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Original Research Article

Evaluation of Role of CTLA4 Immunohistochemistry in the Diagnosis of Colon Cancers

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Conflict of interest: Nil

Abstract:

Aim: To assess role of CTLA4 immunohistochemistry in the diagnosis of colon cancers.

Methodology: Fifty-four resected specimens of colorectal cancer of both genders were selected. From colectomy specimens, paraffin blocks were prepared on samples from the tumour areas and the nearby normal areas. On the sections and on the controls, IHC for CTLA-4 was performed.

Results: Right side was involved in 30 cases and left side in 24. There were 23 males and 23 females. Procedure performed was sigmoidectomy in 7, hemicolectomy in 21, resection in 19, hartmann's procedure in 4 and pancolectomy in 3 cases. The difference was significant (P< 0.05). Grade found to be well differentiated in 13, moderately differentiated in 31 and poorly differentiated in 10 cases. TNM staging was stage 1 in 15, stage 2 in 9, stage 3 in 28 and stage 4 in 2 cases. Perineural invasion was present in 11 and absent in 43 cases. Histopathological diagnosis was mucinous carcinoma in 8, adenocarcinoma in 27 and adenocarcinoma with mucinous component in 19 patients. Lymphovascular invasion was present in 24 and absent in 30 cases. Intensity of CD+4 cell uptake by tumour cell was 1+ in 14, 2+ in 19 and 3+ in 21 cases. The difference was non-significant (P> 0.05). There was a significant increase in the tumor quantity among those with uptake of 3+ with a mean difference of 19.5. The difference was significant (P < 0.05). There was a significantly high tumour infiltration among those with an uptake of 3+, with a mean difference of 19.8.

Conclusion: Authors showed overexpression of CTLA-4 in colorectal cancer specimens.

Keywords: Tumour Infiltration, Immunohistochemistry, Colon Cancers.

Introduction

Colon cancer, also known as colorectal cancer, is a type of cancer that affects the colon or rectum, which are parts of the large intestine. It typically begins as a growth called a polyp, which can be noncancerous (benign) or cancerous (malignant). Over time, malignant polyps can develop into colon cancer.[1]

The exact cause of colon cancer is unclear, but certain risk factors can increase the likelihood of developing the disease. These include age (most cases occur in people over 50), family history of colon cancer or polyps, personal history of inflammatory bowel disease (such as Crohn's disease or ulcerative colitis), unhealthy lifestyle choices (such as a diet high in red meat and low in fiber, lack of physical activity, smoking, and heavy alcohol consumption), and certain inherited gene mutations (such as Lynch syndrome or familial adenomatous polyposis).[2] In the early stages, colon cancer may not cause any noticeable symptoms. However, as the disease progresses, symptoms can include changes in bowel habits (such as diarrhea or constipation), persistent abdominal discomfort, rectal bleeding or blood in the stool, unexplained weight loss, fatigue, and a feeling that the bowel is not completely empty after a bowel movement. Early detection through regular screenings is crucial because it allows for the identification and removal of polyps before they become cancerous or the early detection of cancer when it's most treatable.[3]

Immunohistochemical markers have lately been employed for diagnosis and prognosis evaluation. Epidermal Growth Factor Receptor (EGFR), Ki-67 Proliferative Index, Matrix Metalloproteinase-9, Cytokeratin 7 (CK7), and CDX2 are a few of these markers. The gene for Cytotoxic T Lymphocyte Antigen-4 (CTLA-4) is one such element. On chromosome 2q33, there is a polymorphic gene called CTLA-4. It attaches to B7 molecules and prevents cytokine synthesis, giving T cells unfavourable feedback.[4]Its function in the vulnerability to specific malignancies has long been investigated since the presence of CTLA-4 can drastically change the immune response. The immunosurveillance marker CTLA-4 has been

evaluated for colorectal malignancies, and some studies have shown the immunomodulatory effects.[5] We performed this study to assess role of CTLA4 immunohistochemistry in the diagnosis of colon cancers.

Methodology

After considering the utility of the study and obtaining approval from ethical review committee, we selected fifty- four resected specimens of colorectal cancer of both genders. Patients' consent was obtained before starting the study.

Data such as name, age, gender etc. was recorded. From colectomy specimens, paraffin blocks were prepared on samples from the tumour areas and the nearby normal areas. On the sections and on the controls, IHC for CTLA-4 was performed. Gross discoveries were made in the blocks. For immunohistochemistry, blocks comprising sections containing both tumour and normal epithelium were chosen. For microscopic analysis, for resected lymph nodes, and for surgical margins, hematoxylin and eosin staining was carried out. Intratumoral and peritumoral inflammatory responses were observed as additional histological characteristics. Additionally, the percentage of tumour infiltrating lymphocytes and tumour cells that were positive was calculated. The results were compiled and subjected for statistical analysis using Mann Whitney U test. P value less than 0.05 were set significant.

Results

Table 1: Baseline characteristics

Parameters	Variables	Number	P value
Side	Right	30	0.12
	Left	24	
Gender	Male	23	0.19
	Female	31	
Procedure	Sigmoidectomy	7	0.05
	Hemicolectomy	21	
	Resection	19	
	Hartmann's procedure	4	
	Pancolectomy	3	

Right side was involved in 30 cases and left side in 24. There were 23 males and 23 females. Procedure performed was sigmoidectomy in 7, hemicolectomy in 21, resection in 19, hartmann's procedure in 4 and pancolectomy in 3 cases. The difference was significant (P< 0.05) (Table I).

Table 2: Tumour characteristics

Parameters	Variables	Number	P value
Grade	Well differentiated	13	0.81
	Moderately differentiated	31	
	Poorly differentiated	10	
TNM staging	Stage 1	15	0.04
	Stage 2	9	
	Stage 3	28	
	Stage 4	2	
Perineural invasion	Present	11	0.02
	Absent	43	
Histopathological diagnosis	Mucinous carcinoma	8	0.05
	Adenocarcinoma	27	
	Adenocarcinoma with mucinous component	19	
Lymphovascular invasion	Present	24	0.12
	Absent	30	
Intensity of CD+4 cell uptake by tumour cell	1+	14	0.74
	2+	19	
	3+	21	

Grade found to be well differentiated in 13, moderately differentiated in 31 and poorly differentiated in 10 cases. TNM staging was stage 1 in 15, stage 2 in 9, stage 3 in 28 and stage 4 in 2 cases. Perineural invasion was present in 11 and absent in 43 cases.

Histopathological diagnosis was mucinous carcinoma in 8, adenocarcinoma in 27 and adenocarcinoma with mucinous component in 19

patients.Lymphovascular invasion was present in 24 and absent in 30 cases. Intensity of CD+4 cell uptake by tumour cell was 1+ in 14, 2+ in 19 and 3+ in 21 cases. The difference was non- significant (P> 0.05) (Table II).

Table 3: Uptake of CTLA-4 in tumour cells

Parameters	Mean difference	T value	P value
Tumour quantification	19.5	2.9	0.02
Tumour infiltration	19.8	3.1	0.01

There was a significant increase in the tumour quantity among those with uptake of 3+ with a mean difference of 19.5. The difference was significant (P < 0.05). There was a significantly high tumour infiltration among those with an uptake of 3+, with a mean difference of 19.8.

Discussion

To diagnose colon cancer, various tests and procedures are used, including colonoscopy (insertion of a flexible tube with a camera into the rectum and colon to examine the area), sigmoidoscopy (similar to a colonoscopy but examines only the lower part of the colon), imaging tests (such as CT scans or MRI), and biopsy (removal of a small tissue sample for examination under a microscope).[6] The treatment for colon cancer depends on the stage and extent of the disease. It often involves surgery to remove the cancerous tumour and nearby lymph nodes. In some cases, chemotherapy, radiation therapy, or targeted therapy may be recommended before or after surgery. The treatment plan is determined by a multidisciplinary team of healthcare professionals based on individual factors.[7]

Although it's not always possible to prevent colon cancer, certain lifestyle choices can help reduce the risk. These include maintaining a healthy weight, being physically active, eating a balanced diet rich in fruits, vegetables, and whole grains while limiting red and processed meats, avoiding tobacco and excessive alcohol consumption, and undergoing regular screenings for colon cancer, especially for those at higher risk.[8]

The use of CTLA4 immunohistochemistry in colon cancer diagnosis is primarily for research purposes and not yet established as a routine diagnostic tool.[9] It is mainly employed in clinical trials and studies to assess the expression levels of CTLA4 in tumour tissue samples. This information helps researchers evaluate the potential efficacy of immune checkpoint inhibitors that target CTLA4 in treating colon cancer.[10] Immunohistochemistry for CTLA4 involves staining colon cancer tissue samples with specific antibodies against CTLA4. The presence and intensity of staining are then assessed under a microscope. The results can provide information about the level of CTLA4 expression in the tumour, which may have implications for patient prognosis and response to certain immunotherapies.[11] We assessed the role of CTLA4 immunohistochemistry in the diagnosis of colon cancers.

In our study, right side was involved in 30 cases and left side in 24. There were 23 males and 23 females. Procedure performed was sigmoidectomy in [7], hemicolectomy in 21, resection in 19, hartmann's procedure in 4 and pancolectomy in 3 cases. Narayanan et al[12] analysed the level of CTLA-4 expression in thirty resected colorectal cancer tissues. 43.3% of the tumours showed overexpression of CTLA-4. The tumour infiltration was noticeably higher in the samples that had excessive CTLA-4 expression. Statistics showed that the observed difference was significant (P 0.05). When the tumour grade and CTLA4 uptake intensity were compared, it was found that the majority of the well-differentiated tumours (66.7%) had an intensity of 3+ (66.7%).

Our results showed that grade found to be well differentiated in 13, moderately differentiated in 31 and poorly differentiated in 10 cases. TNM staging was stage 1 in 15, stage 2 in 9, stage 3 in 28 and stage 4 in 2 cases. Perineural invasion was present in 11 and absent in 43 cases. Histopathological diagnosis was mucinous carcinoma in 8, adenocarcinoma in 27 and adenocarcinoma with mucinous component in 19 patients. Lymphovascular invasion was present in 24 and absent in 30 cases. Intensity of CD+4 cell uptake by tumour cell was 1+ in 14, 2+ in 19 and 3+ in 21 cases.

In our study, there was a significant increase in the tumor quantity among those with uptake of 3+ with a mean difference of 19.5. There was a significantly high tumor infiltration among those with an uptake of 3+, with a mean difference of 19.8. Increased CTLA-4 was seen in colon and rectal adenocarcinomas in a study by Kitsou M., et al.[13] The study also found enhanced survival outcomes in the presence of high expression of CTLA-4, indicating efficient immune response.

Conclusion

Authors showed overexpression of CTLA-4 in colorectal cancer specimens.

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