

# Comparative Immunohistochemical Staining for Chromogranin A and Synaptophysin in Intestinal Carcinoids

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## ABSTRACT

**Objective:** To study the immunohistochemical staining for Chromogranin A and Synaptophysin in Intestinal Carcinoids.

**Study Design:** Cross sectional study

**Place and Duration of Study:** This study was conducted at the Ayub Teaching Hospital Abbotabad collected between 2017 to 2019.

**Materials and Methods:** Intestinal wall endocrine glands also seem to be optimistic for immunohistochemical chromogranin A known as CgA and synaptophysin known as SPY, the much more generally utilized stripes for neuron endo chemical cell types as well as tumors. Immunostaining CgA and SPY were conducted with four duodenal, fourteen ideals, five appendices, and seventeen colorectal. CgA with or without invasion of intestinal walls were strongly positive for duodenal and ileal carcinoids. CgA and SPY were weakly immuno-immunized in smaller appendix carcinoids.

**Results:** In particular, colorectal carcinoids with a low-grade tumor less than 1 centimeters with CgA negative coloration were more aggressive, whereas the bigger tumors less than 2 centimeters that infiltrate through the wall with often CIA's positive coloration. Duodenum, small gut, and colorectal CgA-positive carcinoids seem to be much attacking than CgA-negative tumors, most of which were just less than two centimeter, respectively. In the case of CgA with high serum CgA, while rectal hindgut cancers with low polyploidy tumors are CgA-negative but SPY-positive.

**Conclusion:** CgA-positive immunostaining can be used for gastroenteropancreatic neuroendocrine tumors, as an independent marker for potential malignancy, concerning the position and sort of the tumors. The immunostaining of CgA and SPY thus provides important carcinoid information.

**Key Words:** Carcinoid, Chromogranin A, Immunohistochemistry, Synaptophysin

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## INTRODUCTION

The much more frequent place of disease is a stomach, accompanied by the rectum 34 percent, the colon 16 percent, the stomach 11 percent, unfamiliar areas one percent; classic bowel cancer is of slow growth, and

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indolent malignant tumors are the most commonly reported. The Rectosigmoid in the hindgut in the large intestines in the midgut is the numerous generalplaces come after by the brain and transverse colons.<sup>1</sup> The slow-growing colorectal tumors are usually 5 years old with 90 percent colorectal carcinoids. The endocrine cells, which are less than one percent of mucous membranes, are usually dispersed to the exterior and the basic principle of the glandular epithelial organelles, especially gut crypts as well as coincident granules containing carcinoma ChadHurley, whereby different peptide hormone levels are released without hormones.<sup>2</sup> For epithelial and neuroendocrinal markers, the stain is positive for immunohistochemical stain. CEA at apical or lumen location is positive for epithelial markers and CK7 In nearly 10% of tumors and 25% for CK20. The positive markers are chromogranin A, synaptophysin, nerve cell specific enlance, leu seven, PGP 9.5, etc. As the second most commonly used immunochemical markers are CgA and Spy for neuropsychosynthetic and tumorcells, are routinely used in the typical carcinoid

pathology laboratory.<sup>3</sup> The granite family is made up of eight-grain peptides, chromogranin A, B, C, secret graphs 3, 4, 5, 6, and VEGF. The CgA was limited to secretory granules with the immunoelectron microscopy, especially at the granules' peripheries, whereas the SPY cytoplasm was immunostained. CgA is usually good for cells like bowel, thyroid, parathyroid, early pituitary, endocrine pancreatic and other cells. CgA is generally good for cells like intestinal endocrine. CgA- $\beta$  cells are weak to moderately positive in the endocrine pancreas, while non- $\beta$ -CGA cells such as  $\alpha$ - $\beta$ -pancreatic polypeptide and pancreatic polypeptide called PP cells are strongly beneficial.<sup>4</sup> The endocrine cells are CgA and SPY, but they are mostly located through all endocrine cells in the gastric intestinal tract, which is the position of the neurosecretory granules, whilst the SPY occurs more diffusely through the cell cytoplasm outside of the secretory granules, which is equivalent to the distribution throughout the cytoplasm of synaptic vesicles. SPI is a member of a small SV family of proteins, including synaptotagmin, SNAP25, SNAP, syntaxin, Rab3A, and many more. SPY is one of the first synaptic proteins to be detected, but its relationship between structure and function remains ambiguous as SPY does not involve itself in the SV cycle. While neurological markers of both CgA and SPY were used for neuroendocrinal tumors including bowel carcinoids, the place, size of the tumors, and immunochemical stain of the gut carcinoids were not discussed in a thorough comparable immunohistochemical study. Pre-gut tumors with stomach, the first part of the duodenum, and a pancreatic tumor are 88 percent positive, and mid-gut tumors are 100 percent positive.<sup>5</sup>

## MATERIALS AND METHODS

This study was conducted in Ayub Teaching Hospital Abbotabad collected between 2017 to 2019 in all cases of intestinal carcinoids.

**Sample size:** This study has included a total of 40 cases of duodenal carcinoids such as Zollinger Ellison, 14 ileal carcinoids, five appendiceal carcinoids, 11 carcinoids of the colon, and 6 rectal carcinoids.

**Data collection and procedure:** All tumor tissues have been fixed in buffered formalin with neighboring normal tissues. The archives were newly separated for paraffin and within two weeks of separation, the paraffin sections were immune. Deparaffinized sections with the application of a citrate buffer pH 6.2 were treated with monoclonal anti-CgA, and a dilution of 1:100 was applied for SPY.<sup>6</sup> Immunostaining was performed with 20 sections per stain to achieve a good comparative intensity. The +++ ++ + + for CgA and SPY immunostaining control and lower immunostaining controls were listed as well as the

adjacent standard intestinal mucosa in ++ and + and — chromosomes. The SPY in our hands was relatively lower in immune than the CgA. All tumors were well-differentiated WHO-classified neuroendocrine tumors. Even if one or more of the two markers CgA and SPY are positive in every case. Histopathological endocrine patterns were classified by tubular, trabecular, lobular, globular and solid patterns.<sup>7</sup>

## RESULTS

The bulk of intestinal histological patterns present in small tumors less than 2 centimeters and the mix of mid-size solid patterns and lobular tumor peripheries less than 2 centimeters. Duodenal carcinoids were all cubs that clinical were submucosally situated in the distal duodenum of fewer than 2 centimeters. Most of them were trabecular motifs mixed with solid motifs. 9 cases were larger than 2 centimeters among the twelve main small intestinal carcinoids. Small Ileum intestinal carcinoids in ten persons have all been strongly CgA positive and weaker and/or negative. The proposed approach was a metastatic liver lesion, with strong positive CgA and SPY, while invading single tumor cells in sinusoidal diseases. SPY immunostaining, therefore, did not invade tumor cells in the sinusoids immunostaining individually. Incidentally, an appendix carcinoid was found at the top of the appendix submucosa, smooth muscle, in the microscopic sections of the appendectomy, measuring 0.2 to 1 cm. five elevated colons, a cross-colon case, 4 cases of the sigmoid colon, and six cases of the rectum were among seventeen cases of colorectal carcinoids. Of the sixteen primary colorectal carcinoids, nine cases consisted of 0.5 centimeters, six trabecular and two solid patterns, with 2 cases of solid design and five cases, combined with solid or trabecular patterns, consisting of a combined lobular pattern in 7 larger cases. Two cases were a solid pattern. Two cases for CgA, three cases larger than 2cm were negative. All seven were weak to significantly positive for CgA and faintly positive for SPY of five cases of the upward and downward colons. Of the four sigmoidal cases, two have had high CgA and 2 small CgA tumors, 2 of them were very positive 3 moderately to strongly positive for SPY. The smallest tumor was Case ten, 0.2 centimeters, which was negative for CgA and moderately beneficial for SPY. For CgA the case twelve was negative and 0.4 cm in size and for SPY was faintly positive. In less than one percent of tumor cells, strongly and diffusely positive in SPY This tumor invaded the colonic muscle deeper and had a strongly positive CgA positive and a strong positive effect for SPY less than five percent of tumor cells. All six cases of rectal carcinoids, including a large tumor of CgA, and all of them weak and strongly positive for SPY were adverse to CgA.

**Table No.1: Carcinoids of duodenum, small intestine and appendix. Chromogranin A and synaptophysin Immunohistochemical Staining.**

<b>Duodenum</b>					
	Age/Sex	Size (cm)	Histological Pattern	CgA	SPY
1	29/M*	0.8 × 0.5	Trabecular	+++	+++
2	31/M*	0.6 × 0.5	Trabecular > Solid	+++	+++
3	47/F	1.2 × 1.0	Solid	+++	+++
4	52/M	1.2 × 1.0	Solid > Trabecular	+++	+++
<b>Small Intestine</b>					
1	34/M	1.0 × 0.5	Solid > lobular	+++	+
2	34/M	0.3	Lobular	+++	+
3	41/M	0.3	Lobular > Trabecular	+++	-
4	41/F	Liver (Metastasis)	Lobular	+++	+++
5	50/M	0.5 × 0.4	Lobular > Solid	+++	-
6	58/M	3.0 × 2.5 × 2.0	Solid	+++	-
7	59/M	0.5	Lobular	+++	-
8	60/F	2.5 × 1.0 × 1.0	Solid	+++	+
9	65/M	0.8 × 0.5	Lobular > Solid	+++	-
10	66/M	Omentum (Metastasis)	Solid	+++	+++
11	70/M	2.0 × 1.0 × 0.5	Lobular > Solid	+++	-
12	71/F	1.0 × 0.6	Lobular > Trabecular	+++	-
13	76/M	1.2 × 1.0	Solid	+++	++
14	80/F	1.0 × 1.0	Lobular	+++	++
<b>Appendix</b>					
1	21/ F	0.2	Tubular	+	+
2	22/F	0.2	Tubular	+	+
3	25/F	0.3	Tubular	+	+
4	38/M	0.5	Tubular > Globular	+	+
5	62/M	1.0 × 1.0	Lobular > Trabecular	++	+

**Table No. 2: Carcinoids of colon and rectum. Chromogranin A and synaptoph**

	Age/Sex	Size (cm)	Histological Pattern	CgA	SPY
1	45/M A	0.4	Trabecular	+++	+
2	54/M A	0.4	Trabecular	+++	+
3	65/F A	1.5 × 1.0	Lobular > Solid	+++	+++
4	67/M A	0.8 × 0.5	Lobular > Solid	+++	+
5	70/F A	0.5	Trabecular	+++	++
6	80/F T	2.0 × 1.5 × 1.0	Lobular	+++	+
8	70/M S	0.7 × 0.5	Solid	++	++
9	73/F S	1.5 × 1.0	Lobular > Solid	+++	+++
10	74/F S	0.2	Solid	-	+++
11	76/M S	0.5	Solid	-	++
12	47/M R	0.4	Trabecular	-	+
13	54/F R	0.4	Trabecular	-	+
14	63/F R	4.8 × 2.3 × 2.3	Solid	-	+++
15	70/M R	0.8 × 0.5	Solid	-	+++
16	74/M R	0.5	Trabecular	-	++
17	76/M R	0.5	Trabecular	-	+

## DISCUSSION

Carcinoid mediums usually have a carcinoid syndrome that includes washes, nausea, bronchiatic blockage, and correct cardiac failure because of stress hormones, tacykininine, bradykinin, and prostacyclin exudation.<sup>8</sup>

The appendix is considered to be innocuous if the tumors are less than 2 centimeters, where clinical signs are not present. Carcinoids in hindguts are often not active tumors that grow slowly, but without hormone-relating clinical symptoms they can produce PYY, HCG alpha, and β. Carcinoids with a retinal adenocarcinoma have duplication duration of more than

80 months compared to 109 days estimated. Compared to colonic adenocarcinoma at the time of diagnosis, the general appearance of colorectal carcinoids is lower than adenocarcinomas; therefore better predictions are achieved through early colonoscopy detection. For CgA as well as SPY all four duodenal carcinomas were very positive where gastric is co-secreted with CgA. Thus, CgA was very positive for all small intestinal carcinoids, with two cases. This strong, ileal carcinoid-immunostaining CgA is the co-section of carcinoids in CgA and catecholamine's. The aggressive, CgA-positive small intestinal cancers support CgA as an indicator for possible biological malignancy. In the microscopic part of the subaqua appendectomy, all five appendix carcinoids were found without serious extension in the microscopic and smooth muscle layers, all focused positively on CgA and in the tubular histologist, weakly positive, supporting their non-aggressive nature. Of the 16 cases of primary CBC, only 2 cases were larger than two centimeters and six were negative to 0.5 centimeters of the nine small tumors total 0.5 centimeters, but six were positive of the SPY. All 5 CgA colonic midgut carcinoids, including Case 6, have been positive. In hindgut carcinoids, two out of four sigmoid colon tumors were positive for CgA, while another two were negatively affected for CgA. A transverse, intramuscular tumor cell (CBC) less than one percent CgA was found to be positive at the surface when invading CBC >10 percent, suggesting that the CBC is aggressive.<sup>9,10</sup> Immuno-CgA has always been strong for midgut carcinoids, including distal duodenal and ileal.

## CONCLUSION

Neuroendocrine cells, including pancreatic islet cells, have been suggested to secrete pancreatic peptide hormones mainly via exocytose of cell membrane secret granule that is the 2nd step of insulin release, while first insulin spikes are released by sympathetic nerve through SV Endocytosis since neuropathic cells are equipped with Neuroendocrine.

### Author's Contribution:

Concept & Design of Study: Zabihullah  
 Drafting: Rania Hidayat, Shaista Alam  
 Data Analysis: Shabir Ahmed Orakzai, Amara Hayat Awan, Humaira Khan

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**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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