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# Expression of MLH-1, PMS-2, MSH-2, MSH-6 markers and histopathological evaluation in colorectal cancer

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## Abstract

**Background:** The clinicopathological characterization of biomarkers to identify microsatellite instability in patients with colon cancers has not been fully studied. This study aims to evaluate the spectrum of histopathology of MLH1, MSH2, MSH6, and PMS2 markers to identify microsatellite instability in patients with colon cancers.

**Methods:** The study analyzed the association between clinicopathological features and immunohistochemistry MLH-1, MSH-2, PMS-2, & MSH-6 expressions in 36 resected colorectal cancer samples. Patients' data were collected retrospectively, including tumor localization, size, the origin of polyp, mucinous differentiation, tumor stage, lymphovascular and perineural invasion, surgical boundary, and lymph node metastases. The primary antibodies, such as MLH-1, MSH-2, PMS-2, & MSH-6, were tested immunohistochemically.

**Results:** The number of male cases was higher than female cases, 63.89% vs. 36.11%. The highest incidence (27.78%) of colorectal cancer was seen in the age group of 51-60 years. Tumors were predominantly adenocarcinoma NOS (69.44%). Moderately differentiated tumors (55.56%) were higher in number than poorly differentiated and well-differentiated ones. The highest stage seen was stage III in 50%. 80.56% of cases showed perineural invasion, 27.78% showed lymphovascular invasion, and 52.78% showed nodal metastasis. Right colon placement was associated with mucinous adenocarcinoma and a mucinous component. The ulcerous tumors were quite small, and their pathological tumor stage was higher. Tumors developing from polyps were large in size and had a lower pathological tumor stage. The study found that deletion of MSH-2 & MSH-6 expressions was substantially associated with right-colon placement and poor differentiation. In poorly differentiated adenocarcinomas, mucinous, lymphovascular, and perineural invasions were common. Tumors with mucinous differentiation showed less lymphovascular invasion and lymph node metastases. Perineural invasion was not noted in the pT1 or pT2 tumor stages.

**Conclusion**: A strong correlation was found between immunohistochemical markers and clinical characteristics of tumors with microsatellite instability. However, these conclusions should be supported by large-scale investigations involving molecular PCR and other approaches.

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## Highlights

**What is current knowledge?**

Loss of expression of immunohistochemical markers such as MLH-1, MSH2, PMS-2, and MSH-6 was associated with poor differentiation and mucinous adenocarcinomas. There was a significant relationship between the cecum and ascending colon location and lower expression of PMS-2 & MSH-6.

**What is new here?**

The study found that low levels of MSH-2 and MSH-6 were associated with colon location, poor differentiation, and mucinous differentiation. It also found a strong link between MLH-1, MSH-2, PMS-2, and MSH-6 expressions and colorectal cancer and clinical characteristics of tumors with microsatellite instability.

## Introduction

Colorectal cancer accounts for 9% of all cancer cases, making it the third most frequent cancer globally and the second leading cause of cancer-related death (1). Several genetic variations have been proposed as prognostic indicators; however, none have been validated for therapeutic application. Evidence suggests that measuring the level of microsatellite instability can provide important prognostic information in this tumor type (2,3).

Microsatellite instability is characterized by substantial fluctuations in the length of short repeating sequences known as microsatellites in tumor DNA. Mismatch repair is a biological post-replication mechanism that maintains DNA balance. Mutations in mismatch repair genes, including MLH1, PMS2, MSH2, and MSH6, lead to MSI colon cancer (4). Mismatch repair protein deficiency occurs in approximately 15-20% of colorectal tumors. Colorectal cancers are classified as high-frequency microsatellite instability (MSI-H: loss of two or more markers) when instability occurs in at least 30% of the loci studied or low-frequency microsatellite instability (MSI-L: Loss of one marker) when less than 30% of the loci are unstable. Microsatellite-stable tumors exhibit no changes in the length of the DNA sequences analyzed. Microsatellites are stable when no markers are lost. Colorectal cancer develops via the microsatellite instability pathway in 12-15% of cases, with 2-5% being inherited (5,6).

MSI-H colorectal carcinomas are less aggressive than common large bowel tumors. In large patient populations, investigations have shown that the MSI-H phenotype confers a survival advantage independent of the cancer stage and other clinical and pathologic factors (7).

MSI-H is caused in most cancers by DNA mismatch repair abnormalities, specifically the inactivation of the MLH1 and MSH2 genes. The immunohistochemical test for MLH1 and MSH2 expression is a simple, inexpensive, and dependable technique to identify most MMR-defective colorectal cancers (8).

In this work, we examined the histopathological spectrum of MLH1, MSH2, MSH6, and PMS2 markers and their prognostic relevance in identifying microsatellite instability in colon cancer patients.

## Methods

This retrospective study is based on medical records from the Pathology Department of SVS Medical College Hospital, Mahbubnagar. This study included 36 colorectal cancer patients who underwent resections at our institution between 2019 and 2023. Clinical features and tissue characteristics of the tumors were obtained from the hospital records. The study included 36 consecutive patients with TNM stage II (22%), stage III (53%), and stage III (13%) colorectal adenocarcinoma who had received curative surgical resection. The study comprised patients with malignant colon tumors. Benign colon tumors and inflammatory and metastatic lesions were all excluded.

IHC was used to investigate mucosal samples and surgical resections of colon tumors. Microarrays and immunohistochemistry were used to investigate the expression of mismatch repair proteins such as MLH1, MSH2, MSH6, and PMS2.

Two pathologists analyzed hematoxylin and eosin sections, mucinous components, polyp origins, tumor stages, lymphovascular and perineural invasions, lymphocyte densities, surgical margins, and lymph node metastases.

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Tumour staging and histological differentiations were based on the WHO (2010) Classification of Tumours of the Digestive System.

The lymphocyte density in the tumor microenvironment was divided into three categories: 1) stromal lymphocytic infiltration, 2) stromal lymphocyte infiltration and glandular infiltration, and 3) Crohn-like lymphocytic infiltration that occurs independently of lymphocytic infiltration in the tumor.

The presence of a tumor at the surgical margin, either macroscopic or microscopic, was classed as surgical margin positive.

**Immunohistochemical analysis:**

One of the blocks that depicted the tumor's features was chosen. After determining the tumor's proximity to normal mucosa and lymphocytic infiltration, 4 mm of paraffinized tissue was removed, and several blocks were created. Four-micron-thick slices were cut for IHC examination. The primary antibodies MLH-1, MSH-2, PMS-2, & MSH-6 were employed in the immunohistochemistry study. Nuclear staining in normal mucosa and lymphocytic infiltration served as positive controls. Each tumor was inspected in five regions, and the percentage of labeled tumor cells was divided into 3 categories: negative (<1%), 1-50%, and 51-100% positive.

## Results

The highest incidence (27.78%) of colorectal cancer was seen in the age group of 51-60 years, followed by 19.45% in 61-70 years, and 19.45% in the 71-80 years age group. The mean age was 56.5 ± 15.5 years (30-85 years). Males had a higher than female patient population (63.89% vs 36.11%). Tumors were predominantly adenocarcinoma NOS (69.44%) (Table 1). Moderately differentiated tumors (55.56%) were higher in number than poorly differentiated and well-differentiated grading ones. The higher stage was in stage III, which was 50%. 80.56% of cases showed perineural invasion, 27.78% showed lymphovascular invasion, and 52.78% showed nodal metastasis. Colorectal cancer and its distribution cases are illustrated in Table 2.

**Table 1**. Distribution of colorectal cancer cases according to the tumor size and grading

| Characteristics | Frequency | Percentage |
|---|---|---|
| **Histological type** | | |
| Conventional adenocarcinoma | 25 | 69.44% |
| Mucinous adenocarcinoma | 6 | 16.67% |
| Signet ring cell carcinoma | 3 | 8.3% |
| Medullary adenocarcinoma | 1 | 2.7% |
| Squamous cell carcinoma | 1 | 2.7% |
| **Tumor size** | | |
| < 5 cm | 11 | 30.56% |
| ≥ 5cm | 25 | 69.44% |
| **Tumor grading** | | |
| Well-differentiated | 06 | 16.67% |
| Moderately differentiated | 20 | 55.56% |
| Poorly differentiated | 10 | 27.78% |

**Table 2.** Distribution of colorectal cancer cases according to clinicopathological variables

| Variable | Number of cases | Percentage | Variable | Number of cases | Percentage |
|---|---|---|---|---|---|
| Age | | | Mucinous component | | |
| ≤ 50 | 6 | 16.67 | Absent | 8 | 22.22 |
| 51-60 | 10 | 27.78 | Present | 28 | 77.78 |
| 61-70 | 7 | 19.45 | Tumor stage (pT) | | |
| 71-80 | 7 | 19.45 | pT1 | 1 | 2.78% |
| ≥ 81 | 6 | 16.67 | pT2 | 4 | 11.11% |
| Sex | | | pT3 | 18 | 50 |
| Male | 23 | 63.89 | pT4a | 10 | 27.78 |
| Female | 13 | 36.11 | pT4b | 3 | 8.33 |
| Localization | | | Lymphovascular invasion | | |
| Cecum | 8 | 22.22 | Present | 10 | 27.78 |
| Ascending colon | 4 | 11.11% | Absent | 26 | 72.22 |
| Transverse colon | 2 | 5.4% | Perineural invasion | | |
| Descending colon | 1 | 2.78% | Present | 29 | 80.56 |
| Sigmoid colon | 12 | 33.33% | Absent | 7 | 19.44 |
| Rectosigmoid junction | 1 | 2.78% | Lymphocyte density | | |
| Rectum | 8 | 22.23% | Stromal-lymphocytic- infiltration | 7 | 19.45% |
| Growth pattern | | | Glandular- infiltration and stromal- lymphocyte- infiltration | 23 | 63.89% |
| Ulcerous | 7 | 19.45% | Crohn-like- lymphocytic infiltration irrespective of lymphocytic- infiltration within the tumor | 6 | 16.67% |
| Ulcerovegetative | 23 | 63.89 | Tumor in surgical margin | | |
| Polypoid | 6 | 16.67 | Present | 3 | 8.33 |
| Multiple tumors | | | Absent | 33 | 91.67 |
| Absent | 33 | 91.67 | Number of lymph node | | |
| Present | 3 | 8.33 | ≤ 12 | 6 | 16.67% |
| Accompanying polyp | | | 13-24 | 12 | 33.33% |
| Absent | 28 | 77.78 | 25-36 | 10 | 27.78 |
| Present | 8 | 22.22 | 37-48 | 4 | 11.11% |
| Arising in polyp | | | ≥ 49 | 4 | 11.11% |
| Absent | 33 | 91.67 | Lymph node metastasis (pN) | | |
| Present | 3 | 8.33 | pN0 | 17 | 47.22% |
| Differentiation | | | pN1 | 8 | 22.22% |
| Well | 2 | 5.54 | | | |
| Moderate | 28 | 77.78 | pN2a | 4 | 11.11% |
| Poor | 3 | 8.33 | pN2b | 7 | 19.44% |
| Mucinous | 3 | 8.33 | | | |

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In seven cases, there was no staining with MLH-1 or PMS-2. In six cases, MSH-2 & MSH-6 did not stain. Two cases showed only MLH-1 negative. PMS-2 negative was only identified in 3 cases. MSH-2 was positive in the majority of cases (Table 3,4). Immunohistochemical analysis of colorectal cancer samples is depicted in Figure 1, Figure 2, and Figure 3.

**Table 3.** Expression of MMR proteins in colorectal cancers

| MLH1 | PMS2 | MSH2 | MSH6 | No of cases | Percentage |
|---|---|---|---|---|---|
| - | - | + | + | 7 | 19.44% |
| + | + | - | - | 6 | 16.67% |
| - | + | + | + | 2 | 5.56% |
| + | + | - | + | 2 | 5.56% |
| + | + | + | - | 3 | 8.31% |
| + | - | + | + | 1 | 2.78% |
| + | + | + | + | 15 | 41.67% |

**Table 4.** MSI status based on expression of MMR proteins in colorectal cancer

| MSI status | No. of cases | Percentage |
|---|---|---|
| MSS | 15 | 41.67% |
| MSI-L | 8 | 22.22% |
| MSI-H | 13 | 36.11% |
| Total | 36 | 100% |

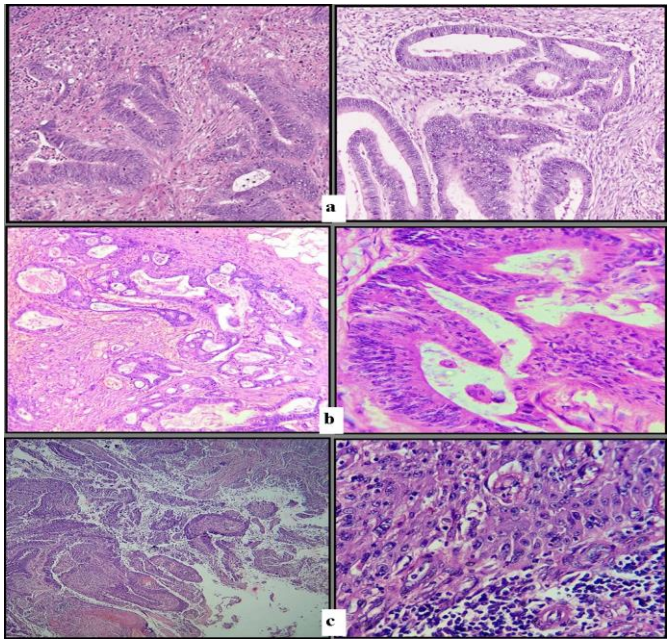


**Figure 1.** a. Well-differentiated adenocarcinoma, well-formed glands. b. Moderately differentiated adenocarcinoma with 50% to 95% gland development. c. Poorly differentiated adenocarcinoma, less than 50% gland development. Most tumors (Excluding the advancing edge) are composed of sheets of cells without gland development.

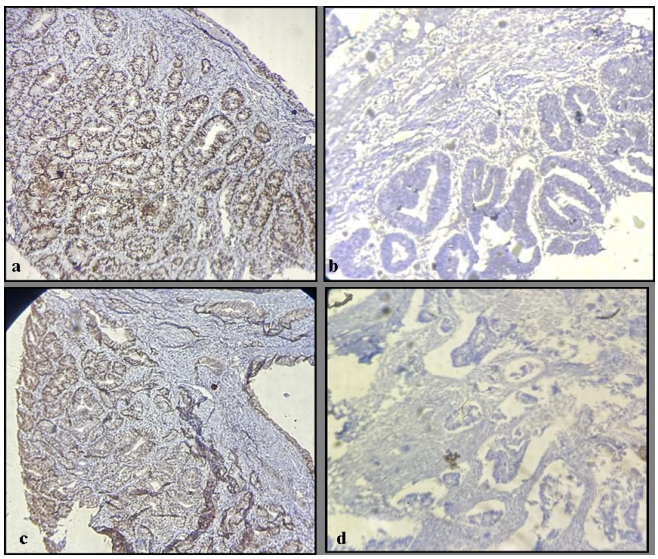


**Figure 2.** a. MLH1 retained +ve. b. MLH1 deficiency (MLH1 -ve). c. PMS2 was preserved (+ve PMS). d. PMS2 deficit (-ve PMS2).

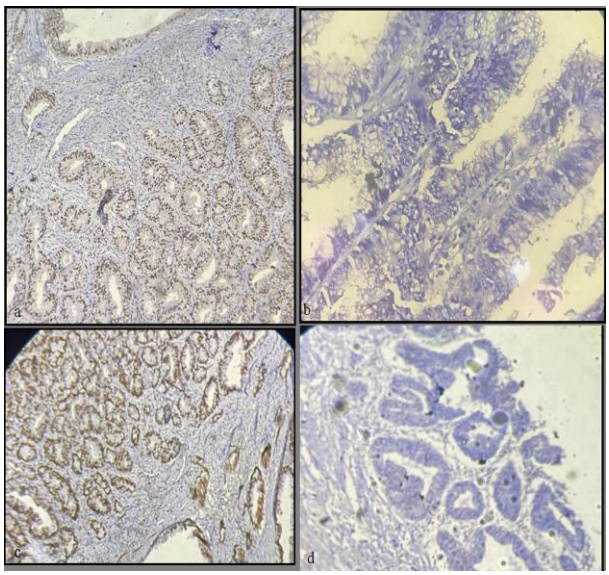


**Figure 3.** a. MSH2 retained (+ve MSH2). b. MSH2 deficient (-ve MSH2). c. MSH6 retained (+ve MSH6). d. MSH6 deficient (-ve MSH6)

Right colon placement was associated with mucinous adenocarcinoma and a mucinous component. The ulcerous tumors were quite tiny, and their pathological tumor stage was higher. Tumors developing in polyps were large and had a lower pathological tumor stage. In poorly differentiated adenocarcinomas, mucinous, lymphovascular, and perineural invasions were common.

## Discussion

The mean age of our study cases was 56.5 years. Due to predisposing circumstances, the incidence of the disease is lower among those under 50. In our study, those aged 51 to 60 comprised 27.78% of colorectal cancer cases. Tumors in the right colon were slightly more common among people aged 51 to 60 than in other age groups.

Colorectal cancer is thought to develop through mechanisms such as chromosomal and microsatellite instability. Microsatellite instability is found in 12% to 15% of colorectal tumors, with 2% to 5% heredity predisposition (9). Tumors induced by microsatellite instability have distinct clinicopathological characteristics, including proximal colon location, mucinous histology, and lymphocyte infiltration.

Due to the complexity and costliness of genetic analysis, it cannot be used to screen for all colorectal malignancies (10,11,12).

We studied the correlation between clinicopathological features and immunohistochemical expressions of MLH-1, PMS-2, MSH-2, & MSH-6 in 36 colorectal cancer resection tissues.

Most malignancies were in the sigmoid colon (33.33%) and rectum (22.23%). Right colon tumors are more likely to have an exophytic growth pattern, be bigger, and be at more advanced stages of development. All left-side tumors were small.

Mucinous adenocarcinomas were detected in young patients, primarily women, with a right colon location, a higher tumor stage, weak differentiation, and advanced lymph node metastases. Our findings revealed a positive correlation between multiple tumor foci and lymph node metastasis, which could be attributable to the tumor advanced stage. Numerous investigations have demonstrated that histological grade, independent of stage, is a major prognostic factor. An advanced tumor grade is associated with increased perineural invasion, peritumoral lymphovascular invasion, lymph node metastases, and tumor invasion (13,14). A significant relationship was noted between high-grade tumors, lymph node metastases, and tumor invasions.

Lymphovascular infiltration was not found in malignant tumors in T1 and T2 stages. The majority of lymphovascular invasion tumors showed lymph node metastases.

A third of tumors without MMR expression were limited to the right colon. According to Chapusot et al., adenocarcinoma in the right colon with poor differentiation has a significant prognostic value for MMR decline (15). In our study, those with negative immunohistochemical markers exhibited right colon location and a poorly differentiated cancer.

No immunohistochemical marker showed a significant correlation with the tumor invasion in our investigation. Our study findings could be supported by large-scale research using molecular approaches.

## Conclusion

The right colon had low levels of MSH-2 and MSH-6 expression, which suggests poor differentiation. The study found a strong correlation between immunohistochemical markers and clinicopathological characteristics. Most subjects were male, most between 51 and 60 years old. As the number of patients

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in advanced stages increases, cost-effective prognostic markers are becoming increasingly necessary. Development of a four-antibody panel containing the IHC markers MLH1, MSH2, MSH6, and PMS2 is recommended to identify MSI-H patients who may benefit from targeted immunotherapy.

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No grant was received for this study.

## Ethical statement

The study followed the Declaration of Helsinki, and the subjects provided written informed consent. The Institutional Ethics Committee, SVS Medical College, Mahbubnagar approved the study protocol.

## Conflicts of interest

None.

## Author contributions

S.A.B.H., S.K., and V.M. analyzed the data; S.A.B.H., P.M.G., A.M., and A.S. conducted the histological analysis; S.K and V.M supervised the work and edited the document. All authors reviewed and approved the final manuscript.

## References

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-91. [View at Publisher] [DOI] [PMID] [Google Scholar]
2. Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001;10(9):917-23. [View at Publisher] [PMID] [Google Scholar]
3. Lanza G, Gafà R, Santini A, Maestri I, Guerzoni L, Cavazzini L. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J Clin Oncol. 2006;24(15):2359-67. [View at Publisher] [DOI] [PMID] [Google Scholar]
4. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol. 2010;7(3):153-62. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol. 2002;20(4):1043-8. [View at Publisher] [DOI] [PMID] [Google Scholar]
6. Cohen R, Buhard O, Cervera P, Hain E, Dumont S, Bardier A, et al. Clinical and molecular characterisation of hereditary and sporadic metastatic colorectal cancers harbouring microsatellite instability/DNA mismatch repair deficiency. Eur J Cancer. 2017;86:266-74. [View at Publisher] [DOI] [PMID] [Google Scholar]
7. Kim JH, Kang GH. Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. World journal of gastroenterology: World J Gastroenterol. 2014;20(15):4230-43. [View at Publisher] [DOI] [PMID] [Google Scholar]
8. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol. 2003;21(6):1174-9. [View at Publisher] [DOI] [PMID] [Google Scholar]
9. Karahan B, Argon A, Yıldırım M, Vardar E. Relationship between MLH-1, MSH-2, PMS-2, MSH-6 expression and clinicopathological features in colorectal cancer. Int J Clin Exp Pathol. 2015;8(4):4044-53. [View at Publisher] [PMID] [Google Scholar]
10. Umar A, Boland CR, Terdiman JP, Syngal S, Chapelle AD, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96(4):261-8. [View at Publisher] [DOI] [PMID] [Google Scholar]
11. Whitehall V, Leggett B. Microsatellite instability: detection and management in sporadic colorectal cancer. J Gastroenterol Hepatol. 2011;26(12):1697-9. [View at Publisher] [DOI] [PMID] [Google Scholar]
12. Yoon YS, Yu CS, Kim TW, Kim JH, Jang SJ, Cho DH, et al. Mismatch repair status in sporadic colorectal cancer: immunohistochemistry and microsatellite instability analyses. J Gastroenterol Hepatol. 2011;26(12):1733-9. [View at Publisher] [DOI] [PMID] [Google Scholar]
13. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer. 2005;4(3):211-8. [View at Publisher] [DOI] [PMID] [Google Scholar]
14. Bolocan A, Ion D, Ciocan DN, Paduraru DN. Prognostic and predictive factors in colorectal cancer. Chirurgia (Bucur). 2012;107(5):555-63. [View at Publisher] [PMID] [Google Scholar]
15. Chapusot C, Martin L, Mungra N, Rageot D, Bouvier AM, Kopp CB, et al. Sporadic colorectal cancers with defective mismatch repair display a number of specific morphological characteristics: relationship between the expression of hMLH1 and hMSH2 proteins and clinicopathological features of 273 adenocarcinomas. Histopathology. 2003;43(1):40-7. [View at Publisher] [DOI] [PMID] [Google Scholar]

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