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# BETA-CATENIN EXPRESSION IN COLORECTAL NEOPLASM AND ITS PROGNOSTIC SIGNIFICANCE- AN OBSERVATIONAL STUDY

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**Abstract**

**Background:** Colorectal cancer ranks third among the most common cancer of male. Globally colorectal adenocarcinoma accounts for approximately 1.2 million new cases, and 600,000 deaths each year1. In India, the incidence of colorectal cancer is 4.3 and 3.4 per 100,000 male and female respectively2. Colorectal carcinoma (CRC) is considered as the most preventable cancers because it arises from benign neoplasms, called polyps which evolve into colorectal carcinomas over many years. The aim of the study is to study the age, sex, site and clinical presentation of various colorectal neoplasm, to study expression of beta catenin in benign, premalignant and malignant tumors of colorectum and correlate the level of expression of beta catenin in various histological grades of colorectal carcinoma. **Materials and Methods:** Paraffin blocks from 23 cases has been selected which includes both polyps and colorectal carcinoma. Colorectal carcinoma has been graded and staged according to the cap protocol. Polyps has been classified in to benign and premalignant, whereas colorectal carcinoma has been graded with respect to degree of differentiation. Beta-catenin immuno-histochemistry has been done. Scoring has done with respect to the localization of beta-catenin in membrane, cytoplasm and nucleus. **Result:** Normal colorectal mucosa and benign polyps shows predominant membranous expression. Premalignant polyp shows predominantly increased cytoplasmic expression with focal nuclear positivity and the colorectal carcinoma shows raised nuclear expression and loss of membranous expression. This proves the gradual translocation of beta catenin from membrane to nucleus in adenoma-carcinoma sequence. Beta-catenin expression has been significantly correlated with depth of invasion (T), nodal metastasis (N) and thereby to the stage of the carcinoma. Beta-catenin expression has been statistically correlated with degree of differentiation (tumor grade). Both recurrence and death were equally distributed in our study, through this observation the morbidity and mortality were greatly correlated with grade of beta-catenin expression. Hence beta-catenin can be used as a marker to identify the malignant potential of premalignant dysplastic polyps which helps in early management. It is used to assess the prognosis of the patients who were in post-colectomy status hence this marker can be used as an additional prognostic marker.

**Keywords:** Beta-catenin, polyps, colonic neoplasms.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

## Introduction

Colo-rectal cancer ranks third among the most common cancer of male. Globally colorectal adenocarcinoma accounts for approximately 1.2 million new cases, and 600,000 deaths each year[1]. In India, the incidence of colorectal cancer is 4.3 and 3.4 per 100,000 male and female respectively[2]. Colorectal carcinoma (CRC) is considered as the most preventable cancers because it arises from benign neoplasms, called polyps, which evolve into colorectal carcinomas over many years. The polyp-carcinoma progression sequence seen in the general population offers an opportunity to detect and remove the polyps before they undergo malignant transformation. Both endogenous and exogenous factors contribute to the risk of colonic carcinoma[1]. Colonic carcinoma mainly affects middle aged and elderly individual[1]. Most common symptoms of patient with colorectal carcinoma are abdominal pain, obstruction, altered bowel habits and anaemia due to chronic blood loss. APC/beta-catenin pathway plays an integral role in the classic adenoma- carcinoma sequence. The inactivation of the (APC) tumour suppressor gene initiates colorectal tumorigenesis. Under physiological conditions the APC protein which acts as a tumour suppressor gene is responsible for the destruction of transcription activators like beta-catenin and T cell (Tcf-4). In cases of mutation in APC gene and its subsequent dysfunction, overactivity of these transcription factors is noted, which results in the uncontrolled proliferation and elevates the survival of those cells. In case of APC mutation, the destruction complex fails to eliminate the beta-catenin, which leads to its accumulationintracellularly and facilitates its nuclear translocation, where it acts as a transcriptional activator to target genes like c– myc and cyclin D1, which subsequently plays an integral role in the development of colorectal neoplasms[7]. Studies have been undertaken to investigate and compare the expression pattern of beta-catenin in normal colorectal tissue and in various colorectal neoplasms: benign, premalignant and malignant. Normal colorectal tissue does not display nuclear or cytoplasmic immunoreactivity with beta-catenin, and an exclusively a diffuse membranous positivity[7]. Gradual decrease in membranous staining and subsequent increase in cytoplasmic and nuclear immunostaining by beta-catenin has been associated with increasing levels of dysplasia in neoplastic lesions of the large intestine[8]. Studies have been undertaken to determine the correlation between beta-catenin expression in colorectal cancer and lymph node metastasis, which is one of the most important prognostic markers for colon cancer[7], as well as the possibility of the future of anti- beta catenin targeted chemotherapy for the treatment of colorectal cancers. In this observational study, age, sex and site distribution of various colorectal neoplasms were analysed and also correlates the expression of beta catenin in various polyps and colorectal carcinoma. This study aims to identify the premalignant polyp and also establishes the relationship between the various grades and stage of colorectal carcinoma with beta-catenin. Thus beta-catenin is used as an additional prognostic marker.

## Aim of the study

1. To study the age, sex, site and clinical presentation of various colorectal neoplasm.
2. To study expression of beta catenin in benign, premalignant and malignant tumors of colorectum.
3. To correlate the level of expression of beta catenin in various histological gradesof colorectal carcinoma.
4. To evaluate its role as an effective prognostic marker in colorectal carcinoma bycorrelating with staging.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

## Materials And Methods

Observational study conducted among 60 cases of colorectal neoplasm were diagnosed in the department of pathology, Thanjavur medical college during the period of June 2019 to September 2021.

**SAMPLE:** A total of 60 colectomy specimens received from general surgery and surgical gastroenterology department of Thanjavur medical college.

**Inclusion Criteria**

Specimen including small biopsy, segmental resection, hemicolectomy and total colectomy were included.

**Exclusion Criteria**

Infective Lesions, Inflammatory Lesions, Inadequate Sampling and Post chemoradiotherapy with no residual tumor tissue (complete response**)**

Methodology

Cases were evaluated based on inclusion criteria. Information regarding age, sex, clinical presentation were collected. Specimens were fixed in 10% neutral buffered formalin. Colorectal neoplasms were grossed and bits were taken from the representative areas. Sections were of 4 to5 micron thickness sections were taken and stained with routine haematoxylin and eosin stain for histopathological expression. Colo-rectal neoplasms were assessed for site, size, extent of invasion, histological grade and lymph nodal status. TNM staging were used to stage the malignant neoplasm.

## BETA CATENIN IMMUNOHISTOCHEMISTRY

Expression of beta-catenin in cells was compared between normal mucosa and neoplastic tissue. The criterion for a positive immune reaction was brown membranous staining in histologically normal colorectal tissue (internal control) and dark brown to black nuclear staining in the fibroblasts of Desmoid tumour (external control). Positive findings were evaluated in four fields (1000 cells) under a light microscope at 400X magnification, without knowledge of the clinical outcome.

Scoring of immunohistochemistry

The scoring of positive beta-catenin expression was performed according to Mauri et al[4] separately for membrane, cytoplasm and nucleus. The final score was expressed as immune-histochemical staining score (IHC score) obtained by multiplying the percentage of positive cells with the staining intensity. Individual membranous, cytoplasmic and nuclear score has been generated and mean has been calculated for each type of neoplasm which includes benign, pre- malignant and malignant lesions.

Other Scoring Method - Jass et al scoring system[5]

The β - catenin activation score was calculated as the sum of nuclear score, cytoplasmic score and the membrane score. Total score then collapsed in to grade I, grade II and grade III. Total score of 0 reflecting normal colonic mucosa and score of 5 for tumours with strong nuclear staining (n = 2), strong and diffuse cytoplasmic staining (n = 2), loss of cell membrane staining (n = 1). This method has been exclusively used of carcinomas. Both methods of scoring were equally effective.

## Statistical analysis

The collected study data was entered in Microsoft Office Excel 2013 and analyzed using SPSS software version 16. Continuous variables was expressed as mean and standard

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

deviation. Description of categorical variables was expressed as frequency and proportion. Unpaired t test was used to compare two means. Chi Square Test and Fishers exact test was used to compare two categorical variables. All tests will be two-tailed, with results considered statistically significant if the p-value is less than 0.05.

**Ethical consideration**

Ethical principles such as respect to the patient, beneficence and justice were strictly adhered. Ethical committee approval was obtained before starting the study. The approval to conduct the present study was obtained from the Institutional Ethical Committee. Confidentiality of the study participants was maintained throughout the study.

**Observation And Results**

Histopathological examination of 60 colorectal neoplasms were done. Out of which 4 were benign, 4 were premalignant and 52 malignant lesions were studied.

**Table 1: Frequency distribution of type of lesion observed in patient with colorectal lesions.**

| Type of lesion | n | % |
|---|---|---|
| PJ POLYP | 1 | 1.6 |
| JUVENILE POLYP | 1 | 1.6 |
| HYPERPLASTIC POLYP | 1 | 1.6 |
| TUBULAR ADENOMA | 4 | 6.7 |
| ADENOMA | 1 | 1.6 |
| ADENOCARCINOMA | 45 | 75 |
| MUCINOUS CARCINOMA | 5 | 8.3 |
| NEUROENDOCRINE | 1 | 1.6 |
| LYMPHOMA (DLBCL) | 1 | 1.6 |

Data are expressed as n with %. Total N=60

From Table 1 it is evident that colorectal adenocarcinoma is the highly prevalent lesion among the colorectal lesion as it occupies around 75% of the lesion.

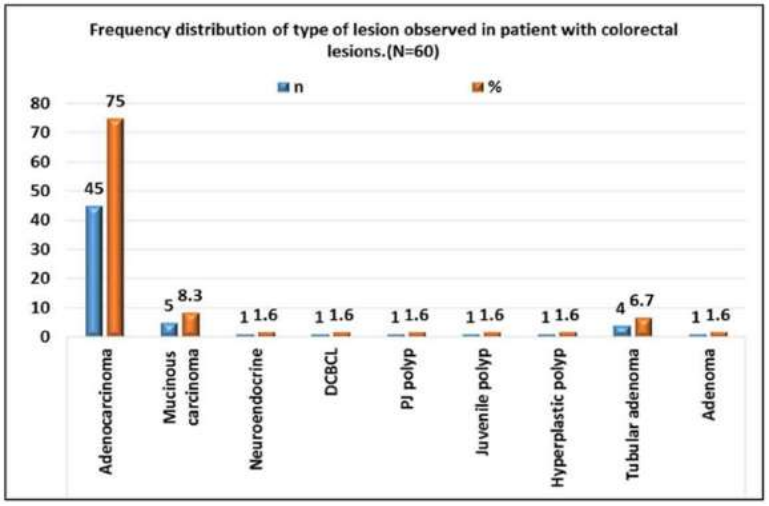


**Figure 1**

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

**Table 2: Frequency distribution of type of clinical presentation observed in patient with colorectal carcinoma.**

| Variables | n | % |
|---|---|---|
| **Age category** | | |
| <30 years | 1 | 2% |
| 31 – 40 years | 9 | 18% |
| 41 – 50 years | 8 | 16% |
| 51 – 60 years | 13 | 26% |
| 61 – 70 years | 12 | 24% |
| 71 – 80 years | 7 | 14% |
| **Gender** | | |
| Female | 20 | 40% |
| Male | 30 | 60% |
| **Clinical presentation** | | |
| Chronic constipation | 2 | 4% |
| Abdominal Pain | 1 | 2% |
| Altered bowel habits | 6 | 12% |
| Anaemia | 2 | 4% |
| Bleeding per rectum | 15 | 30% |
| Caecal growth | 1 | 2% |
| Constipation with haemorrhoids | 1 | 2% |
| Intussusception | 1 | 2% |
| Obstruction | 12 | 24% |
| Perforation | 3 | 6% |
| Polypoidal growth | 1 | 2% |
| Weight loss with abdominal pain | 1 | 2% |
| Weight loss with anaemia | 4 | 8% |
| **Site of lesion** | | |
| Ascending Colon | 7 | 14% |
| Caecum | 8 | 16% |
| Descending Colon | 9 | 18% |
| Hepatic Flexure | 1 | 2% |
| Rectum | 16 | 32% |
| Sigmoid Colon | 7 | 14% |
| Transverse Colon | 2 | 4% |
| **Distribution of gross type** | | |
| Circumferential | 5 | 10% |
| Polypoidal | 7 | 14% |
| Ulcerative | 5 | 10% |
| Ulcero-proliferative | 33 | 66% |
| **Distribution of size category of lesion** | | |
| <5 cm | 22 | 44% |
| 5 – 10 cm | 25 | 50% |
| >10 cm | 3 | 6% |
| **Tumour stage** | | |
| T1 | 2 | 4% |
| T2 | 25 | 50% |

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

| | | |
|---|---|---|
| T3 | 15 | 30% |
| T4 | 8 | 16% |
| **Nodes** | | |
| N0 | 27 | 54% |
| N1 | 14 | 28% |
| N2 | 9 | 18% |
| **Presence of LVI** | 4 | 8% |
| **Presence of PNI** | 1 | 2% |
| **Total** | **50** | **100%** |

Data are expressed as n with %. Total N=50

Majority of the colorectal carcinoma were occurred between 5th decade of life (28.9%) and mucinous carcinoma shows predominant age presentation found to be 3rd decade of life (40%). Dominant clinical presentation is bleeding per rectum (30%) and second common presentation was obstruction (24%). Predominant site involved in the present study is found to be rectum (32%) followed by descending colon (18%). In predominant gross presentation of the colorectal carcinomas is found to be ulcero-proliferative growth (66%) followed by polypoidal growth (14%). Size of the lesion observed predominantly in the study population is found to be between 5 to 10 cm. Majority of adenocarcinoma falls into T2 stage (50%) and no nodal involvement (54%) has been observed at the time of presentation. Only 8% of the study population shows lympho-vascular invasion and 2% shows perineural invasion.

**Table 3: Descriptive assessment of beta-catenin subcellular localization and scores (mean) across colorectal neoplasms (Total N=23)**

| **Colorectal neoplasms** | **n** | **Beta-Catenin Expression** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Membranous** | | **Cytoplasmic** | | **Nuclear** | |
| | | **Mean** | **± SD** | **Mean** | **± SD** | **Mean** | **± SD** |
| **Benign Neoplasms** | | | | | | | |
| Polyp (PJ, Juvenile, HP and adenoma) | 4 | 200 | ± 81.6 | 62.5 | ± 43.4 | 12.5 | 2.08 |
| **Premalignant** | | | | | | | |
| Tubular adenoma-Low grade dysplasia | 2 | 130 | ± 42.4 | 166.5 | ± 40.3 | 57 | 1.4 |
| Tubular adenoma – High grade dysplasia | 2 | 110 | ± 14.1 | 202 | ± 31.8 | 62.5 | 3.5 |
| **Malignant** | | | | | | | |
| Adenocarcinoma | 15 | 52 | ± 8.8 | 142.3 | ± 26.1 | 219 | 42.8 |

According to table 3, the numerical beta catenin score has given to each of the polyps and adenocarcinoma. Expression of beta-catenin is predominantly membranous in benign lesions, predominantly cytoplasmic in premalignant lesion and nuclear in malignant lesions.

**Table 4: Comparison of mean beta-catenin score between benign/premalignant and malignant neoplasms in colorectal carcinoma**

| **S.No** | **Beta-Catenin Score** | **Benign/ Premalignant (N=8)** | | **Malignant (N=15)** | | **T value** | **df** | **P value** |
|---|---|---|---|---|---|---|---|---|
| | | **Mean** | **SD** | **Mean** | **SD** | | | |
| 1 | Membranous | 160 | 70.9 | 52 | 8.9 | 5.9 | 21 | <0.001* |

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | Cytoplasmic | 123.5 | 75 | 142 | 26.1 | 0.88 | 21 | 0.385 (NS) |
| 3 | Nuclear | 36.1 | 25.4 | 219 | 42.8 | 11.05 | 21 | <0.001* |

Data are expressed as mean with SD. Unpaired't' test was used to compare the mean between the groups. *indicates $p<0.05$ and considered statistically significant. NS = Not significant.

According to table 4, the correlation between the membranous scoring and the nuclear beta catenin scoring has found to be statistically correlated

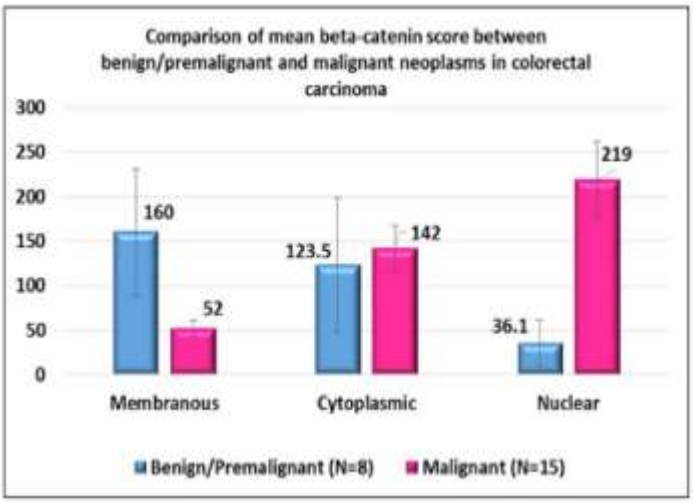


**Figure 2**

**Table 5: Correlation of beta-catenin IHC score grade with respect to tumor grade, tumor stage in patient with colorectal carcinoma in the study.**

| | **Beta-catenin scoring grade** | | | | | | **p value** |
|---|---|---|---|---|---|---|---|
| | **Grade I (N=3)** | | **Grade II (N=7)** | | **Grade III (N=5)** | | |
| | **n** | **%** | **n** | **%** | **n** | **%** | |
| **Tumor grade** | | | | | | | |
| Well differentiated | 3 | 100 | 2 | 28.6 | 0 | 0 | 0.049 |
| Moderately differentiated | 0 | 0 | 3 | 42.8 | 2 | 40 | |
| Poorly differentiated | 0 | 0 | 2 | 28.6 | 3 | 60 | |
| **Tumour stage** | | | | | | | |
| T2 | 3 | 100 | 4 | 57.1 | 0 | 0 | 0.008 |
| T3 | 0 | 0 | 3 | 42.9 | 1 | 20 | |
| T4 | 0 | 0 | 0 | 0 | 4 | 80 | |
| **Node status** | | | | | | | |
| N0 | 3 | 100 | 3 | 42.9 | 0 | 0 | 0.001 |
| N1 | 0 | 0 | 4 | 57.1 | 0 | 0 | |
| N2 | 0 | 0 | 0 | 0 | 5 | 100 | |

Note: Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. *indicates $p<0.05$ and considered statistically significant.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

The degree of tumor differentiation has been statistically correlated with grade of beta catenin score. Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. *indicates $p<0.05$ and considered statistically significant. Regarding stage of the tumour has been correlated with degree of beta catenin expression in grades and are found to be statistically correlated. The degree of nodal involvement is found to be statistically correlated with grade of beta catenin expression.

**Table 6. Comparison of frequency distribution of survival with respect to the IHC grade in colorectal adenocarcinoma patients.**

| S.No | Beta catenin IHC scoregrade | Alive (N=9) | | Expired (N=3) | | Recurrence (N=3) | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| 1 | Grade I | 3 | 33.3 % | 0 | 0 | 0 | 0 |
| 2 | Grade II | 5 | 55.6 % | 1 | 33.3 % | 1 | 33.3 % |
| 3 | Grade III | 1 | 11.1 % | 2 | 66.7 % | 2 | 66.7 % |
| Chi square value = 5.71; Degree of freedom = 4; p value = 0.222 | | | | | | | |

Note: Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. NS = Not significant.

According to the table 6, beta catenin expression has been correlated with survival of the patient. As the recurrence rate and the death were equally distributed both are statistically not correlated in the present study.

**Discussion**

Colorectal carcinoma is the one of the most common cancer worldwide. In order to reduce the mortality, we need to understand the basics of carcinogenesis and various steps in it. Most of the colorectal carcinoma develops from the polyp and thus follows the adenoma-carcinoma sequence. There lies the complex interaction between the genetic and environmental factors. Regarding the incidence observed in the colorectal lesion of the present study, malignant lesion constitutes about 86.5% and polyp constitutes about 13.5% and this has been correlated with Bhattacharya et al study where the malignant lesion forms 66.6% and polyp forms about 33.3%. Among the polyps, Tubular adenoma predominates as it forms about 6.7% and rest of the other polyps occupies 1.6% each. Similar results have been observed in Eshghi MJ et al[20] Bhattacharya et al[10] and Dakshitha Praneeth wickrame singh et al[89] study. Among the malignant lesion, conventional adenocarcinoma constitutes 75% of the lesion followed by mucinous adenocarcinoma (8.3%) and other lesion such as lymphoma and neuroendocrine carcinoma forms about 1.6% each. This type of observation has been documented in various studies such as Gao et al[16](59.8%), Brunn et al[18] (72.5%), Kazem et al[17] (68%) and Bhattacharya et al[10] (79.5%) study.

Incidence of polyp with respect to age in the study population has found to be $4^{th}$ and $5^{th}$ decade of life (table 2) as it constitutes about 50% and this observation has not been seen in other studies as they all shows the predominant age involved to be $5^{th}$ and $6^{th}$ decade, this is mainly due to the increasing awareness and early colonoscopy investigation that facilitates the detection of polyp at earlier age. The pre- dominant gender involvement is male which constitutes about 62.5% (table 2). Sim ilar results have been noted in study such as Eshghi MJ et al[90] (65%) male Dakshitha Praneeth wickrame singh et al[19] (74% male), Bhattacharya et al[10](71% male). The dominant clinical presentation found to be pedunculated growth 75%

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

(table 2.) Sim ilar observation has been made in Bhattacharya et al[10], 66% shows pedunculated growth.

The predominant site involved to be distal colon (sigmoid colon and rectum) as it constitutes 75% (table 2) and this has been correlated with study conducted by Dakshitha Praneeth wickrame singh et al[19] where the rectum involvement has found to be 68%.Regarding the incidence of size (table 3), majority of the polyps were less than 1cm (62.5%) followed by size between 1 to 2cm (25%),only 12.5% of polyp is more than 2cm.Similar observations has been noted in M.H.Vatyn et al (58%) and Dakshitha Praneeth wickrame singh et al[19] (64%) as in both studies the predominant size of the polyp were less than 1 cm. The distribution of both benign and pre-malignant polyps in the present study were found to be equally distributed likewise the sessile and pedunculated polyps were also equally distributed 50% (table 3).This observation has been correlated with Bhattacharya et al as it shows 54% of premalignant polyp and 45% of benign polyp. Tatsuhiro ishii[15] et al, according to this study 269 polyps were studied and advanced colorectal polyps were identified based on the size more than 10mm and high-grade dysplasia and the presence of villous histology, the concept of villous change may indicate the increased malignant potential. Polyps were divided into two types depending on its attachment with colonic mucosa in sessile polyps the base of the polyp directly attached to the colonic mucosa whereas in pedunculated polyps the base is attached with the colonic mucosa with a mucosal stalk. The premalignant potential of the sessile polyp is more when compared to the pedunculated polyp as it can directly invades the colonic tissue. The presence of dysplasia either low grade or high grade also contributes to the premalignant potential of the polyp. In the present study, we observed 2 polyps having low grade dysplasia and 2 polyps having high grade dysplasia[20].

The distribution of colorectal carcinoma with respect to age has been studied in the table 2 in which the conventional adenocarcinoma has been more common in the 5$^{th}$ decade of life (26%) whereas majority of mucinous adenocarcinoma found to be presented in 3$^{rd}$ to 5$^{th}$ decade of life (6%). This observation shows the early age of presentation of mucinous adenocarcinoma when compared to that of conventional adenocarcinoma.

Regarding the gender affected in the present study population (table 2) shows male predominance as it constitutes about 60% and affected female shows only 40% and this observation has been correlated with Gao et al[16], Peker et al, Kazem et al[17], Wong et al[13], Rashifa vakiyath et al[11]. Only one study Brunn et al[18] shows female dominance (60%) and affected male (40%) as most of study population belongs to Caucasian ethnic groups where the predominant population undergo screening was female more than 60%

Although patient present with many clinical symptoms, Bleeding per rectum which occupies about 30% followed by obstruction (24%) and altered bowel habits (12%). Similar results had been correlated with study conducted by Bhattacharya et [10] study in which bleeding per rectum constitutes 77.7%.

Distribution of colorectal carcinoma with respect to site (table 2) shows, pre- dominant site involved found to be Rectum (32%) followed by descending colon (18%) and has been correlated with study conducted by Gao et al[16] (60.2%) and Kazem et al[17] (33%) and other studies such as Bhattacharya et al[80] shows proximal colon was the dominant site.

Age range of the affected population has been correlated with site involved and the result found to be predominant site rectum and affected groups belong to 5$^{th}$ decade, both were statistically correlated as the p value is less than 0.003 by using Fisher's exact test. In similar way, age has been compared with gender and were found to be not statistically correlated.

Colorectal carcinoma grossly presented as an ulcero-proliferative growth (table 2) in 33 cases

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

and it forms the majority 66% followed by polypoidal which constitutes about 14%. This type of observation is seen in Kazem et al[17] study where the ulceroproliferative lesion constitutes 52%. Other study such as Bhattacharya et al[10] shows 66% of polypoidal growth and are found to be discordant with present study.

Incidence of colorectal carcinoma with respect to size has been studied which shows 50% of the carcinoma lies between 5 to 10 cm with mean size of 4cm followed by less than 5cm, it constitutes about 44% and this has been correlated with Bhattacharya et al study[10] (table 2) as it shows the size range between 2 to 10 cm with mean size of 3.5cm Whereas Gao et al[16] shows the predominant size involved is less than 5cm.

Distribution of various histological grades of adenocarcinoma has been studied which shows the dominant population belongs to Grade 1-well differentiated carcinoma as it occupies about 66.77% followed by Grade 2-moderately differentiated carcinoma (22.2%) and Grade 3-poorly differentiated carcinoma constitutes only 11.1%.This has been correlated with Peker k et al[12] study which shows 44% of well differentiated carcinoma,22% shows moderately differentiated carcinoma and 33% shows poorly differentiated carcinoma. Other study such as Kazem et al[17](53%), Wong et al[83] (73.3%) and Rashifa vakiyath et al[11] (52%) shows predominance of moderately differentiated carcinoma. Degree of differentiation has been correlated with age in the present study reveals that age group affected to be 5$^{th}$ decade (33%).

Distribution of colorectal carcinoma with respect to stage that includes the depth of penetration and nodal status has been studied (table 2), the predominant T stage was found to be T2 as it constitutes 50% followed by T3 which constitutes 30% and this has been correlated with Kazem et al[17](T2-60%). Other study such as Wong et al[13](T3-56%), Bhattacharya et al[10] (T3-45%), Rashifa vakiyath et al[11](T3- 36%) shows predominant T stage is T3. Predominant nodal status was found to be N0 in our present study as it constitutes 54%, N1 occupies about 28%, N2 occupies about 18% and has been correlated with Rashifa vakiyath et al[11] (N0-37%) and Kazem et al[17] (N0-60%) and other study shows predominantly of N1.

In the present study population, the dominant histological grade and stage is found to be G1 and T2N0. This is comparatively early in presentation which is due to the community awareness and establishment of colonoscopy investigation that has resulted in the detection of colorectal carcinoma at earlier stage before metastasis.

Among the total colorectal carcinoma studied only 8% shows lympho-vascular invasion and 2% only shows perineural invasion (table2). Among the various study, Bhattacharya et al[80] shows 56% shows lympho-vascular invasion and 40% shows perineural invasion.

Beta-catenin (CTNNB1) plays an important role in the WNT signaling pathway which is controlled by APC (adenomatous polyposis coli) gene. Beta- catenin is the cell adhesion molecule and it is a member of signal transduction pathway. Mutation of the beta-catenin gene results in the activation of WNT signaling pathway. Normally beta-catenin is situated in the colonic mucosa, intracellular accumulation of beta-catenin has been prevented by the APC activity thus it acts as tumor suppressor gene. The APC gene maps at position 21q in chromosome 5 and it codes for the multiple functional domain that interact with proliferation and apoptosis regulators. Mutation in the APC gene results in the intracellular accumulation of beta- catenin which leads to activation of transcription factors with subsequent nuclear translocation which results in the development of colorectal neoplasm. The adenoma-carcinoma sequence of colorectal tumorigenesis requires accumulation of genetic alteration.

Normal colonic mucosa shows diffuse membranous positivity and no cytoplasmic and nuclear accumulation exclusively studied by Miet *et al*. Gradual decrease in membranous staining and subsequent increase in cytoplasmic and nuclear staining has been associated

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with increasing level of dysplasia in the neoplastic lesion of large intestine studied by Iwamoto M *et al.* Increased nuclear staining of beta- catenin has been noted in the colorectal carcinoma. In the present study, localization of beta-catenin has been studied in various colorectal neoplasm which includes benign polyps, pre-malignant polyps and carcinomas. This shows increased membranous expression in benign polyps with mean score of 200, premalignant polyps such as tubular adenoma with low grade dysplasia and tubular adenoma with high grade dysplasia shows increased cytoplasmic expression of beta-catenin with mean score of 166.5 and 202 respectively. Adenocarcinoma shows loss of membranous expression with mean of 52, slightly raised cytoplasmic score with mean of 142.3 and increased nuclear score of 219 has been observed.

Present study conducted on the colorectal neoplasm correlates the mean score of membranous, cytoplasmic and nuclear expression of polyps and carcinoma by using unpaired "t" test and results obtained shows a significant correlation between the increased membranous score in benign polyps and increased nuclear score in colorectal carcinoma. Similar results had been observed in Bhattacharya et al [10], Wong et al[13] study.

The scoring system adapted by both of this study follows Mauri et al[4] in which membrane, cytoplasm and nucleus were scored separately by multiplying the percentage of positive cells with intensity of staining where the percentage has been obtained by evaluating 1000 cells in 4 fields under 400X magnification and the staining intensity has been scored as follows 0=no expression 1+=weak expression 2+=moderate expression 3+=strong expression 4+=very strong expression. Colorectal carcinoma has been graded histologically by means of degree of differentiation as well, moderate and poorly differentiated carcinoma and this has been correlated with beta-catenin expression which also graded in to grade 1,2 1nd

This method of scoring is based on Jass et al[5] scoring system. The β - catenin activation score was calculated as the sum of nuclear score, cytoplasmic score and the membrane score. Total score then collapsed in to grade I, grade II and grade III. For nuclear scoring three categories are given 0-no expression, + 1 - weak expression, + 2 - positive expression, for cytoplasmic staining–0 no expression, + 1 - weak expression, + 2 - positive expression, for membrane scoring, 0 – positive membrane expression, + 1 - negative membrane expression. Total score collapsed in to three grades. GRADE I = 0 - 1 GRADE II = 2 - 3 GRADE III = 4 to 5. Total score of 0 reflecting normal colonic mucosa and score of 5 for tumours with strong nuclear staining (n = 2), strong and diffuse cytoplasmic staining (n = 2), loss of cell membrane staining (n = 1).

Initially individual membranous, cytoplasmic and nuclear scoring has been made in many studies which includes Wong et al and Bhattacharya et al[10] they have encountered many controversies like histological grade has not been correlated with beta-catenin score, in order to overcome those shortcomings, grading of beta-catenin has been introduced especially for studying malignant lesions. In the present study we use both methods of scoring as the study population includes both benign and malignant lesions.

The histological grade of colorectal carcinoma has been statistically correlated with the beta-catenin scoring grade and has found to be statistically correlated as the fisher's test indicates the p value of 0.049. Predominant grade of beta-catenin expression in the present study population have found to be grade 2. The predominant grade of beta-catenin expression in the present study population has found to be grade 2 (table 6) and similar observation has been seen in 2 studies Kazem et al[17](83.3%) and Rashifa vakiyath et al[11] (73.3%). Expression of beta-catenin has been correlated with regards to depth of invasion i.e .T stage of colorectal carcinoma and are found to be statistically correlated by obtaining the p value of 0.008% by using Fisher's exact t test. Similar results had been obtained in Rashifa vakiyath et al[11], Gao et al[16] and Kazem et al[17]. Expression of beta-catenin has been

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

compared with the nodal status which shows significant correlation and has been observed in Rashifa vakiyath etal[11], Gao et al[16] and brunn et al[18]. The survival range of the patients with colorectal carcinoma has been studied which reveals the population having low beta-catenin grade constitutes about 20% they were followed up for 12 months and were found to be no recurrence and death. Population with high grade of beta-catenin expression which constitutes about 33% recurrence rate , death documented in 40% and only 20% found alive in the present observation study. Similar results has been found in Gao et al[16] study which proves survival rate of the patients with high grade of expression over the period of 5 years is 52.6% and in patients with low grade of beta-catenin expression has survival rate of 70.1%

Among all the study, only Kazem et al[17] study had observed the recurrence of carcinoma in 16.66% of population over the period of follow up of 2 years exhibiting the grade 3 expression of beta-catenin which approximately correlates with our present study which constitutes about 20% of recurrence with grade 3 expression of beta-catenin over the follow up period of 1 year. β - catenin to detect the malignant potential of colorectal lesions and this β - catenin study will help in the early management and prevention of the development of a subsequent malignancy. The deeply invasive and high - grade colorectal carcinoma shows intense β - catenin expression, as this study highlights the possibility of use of immunomarker to assess the prognosis of the patient. The statistically significant correlation of β - catenin with TNM staging and histological grading may supports its use as an additional prognostic marker in colorectal adenocarcinoma. In future β - catenin marker could also be used in small biopsies to predict tumour stage and grade to plan appropriate treatment strategy.

**Conclusion**

Males were predominantly affected than females. The commonest presenting symptoms was bleeding per rectum. Commonest pattern of growth is ulcero-proliferative growth. Rectum was the commonest site to be involved followed by descending colon and caecum. The predominant polyps in the present study was found to be tubular adenoma as it constitutes 50% and it is associated with dysplasia (both low and high). Among the carcinoma studied, conventional adenocarcinoma forms the major population as it constitutes about 75%. Regarding the degree of differentiation- Well differentiated adenocarcinoma (grade 1) dominates as constitutes 66.7% of conventional adenocarcinoma. Most of the colorectal carcinoma presents at T2N0 stage.

**Beta Catenin Expression**

Normal colorectal mucosa and benign polyps shows predominant membranous expression. Premalignant polyp shows predominantly increased cytoplasmic expression with focal nuclear positivity and the colorectal carcinoma shows raised nuclear expression and loss of membranous expression. This proves the gradual trans location of beta catenin from adenoma carcinoma sequence. Beta-catenin expression has been significantly correlated with depth of invasion (T), nodal metastasis (N), degree of differentiation (tumor grade) and to the stage. Increased degree of beta-catenin expression has been associated with reduced disease- free interval. Hence it can be used as an additional prognostic marker in colorectal carcinoma.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

**Fig 1: Multiple polyps in sigmoid colon**

**Fig 2: Ulceroproliferative growth of the rectum**

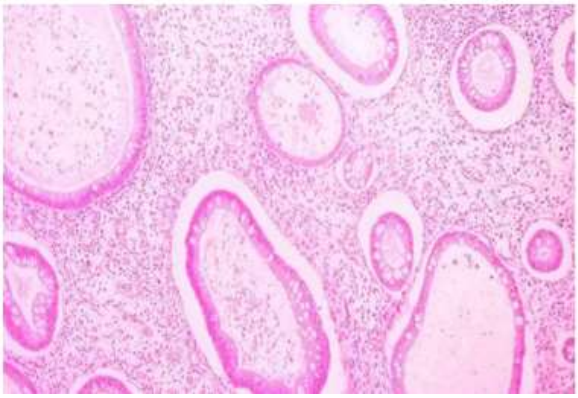

**Fig 3: Juvenile polyp 10x**

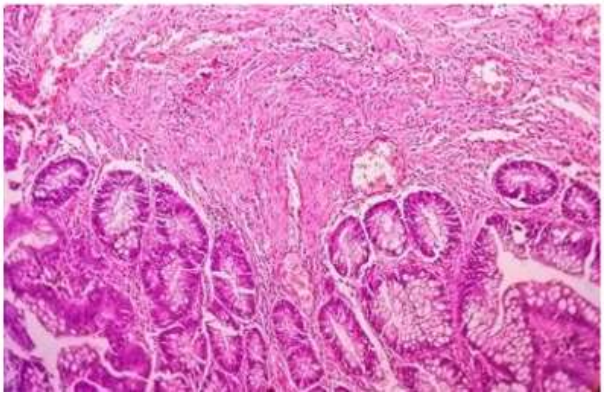

**Fig 4: Peutz Jegher polyp 10x**

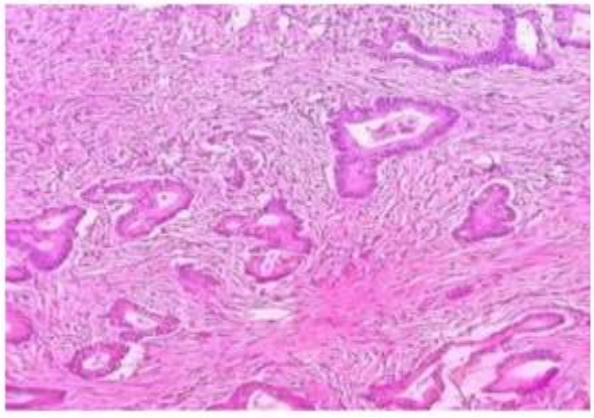

**Fig5: Well differentiated adenocarcinoma**

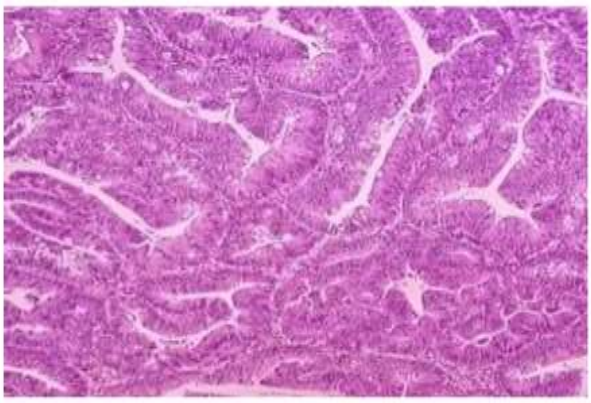

**Fig 6: Moderately differentiated adenocarcinoma**

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

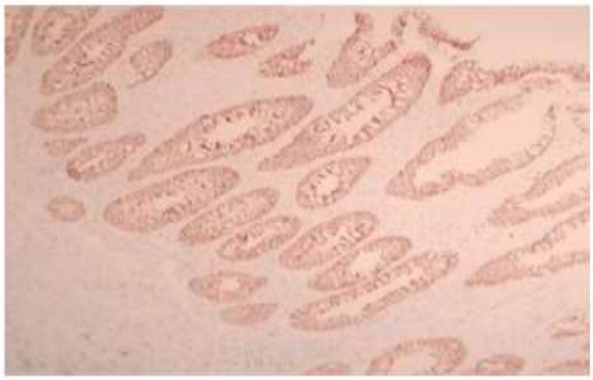

**Fig 7: Beta catenin showing diffuse membranous positivity**

**in normal colonic mucosa**

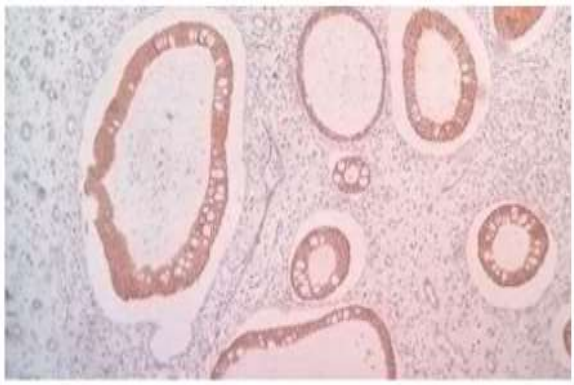

**Fig 8: Beta catenin showing diffuse membranous positivitiy in Juvenile polyp.**

**Fig 9: Patchy loss of membranous expression with nuclear positivity diffuse in tubular adenoma with low grade dysplasia**

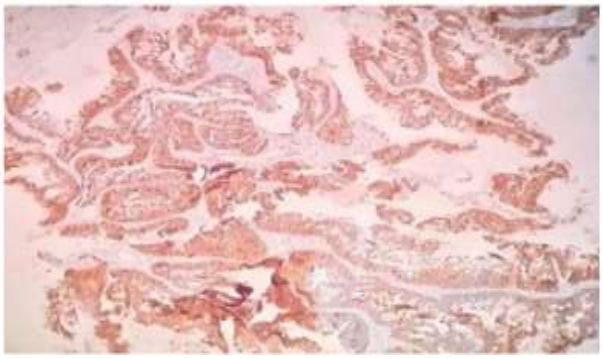

**Fig 10 Diffuse cytoplasmic and focal nuclear positivity in tubular adenoma with high grade dysplasia**

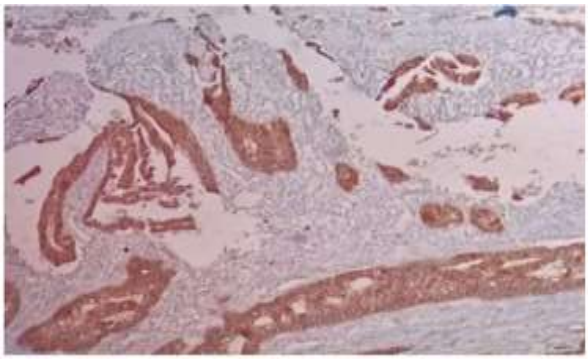

**Fig 11: predominant nuclear positivity and variable cytoplasmic positivity in well differentiated adenocarcinoma**

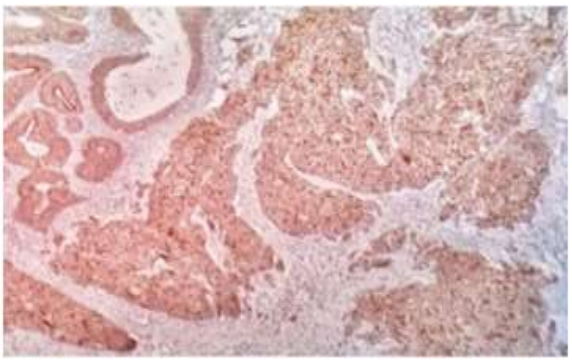

**Fig 12: predominant nuclear positivity in moderately differentiated adenocarcinoma**

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

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ISSN: 0975-3583,0976-2833 VOL15, ISSUE 07, 2024

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