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EZH2 and MMP1 Overexpression in Colorectal Cancer: Correlation with Tumor Aggressiveness and Clinicopathologic Features

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ABSTRACT

Background & Objective: One of the most lethal as well as prevalent malignancies across the globe is colorectal cancer (CRC). MMP1, a certain type of matrix metalloproteinase, as well as EZH2, a histone methyltransferase, are gaining attention for their part in metastasis and tumor progression. This research analyses the expression of immunohistochemistry EZH2 and MMP1 in relation to colorectal carcinoma, and how these two proteins correlate with other clinicopathological features.

Methods: An analysis was done retrospectively on 38 cases of colorectal cancer, which were formalin-fixed and paraffin-embedded. MMP1 and EZH2 staining were assessed immunohistochemically and correlated with the tumor's stage, lymph nodes, clinical stage, and prognosis. Statistical analysis was conducted with SPSS version16.0 and a level of significance of $p < 0.05$.

Results: Markedly increased expression of EZH2 and MMP1 had notable correlation with advanced T stage ($p = 0.01$ and0.03) along with metastasis to the regional lymph nodes ($p = 0.027$) and clinical stages III or IV ($p = 0.04$ for EZH2 and $p = 0.023$ for MMP1). Although both markers demonstrated a trend for decreased disease-free survival (DFS), neither achieved statistical significance for DFS or overall survival (OS).

Conclusion: EZH2 and MMP1 overexpression were associated with aggressive clinicopathologic features (higher T stage, nodal involvement, and advanced clinical stage). However, neither marker showed a statistically significant association with overall survival (OS) or disease-free survival (DFS) in this cohort. Larger, adequately powered studies are required to clarify any potential prognostic value.

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Introduction

Following breast, lung, and prostate cancer, colorectal cancer (CRC) remains the third most prevalent malignant tumor and holds the fourth most common cause of cancer mortality across the globe. In Egypt, colorectal cancer constitutes roughly3.47 % of all cancer cases, ranking as the seventh most frequent cancer type. Over 1.9 million new CRC cases and 930 000 deaths were estimated in 2020 (2).

The adenoma-carcinoma sequence, initially posited by Fearon and Vogelstein, delineates the genetic evolution of colorectal carcinogenesis. They proposed that colorectal cancer arises from adenomatous polyps by a sequence of genetic changes, including the inactivation of tumor suppressor genes (3). Nonetheless, the epigenetic processes that govern CRC formation are still not well appreciated (1).

Enhancer of Zeste Homolog 2 (EZH2) is a component of the polycomb repressive complex 2 (PRC2) and acts as a histone methyltransferase, silencing gene expression by chromatin compaction. Overexpression of EZH2 has been associated with increased cell proliferation and tumorigenesis across several cancer types (1,5). EZH2 is known to drive oncogenesis through modulation of both cell cycle and apoptotic pathways (27). Nonetheless, the specific function of EZH2 in colorectal carcinogenesis remains under examination.

Matrix metalloproteinases (MMPs) constitute a group of enzymes that degrade the extracellular matrix (ECM), hence promoting tumor invasion and metastasis. MMP-1 (collagenase-1) specifically targets collagen types I, II, and III, which constitute the principal structural elements of the interstitial stroma (7). In many cancers, higher MMP levels have been linked with poorer prognosis because of the increased angiogenesis, invasion, and metastasis that they facilitate.)13 .(Increased MMP-1 expression has been linked to aggressive behavior and advanced stages of colorectal cancer.

These findings may have implications in clinical oncology. EZH2 and MMP1 molecular biomarkers may support characterization of tumour aggressiveness & progression, and the molecular patterns delineated in this study may help clarify their roles and inform future therapeutic research.

This study is to evaluate the immunohistochemical expression of EZH2 and MMP1 across different histological subtypes of colorectal cancer and to connect their expression with clinicopathologic factors.

Materials and Methods

This research utilized a retrospective analytical cohort design, 50cases with colorectal carcinoma were retrieved from the archives of the pathology laboratory at the Oncology Center, Mansoura University, during the period from January 2014 to June2017. 12cases were excluded due to unavailable paraffin blocks (9cases) and 3 were excluded due to unavailable clinical data. The remaining 38 formalin-fixed were reviewed microscopically for recording of histopathological parameters including grading, staging, size of tumor and clinicopathologic data The Medical and Bioethics Committee of Mansoura University authorized the investigation (Approval ID: RP.22.09.141) and conducted it according to the Declaration of Helsinki.

Tissue Preparation and Immunohistochemistry

Paraffin-embedded tissue blocks were sectioned to a thickness of 4 μm, deparaffinized using xylene, and rehydrated through a series of graded alcohols. Antigen retrieval was conducted as outlined below.

• Sections were autoclaved in Histofine solution (pH9.0; Nichirei Biosciences, Tokyo, Japan) for 15 minutes for EZH2.

• Sections were incubated in citrate buffer (Nichirei Biosciences) at 95°C for 15 minutes for MMP1. For ten minutes,0.3 % hydrogen peroxide (H_2O_2) was used to inhibit endogenous peroxidase activity, and afterward the mixture was washed with phosphate-buffered saline (PBS). The specified primary antibodies were applied to the sections, and then they were placed at 4°C overnight.

• Mouse monoclonal antibody against EZH2 (Cat. no.612666; BD Biosciences, San Jose, CA, USA)
• Rabbit polyclonal antibody against MMP1 (diluted1:30; Thermo Fisher Scientific) Subsequently, a primary antibody enhancer was applied at room temperature for 10 minutes to mitigate non-specific binding. This was followed by a 15-minute incubation of the sections with horseradish peroxidase (HRP) polymer.3,3 -diaminobenzidine tetrahydrochloride (DAB; Zymed) was employed to observe the reaction. Hematoxylin was employed for counterstaining, and Tissue-Tek Glas 6419 (Sakura Finetek) was employed to mount the transparencies. The primary antibody was omitted to generate negative controls. Reactive lymph node tissue for EZH2 and scar tissue for MMP1 were used as positive controls.

Immunohistochemical Scoring

Two expert pathologists examined and scored all stained sections independently and blinded to the patients' clinicopathologic and outcome data. Discrepant cases were subsequently reviewed jointly to reach a consensus score, which was used for the final analysis. A formal concordance metric (e.g., Cohen's kappa) could not be calculated because individual rater-level scoring records were not retained in a format suitable for κ computation.

- **EZH2 expression** was scored semi-quantitatively by multiplying the staining intensity and extent:
- Intensity: 0(negative), 1(weak), 2(moderate), 3(strong)
- Extent: 0(0%), 1 (<10%), 2 (10–50%), 3 (50–80%), 4 (>80%)
- Final score ranged from 0 to12. Scores <4 were classified as low expression and scores ≥4 as high expression, as previously described (9). This prespecified dichotomization was used to distinguish low versus overexpression and to enable categorical analyses given the limited sample size.
- MMP1 expression was evaluated based on the percentage of tumor cells with positive cytoplasmic staining: Negative (<10%), Weak (10–50%), Strong (50–80%), and Intense (>80%). For categorical analyses, strong and intense staining were grouped as high expression (versus negative/weak as low), following previously reported methodology (8) and reflecting a biologically meaningful higher-expression category.

Statistical Analysis

Statistical analysis was performed using SPSS version16.0. Normality of the data was assessed by the Shapiro-Wilk test. Categorical variables were described as counts and proportions, while continuous variables were expressed as mean ± SD or median (range).

For analysis purposes, clinical stage was dichotomized as early stage (I/II) versus advanced stage (III/IV) to enable categorical comparisons given

the limited sample size across individual stages. Missing data were handled by available case analysis. For each clinicopathologic or laboratory variable, analyses were performed using all cases with non-missing data for that variable. Where subgroup analyses were performed on fewer than 38 cases, the denominator (n) is explicitly reported in tables and relevant Results text.

For comparison of groups, the Mann–Whitney U test or independent t-test was used for continuous variables.

• Fisher's exact test or Chi-square test for categorical variables

• The time from date of diagnosis to date of death was termed Overall Survival (OS).

• Disease-Free Survival (DFS) was defined as the time interval from surgery to recurrence or metastasis.

Because of the limited sample size and number of survival events, multivariable Cox proportional hazards modeling was not performed; therefore, survival analyses should be interpreted as exploratory univariate findings. Given the limited cohort size and number of survival events, multivariable Cox proportional hazards regression (adjusted for stage, grade, and nodal status) was not performed; therefore, survival analyses should be interpreted as exploratory univariate findings only.

Results

Clinicopathological features of all cases:

The study included 38 patients diagnosed with colorectal cancer, with a mean age of56.1 years (SD ±13). The predominant portion was female (63.2 %), and most had no history of diabetes mellitus or hypertension. Abdominal pain was the most frequent presenting complaint (71%), and the predominant portion of patients (74.1 %) underwent hemicolectomy (Table1).

Some clinicopathologic and laboratory variables were not available for all cases; therefore, percentages and subgroup comparisons were calculated using the number of evaluable cases for each variable (variable-specific denominators are indicated in the corresponding tables).

Table 1. Sociodemographic characteristics of all patients

		N (%)
Age Ys	Mean (SD)	56.1 (13)
Gender	Male	14 (36.8)
	Female	24 (63.2)
Family history (N=28)	No	26 (92.9)
	Yes	2 (7.1)
M/HTN (N=28) *	No	20 (71.4)
	Yes	8 (28.6)
Complaint (N=28)	Abdominal pain	20 (71.4)
	Bleeding per rectum	3 (10.7)
	Intestinal obstruction	2 (7.1)
	Others	3 (10.7)
Surgery	Hemicolectomy	20 (74.1)
	Sigmoidectomy	3 (11.1)
	Others	4 (14.8)

* Chronic diseases: Diabetes or Hypertension

Note: Denominators (n) may vary due to missing data; where analyses were performed on fewer than 38 cases, the applicable denominator is indicated in the parameter label (e.g., DM/HTN (N=28)) and reflected in the corresponding counts/percentages.

Correlation Between EZH2 Expression and Clinicopathologic Parameters

Regarding immunohistochemical findings, **EZH2 expression** was classified as low in 13 patients (34.2 %) and high in 25 patients (65.8 %) (Figure1).

Table 2 indicates that no substantial correlations were identified between EZH2 expression and the majority of sociodemographic or clinical characteristics, except T stage and overall clinical stage.

Table 2. The association between Characteristics of the studied groups and EZH2 expression (N=38)

Parameter		EZH2		
		Low N (%) 13 (34.2%)	High N (%) 25 (65.8)	Significance
Age Ys	Mean (SD)	53.8 (11.3)	57.4 (13.9)	t: -0.75, p=0.462 [a]
Gender	Male	7 (29.2)	17 (70.8)	P=0.486 [b]
	Female	6 (42.9)	8 (57.1)	
Family history	No	9 (34.6)	17 (65.4)	P=1 [b]
	Yes	1 (50)	1 (50)	
DM/HTN (N=28)	No	8 (40)	12 (60)	P=0.669 [b]
	Yes	2 (25)	6 (75)	
Complaint (N=28)	Abdominal pain	8 (40)	12 (60)	P=0.669 [b]
	Others	2 (25)	6 (75)	
Surgery	Hemicolectomy	8 (40)	12 (6)	P=0.678 [b]
	Others	2 (28.6)	5 (71.4)	
Site	Right	4 (25)	12 (75)	X^2=1.27, p=0.260
	Left	9 (42.9)	12 (57.1)	
Pathology type	Adenocarcinoma	10 (41.7)	14 (58.3)	P=0.294 [b]
	Mucinous/signet ring	3 (21.4)	11 (78.6)	
Pathology grade	G1/2	10 (34.5)	19 (65.5)	P=1 [b]
	G3/4	3 (33.3)	6 (66.6)	
Margin infiltration (N=27)	No	9 (36)	16 (64)	P=0.693 [b]
	Yes	1 (50)	1 (50)	
T stage	TI/II	5 (83.3)	1 (16.7)	P=0.012 [b]
	TIII/IV	8 (25)	24 (75)	
N stage	N0	9 (50)	9(50)	P=0.087 [b]
	N1-2	4(20)	16 (80)	
M stage	M0	13 (41.9)	18 (58.1)	P=0.072 [b]
	M1	0 (0)	7 (100)	
Clinical Stage	Stage ½	9 (52.9)	8 (47.1)	P=0.042 [b]
	Stage ¾	4 (19)	17 (81)	
Lymphovascular Invasion	No	11 (35.5)	20 (64.5)	P=1 [b]
	Yes	2 (33.3)	4 (66.7)	
Perineural Invasion	No	9 (37.5)	15 (62.5)	P=1 [b]
	Yes	1 (33.3)	2 (66.7)	
Adjuvant chemotherapy	No	5 (55.6)	4 (44.4)	
	Yes	5 (27.8)	13 (72.2)	P=0.219 [b]
Overall survival	Dead	1 (16.7)	5 (83.3)	P=0.643 [b]
	Live	12 (37.5)	20 (62.5)	
CEA	Median (min-max)	2.4 (0.1-68)	4 (0.1-100)	Z:0.77, p=0.444 [c]
CA99	Median (min-max)	16 (2.5-36)	13 (0.1-86.6)	Z: -0.63, p=0.527 [c]
lymphocytes	Median (min-max)	2.1(1.2-4.5)	2.3 (0.9-7.9)	Z: -0.34, p=0.735 [c]
Neutrophils	Median (min-max)	5.8 (3-9.7)	5 (3.4-12.5)	Z: -0.49, p=0.624 [c]
Monocytes	Median (min-max)	0.5 (0.2-0.9)	0.5 (0.003-2.6)	Z:0, p=0.1 [c]

[a:] Levene's Test, [b]: Fisher's Exact Test, [c]: Mann-Whitney U

CA: Cancer antigen 99

CEA: Carcinoembryonic antigen

Note: Denominators (n) may vary due to missing data; where analyses were performed on fewer than 38 cases, the applicable denominator is indicated in the parameter label (e.g., DM/HTN (N=28)) and reflected in the corresponding counts/percentages.

High EZH2 expression was observed in 75% of tumors classified as T stage4.3, compared to only16.7 % of T stage 2.1 tumors (p =0.012). Similarly, high EZH2 expression was significantly more prevalent among patients with clinical stage III/IV (81%) than those with stage I/II (47.1 %) (p =0.042) (Figure1).

Correlation Between MMP1 Expression and Clinicopathologic Parameters

Immunohistochemical investigation revealed that MMP1 expression was low in 10 instances (26.3 %) and high in 28 cases (73.7 %). (Figure2).

Table 3 indicates that elevated MMP1 expression was substantially correlated with advanced tumor stage (T), nodal involvement (N), and clinical stage.

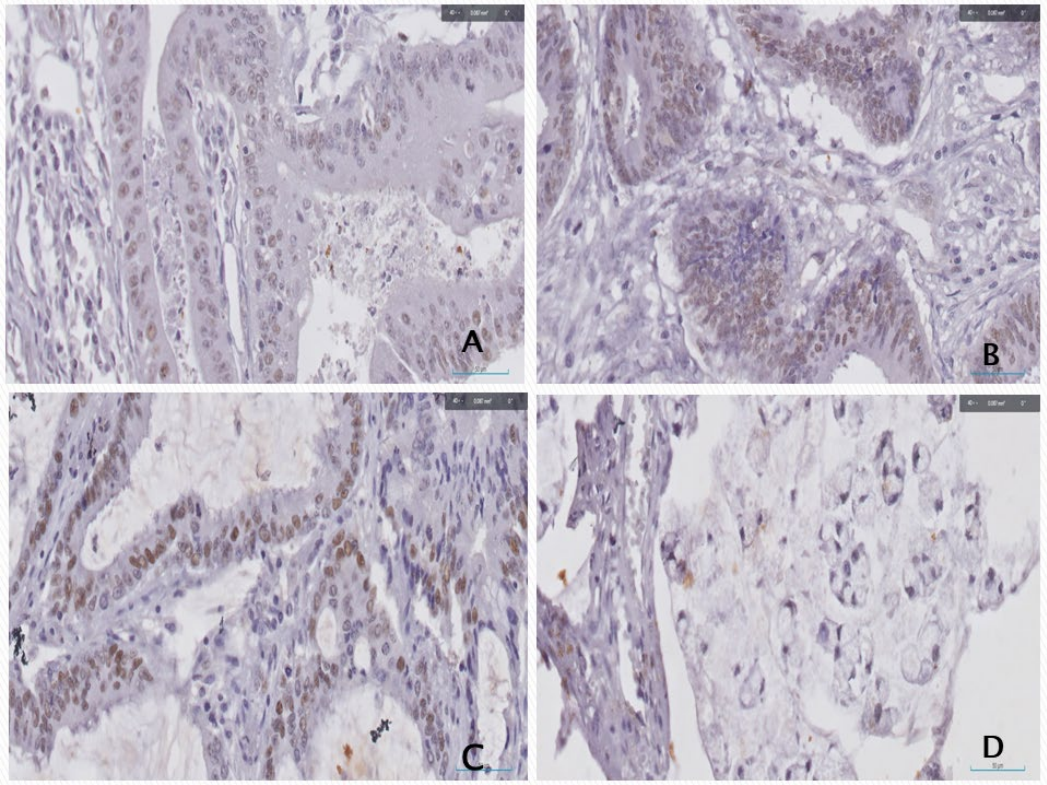

Fig. 1. Immunohistochemical staining for EZH2 in colonic adenocarcinoma showing:
(A) Weak nuclear expression (B) Moderate nuclear expression
(C) Strong nuclear expression (D) Negative expression in signet ring carcinoma.
All images captured at ×400 magnification.

Table 3. The association of different patients' parameters and MMP1 expression

Parameter		MMP1		
		Low N (%) 10 (26.3%)	High N (%) 28 (73.7%)	Significance
Age Ys	Mean (SD)	65.5 (13.3)	56 (13.2)	T:0.09, p=0.930[a]
Sex	Male	5 (20.8)	19 (79.2)	P=0.449[b]
	Female	5 (35.7)	9 (64.3)	
Family history	No	7 (26.9)	19 (73.1)	P=0.497 [b]
	Yes	1 (50)	1 (50)	
Complaint (N=28)	Abdominal pain	7 (35)	13 (65)	P=0.371 [b]
	Others	1 (12.5)	7 (87.5)	
Surgery	hemicolectomy	8 (40)	12 (60)	P=0.068 [b]
	Others	0	7 (100)	
DM/HTN (N=28)	No	6 (30)	14 (70)	P=1 [b]
	Yes	2 (25)	6 (75)	
Site	Right	5 (31.3)	11 (68.8)	P=0.716 [b]
	Left	5 (23.8)	16 (76.2)	
Pathology type	adenocarcinoma	6 (25)	18 (75)	P=1 [b]
	Mucinous/signet ring	4 (28.6)	10 (71.4)	

Parameter		MMP1		
		Low N (%) 10 (26.3%)	High N (%) 28 (73.7%)	Significance
Pathology grade	G1/2	8 (27.6)	21 (72.4)	P=1 [b]
	G3/4	2 (22.2)	7 (77.8)	
Margin infiltration (N=27)	No	7 (28)	18 (72)	P=0.513 [b]
	Yes	1 (50)	1 (50)	
T stage	TI/II	4 (66.7)	2(33.3)	p=0.031 [b]
	TIII/IV	6 (18.8)	26 (81.3)	
N stage	N0	8 (44.4)	10 (55.6)	P=0.027 [b]
	N1-N2	2 (10)	18 (90)	
M stage	M0	10 (32.2)	21 (67.7)	
	M1	0	7(100)	p=0.156 [b]
Clinical Stage	Stage ½	8 (47.1)	9 (52.9)	p=0.023 [b]
	Stage ¾	2 (9.5)	19 (90.5)	
Lymphovascular Invasion	No	8 (25.8)	23 (74.2)	P=0.653 [b]
	Yes	2(33.3)	4 (66.7)	
Perineural invasion	No	7 (29.2)	17 (70.8)	P=1 [b]
	Yes	1 (33.3)	2 (66.7)	
Adjuvant chemotherapy	No	4 (44.4)	5(55.6)	P=0.375 [b]
	Yes	4(22.2)	14 (77.8)	
Overall survival	Dead	1(16.7)	5(83.3)	P=1 [b]
	Live	9 (28.1)	23 (71.9)	
CEA	Median (min-max)	1.4 (0.1-5.5)	5.3(0.1-100)	Z: 1.9, p=0.05 [c]
CA99 *	Median (min-max)	11.7(0.1-36)	14 (0.6-86.6)	Z: 0.23, p=0.821 [c]
lymphocytes	Median (min-max)	2.5 (1.9-4.5)	2.1 (0.9-7.9)	Z: -1.4, p=0.176 [c]
Neutrophils	Median (min-max)	5.7 (3.1-8.5)	5.1 (3.4-12.5)	Z: -0.16, p=0.874 [c]
Monocytes	Median (min-max)	0.35(0.01-0.7)	0.56 (0.003-2.63)	Z: 0.83, p=0.405 [c]

[a]: Levene's Test, [b]: Fisher's Exact Test, [c]: Mann-Whitney U

Note: Denominators (n) may vary due to missing data; where analyses were performed on fewer than 38 cases, the applicable denominator is indicated in the parameter label (e.g., DM/HTN (N=28)) and reflected in the corresponding counts/percentages.

Specifically, high expression was observed in81.3 % of tumours with T stage ¾, compared to33.3 % of tu0mours with T stage ½ (p =0.031). Similarly, it was more frequent among patients with nodal metastasis (N stage1–3; 90%) versus those without (N0;55.6 %) (p =0.027). In terms of clinical stage, high MMP1 expression was detected in90.5 % of stage III/IV tumours, compared to52.9 % of stage I/II tumours (p =0.023) (Figure2).

Association Between EZH2 Expression and Survival Outcomes

Table 4 shows that people with higher mean EZH2 expression suffered a lower mean overall survival (OS) and mean disease-free survival (DFS) compared to those with lower expression. Among participants in the high-expression cohort, the average overall survival was88.1 months compared to91.9 months for the low-expression cohort. Mean DFS in the high-expression group was63.8 months, while in the low-expression group it was92.4 months.

Although the high-EZH2 group showed numerically lower mean OS and DFS, the differences did not reach statistical significance (OS: p = 0.357; DFS: p = 0.052, log-rank test) (Figure 3). These findings should be interpreted cautiously given the limited sample size and the univariate nature of the analysis.

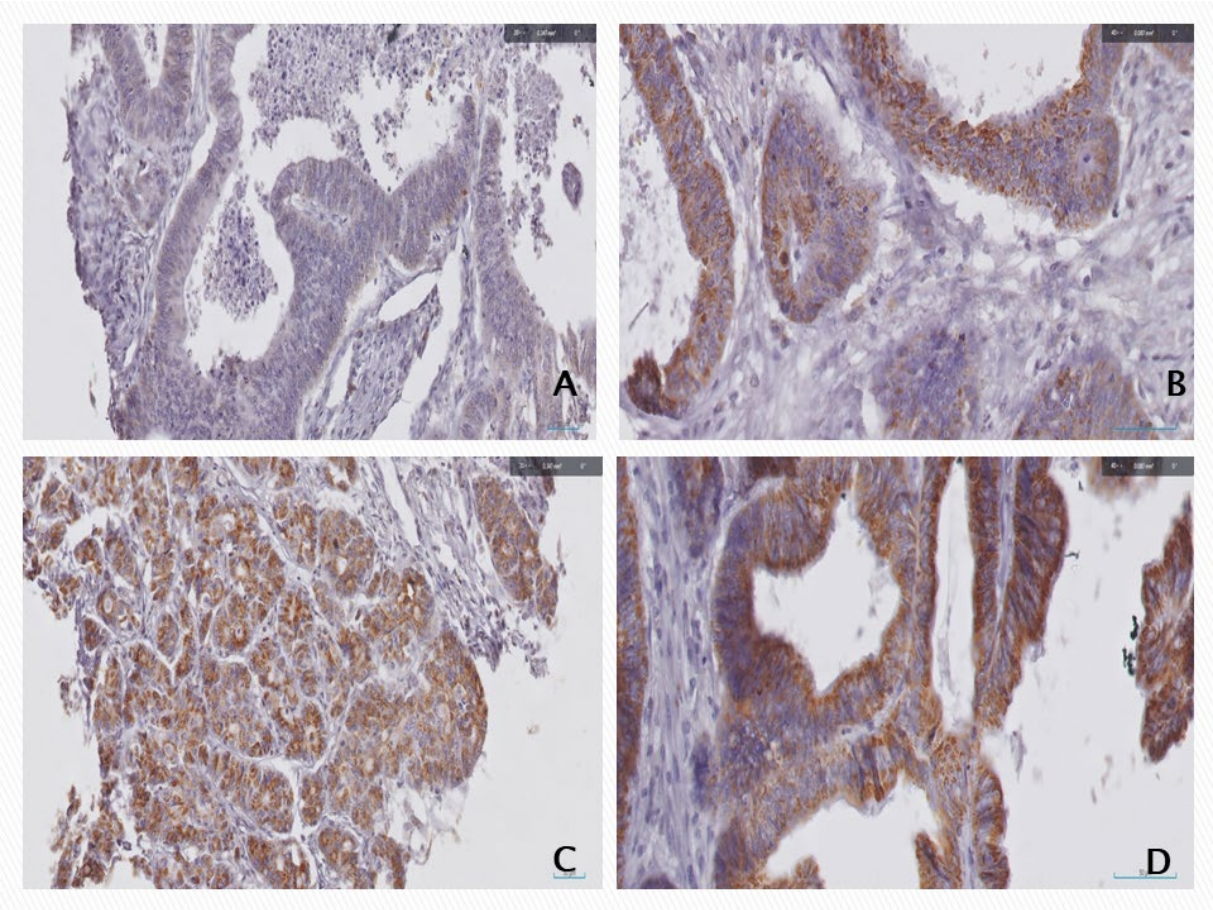

Fig. 2: Immunohistochemical staining for MMP1 in colonic adenocarcinoma showing:
(A) Weak cytoplasmic expression at ×200 magnification (B) Moderate cytoplasmic expression at ×400
(C) Strong cytoplasmic expression at ×100 (D) Negative cytoplasmic expression at ×400

Table 4. Analysis of survival data of the patients about EZH2 expression: Survival Analysis Results

Survival Type	Group	Total (N)	Events (N)	Censored N (%)	Survival Time Mean (95% CI)	X^{2}*	p-value
Disease-Free Survival	Low	13	1	12(92.3 %)	92.4 (78.1 -106.7)	3.8	0.052
	High	25	11	14(56.0 %)	63.8 (42.4 -85.2)		
Overall Survival	Low	13	1	12(92.3 %)	91.9 (76.6 -107.1)	0.84	0.357
	High	25	5	20(80.0 %)	88.1 (69.3 -107.9)		

*: Log Rank (Mantel-Cox)

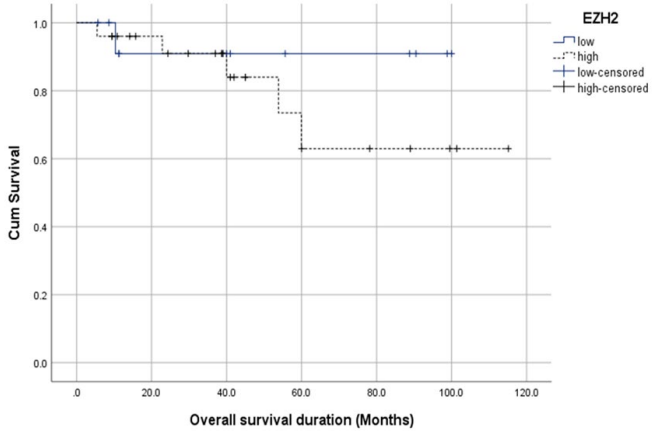

Figure 3: Analysis of survival data of the patients about EZH2 expression.

Association Between MMP1 Expression and Survival Outcomes

Table 5 summarizes survival outcomes by MMP1 expression level. Neither overall survival (OS) nor disease-free survival (DFS) differed significantly between high and low MMP1 expression groups (OS: p = 0.722; DFS: p = 0.052, log-rank test) (Figure 4). These results did not reach statistical significance and should be interpreted cautiously.

Table 5: Analysis of survival data of the patients about MPP1 expression:

Survival Type	Group	Total (N)	Events (N)	Censored N (%)	Survival Time Mean (95% CI)	X^2 (Log Rank)	p-value
Disease-Free Survival	Low	10	1	9(90.0 %)	90.8 (73.8 -107.9)	3.8	0.052
	High	28	11	17(60.7 %)	63.6 (48.2 -88.9)		
Overall Survival	Low	10	1	9(90.0 %)	87.2 (63.9 -110.4)	0.126	0.722
	High	28	5	23(82.1 %)	92.2 (74.9 -109.5)		

The Log Rank (Mantel-Cox) test was used for survival analysis.

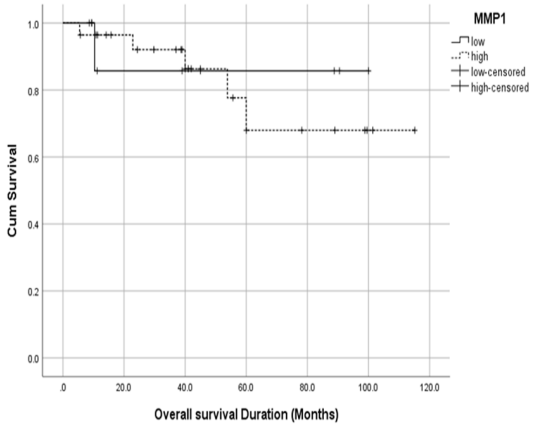

Figure4: Analysis of survival data of the patients about MMP1 expression.

Discussion

Colorectal cancer (CRC) is one of the deadliest oncological diseases, with the third highest incidence and fourth highest mortality rate internationally.

Colorectal carcinogenesis involves multistep and various mechanisms starting from ordinary epithelial cells into malignant cells, undergoing local invasion, intravasation into the vasculature, and metastasising to other organs, most commonly the liver (10).

The extracellular matrix has a significant role in the emergence of cancer by providing tumour cells with organisation and information concerning the activity of cells. The remodelling of the extracellular matrix is critical to enable the dispersion of tumour cells from the primary site. This is brought about predominantly through the action of proteolytic enzymes by MMPs, which cleave extracellular matrix elements and facilitate tumour progression. MMP-1 is designated as interstitial collagenase due to its activity in the cleavage of collagen types I, II and III, primary constituents of the interstitial stroma (11).

MMPs cleave matrix proteins as one of the primary processes in matrix degradation as well as the mediation of bioactive molecules (12). They control proteolytic 'shedding' of numerous surface proteins such as growth factors, chemokines, and adhesion molecules. In many cancers, higher MMP levels have been linked with poorer prognosis because of the increased angiogenesis, invasion, and metastasis that they facilitate. There are recent studies suggesting that certain MMPs may also have some tumour-suppressive functions (13).

In carcinoma lesions, MMP-1 has been reported in the stromal cells bordering the malignancy, as well as in the carcinoma cells themselves. In colorectal cancer patients, higher MMP-1 expression in tumour tissues is associated with more aggressive disease and poor prognosis (14). This enzyme facilitates stromal destruction by augmenting the interstitial collagen cleavage and increasing the collagenolytic activity within the tumour, thus promoting tumour invasiveness (7).

Among other factors in this study of 38 patients with colorectal cancer, high MMP-1 expression was significantly associated with more advanced tumour

stage (T4.3), Regional lymph node metastases N stage (N1-3), and clinical stage III/IV.

The data support the prior study of the expressive correlation between the increase in MMP-1 and the aggressive nature of the tumour (7). Other studies indicate possible involvement of MMP-1 in the phenomenon termed “tumour self-seeding,” which refers to the re-infiltration of circulating tumour cells back to the primary tumour, thereby increasing the tumour’s malignancy (9).

Experimental studies have shown that stimulation of muscarinic receptors can markedly increase MMP-1 mRNA expression in colon cancer cell lines, supporting its role in invasion and metastasis (8). Additionally, higher levels of MMP-1 have been linked to increased tumour invasiveness and lymph node involvement in clinical specimens (16). Interestingly, discrepancies in MMP-1 expression between primary tumours and metastatic lesions have been reported. While high expression is observed in node-positive primary tumours, lower levels are noted in metachronous metastases, suggesting a time-dependent role for MMP-1 in early invasion (17).

Although molecular markers of tumor aggressiveness may eventually be explored in broader interdisciplinary contexts, our study did not evaluate medicolegal or forensic applications. Therefore, any such implications are purely hypothesis-generating and require dedicated studies before any forensic applicability can be considered.

Moreover, genetic polymorphisms in the MMP-1 promoter region have been linked to increased CRC risk, particularly the 2G allele, which enhances transcriptional activity (17). These findings highlight MMP-1 as a potential marker for tumour aggressiveness and metastatic potential.

Historically, prognosis in colorectal cancer (CRC) has relied on tumour dimensions, lymph node involvement, and the existence of metastases. (18). Our study demonstrated that while high MMP-1 expression correlated with aggressive pathological features, it did not substantially influence overall survival (OS) or disease-free survival (DFS), aligning with results from earlier studies (16).

In our series, MMP-1 immunoreactivity was absent in normal mucosa and adenomas but present in invasive carcinoma cases (T1–T4). These findings support previous observations by Shiozawa et al. (16), who reported strong MMP-1 expression in tumours exhibiting infiltrative growth patterns.

Type I and III collagens constitute the structural framework of the gastrointestinal stroma (19), and their breakdown is essential for tumour penetration through the bowel wall. MMP-1 is believed to play a critical role in this process (20), thus supporting its involvement in local tumour spread.

Although the exact cellular source of MMP-1 remains under debate, both carcinoma and stromal cells have been shown to express the enzyme (21–24). Some studies suggest that variation in immunolocalisation may be due to differences in antibody specificity (25).

EZH2 was discovered to be considerably elevated in colorectal cancer tissues compared to neighbouring normal mucosa. EZH2, an essential element of the polycomb repressive complex 2 (PRC2), promotes gene silencing via histone methylation and is known to drive oncogenesis through modulation of cell cycle and apoptotic pathways (26–27).

Prior studies have demonstrated that EZH2 overexpression facilitates proliferation, invasion, angiogenesis, and metastasis in several tumour types, including breast cancer (32), bladder (33), ovarian (34), and lung cancers (35–36). Its oncogenic potential in CRC, although less understood, is gaining attention (31,43).

In our cohort, higher EZH2 expression was associated with adverse clinicopathologic features. However, EZH2 expression was not significantly associated with OS or DFS, and thus its prognostic relevance remains inconclusive in this sample (43).

Importantly, because multivariable Cox regression adjusting for stage, grade, and nodal status was not performed, we cannot conclude that EZH2 or MMP1 provides independent prognostic information beyond established clinicopathologic factors. In our cohort, the high EZH2 expression group showed numerically lower OS and DFS; however, these differences were not statistically significant (OS: $p = 0.357$; DFS: $p = 0.052$). Although some prior studies have linked EZH2 overexpression to adverse features and poorer outcomes in CRC (43), our survival analyses did not reach statistical significance; thus, our findings should be interpreted cautiously and do not confirm prognostic utility. To enhance scoring reliability, two pathologists assessed slides independently and discrepancies were resolved by consensus; however, formal inter-observer concordance statistics (e.g., κ) were not available.

In conclusion, EZH2 and MMP1 overexpression were associated with aggressive clinicopathologic characteristics in this cohort. Survival analyses did not demonstrate statistically significant associations with OS or DFS, and all survival analyses were univariate. Because multivariable Cox regression adjusted for established prognostic factors was not performed, independent prognostic utility cannot be inferred. Larger, adequately powered studies with multivariable modeling are warranted. Survival findings were not statistically significant and should be interpreted cautiously; independent prognostic utility cannot be inferred without multivariable modeling in larger cohorts.

This study proposes that future studies focus on delineating the exact molecular pathways involved and extending the clinical scope of these biomarkers to include larger sample sizes.

Additional research involving larger cohorts is necessary to confirm these results and to investigate the

underlying molecular mechanisms. Furthermore, EZH2 and MMP1 could function as potential prognostic biomarkers and therapeutic targets in the treatment of colorectal cancer.

Study Limitations and Practical Constraints

This study is limited by its retrospective design and modest cohort size (n = 38), which reduces statistical power, particularly for subgroup and survival analyses. Some clinicopathologic variables were missing for a subset of cases; therefore, analyses were performed using available cases for each variable and denominators (n) are reported accordingly in the Results and tables. Due to the small sample size, stage was analyzed in two categories (I/II vs III/IV) as described in the Methods; however, survival findings remained not statistically significant and should be interpreted cautiously. Larger, prospective studies with adequately powered cohorts and multivariable modeling are warranted to validate these observations.

Inter-observer agreement between pathologists was not formally quantified (no κ statistic), as rate-level scoring data were not preserved for concordance analysis; however, all discrepant cases were resolved by consensus review.

Conclusion

In this retrospective cohort of 38 colorectal carcinoma cases, high EZH2 and MMP1 immunoexpression was significantly associated with aggressive clinicopathologic features, including advanced T stage, nodal involvement, and clinical stage III/IV. However, neither marker showed a statistically significant association with overall survival (OS) or disease-free survival (DFS) in univariate analyses. Larger, adequately powered prospective studies with standardized scoring and multivariable modeling are warranted to clarify any independent prognostic value and to explore potential therapeutic relevance.

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None

Authors' Contributors

All authors contributed equally to the conceptualization, design, and execution of this study.

Data Availability

The datasets generated and analyzed during the current study are not publicly available; however, the data can be shared for research and authentication purposes upon reasonable request.

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Ethics Approval

Approval was obtained from the ethical committee of Mansoura Faculty of Medicine, ID (RP.22.09.141). The Institutional Review Board approved the request for a waiver of informed consent.

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Conflict of Interest

The authors declared no conflict of interest.

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