

Histopathological Spectrum of Lower Gastro-Intestinal Colonoscopic Biopsy Lesion With Special Reference To Her-2/Neu Expression In Carcinoma Colon

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Abstract:

Introduction :

Cancer associated with colon is one of the principal risk factors from decease in women and men. Although importancegrowing aspect of human epidermis receptor2 (Her2)as atherapeutic target is rising butits role as a biomarker in form of predicting indicator indicatorwithin colorectal cancer(CRC)is stilla mystery.Present research isundertakenfor evaluating the of Her2/neu description in cancer of colon.

Material & Method :

This research comprises 256patientswith spectrum of histopathological treatment ranging from colitis to colorectal carcinoma at our department between 2015- 2017. Her2/neu Immunohistochemistry was done in the colorectal carcinomaand scores based on Ruschoff et al (2012) Her-2 testing in gastric cancer.

Result :

Out of total number of 256 cases enrolled in our study group, majority belonged to the age group of 40-60 years, with M:F ratio being 1.4:1. Commonest site of lesion occurred in rectum (43.75%) followed by ascending colon and caecum (12.08%). Non neoplastic lesions constituted about two third of all cases, the commonest being inflammatory bowel disease(21.48%). In benign neoplastic lesions of tubular adenoma was thecommonest type, and in malignant commonest type was colorectal adenocarcinoma NOS(64.44%) followed by mucinous adeno carcinoma (22.22%). Because of more prominent membranous staining observed in high grade colorectal cancers, Her2neu expression is found to be an important predictive marker of carcinoma colon, especially the adenocarcinoma,NOS.

Conclusion : Like Breast carcinoma, target oriented therapy can be instituted especially in Her 2/neu positive high grade and metastatic tumors.

Keywords: Colonoscopic biopsy, Inflammatory bowel disease, Tubulovillous adenoma, Colorectal cancer, Her2/neu.

1. INTRODUCTION:

A variety of inflammatory disorders affect colon and rectum, accompanying acute and chronic conditions. The non-neoplastic conditions include colitis of various etiology andbowel disease & syndrome diverticular disorder, whereas the neoplastic disease include benign and malignant polyps along with colorectal carcinoma(CRC)[1].

Colorectal carcinoma (CRC) is considered to be very frequently happening carcinomas & remains a major reason of death [2]. Colon cancer being the third prevalent cancer irrespective of gender predilection and was main factor of both class incidence & death. The rising overall incidence of colorectal cancer globally is around 3 percent per annum [3]. The incidence in rural areas in India are approximately half of that in urban population, less incident of CRC in our country is attributable to diet which contains more percentage of fibers [4]. Chemotherapy has proven to be an efficient strategy for adjuvant therapy, but is still incapable of preventing recurrence in all patients [5]. In the era of tailored therapy, monoclonal antibody is now playing a key in the treatment of metastatic CRC [2]. This one goal is the transmitter of human aspect of epidermal growth-2 Her2/neu, primarily being associated with breast carcinoma. Although the comprehensive characterization in the molecular histopathogenesis of colon cancer established ErbB2 enhancement as an effective therapeutic goal but its under-expression was related to its prediction of treatment's outcome [6].

HER1 human epidermal growth factor (EGFR), (Her2/neu or ErbB2), Her-3 & Her-4 associated with growing aspect of human epidermal receptor family and were membranous bound G-protein coupled receptor present upon chromosome 17q21 which encodes an intracellular protein 185KD that lacks a normal ligand. Mitogen activated protein kinase (MAPK) is activated by Her-2 that in turn initiates signal cascades and phosphoinositide-3-kinase pathways PI3K/AKT those are essential for differentiation and proliferation of cells [7].

The dysregulation in the form of mutations, overexpression by Her2/neu encourages the development & transfer of tumors [8]. In the tumor model system, the gene of Her-2/neu overexpression avidly compared to mutagenesis, improved motility of cell, malignant transformation, metastasis and invasion. Other epithelial malignancies such as carcinoma lung, prostate and bladder have shown overexpression of Her-2/neu in several studies [7]. Thus potential new adjuvant monoclonal antibody can be used which causes direct inhibition of Tyrosine kinase (TRK) activity resulting in proapoptotic, antiangiogenic and anti-invasive effect [9].

2. AIM & OBJECTIVE :

- a) To study the incidence of various lesions of lower gastro-intestinal colonoscopic biopsy.
- b) To study histopathology of lower GI lesions and find out incidence of various non-neoplastic as well as neoplastic lesions.
- c) To study the Her2/neu expression in carcinoma of colon with respect to age, sex, site, type and grade

3. Materials and methods :

A study was conducted prospectively in a tertiary care teaching Hospital from July 2015 till Oct 2017, on the lower GI colonoscopic biopsies, sent from the department of Gastroenterology of the same institution. Inclusion criteria included patients of either sex of all age groups with lower gastrointestinal signs and symptom as well as visible mucosal lesions.

The exclusion criteria was patients with perforated viscus, cases with prior chemo and radio therapy and recent history of myocardial infarction. All histologically confirmed cases of CRC were included for Her2neu study with a follow-up of one year. After obtaining the

well informed consent and thorough health experience, endoscopy with a compact forward facing camera endoscope & five to eight colonoscopies from different quadrant of the surface and margins of the mucosal lesions. All clinico-radiologic and endoscopic reports were procured for subsequent histopathologic examination. Full thickness biopsy of mucosa without much submucosal tissue was obtained from the margin (all four quadrants) surface and surrounding mucosa of suspicious lesions, as well as 4-6 biopsy from different segments of colon were taken. Tissue processing was followed by Harris Hematoxylin and eosin (H & E) staining followed by special staining was done, for establishing final diagnosis. All cases of the CRC were subjected to IHC stain of Her2neu using DAKO polyclonal rabbit anti-human cerb-2 oncoprotein. Patients especially with IHC of Her-2/neu score 2+ tumors were enrolled with fluorescent in-situ hybridization (FISH) testing.

4. PROCEDURE :

IHC is a method based on Antigen- Antibody reaction for identifying antigenic substance in the tissue. The primary antibody binds to specific tissue antigens. The biotinylated secondary antibody is directed to primary antibody. The streptavidin/horse radish peroxidase complex is then applied. Streptavidin then binds to biotin on the secondary antibody and Horseradish peroxidase (HRP) acts as an indicator enzyme. On addition of Diaminobenzidine (DAB) substrate, free oxygen radicals are released which oxidize DAB to a brown precipitation. The precipitate gets deposited on the antigen site and can be detected by microscopy [5]

The four tier scoring system used by Ruchoff J et al. (2012) [10], for Her2 expression in gastric carcinoma was used in our study for scoring Her2/neu of all the colorectal carcinoma Table 1.

Table 1. Guideline for colorectal carcinoma test of Her-2/neu, Ruchoff J et al [10].

Score	Observation	Staining
0	No sign of cell staining in < 12 percent	Negative
One+	Light, scarcely noticeable in > 12 per cent of the cells;	Negative
Two+	In > 12 percent of cells, low to medium staining; circumferential, basolateral or lateral.	Equivocal
Three+	Stressful in > 12% of the cell; transverse, horizontal, or basolateral.	Positive

5. OBSERVATION:

In this study the wide age range was covered from 2 years to 85 years with maximum number of patients who underwent colonoscopy belonged to 41- 60 years of age group in both sexes. Sex ratio is 1.4:1, male (58.88%) and female (41.11%). The incidence of disease was more commonly found in rectum (43.7%) in comparison to other sites of lower GI tract. Out of 256 cases the number of patients suffering from colorectal carcinoma was 35.15%, followed by IBD 21.48% , non-neoplastic polyp 14.84 % and neoplastic polyp 5.85%. Among 55 cases of idiopathic colitative ulcers, inflammatory bowel syndrome, was 19.6%, disease of crohn was only 3.4% revealed different stages of colitis (acute, chronic acute and inactive) together constitute 21.48%. The incidence of Inflammatory polyp (39.47%) is highest among the group of non-neoplastic polyps Table-2. Adenomas are

localized in the rectum (46.67%) followed by sigmoid colon (26.6%). Tubular adenomas constituted 80.0% Figure 1. tubulovillous adenoma comprised 13.33% and villous adenoma is least with 6.67%. the distribution of adenoma according to different site is depicted in Table 3.

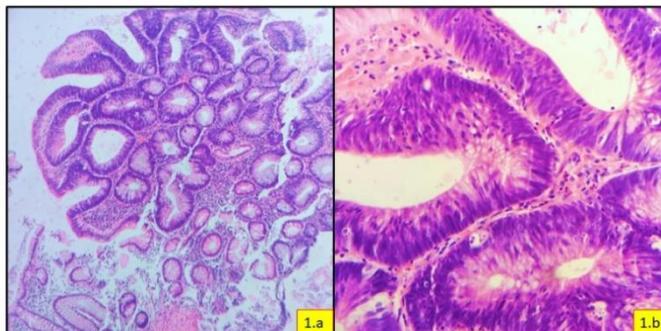


Figure 1: 1a. showing tubulovillous adenoma with high grade dysplasia(100x) , 1b. showing nuclear crowding and hyperchromasia(400x).

Table 2 – Histopathology findings in various lesion of lower gastrointestinal tract.

HPS	No. of pts.	%
Non-specific ileitis	14	5.40
Other- (Melanosis coli, infective, pseudomembranous colitis,	8	3.12
Idiopathic Bowel Disease (Acute colitis, Chronic Active, chronic inactive, indeterminate UC, and Crohn’s disease)	55	21.48
Intestinal tuberculosis, Solitary Rectal syndrome, Proctitis, Fistula in ano)	36	14.06
Colorectal polyp	38	14.84
Adenomas	15	5.85
Colorectal carcinomas	90	35.15
Total	256	100.00

Table -3: Distribution of Types of Adenomas (Neoplastic polyps) large intestine (n=15)

Region of large bowel	Tubulovillous		Tubular		Villous		Total	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%
Rectum	1	6.67	5	33.33	1	6.67	7	46.67
Sigmoid colon	1	6.67	3	20	0	-	4	26.67
Descending colon	-	-	-	-	-	-	-	-
Transverse colon	-	-	1	6.67	0	-	1	6.67

Right colon	0	-	3	20	0	-	3	20.00
Total	2	13.33	12	80	1	6.67	15	100.00

Age sex distribution of colon carcinomas are given in Table 4. The different subtypes of CRC included were adenocarcinoma – NOS (64.44%) followed by mucinous signet ring type, basaloid and adenosquamous type. The Histopathological spectrum of carcinoma as per site and type are depicted in Table 5. The distribution of Her2 scoring according to site of lesion is provided in Table 6 and Her2/neu scoring according to Histopathological type are given in Table 7.

Table 4- Age and sex distribution of cancer colon in accordance to site (n=90)

Age group	Colon				Rectum			
	Male	%	Female	%	Male	%	Female	%
0-20	0	-	0	-	1	1.11	0	-
21-40	3	3.33	4	4.44	4	4.44	7	7.77
41-50	4	4.44	2	2.22	2	2.22	6	6.66
51-60	11	12.22	2	2.22	7	7.77	5	5.55
61-70	3	3.33	4	4.44	10	11.11	2	2.22
>71	4	4.44	1	1.11	4	4.44	4	4.44
Total	25	27.77	13	14.44	28	31.33	24	26.66
Mean age	56.91		52.0		56.30		49.72	

Table 5- Histopathological carcinomaspectrum colon depending upon the form & location (n=90)

Site	Signet ring type	Adenocarcinoma	Basaloid	Adenosquamous	Mucinous
Colon	2(1.11%)	29(31.11%)	-	-	8(7.77%)
Rectum	6(6.66%)	31(33.33%)	3 (3.33%)	2(2.22%)	14 (14.44%)
Total	7(7.77%)	58(64.44%)	3 (3.33%)	2(2.22%)	20 (22.22%)

Table 6- Distribution of Her2/neu scores according to site of lesion (n=90)

Expression at location of Her2/neu	Her2/neu positive and equivocal		Her2/neu negative		Total in CRC	
	%	N	%	N	%	N
Colon	10.00	10	31.11	28	41.11	37.
Rectum	18.88	17	40	36	58.8	53
Total	28.88	26	71.11	64	100.00	90

Table 7- Immunohistochemistry :- Score of Her2/neu score as per Histopathological form (n = 90)

Score	Adenocarcinoma		Mucinous		Signet ring type		Basaloid		Adenosquamoid		Total	
	n	percentage	n	percentage	n	percentage	n	percentage	n	percentage	n	percentage
Her2/neu +ve score 3+	6	7.66	-	-	-	-	-	-	-	-	6	7.66
Her2/neu equivocal score 2+	16	17.77	2	2.22	2	2.22	0	-	0	-	20	22.22
Her2/neu negative score 1+	10	11.11	5	5.55	2	2.22	1	1.11	0	-	18	20
Her2/neu negative score 0	26	28.88	13	14.44	3	3.33	2	2.22	2	2.22	46	51.01
Total	58	64.44	20	22.22	7	7.77	3	3.33	2	2.22	90	100.00

For ninety cases reported into Her-2neu IHC, 6 cases (6.66%) for adenomas were optimistic with a rating of 3+Figure 2, 16 cases (17.77%) of adenomas displaying an equivocal rating of 2+Figure3, two cases (2.22%) of mucinous form displayed an equivocal rating of 2 +., whereas 10adeno-carcinoma cases, 5 cases (5.55%) of mucinous carcinoma tworing form signet& single carcinoma basaloid was score 1+Figure 4, and twenty six adeno-carcinoma cases, thirteen mucinous scenarios, three cases each of signet ring type, two cases each of basaloid, & of adeno-squamous carcinomas found -veby rating 0. The score 2+ positive cases were subjected to FISH study.Out of 20 cases of Her2neu 2+ score 4 (33.33%) cases was found to be Her2neu 3+ score in FISH study (33.3%).Both high positive and negative score (18.88 & 40.0%) were found in cancer located in rectum.

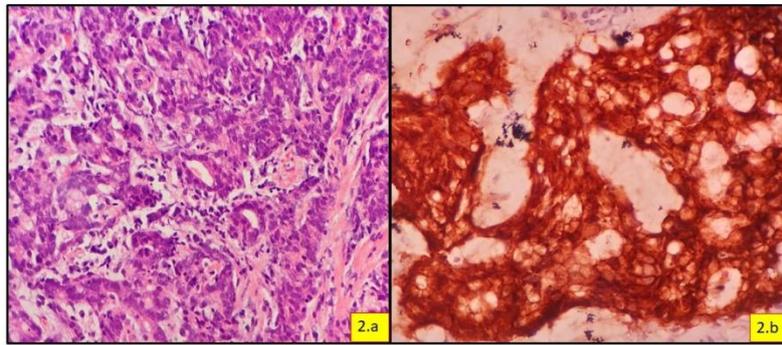


Figure 2: 2a. photomicrograph showing moderately differentiated adenocarcinoma NOS,(100x)
2b . photomicrograph showing Her2 neu score 3+ ,intense circumferential membrane staining
in >10% of cells(100x)

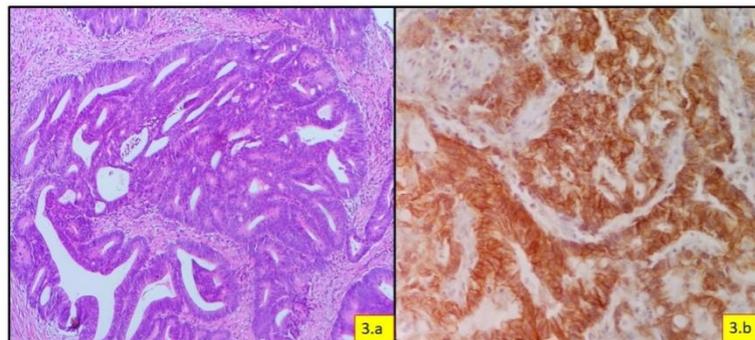


Figure 3: 3a. Photomicrograph showing moderately differentiated adenocarcinoma
NOS,(100x) ,3b. Photomicrograph showing Her2/neu rating 2+, weakened to fine staining in
cells of >12% c(100x).

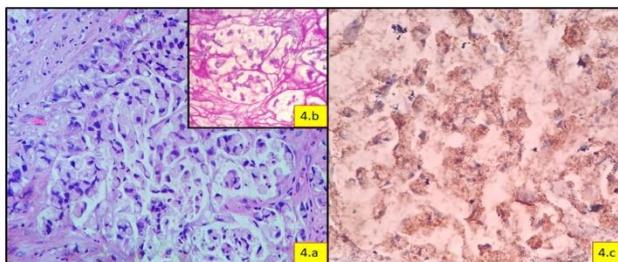


Figure 4:4a. Photomicrograph showing signet ring cell carcinoma (100x),4b inset showing positivity for special stain PAS,4c. Photomicrograph showing Her2/neu rating 1+ with light and scarcely visible granular staining in cells >12% (100x).

6. DISCUSSION

Colonoscopy biopsy provides useful information for diagnosis of various lesion of lower gastrointestinal tract. In this study, 256 patients were evaluated, most of which were in the 41-60 years age group, mean age being 45 years showing male sex predominance of about 58.88%, while females were 41.11%. The statistics coincided with that of Deoetal (2001)[11] whose study reported male predominance 64% and mean age of presentation being 48.5 yrs.

Within present research predominant abrasions are into rectum (60.0%) followed by pan colon (28.75%), ascending colon and caecum (12.8%), sigmoid colon (6.25%) transverse colon (2.92%) and descending colon, anus (1.25%). This observation correlated with the study of Robert et al (1912)[12] who represented that colonic diseases were prevalent in left side (60%).

The incidence of different lesions constituted 35.15% of colorectal cancer, followed by inflammatory bowel disease (21.48%), non-neoplastic polyps (14.06%), This is compared with the following authors: Koseoglu et al (2012)[13] showing IBD (16.3%), carcinoma (10%) and proctitis (6%) and Rajbandhari M et al (2013)[14] whose study showed IBD (27%), carcinoma (20%) and non neoplastic polyp (16.7%). We had a higher incidence of CRC, attributed possibly to being a referral centre for oncology in eastern part of India.

Inside the class of intestinal disorder which is inflammatory (21.48%) out of which ulcerative colitis constituted (21.48%) and Crohn's disease (2.4%). The study well correlated with that of A Sood et al (1999)[15] who found ulcerative colitis (16%), infective colitis (18.04%) and no cases of Crohn's disease. Similarly Quejang Q et al (2006)[16] found Superior incidence of colitis ulcer (65%) than disease of Crohn (35%).

Current study shows the incidence of non-neoplastic colorectal polyps constituted 14.84% of total cases out of which, inflammatory polyp (39.47%), juvenile polyp were (31.57%), hyperplastic polyp (23.68%) and least was hamartomatous polyp (5.26%). Adenoma (neoplastic polyps) constituted about 6.0% of total cases of which, tubular adenoma was (80%), tubulovillous adenoma (13.33%) and villous adenoma (6.67%). The commonest location of adenoma was rectum (47%) followed by sigmoid colon (27%) showing male predominance. In the study of Ali Zare Mirzare et al (2012)[17] stated that malignant polyps were low compared to non-neoplastic polyp of which hyperplastic polyps were commonest (23.22%) and tubulovillous adenoma was 8.11%. Study by Sebaretal

(2014)[18] also found that hyperplastic polyps were commonest and tubular adenoma commonest amongst neoplastic polyps (16.3%).

Colorectal carcinoma constituted 35.15% majority of which located in rectum (55.55%). The age incidence (>50 years), was similar to Boyle et al. (2002)[19] While cancers of colorectal have reported to be two times higher in males as compared with female and median age being 50 years. CRC in current study was mostly adenocarcinoma NOS type (64.44%) that correlated with Ocha M. et al (2003)[20] showing 98% of colon cancer to be adenocarcinoma, NOS type.

The Her2/neu expression was more found in left sided colon compared to rightsided colon. Strong Her2neu (score 3+) was found in 6.66% cases which were moderately differentiated adenocarcinoma. Score 2+ was observed in 22.22% of colorectal carcinoma out of which 33.3% were found to be score 3+ on FISH study, were found mostly in high grade colon adenocarcinoma. The subtypes of adenocarcinoma like mucinous, signet ring type, cloacogenic and adenosquamous type showed negative score for Her2neu.

The percentage of positivity of Her2neu in CRC was well correlated with other authors given in Table 8.

Table -8 Her2/ neu overexpression by different authors:

7. CONCLUSION

Commonest type of CRC was adenocarcinoma (NOS) followed by mucinous, adenosquamous, basaloid & signet ring form. Because of more prominent membranous staining observed in high grade colorectal cancers like breast carcinoma, Her2neu expression is found to be an important predictive marker of carcinoma colon.

Thus in a nutshell this study concludes that both conventional as non-conventional adenocarcinoma among colorectal cancer especially those by lymphovascular infiltration metastasis, Her-2 / neu expression must be evaluated in form of a different therapeutic approach. Due to much stained membranes in higher grades of CRC (grade II, grade III). Treatment of Herceptin may assist individuals suffering from disease especially in adenocarcinoma, NOS category. Other poorly differentiated carcinoma (grade III) like mucinous and signet ring cell type do not well respond to Herceptin therapy.

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