: Moderately differentiated colorectal adenocarcinoma demonstrating high expression of HDC (A, B) while low expression of both CgA (C) and Synaptophysin (D). (A: 400X; B, C, D: 200X)



When NE markers status was assessed, immunohistochemical staining of 21%, 15% and 11% of colorectal adenocarcinomas demonstrated diffuse strong cytoplasmic staining of varying proportion of tumour cells with HDC, CgA and NSE respectively. Individual subtypes when assessed revealed that 8%, 6% and 5% mucinous, 11%, 8% and 6% non mucinous while 2%, 1% and none of the signet ring cell adenocarcinomas demonstrated positive staining with HDC, CgA and NSE respectively (Table 2).

When these NE marker positive tumours were segregated according to histological grades, most of the PD CRC(s) (n=12, 19.0%) followed by MD (n=6, 25%) and WD (n=3, 23.0%) CRC(s) demonstrated NED (p=0.021, Post Hoc Tukey).Also both low (score 1) and high expression (score 2) of NE markers were observed. High expression with increasing histological grades was shown by HDC and NSE (p=0.101) (Table 2)

HDC expression in detecting the NE foci in colorectal adenocarcinomas was stronger and more sensitive than CgA and NSE (P=0.006) and that too even in PD tumours of which 13% were strongly positive with HDC as compared to CgA (9%) and NSE (2%) (P=0.001). No significant correlation (P-value > 0.05) could be appreciated between the NED in CRCs and grades or other variables like age, gender, site of involvement, clinical symptoms etc..

Note that most of the females (n=12, 54.5%) and males 51(65.3%) presented with PD CRC(s). (p=0.015, Post Hoc Tukey). Where WD: well differentiated, MD: moderately differentiated, PD: poorly differentiated, CRC: CRC

Gender	Non Mucinous (74)			Mucinous (22)			Signet(4)	Total
	WD	MD	PD	WD	MD	PD	PD	
Male	4	13	42	6	4	6	3	78
Female	1	5	9	2	2	2	1	22
Total	5	18	51	8	6	8	4	100

Table: 2 - NED w.r.t histological subtypes and grades of CRC in n=100 cases.

Note that high expression (score 2) of NE markers was observed more frequently with HDC and NSE in contrast to CgA.

NE Marker	Histological Subtype							Total
	Mucinous (22)			Non Mucinous (74)			Signet (4)	
	WD (8)	MD (6)	PD (8)	WD (5)	MD (18)	PD (51)	PD (4)	
HDC n(%)	2 (25)	2 (33.3)	4 (50)	1 (20)	4 (22.2)	6 (11.6)	2 (50)	21 (21)
Score 1	2 (100)	1 (50)	1 (25)	1 (100)	2 (50)	2 (33.4)	0	9 (42.8)
Score 2	0	1 (50)	3 (75)	0	2 (50)	4 (66.6)	2 (100)	12 (57.2)
CgAn(%)	1 (12.5)	3 (50)	2 (25)	2 (40)	2 (11.1)	4 (7.8)	1 (25)	15 (15)
Score 1	1 (100)	2 (75)	1 (50)	1 (50)	1 (50)	2 (50)	0	8 (53.3)
Score 2	0	1 (25)	1 (50)	1 (50)	1 (50)	2 (50)	1 (100)	7 (46.6)
NSE n(%)	2 (25)	3 (50)	0	1 (20)	3 (16.6)	2 (3.9)	0	11 (11)
Score 1	1 (50)	1 (25)	0	1 (100)	1 (25)	1 (50)	0	5 (45.4)
Score 2	1 (50)	2 (75)	0	0	2 (75)	1 (50)	0	6 (54.5)

DISCUSSION

This study is the first ever in Pakistan to reveal the NED in CRCs taking in account other important clinic-pathological parameters as a continuation of our earlier reported study¹². A total of n=100 biopsies from patients with different histological grades and TNM stages of CRCs were included. Male to female ratio was 3.5:1 (p=0.010). A quite lower ratio, but significant male preponderance of 1.68:1 (p = 0.020) was reported by Zubair et al from Karachi, Pakistan¹³. A male preponderance was also shown in an Asian study¹⁴. However, a recent study from Pakistan found an equal male to female ratio². Similarly, an Iranian study reported 47% males with a male-to-female ratio of 0.88:1¹⁵.

The mean age of patients in the present study was 57.5±12.31 years with 6% cases below the age of 40 years. Studies from Pakistan notified much increased frequency of 40% of patients below the age of 40 years^{13,16} while another 3.5% patients below 20 years of age. Similar mean ages ranging from 43.7-51.2 years have also been reported from Pakistan^{2,17} and Iran¹⁵. Almost 82% of CRCs were located in the left colon in the present study which is quite consistent with the reports by Zubair et al¹³.

The present study reports 74% mucinous, 22% non-mucinous and 4% signet ring cell carcinomas. A total of 11.9% mucinous while 7.0% signet ring carcinomas were reported in another local study and 5 (2.7%) cases with neuroendocrine differentiation¹³. Similarly, 19.7% mucinous adenocarcinomas, and 3% signet ring adenocarcinomas were reported by another local study from Karachi and 1.5% had adenocarcinoma with neuro-endocrine differentiation¹⁸. Most of our study patients (63%)

presented with grade III carcinomas including the mucinous, non mucinous and signet ring cell adenocarcinomas. This is in contrast with the reports from local (31.9%)¹³, Iranian¹⁵ (10.8%) and Asian (2.2%)⁴ studies where moderately differentiated tumours were reported most frequently (40-54%).

As regards tumour stage, all the 23 resection specimens in a local study showed advanced disease of T3 (78.9%) or T4 (15.8%) where almost 80% cases were lymph node positive¹³. Similarly, 49% T3 while only 2% T4 cases were reported by Nasrin et al.¹⁵ which is quite consistent with our findings (53% T3, 2% T4). In line with this notion, Vortmeyer et al observed identical genetic alterations in synchronous poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas with probably therapeutic implications in the near future¹⁹. NED is often seen as an independent prognostic factor in stage III-IV CRCs⁵. This is consistent with our findings where most of the PD CRC(s) (19%) followed by MD (25%) and WD (23%) CRC(s) demonstrated NED (p=0.021).

In the present study, applying immunohistochemical staining with HDC, CgA and NSE we demonstrated NED in 21%, 15% and 11% CRCs respectively (Fig:1). In contrast, Tumori et al observed CgA as the most frequently and strongly expressed marker (38%) in comparison with NSE (26%) and synaptophysin (6%)²⁰. Similarly, Nasrin et al. and Tumori et al. reported higher frequency of CgA positive CRCs as compared to NSE^{15, 20}. Shinji from Japan, however, detected NED in equal number (16.7%) of PD adenocarcinomas by both CgA and NSE⁴. On the other hand, CHO Y reported 77.5% NSE positive samples in comparison to 30.3% CgA positive CRCs¹¹.

NED was more prevalent in mucinous tumors (66%) in a study by Nasrin et al whereas in the present study non mucinous tumours (11%) more frequently demonstrated NED and that too in a much lower frequency¹⁵.

Chromogranin A expression correlated with grade and stage of the CRCs in various reports^{15,20} in contrast to the present study where high expression of NE markers with increasing histological grades was shown by HDC and NSE ($p=0.101$). Contrary to this Schwander et al and Cho did not find any significant association between NE markers expression and clinical or histological variables of CRCs^{11, 21}.

One of our previous reports also supports HDC as a reasonably reliable marker for demonstrating NE differentiation in pulmonary neuroendocrine carcinomas, regardless of their degree of differentiation as compared to the dependence of CgA positivity on the differentiation of a particular malignancy²².

These results clearly indicate the experimental accord by Garcia-Caballero and Masini who reported that changes in the enzymic activity of HDC may have a significant role in the development of tumour cells including the CRCs^{23, 24}.

CONCLUSION

Supporting the previous literature, we demonstrated the role of HDC as a reasonably reliable marker for demonstrating NE differentiation in CRCs regardless of their degree of differentiation. More prospective analysis relating to the prognostic significance of NE differentiation in CRC and other NE malignancies with respect to HDC immunoreactivity should be followed.

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