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Research Article

Immunohistochemical Expression of MLH1, PMS2 and P53 in Colorectal Carcinoma with Clinicopathologic Correlation

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Abstract

Background: Proteins of the mismatch repair system and p53 are important for prognosis and treatment of colorectal carcinoma. This raises the need for a better definition of clinical criteria that can be used to detect patients who have defects in these proteins. ***Objective***: To detect the correlations between clinicopathologic features and the expressions of MLH1, PMS2, and p53 in colorectal carcinoma. ***Methods***: This is a cross-sectional analytical study. Tissue samples of 102 colorectal carcinomas were collected in the hospitals of Baghdad Medical City. Archived reports of patients provided clinical and pathological data. The study was done during 2023 and 2024. Immunohistochemical staining results for MLH1, PMS2 and p53 proteins were compared to clinicopathologic criteria and to each other. ***Results***: MLH1 loss was more frequent in tumors of the right colon (p=0.019) and tumors with T3 extension (p=0.05). PMS2 absence was predominant in tumors of moderate differentiation (p=0.04), adenocarcinoma, NOS (p=0.05), tumor-free resection margins (p=0.03) and absence of perineural invasion (p=0.04). The wild expression of p53 was more frequent with the absence of lymphovascular tumor invasion (p=0.04). Aberrant p53 is associated with resection margins clear of tumor invasion (p=0.03), adenocarcinoma, NOS (p=0.05) and grade 2 differentiated tumors (p=0.04). PMS2 is associated with MLH1 (p=0.0001). p53 is associated with PMS2 (p=0.04). ***Conclusions***: A number of CRC clinicopathological variables are related to MLH1, PMS2 and p53 expression status. p53 is correlated with PMS2 status. Consequently, p53 may affect the prognosis of CRC with normal PMS2.

Keywords: Colorectal carcinoma, MLH1, PMS2, p53, Clinicopathologic features, Immunohistochemistry.

التعبير المناعي الكيميائي عن MLH1, PMS2 and P53 في سرطان القولون والمستقيم مع الارتباط السريري المرضي

الخلاصة

الخلفية: بروتينات نظام اصلاح عدم التطابق و P53 مهمة للتنبؤ بسلوك سرطان القولون والمستقيم وعلاجه. هذا الامر اثار الحاجة الى تعريف افضل للمعايير السريرية التي يمكن ان تستعمل لكشف المرضى الذين يمتلكون عيوبا في هذه البروتينات. **الهدف**: الكشف عن الروابط بين السمات السريرية المرضية والتعبير عن MLH1, PMS2 وP53 في سرطان القولون والمستقيم. **الطرائق**: تم جمع عينات نسيجية من ١٠٢ حالة اصابة بسرطان القولون والمستقيم في مستشفيات مدينة بغداد الطبية. التقارير المؤرشفة للمرضى قدمت المعلومات السريرية والمرضية. تمت مقارنة نتائج الصبغ المناعي الكيميائي للبروتينات MLH1, PMS2 and p53 مع المعايير السريرية والمرضية. **النتائج**: فقدان MLH1 كان اكثر شيوعاً في اورام الجانب الايمن من القولون (p=0.019) و في الاورام التي تترافق مع مرحلة T3 من الامتداد الى النسيج المحاذي (p=0.05). فقدان PMS2 كان سائداً في الاورام ذات التمايز المتوسط (p=0.04) والسرطانات الغدية غيرالمحددة خلاف ذلك (p=0.05) والاورام ذات هامش استئصال خالي من الورم (p=0.03) وفقدان الغزو الورمي للنسيج حول العصبي (p=0.04). التعبير النمطي للبروتين p53 كان اكثر شيوعاً مع انعدام غزو الورم للأوعية اللمفاوية والدموية (p=0.04). الشذوذ التركيبي للبروتين p53 ارتبط مع هامش الأستئصال الخالي من الغزو الورمي (p=0.03) والسرطانات الغدية غيرالمحددة خلاف ذلك (p=0.05) والأورام التي تظهر الدرجة 2 من التمايز (p=0.04). PMS2 يكون مرتبطاً مع MLH1 (p=0.0001). p53 يكون مرتبطاً مع PMS2 (p=0.04). **الاستنتاجات**: المتغيرات السريرية المرضية لسرطان القولون والمستقيم تكون مرتبطة بحالة التعبيرعن MLH1, PMS2 و p53. p53 مرتبطة مع حالة PMS2. بالتالي, p53 قد تؤثر على التنبؤ بمسار حالة سرطان القولون والمستقيم الذي يكون فيه PMS2 طبيعي.

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INTRODUCTION

Colorectal carcinoma (CRC) is ranked third among cancers of highest incidence in the world [1]. In Iraq, CRC is the second most frequent cancer in the country. Nevertheless, physicians show variable attitudes and practice levels concerning the CRC detection program [2,3]. CRC occurs mostly in elderly patients [4]. Symptoms were found to be mostly similar in young and elderly patients [5]. Often, polyps are the primary lesions that give rise to CRC. They are related to bleeding from the lower gastrointestinal tract and positive family history [6]. Using colonoscopy, colorectal polyps and cancers were shown to be the second most frequent source of lower gastrointestinal bleeding [7]. Genetic and epigenetic changes can be involved in CRC formation [8]. These mutations can be hereditary or sporadic [9]. Impaired capability to repair deoxyribonucleic acid (DNA) increases the risk of

developing cancer [10]. Malignant changes need to be accumulated over an extended period of time, reaching 10-20 years, to produce CRC [11]. Many environmental factors contribute to CRC development. These factors include being overweight, smoking, alcohol, and a diet with a high percentage of red meat and low in fiber. Some bacterial species in the colon and rectum may contribute to the pathogenesis of the tumor [12]. Mismatch repair system (MMR) is an important system in the repair of DNA defects that occur at the time of DNA replication, methylation, and oxidative stress. The main proteins of the MMR system are MLH1, MSH2, PMS2, and MSH6. In the process of DNA mismatch repair, MLH1 is coupled to PMS2, and MSH2 is coupled to MSH6. They work as heterodimers to repair DNA defects. Loss of MLH1 expression is joined by loss of PMS2 expression. Likewise, when MSH2 expression is lost, MSH6 expression is also lost. PMS2 and MSH6 expression loss is more commonly isolated, as compensatory proteins attach to MLH1 and MSH2 and prevent their expression loss [13]. Nevertheless, loss of MLH1 or MSH2 is not the case at all times. Hence, in many cases all of the four major MMR proteins are tested [14]. MLH1 loss is related to chemotherapy resistance [15]. P53 expression was found to be related to higher TNM stages of colorectal tumors; hence, p53 expression is related to CRC prognosis. [16]. Tumor suppressor protein p53 is an important protein for cell cycle control. It functions to prevent tumor formation through inhibiting cell cycle, inducing cellular senescence, and initiating cell apoptosis. p53 gives its cellular effects when the cell is affected by stress that may cause damage to the DNA material of the cell. TP53 is the gene responsible for encoding the protein of p53. Mutation of TP53 gene is found in 43.28% of colorectal cancers. The missense type of mutation generates an abnormal p53, which shows overexpression by immunohistochemical staining and oncogenic characteristics, namely, proliferation enhancement of malignant cells. Nonsense/frameshift mutations yield decreased expression or even absence of expression of p53. Overexpression of p53 indicates a more aggressive and chemotherapy-resistant tumor. Tumors have a more desirable prognosis when they display expression loss of p53 protein [17,18]. CRC prognosis is typically related to TNM system stage of the tumor; still, Survival rate in some cases is affected by other factors. As a result, biomarker tests and pathological features may represent additional prognostic criteria. These criteria help to determine the prognosis and provide guidance to select the most effective treatment [19]. The present study aims to evaluate the relation of MLH1, PMS2, and p53 to a number of clinicopathologic features in CRC cases. This can assist in understanding the effect of the status of these proteins on the biological behavior of CRC.

METHODS

Study design and setting

This study was performed as a retrospective cross-sectional analytical study. 102 cases with a histopathologically confirmed diagnosis of colorectal carcinoma were studied. Hematoxylin and eosin stained slides of the cases were examined. Tissue samples in paraffin blocks and clinical reports of patients were collected from the archives of the National Center for Teaching Laboratories, the laboratories of Baghdad Teaching Hospital, and the laboratories of Gastroenterology and Liver Disease Hospital throughout 2023 and 2024.

Data collection and outcome analysis

The data collected from clinical reports include patient age, patient sex, tumor site, tumor histopathological type, tumor grade, lymph node involvement, TNM stage of the tumor, extension of the tumor, tumor wall perforation, perineural invasion, lymphovascular invasion, and if the patient received neoadjuvant therapy. This study includes tumors that primarily originated in the colon and rectum, surgically resected tumors, and tumors that were confirmed to be colorectal carcinomas by histopathological examination. Tumors that have T1 or T2 extension stage with no involvement of lymph nodes, tumors primarily originating from the anal canal or appendix, and tumors diagnosed as neuroendocrine carcinoma were excluded from the study. Tissue slices of 4 micrometers thickness were taken from paraffin blocks. These tissue samples were fixed to positive slides and deparaffinized using xylene; thereafter, the tissue was rehydrated using different concentrations of alcohol in a gradually decreasing manner, and then antigen retrieval was done using Tris-EDTA buffer (pH 9.0), which is diluted and heated to 95-97°C for 20 minutes and then washed with phosphate buffer saline. After antigen retrieval, tissue samples were stained with immunohistochemical staining technique using Peroxidase blocking reagent (Envision FLEX Peroxidase blocking reagent (ready to use) SM801) 100 µL for 5 minutes in humid chamber for blockage of tissue endogenous peroxidase, then antibody reagents (FLEX PMS2 Monoclonal Rabbit Anti-Human Clone EP51 (ready to use), DAKO, reference number:IR087), (FLEX Monoclonal Mouse Anti-Human MLH1 Clone ES05 (ready to use), DAKO, reference number: IR079) and (FLEX P53 Clone DO-7 (ready to use), DAKO, reference number: IR616) were added to PMS2, MLH1 and p53 antigens respectively and placed in humid chamber for a period of 30 minutes, thereafter, linker reagent 100 µL (Envision FLEX+, Mouse (LINKER) SM804) or (Envision FLEX+, Rabbit (LINKER), SM805, DAKO, code: K8009) was applied as appropriate and the slide placed for 20 minutes in humid chamber, and then Horseradish peroxidase (Envision FLEX/Horseradish peroxidase (HRP) (ready to use) SM802) 100 µL was added to the tissue and placed for 20 minutes in the humid

chamber . Envision FLEX Diaminobenzidine (DAB) + Chromogen SM827 was mixed with a substrate buffer, and 100 µL of the mixture was used and kept for 10 minutes in a humid chamber, followed by counterstaining using Hematoxylin stain. Tissue dehydration was done using increasing concentrations of alcohol. Then, the tissue sample was covered with Dibutylphthalate Polystyrene Xylene and a cover slip. Each step, starting from antigen retrieval to the use of DAB + Chromogen, was followed by two washes in washing buffer, each washing last for 5 minutes. Immunohistochemically stained samples were examined. In cases of MLH1 and PMS2 expressions, the following factors were considered. Positive internal control included stromal cells, basal crypt cells in the colon, and lymphocytes. Protein expression was considered present when its expression was nuclear with equal or more intensity in tumor tissue than in control normal tissue. Protein expression was considered lost when the stain was completely absent or weaker in tumor cell nuclei compared to internal control, or when the stain was present in cellular cytoplasm with no nuclear staining [20]. The stain of p53 was considered aberrant when it showed overexpression (strong diffuse nuclear staining in 80 percent or more of tumor cells), absent expression (no nuclear or cytoplasmic staining), or cytoplasmic expression (stain is expressed in cellular cytoplasm but not in its nucleus). Wild type p53 was identified when the stain was expressed with variable intensity in different nuclei [21]. MLH1, PMS2 and p53 expressions were statistically analyzed and correlated to clinicopathologic information of archived clinical patients' reports.

Ethical considerations

This study received ethical approval by the research ethics committee (REC) of the Department of Pathology and Forensic Medicine, College of Medicine, University of Baghdad. All procedures followed were in accordance with institutional guidelines.

Statistical analysis

Results were analyzed statistically using the IBM SPSS 22 program. An initial description of the data was conducted. Categorical variables were expressed using frequencies and percentages. Relations of categorical variables were assessed using the chi-square test. In cases that were expected to have low frequencies, Fisher's exact test was employed to guarantee accuracy in significance testing. Continuous variables were described utilizing means and standard deviations. Relationship direction and strength of continuous variables were assessed utilizing the Pearson correlation coefficient. The potential impact of clinicopathologic and demographic variables on dMMR was evaluated through binary logistic regression analysis. Two tailed statistical tests were employed; confidence intervals were 95%. *p*-value was considered statistically significant when it was < 0.05. Adjusted odds ratios were calculated using multivariate and univariate models.

RESULTS

Case distribution according to demographic features and other variables is shown in Table 1. Most of the cases (47.1%) were greater than or equal to 60 years of age. Females (53.9%) were more than males (46.1%). Tumors were most frequently located in the left colon (71.6%); a lower percentage of cases was found in the right colon (27.5%) and transverse colon (1.0%). T3 tumor extension shows the highest prevalence (83.3%).

Table 1: Distribution of Cases by Demographic and Tumor Variables (n= 102)

Variable	Category	n(%)
Age group (years)	≤ 29	5(4.9)
	30–39	8(7.8)
	40–49	17(16.7)
	50–59	24(23.5)
	≥60	48(47.1)
Sex	Female	55(53.9)
	Male	47(46.1)
Histopathological type	Adenocarcinoma, NOS	91(89.2)
	Mucinous adenocarcinoma	11(10.8)
Tumor site	Left colon	73(71.6)
	Right colon	28(27.5)
	Transverse colon	1(1.0)
Tumor Grade	G1	10(9.8)
	G2	82(80.4)
	G3	10(9.8)
Tumor Extension (T-Stage)	T2	6(5.9)
	T3	85(83.3)
	T4a	8(7.8)
	T4b	3(2.9)
Wall Perforation	Not identified	88(86.3)
	Seen	14(13.7)
Lymphovascular Invasion	Not identified	56(54.9)
	Seen	46(45.1)
Perineural Invasion	Not identified	64(62.7)
	Seen	38(37.3)
Regional Lymph Node Involvement	1–3 Nodes	24(23.5)
	>3 Nodes	23(22.5)
	0 Nodes	55(53.9)
TNM Tumor Stage	II	39(38.2)
	III	52(51)
	IV	11(10.8)
Receiving Neoadjuvant Therapy	Not identified	86(84.3)
	Received	16(15.7)
Resection margin	negative	84(82.4)
	positive	18(17.6)

Concerning MLH1, PMS2, and p53 frequencies, 16 cases (15.7%) show MLH1 expression absence (Figure 1), while 23 cases (22.5%) display absence of PMS2 expression (Figure 2) and 78 cases (76.5%) exhibit p53 aberrant expression (Figure 3).

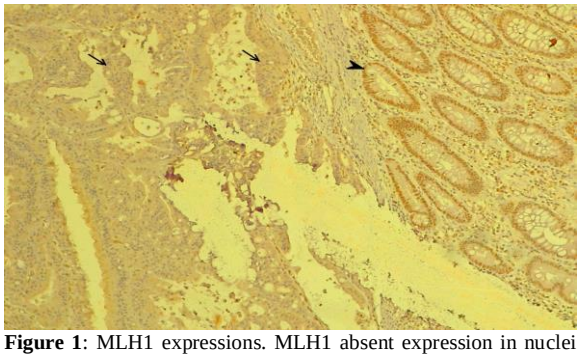

Figure 1: MLH1 expressions. MLH1 absent expression in nuclei of the tumor, while nuclei of the normal glands show the presence of nuclear expression (10X).

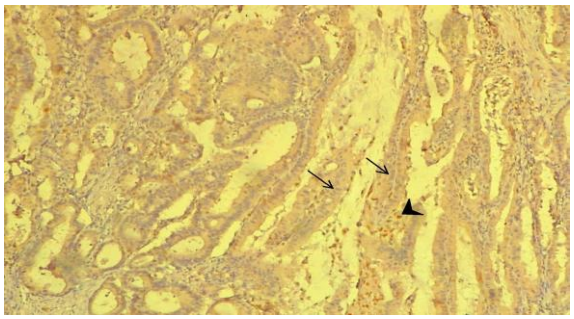

Figure 2: PMS2 expressions. PMS2 expression is absent in tumor tissue nuclei. PMS2 expression can be seen in normal tissue nuclei (40X).

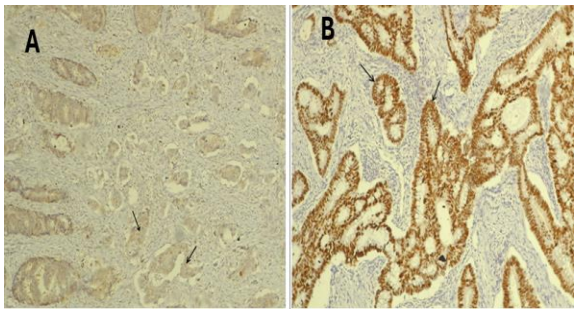

Figure 3: p53 expressions. **A**) p53 absent expression can be noticed in tumor cells nuclei (10X). **B**) p53 overexpression is evident in the nuclei of tumor tissue (10X).

The correlation of MLH1 to clinicopathologic features is displayed in Table 2.

Table 2: Relationship of MLH1 expression to tumor variables (n= 102)

Variable	Category	MLH1 Lost	MLH1 Present	*p*-value
Age Group (years)	≤ 29	2(12.5)	3(3.5)	
	30–39	1(6.3)	7 (8.1)	
	40–49	2(12.5)	15(17.4)	0.4
	50–59	2(12.5)	22(25.6)	
	≥60	9(56.3)	39(45.3)	
Sex	Female	10(62.5)	45(52.3)	0.6
	Male	6(37.5)	41(47.7)	
Histopathological Type	Adenocarcinoma, NOS	13(81.3)	78(90.7)	0.4
	Mucinous Adenocarcinoma	3(18.8)	8(9.3)	
Tumor Site	Left Colon	7(43.8)	66(76.7)	
	Right Colon	9(56.3)	19(22.1)	0.019
	Transverse Colon	0(0.0)	1(1.2)	
Tumor Grade	G1	3(18.8)	7(8.1)	
	G2	10(62.5)	72(83.7)	0.15
	G3	3(18.8)	7(8.1)	
Tumor Extension	T2	3(18.8)	3(3.5)	
	T3	13(81.3)	72(83.7)	0.05
	T4a	0(0.0)	8(9.3)	
	T4b	0(0.0)	3(3.5)	
Tumor Wall Perforation	Not identified	16(100)	72(83.7)	0.11
	Seen	0(0.0)	14(16.3)	
Lymphovascular Invasion	Not identified	8(50)	48(55.8)	0.8
	Seen	8(50)	38(44.2)	
Perineural Invasion	Not identified	14(87.5)	55(64)	0.08
	Seen	2(12.5)	31(36)	
Regional Lymph Nodes	1–3 nodes	7(43.8)	17(19.8)	
	>3 nodes	3(18.8)	20(23.3)	0.11
	0 nodes	6(37.5)	49(57.0)	
TNM Tumor Stage	II	6(37.5)	33(38.4)	
	III	10(62.5)	42(48.8)	0.3
	IV	0(0.0)	11(12.8)	
Receiving Neoadjuvant Therapy	Not Identified	14(87.5)	72(83.7)	0.8
	Received	2(12.5)	14(16.3)	
Resection Margins	Negative	15(93.8)	69(80.2)	0.3
	Positive	1(6.3)	17(19.8)	

Values were expressed as frequency and percentage.

MLH1 loss is significantly more in the right colon (p= 0.019) and T3 tumor extension (p= 0.05). Loss of MLH1 expression was found to be more frequent in the age group of ≥60 (56.3%) and in females (62.5%). However, these relations were not significant. Furthermore, MLH1 did not show significant association with tumor grade, lymphovascular invasion, perineural invasion, lymph node involvement, TNM stage, receiving neoadjuvant therapy, and resection margin involvement. PMS2 correlation to clinicopathologic features is shown in Table 3. PMS2 loss was significantly more common with adenocarcinoma, NOS (p= 0.05), grade 2 tumors (p= 0.04), absence of perineural invasion (p= 0.04), and free resection margins (p= 0.03). PMS2 expression loss was identified more in females (69.6%) and in the age group of greater than or equal to 60 (52.2%). Relations of PMS2 to age and sex were nonsignificant. Likewise, PMS2 was statistically not significantly correlated with the remaining clinicopathological variables. The association of p53 to clinicopathologic features is shown in Table 4. Aberrant p53 was significantly more frequent in relation to adenocarcinoma, NOS (p= 0.05). Wild p53 was more frequent with the absence of lymphovascular invasion (p= 0.04), aberrant p53 was more frequent with G2 tumors (p= 0.04), and there was no resection margin involvement (p= 0.03). Aberrant p53 was non-significantly more prevalent in elderly patients who are ≥60 years (42.3%) and in females (42.3%). Similarly, p53 correlation to other clinicopathological variables of the study was not statistically significant. Table 5 shows the association

between expressions of PMS2 and MLH1 (p= 0.0001). Loss of PMS2 expression was significantly more frequent in cases with MLH1 lost expression (69.6%). Relations between MLH1, PMS2 and P53 are displayed in Table 6. Aberrant p53 was significantly related to PMS2 expression loss (p= 0.04). In cases of aberrant p53, the prevalence of present PMS2 was higher (80.8%) compared to PMS2 loss **(**19.2%). The relation noticed between p53 expression and MLH1 was statistically not significant (p= 0.32).

Table 3: Relationship of PMS2 expression to tumor variables (n=102)

Variable	Category	PMS2 Lost	PMS2 Present	Adjusted *p*-value
Age Group (year)	≤ 29	2(8.7)	3(3.8)	
	30–39	2(8.7)	6(7.6)	
	40–49	5(21.7)	12(15.2)	0.22
	50–59	2(8.7)	22(27.8)	
	≥60	12(52.2)	36(45.6)	
Sex	Female	16(69.6)	39(49.4)	0.07
	Male	7(30.4)	40(50.6)	
Histopathological Type	Adenocarcinoma, NOS	20(87)	71 (89.9)	0.05
	Mucinous Adenocarcinoma	3(13)	8 (10.1)	
Tumor Site	Left Colon	13(56.5)	60(75.9)	
	Right Colon	10(43.5)	18(22.8)	0.10
	Transverse Colon	0(0.0)	1(1.3%)	
Tumor Grade	G1	3(13)	7(8.9)	
	G2	16(69.6)	66(83.5)	0.04
	G3	4(17.4)	6(7.6)	
Tumor Extension	T2	3(13)	3 (3.8)	
	T3	19 (82.6)	66(83.5)	0.08
	T4a	1(4.3)	7(8.9)	
	T4b	0(0.0)	3(3.8)	
Tumor Wall Perforation	Not identified	22(95.7)	66(83.5)	0.15
	Seen	1(4.3)	13 (16.5%)	
Lymphovascular Invasion	Not identified	13(56.5)	41(51.9)	0.09
	Seen	10(43.5)	38(48.1)	
Perineural Invasion	Not identified	17(73.9)	47(59.5)	0.04
	Seen	6(26.1)	32(40.5)	
TNM Tumor Stage	II	9(39.1)	30 (38)	
	III	12(52.2)	40(50.6)	0.12
	IV	2(8.7)	9(11.4)	
Neoadjuvant Therapy	Not Identified	20(87)	66(83.5)	0.11
	Received	3(13)	13(16.5)	
Resection Margins	Negative	20(87)	64(81)	0.03
	Positive	3(13)	15(19.0)	

Values were expressed as frequency and percentage.

Table 4: Relationship of p53 expression to tumor variables (n= 102)

Variable	Category	Aberrant p53	Wild p53	Adjusted *p*-value
Age Group (years)	≤ 29	4(5.1)	1(4.2)	
	30–39	7(9)	1(4.2)	
	40–49	12(15.4)	5(20.8)	0.18
	50–59	22(28.2)	2(8.3)	
	≥60	33(42.3)	15(62.5)	
Sex	Female	41(52.6)	14(58.3)	0.09
	Male	37(47.4)	10(41.7)	
Histopathological Type	Adenocarcinoma, NOS	70(89.7)	21(87.5)	0.05
	Mucinous Adenocarcinoma	8(10.3)	3(12.5)	
Tumor Site	Left Colon	56(71.8)	17(70.8)	
	Right Colon	21(26.9)	7(29.2)	0.14
	Transverse Colon	1(1.3)	0(0.0)	
Tumor Grade	G1	6(7.7)	4(16.7)	
	G2	63(80.8)	19(79.2)	0.04
	G3	9(11.5)	1(4.2)	
Tumor Extension	T2	5(6.4)	1(4.2)	
	T3	65(83.3)	20(83.3)	0.10
	T4a	5(6.4)	3(12.5)	
	T4b	3(3.8)	0(0.0)	
Tumor Wall Perforation	Not identified	68(87.2)	20(83.3)	0.12
	Seen	10(12.8)	4(16.7)	
Lymphovascular Invasion	Not identified	39(50)	15(62.5)	0.04
	Seen	39(50)	9(37.5)	
Perineural Invasion	Not identified	52(66.7)	17(70.8)	0.11
	Seen	26(33.3)	7(29.2)	
TNM Tumor Stage	II	28(35.9)	11(45.8)	
	III	41(52.6)	11(45.8)	0.09
	IV	9(11.5)	2(8.3)	
Neoadjuvant Therapy	Not Identified	64(82.1)	22(91.7)	0.12
	Received	14(17.9)	2(8.3)	
Resection Margins	Negative	63(80.8)	21(87.5)	0.03
	Positive	15(19.2)	3(12.5)	

Values were expressed as frequency and percentage.

DISCUSSION

CRC represents a growing challenge. Incidence is increasing worldwide. By the year 2035, the mortality rate of colon cancer is expected to increase by 71.5%, while the prediction for the mortality rate of rectal cancer is to increase by 60%. It mostly affects elderly people. Nevertheless, CRC can also be diagnosed in young patients. MMR status of a tumor affects its response to certain treatments and its prognosis [22]. Deficient MMR (dMMR) shows specific features in comparison to the same type of tumor with proficient MMR (pMMR) status [23]. MLH1 and PMS2 are the most frequently defected proteins among dMMR proteins [24].

Table 5: PMS2 and MLH1 expression relation (n= 102)

Variable	Category	PMS2 Lost	PMS2 Present	*p*-value
MLH1	Lost	16(69.6)	0(0.0)	0.0001
	Present	7(30.4)	79(100)	

Values were expressed as frequency and percentage.

Table 6: Relations between the expressions of p53, MLH1 and PMS2 (n= 102)

MMR Marker	Aberrant p53	Wild p53	Adjusted *p*-value
MLH1 Expression			
Lost	12(15.4)	4(16.7)	0.32
Present	66(84.6)	20(83.3)	
PMS2 Expression			
Lost	15(19.2)	8 (33.3)	0.04
Present	63(80.8)	16(66.7)	

Values were expressed as frequency and percentage.

Favorable responses to neoadjuvant immunotherapy were found in CRC with dMMR [25]. Despite the encouraging response of the dMMR tumors to immune checkpoint inhibitor (ICI) therapy, approximately half of the cases show acquired or primary resistance [26]. CRC poor prognosis was shown to be related to overexpressed p53 [17]. TP53 mutation may induce inhibition to the microenvironment of dMMR CRC tumor. The inhibition of microenvironment can cause resistance to immunotherapy [27]. Hence the importance of investigating the relations of dMLH1/dPMS2 and mutated p53 to clinicopathologic features. Immunohistochemical (IHC) staining was used to stain tissue samples in this study. IHC for MMR protein detection gives the ability to identify each of the MMR proteins separately. It is relatively simple and not expensive to detect MMR and p53 proteins. A study done on CRC revealed that compatibility between MMR and MSI status was 98.4% [28]. In this study, CRC was found to be more prevalent in patients aged 60 years or older (47.1%). A similar observation was made by Soliman and Mohamad, who indicate that most of the cases in the study cohort were in the range of 66-90 years of age [11]. In addition, Farhad *et al.* have found that the largest number of cases studied were above 50 years of age [5]. The percentage of female patients was 53.9% and was identified to be more than that of male patients (46.1%). This finding was not in consensus with the findings of Farhad *et al.* [10] and Ye *et al.* [28], who have indicated that the majority of patients in their studies were males. This inconsistency can be due to the exclusion of tumors with TNM stage I, which is represented by extension stages T1 or T2 with no lymph node involvement in this study. This can be clarified by the observations of White *et al.* [29], as they noticed that TNM stage I cancers show more male prevalence, while stage II cancers show more female prevalence, and the ratios of males to females in stages III and IV displayed no variation. Consequently, excluding stage I tumors in the present study can be the cause for the higher percentage of females found. The significant relation between MLH1 and T3 tumor extension in this study was found to be in concordance with the results of Hashmi *et al.* [30]. The work of Jiang *et al.* has shown that the frequency of MLH1 alone or in combination with PMS2 was determined to be more on the right side of the colon [31]. This agrees with the present work, as MLH1 loss was significantly more in right colon tumors. The defect of PMS2 in this study was significantly more in tumors of moderate differentiation, absence of perineural invasion, and adenocarcinoma, NOS. Zeng *et al.* showed similar findings [32]. Furthermore, PMS2 defect was significantly predominant in cases with tumor-free resection margins. The results of the present study displayed a significant relation of p53 to moderately differentiated CRC and colorectal adenocarcinoma, NOS. It was noticed by Kim *et al.* that aberrant p53 expression was more prevalent in cases of well to moderate histopathological grade [17]. A previous study by Cao *et al.* showed that negative expression of p53 was determined to be significantly more in colorectal adenocarcinoma [33]. Zarbaliyev *et al.* have noticed that lymphovascular invasion was an independent poor prognostic factor for CRC cases with negative involvement of lymph nodes [34]. Kataoka *et al.* have mentioned that the invasion of lymphovascular tissue involves lymphatic along with venous invasion. Additionally, venous invasion is rarely differentiated from invasion of lymphatic vessels [35]. Our results elucidate that wild p53 is significantly more prevalent in tumors without invasion into lymphovascular tissue. Oh *et al.* study shows that mutant TP53 is predominant in tumors that did not show invasion of lymphovascular tissue [36]. Our study observations indicate that aberrant p53 is more frequent, although non-significant, in cases of no perineural invasion. In addition, the Linin *et al.* study displays significantly more frequent absence of lymphovascular tissue invasion with perineural invasion absence [37]. Combining these findings support the relation between aberrant p53 and absent lymphovascular invasion. The presence of tumor cells in postresection margins increases the chance of local as well as distant recurrence. Surgical margin status concerning tumor cell existence serves as a prognostic factor. It affects the decision of using adjuvant chemotherapy [38]. Moreover, positive tumor cells involvement of vertical surgical margins represents a greater risk of complications in

comparison to horizontal margin involvement [39]. Therefore, it is essential to define the presence of tumor cells in postoperative margins. Results of the present study indicate that aberrant p53 was identified significantly more in cases with tumor free resection margins. This can be attributed to the relation of aberrant p53 to moderately differentiated CRC but not to the absence of vascular invasion. As the absence of lymphovascular invasion is related to wild p53 in the present study; and to the relation of free resection margin to the two variables of moderately differentiated tumors and the absence of vascular invasion that were indicated by Balba *et al.* [40].

Study limitations

Limitations of this study include the relatively small number of samples and the proportionally short period of time. Another limitation is the lack of molecular testing. Concordance with molecular testing can help in increasing the accuracy of the observations.

Conclusion

Based on the data of this study, the number of CRC histopathological variables, involving histopathological type, resection margin involvement, grade, and lymphovascular invasion, are correlated to the status of p53. In addition, histopathological type, grade, resection margin involvement, and perineural invasion of CRC have shown correlation to PMS2 status. Similarly, CRC site and tumor extension can be related to MLH1 status. Additionally, due to the significant relation between aberrant p53 expression and present PMS2 expression, mutant p53 may play a role in the prognosis of CRC with present PMS2 expression.

Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Hossain S, Karuniawati H, Jairoun AA, Urbi Z, Ooi DJ, John A, et al. Colorectal Cancer: A review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers*. 2022;14(7):1732. doi: 10.3390/cancers14071732.
2. Annual Report, Iraqi Cancer Registry 2022. Iraqi Cancer Board, Ministry of Health, Republic of Iraq, 2022.
3. Abbas MM, Alkhazrajy LA. Knowledge, attitudes and practices regarding the screening of colorectal cancer among primary care physicians in Baghdad during 2022. *J Fac Med Baghdad*. 2024;66(1):45–50. doi: 10.32007/jfacmedbagdad.2162.
4. Shakir A, Al-Rubaiawi H, Haba MK, Raouf L, Al-Hadad A. Evaluation of CEA,CA19-9, and CA242 tumor markers in patients with colorectal cancer by ELISA technique. Indian J Forensic Med Toxicol. 2020. doi: 10.37506/ijfmt.v14i2.2809.
5. Farhad RM, Saleh ES, Alsammarraie AZ. Clinicopathological features of colorectal cancer in the Iraqi population focusing on age and early-onset of malignancy: A descriptive cross-sectional study. *Al-Rafidain J Med Sci*. 2023;5:86–91. doi: 10.54133/ajms.v5i.158.
6. Alezzi MJ, Alani KH, Alezzi JI. Epidemiology of colorectal polyps in Iraqi patients. *Med J Babylon*. 2024;21(3):506–510. doi: 10.4103/mjbl.mjbl_348_22.
7. Abdulhassan BA, Mahdi QA, Meeshal MT, Ali MH, Kadhim SMA, Noori A. Descriptive study of patients referred for colonoscopy at gastro-enterology unit at Al-Imamain Al-Kadhemain Medical City in Baghdad. *J Fac Med Baghdad*. 2024;66(1):11–17. doi: 10.32007/jfacmedbagdad.6612163.
8. Bresam S. Phenotypic and prevalence correlation with recurrent KRAS G12C, G13D, and G22E mutations in colorectal adenocarcinoma. Egypt J Basic Appl Sci.. 2024;11(1):616–625. doi: 10.1080/2314808x.2024.2375091.
9. Kadhem Mallakh M, Mohammed Mahmood M, Hasan Mohammed Ali S. Immunomolecular investigation of human papillomavirus genotypes (16, 18) and P63 expression in patients with malignant and non-malignant colorectal tumors. *Arch Razi Inst*. 2022;77(1):383-390. doi: 10.22092/ARI.2021.356608.1879.
10. Farhad R, Saleh E, Alsammarraie A. No evidence of relationship between colorectal cancer susceptibility and ERCC2 gene polymorphisms. *J Adv Biotechnol Exp Ther*. 2023;6(3):638. doi: 10.5455/jabet.2023.d155.
11. Falih Soliman N, Jasim Mohamad B. Clinical and histopathological characteristics of colorectal cancer in Iraq between 2015-2021. *Arch Razi Inst*. 2022;77(6):2407-2413. doi: 10.22092/ARI.2022.358613.2263.
12. Khlf GK, Alash SA. Study the bacterial activity isolated from colon and rectal cancer biopsy in cell lines culture. *Biomed Pharmacol J*. 2023;16(2):1245–1255. doi: 10.13005/bpj/2705.
13. Nádorvári ML, Lotz G, Kulka J, Kiss A, Tímár J. Microsatellite instability and mismatch repair protein deficiency: equal predictive markers? Pathol Oncol Res. 2024;30. doi: 10.3389/pore.2024.1611719.
14. Bateman AC. DNA mismatch repair proteins: scientific update and practical guide. *J Clin Pathol*. 2021;74(4):264–268. doi: 10.1136/jclinpath-2020-207281.
15. Salman AM, Babaei E, Al-Khafaji ASK. Exploring the modulation of MLH1 and MSH2 gene expression in hesperetin-treated breast cancer cells (BT-474). *J Adv Pharm Technol Amp Res*. 2024;15(1):43–48. doi: 10.4103/japtr.japtr_279_23.
16. Qaddoori YB, Al-Khafaji ASK, Khashman BM, Abdulghafour KH. The potential role of HDAC1 and HDAC3 immunoexpression in P53 downregulation and tumor aggressiveness of colon and rectum carcinomas patients. *Exp Oncol*. 2025;46(4):393–401. doi: 10.15407/exp-oncology.2024.04.393.
17. Kim KM, Ahn AR, Park HS, Jang KY, Moon WS, Kang MJ, et al. Clinical significance of p53 protein expression and TP53 variation status in colorectal cancer. *BMC Cancer*. 2022;22(1). doi: 10.1186/s12885-022-10039-y.
18. Yahya A, Alhamadani ZR, Mundher M. Immunohistochemical expression of retinoblastoma gene product and p53 protein in transitional cell carcinoma of the urinary bladder and its relationship to different clinicopathological parameters. *Open Access Macedonian J Med Sci*. 2021;9(A):595–609. doi: 10.3889/oamjms.2021.6559.
19. Chen K, Collins G, Wang H, Toh JWT. Pathological features and prognostication in colorectal cancer. *Curr Oncol*. 2021;28(6):5356–5383. doi: 10.3390/curroncol28060447.
20. Chen W, Frankel WL. A practical guide to biomarkers for the evaluation of colorectal cancer. *Modern Pathol*. 2018;32:1–15. doi: 10.1038/s41379-018-0136-1.
21. Osakabe M, Yamada N, Sugimoto R, Uesugi N, Nakao E, Honda M, et al. The pattern-based interpretation of p53 immunohistochemical expression as a surrogate marker for TP53 mutations in colorectal cancer. *Virchows Arch*. 2024; doi: 10.1007/s00428-024-03790-z.

22. Fan WX, Su F, Zhang Y, Zhang XL, Du YY, Gao YJ, et al. Oncological characteristics, treatments and prognostic outcomes in MMR-deficient colorectal cancer. *Biomarker Res*. 2024;12(1). doi: 10.1186/s40364-024-00640-7.
23. Mei WJ, Mi M, Qian J, Xiao N, Yuan Y, Ding PR. Clinicopathological characteristics of high microsatellite instability/mismatch repair-deficient colorectal cancer: A narrative review. *Front Immunol*. 2022;13. doi: 10.3389/fimmu.2022.1019582.
24. Nádorvári ML, Kenessey I, Kiss A, Barbai T, Kulka J, Rásó E, et al. Comparison of standard mismatch repair deficiency and microsatellite instability tests in a large cancer series. *J Transl Med*. 2024;22(1). doi: 10.1186/s12967-024-04960-y.
25. Deng Z, Luo Y, Chen X, Pan T, Rui Y, Hu H, et al. Pathological response following neoadjuvant immunotherapy and imaging characteristics in dMMR/MSI-H locally advanced colorectal cancer. *Front Immunol*. 2024;15. doi: 10.3389/fimmu.2024.1466497.
26. Guan J, Li GM. DNA mismatch repair in cancer immunotherapy. NAR Cancer [Internet]. 2023 Jun 8;5(3). Available from: https://doi.org/10.1093/narcan/zcad031
27. Lu Z, Shen L, Zhang H, Jiao X, Wang Y, Chen H, et al. Effect of TP53 mutation on antitumor immunity and responsiveness to immunotherapy in colorectal cancer. *J Clin Oncol*. 2020;38(15_suppl):e16014. doi: 10.1200/jco.2020.38.15_suppl.e16014.
28. Ye M, Ru G, Yuan H, Qian L, He X, Li S. Concordance between microsatellite instability and mismatch repair protein expression in colorectal cancer and their clinicopathological characteristics: a retrospective analysis of 502 cases. *Front Oncol*. 2023;13. doi: 10.3389/fonc.2023.1178772.
29. White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. *BMC Cancer*. 2018;18(1). https://doi.org/10.1186/s12885-018-4786-7
30. Hashmi AA, Bukhari U, Rizwan R, Faisal F, Kumar R, Malik UA, et al. Mismatch repair deficient (DMMR) colorectal carcinoma in a Pakistani cohort: association with clinical and pathological parameters. *Cureus*. 2023. doi: 10.7759/cureus.42781.
31. Jiang W, Sui QQ, Li WL, Ke CF, Ling YH, Liao LE, et al. Low prevalence of mismatch repair deficiency in Chinese colorectal cancers: a multicenter study. *Gastroenterol Rep*. 2020;8(5):399–403. doi: 10.1093/gastro/goaa006.
32. Zeng Z, Yan Q, Chen G, Zhang X, Huang J, Fu K, et al. Characteristics of colorectal carcinoma patients with PMS2 defects detected by immunohistochemistry. *Eur J Cancer Prevent*. 2020;30(3):251–257. doi: 10.1097/cej.0000000000000620.
33. Cao DZ, Ou XL, Yu T. The association of p53 expression levels with clinicopathological features and prognosis of patients with colon cancer following surgery. *Oncol Lett*. 2017;13(5):3538–3546. doi: 10.3892/ol.2017.5929.
34. Zarbaliyev E, Turhan N, Çelik S, Çağlıkülekçi M. Lymphovascular invasion in colorectal cancers: can we predict it preoperatively? *Ann Coloproctol*. 2024;40(3):245–252. doi: 10.3393/ac.2023.00458.0065.
35. Kataoka M, Hirano Y, Ishii T, Kondo H, Asari M, Ishikawa S, et al. Impact of lymphovascular invasion in patients with Stage II colorectal cancer: a propensity score-matched study. *In Vivo*. 2021;35(1):525–531. doi: 10.21873/invivo.12287.
36. Oh HJ, Bae JM, Wen X, Jung S, Kim Y, Kim KJ, et al. p53 expression status is associated with cancer-specific survival in stage III and high-risk stage II colorectal cancer patients treated with oxaliplatin-based adjuvant chemotherapy. *Br J Cancer*. 2019;120(8):797–805. doi: 10.1038/s41416-019-0429-2.
37. Lin Z, Zheng Y, Yang J, Jin W, Wang J, Wang W, et al. Prognostic analysis of lymphovascular invasion in stages I–III colorectal cancer. *Am J Clin Oncol*. 2023;46(8):366–373. doi: 10.1097/coc.0000000000001015.
38. Smith HG, Schlesinger NH, Qvortrup C, Chiranth D, Lundon D, Ben-Yaacov A, et al. Variations in the definition and perceived importance of positive resection margins in patients with colorectal cancer – an EYSAC international survey. *Eur J Surg Oncology*. 2023;49(11):107072. doi: 10.1016/j.ejso.2023.107072.
39. Oh HH, Kim JS, Lim JW, Lim CJ, Seo YE, You GR, et al. Clinical outcomes of colorectal neoplasm with positive resection margin after endoscopic submucosal dissection. *Sci Rep*. 2024;14(1). doi: 10.1038/s41598-024-63129-1.
40. Balbaa MA, Elkady N, Abdelrahman EM. Predictive factors of positive circumferential and longitudinal margins in early T3 colorectal cancer resection. *Int J Surg Oncol*. 2020;2020:1–8. doi: 10.1155/2020/6789709.