Iranian Journal of Pathology | ISSN: 2345-3656

Immunohistochemical Expression of Metastasis-Associated Gene 3 (MTA3) and Epithelial-Mesenchymal Transition (EMT) Markers in Colorectal Carcinoma: A Cross-sectional Study

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KEYWORDS

Metastasis-Associated Gene 3; Cadherins; SNAIL1/2; Colorectal Neoplasms

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Main Subjects:
GI, Liver & Pancreas Pathology

Received 02 Dec 2025;
Accepted 25 Feb 2026;
Published Online 10 May 2026;

10.22034/ijp.2026.2079960.3600

ABSTRACT

Background & Objective: Colorectal carcinoma (CRC) is a main cause of cancer-related deaths worldwide. Studying genes involved in CRC progression helps predict prognosis and develop new therapeutic modalities. Metastasis-Associated Gene 3 (MTA3) is one of these studied genes. MTA3 could regulate tumor carcinogenesis and modulate the epithelial-mesenchymal transition (EMT) pathway due to its ability to promote or suppress target gene expression. This study evaluated the immunohistochemical expression of MTA3 and EMT markers (E-cadherin and Slug) in CRC specimens.

Methods: This retrospective cross-sectional study included 81 cases of primary CRC. All cases were stained with MTA3, E-cadherin, and Slug antibodies. The relations between their expression and clinicopathological parameters, as well as the relation between MTA3 and EMT markers, were assessed.

Results: High MTA3 expression and preserved membranous E-cadherin were significantly related to low tumor grade, absence of lymphovascular and perineural invasion, low depth of invasion, absence of lymph node and distant metastasis, as well as early tumor stage. High Slug expression was related to higher tumor grade, high depth of invasion, presence of lymph node and distant metastasis, as well as advanced tumor stage. There was a significant correlation between high MTA3 expression and both preserved E-cadherin ($r_s = 0.520$, $P < 0.001$) and low Slug expression ($r_s = -0.290$, $P = 0.009$) in the studied cases.

Conclusion: MTA3 is related to favorable clinicopathological parameters in CRC and could regulate EMT marker expression, suggesting that MTA3 may play a possible role in controlling tumor cell invasion and metastasis and could exhibit a potential tumor-suppressive role.

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Introduction

Colorectal carcinoma (CRC) is the 2nd most common cause of cancer mortality for both men and women globally (1). According to the GLOBOCAN 2022 estimates provided by the International Agency for Research on Cancer, an approximate number of 935,173 deaths and 1,931,590 newly diagnosed cases are reported worldwide (2). Its incidence is predicted to increase by over 60% by 2030 (3).

Cancer begins locally and then spreads to other organs. Tumor metastasis is responsible for more than 90% of cancer-related deaths worldwide (4). The members of the metastasis-associated gene (MTA) family have been recognized as some of the most crucial regulatory molecules in physiological processes as well as cancer progression and metastasis. Three genes, MTA1, MTA2, and MTA3, constitute the MTA family and are located on chromosomes 14q32, 11q12–q13.1, and 2p21, respectively (5).

Metastasis-Associated Gene 3 (MTA3) plays a vital role in cancer development, cellular proliferation, and metastasis by being an integral subunit of the nucleosome-remodeling and histone deacetylase (NuRD) complex, which serves as a transcriptional repressor and regulates the expression of epithelial-mesenchymal transition-associated genes (6). Studies about the role of MTA3 in human malignancies, as an oncogene or tumor suppressor gene, are still limited, and its mechanistic role in CRC needs further characterization (5).

Epithelial-mesenchymal transition (EMT), as an important biologic process, allows epithelial cells to gain multiple biogenetic changes, thus acquiring a mesenchymal cell phenotype (7). EMT contributes to tumor development, resistance to apoptosis, invasion, metastasis, and chemoresistance (8).

The main events of EMT include loss or reduced epithelial marker expression, such as E-cadherin and Claudin-1, and gain of mesenchymal marker expression, such as N-cadherin, vimentin, and Snail1/2 (7, 9).

The aim of this work was to study the immunohistochemical expression of MTA3 and EMT markers (E-cadherin and Slug) in colorectal carcinoma and their relation to different clinicopathological parameters, and to evaluate the relation between MTA3 and EMT markers in colorectal carcinoma.

Materials and Methods

This retrospective cross-sectional study was carried out on 81 cases of primary colorectal carcinoma, collected from the Pathology Department, Faculty of Medicine, Tanta University, and Tanta Cancer Centre archives during the period from December 2022 to December 2024. The Research Ethics Committee, Faculty of Medicine, Tanta University, approved this study (approval code: 36068/11/22).

Inclusion criteria were known primary colorectal carcinoma cases without any other malignancies, cases with adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks, and complete clinical data. Exclusion criteria were cases with other malignancies, as well as cases with insufficient FFPE blocks and inadequate clinicopathologic data. At the time of inclusion in the study, all patients underwent colectomy with lymph node dissection without receiving chemotherapy or any preceding treatment.

Clinicopathological data

The data were collected from the pathology report of each case, including patient age and sex. The tumor site of each lesion was obtained from the pathology request, operative sheet, and the available gross specimens. The site of studied cases was divided into right side and left side.

Histopathological evaluation

Histopathological evaluation of the studied cases was done independently by two pathologists. The gross picture of each case was obtained from the pathology report, colonoscopy report, or available specimens for gross appearance of the tumor, either fungating, infiltrating, or ulcerating masses; tumor size; anatomic depth of invasion; distance from margins (proximal, distal, and circumferential); presence of perforation; and number of dissected lymph nodes (should be ≥ 12) and number of lymph nodes involved by tumor deposits. Microscopic examination was done to evaluate various histopathological features, such as histopathological types, tumor grade, lymphovascular and perineural invasion, as well as lymph node metastasis.

Pathological grading and staging of the studied cases were done according to the World Health Organization classification of colorectal carcinoma and the American Joint Committee on Cancer tumor-node-metastasis (TNM), 2019 (9th edition) (10).

Immunohistochemical staining

Five µm thickness tumor sections were mounted on positively charged slides, then left to dry for 30 minutes at 37 °C. Deparaffinization and antigen retrieval were performed in a Dako PT Link unit using high- and low-pH EnVision FLEX antigen retrieval solution (at 97 °C for 20 minutes). Immunostaining was done using Dako Autostainer (Link 48). Peroxidase blocking reagent was applied, then incubation with primary antibodies for 30 minutes was done: MTA3 (rabbit polyclonal antibody, Abclonal, USA, catalog no. A2328: AB_2764293, dilution 1:500), E-cadherin (rabbit polyclonal antibody, Genetex, USA, catalog no. GTX100443, dilution 1:500), and Slug (mouse monoclonal antibody, Santa Cruz Biotechnology, Inc, USA, catalog no. A-7: sc-166476, dilution 1:100). Horseradish peroxidase polymer and then diaminobenzidine chromogen were applied. Counterstaining with hematoxylin was done.

Immunohistochemical scoring was done with no significant interobserver differences (high agreement with kappa statistics of 0.9).

Evaluation of MTA3 Immunostaining

MTA3 nuclear staining was scored semiquantitatively by multiplying the intensity score and the percentage of immunostained cells. The intensity score of MTA3 staining was as follows: 0, negative; 1, weak; 2, strong. The percentage of stained cells was scored as follows: 0, no staining; 1, 1%-25% of tumor cells; 2, 26%-50% of tumor cells; 3, 51%-75% of tumor cells; 4, 76%-100% of tumor cells. Giving a final score (0-8), cases were classified as low MTA3 expression (total score < 4) and high MTA3 expression (total score ≥ 4) (11). Positive control for MTA3 was sections of invasive ductal breast carcinoma. Negative control was achieved by replacing the primary antibody with phosphate-buffered saline (PBS).

Evaluation of E-cadherin Immunostaining

Reduced membranous E-cadherin expression was defined as no or weak-to-moderate complete membranous staining in more than 10% of tumor cells. However, preserved E-cadherin expression was observed as strong membranous staining in more than 10% of tumor cells (12). Positive control for E-cadherin was sections of invasive ductal breast carcinoma. Negative control was achieved by replacing the primary antibody with PBS.

Evaluation of Slug Immunostaining

Perinuclear and/or cytoplasmic Slug immunoreactivity was assigned as follows: 0, no staining; +1, 1%-10% positive tumor cells; +2, 11%-

50% positive tumor cells; +3, ≥ 51% positive tumor cells. Specimens exhibiting +2 and +3 immunostaining were classified as high Slug expression (13). Positive control for Slug was sections of invasive gastric adenocarcinoma. Negative control was achieved by replacing the primary antibody with PBS.

Statistical analysis

Data were analyzed using SPSS software package version 20.0 (Armonk, NY: IBM Corp). Qualitative data were expressed as number and percent. The Kolmogorov-Smirnov test was used to verify the normal distribution of data. Quantitative data were described using range, mean, and standard deviation. Significance of the results was set at the 5% level. The tests used were the chi-square test, Fisher exact test or Monte Carlo test, Mann-Whitney test, and Spearman correlation test (rs). Correlation graphs were created using SPSS software package version 20.0 (Armonk, NY: IBM Corp).

Results

Clinicopathological results

The current study included 81 cases of CRC; the clinicopathological features are demonstrated in Table 1.

Table 1. Clinicopathological characteristics of the studied cases (N = 81)

		N. (%)
Age		50.0 ± 12.09
Sex	Male	53 (65.4%)
	Female	28 (34.6%)
Tumor Size	< 5 cm	47 (58.0%)
	≥ 5 cm	34 (42.0%)
Tumor Site	Right side	27 (33.3%)
	Left side	54 (66.7%)
Perforation	Present	8 (9.9%)
	Absent	73 (90.1%)
Histopathological Types	Adenocarcinoma NOS	47 (58.0%)
	Mucinous adenocarcinoma	15 (18.5%)
	Adenocarcinoma with Mucinous differentiation	10 (12.3%)
	Signet ring cell carcinoma	5 (6.2%)
	Medullary carcinoma	2 (2.5%)
	Adenosquamous carcinoma	2 (2.5%)
Histopathological grade	Grade 1	4 (4.9%)
	Grade 2	41 (50.6%)
	Grade 3	24 (29.6%)
	Grade 4	12 (14.9%)
Lymph vascular invasion		
Present		29 (35.8%)
Absent		52 (64.2%)
Perineural invasion		
Present		8 (9.9%)
Absent		73 (90.1%)
Depth of invasion (T)	T1	8 (9.9%)
	T2	25 (30.9%)
	T3	40 (49.3%)
	T4	8 (9.9%)
Lymph node status (N)	N0	54 (66.7%)
	N1	16 (19.8%)
	N2	11 (13.5%)
Distant metastasis (M)	M0	69 (85.2%)
	M1	12 (14.8%)
TNM staging	Early stages Stage I	32 (39.5%)
	Stage II Advanced stages	20 (24.7%)
	Stage III	17 (21.0%)
	Stage IV	12 (14.8%)

NOS: Not otherwise specified, TNM: Tumor-Node-Metastasis

Table 2. Relation between MTA3 immunohistochemical expression and clinicopathologic characteristics of studied cases (N = 81)

Clinicopathologic characteristics		Low MTA3 expression (n=25)	High MTA3 expression (n=56)	P value
Age (Years)				
Mean ± SD **Min. – Max.** **Median (IQR)**		54.7 ± 12.15 30.0 – 72.0 55.0 (47.5 – 66.0)	47.9 ± 11.57 30.0 – 70.0 46.5 (35.5 – 56.0)	0.029*
Sex				
Male **Female**		19 (35.8%) 6 (21.4%)	34 (64.2%) 22 (78.6%)	0.181
Tumor Size	< 5	15 (31.9%)	32 (68.1%)	0.810
	≥ 5	10 (29.4%)	24 (70.6%)	
Tumor Site	Right side	8 (29.6%)	19 (70.4%)	0.865
	Left side	17 (31.5%)	37 (68.5%)	
Perforation	Present	8 (100%)	0 (0%)	<0.001*
	Absent	17 (23.3%)	56 (76.7%)	
Histopathological types	Adenocarcinoma NOS	13 (27.7%)	34 (72.3%)	0.069
	Mucinous adenocarcinoma	6 (40%)	9 (60%)	
	Adenocarcinoma with Mucinous differentiation	1 (10%)	9 (90%)	
	Signet ring cell carcinoma	4 (80%)	1 (20%)	
	Medullary carcinoma	0 (0%)	2 (100%)	
	Adenosquamous carcinoma	1 (50.0%)	1 (50.0%)	
Histopathological grade	Grade 1	0 (0%)	4 (100%)	0.001*
	Grade 2	6 (14.6%)	35 (85.4%)	
	Grade 3	15 (62.5%)	9 (37.5%)	
	Grade 4	4 (33.3%)	8 (66.7%)	
Lymph vascular invasion				
Present **Absent**		18 (62.1%) 7 (13.5%)	11 (37.9%) 45 (86.5%)	0.001*
Perineural invasion				
Present **Absent**		7 (87.5%) 18 (24.7%)	1 (12.5%) 55 (75.3%)	0.001*
Depth of tumor invasion (T)	T1	0 (0%)	8 (100%)	0.001*
	T2	0 (0%)	25 (100%)	
	T3	17 (42.5%)	23 (57.5%)	
	T4	8 (100%)	0 (0%)	
Lymph node status (N)	N0	5 (9.3%)	49 (90.7%)	0.001*
	N1	10 (62.5%)	6 (37.5%)	
	N2	10 (90.9%)	1 (9.1%)	
Distant metastasis (M)	M0	14 (20.3%)	55 (79.7%)	0.001*
	M1	11 (91.7%)	1 (8.3%)	
TNM staging	Early stages Stage I	0 (0%)	32 (100%)	0.001*
	Stage II Advanced stages	4 (20%)	16 (80%)	
	Stage III	10 (58.8%)	7 (41.2%)	
	Stage IV	11 (91.7%)	1 (8.3%)	

NOS: Not otherwise specified, TNM: Tumor-Node-Metastasis, MTA3: Metastasis-Associated Gene 3, * Significant as P-value ≤ 0.05.

Immunohistochemical results

Relation between MTA3 immunohistochemical expression and clinicopathologic characteristics of studied cases:

Tissue samples from normal colonic mucosa at the tumor margins were assessed during the study. All specimens of normal colonic mucosa showed high MTA3 expression (Fig. 1).

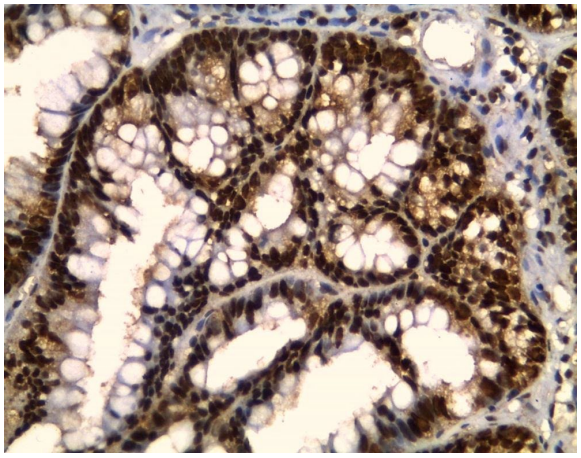

Fig. 1. Metastasis-Associated Gene 3 (MTA3) immunohistochemical high nuclear expression in normal colonic mucosa (x400)

Regarding CRC specimens, high MTA3 expression was detected in 69.1% of cases. High MTA3 expression was significantly related to absence of perforation (P < 0.001), lower tumor grade (P = 0.001), absence of lymphovascular invasion and perineural invasion (P = 0.001), low depth of invasion (P = 0.001), absence of lymph node and distant metastasis (P = 0.001), as well as early TNM staging (P = 0.001) (Table 2, Fig. 2).

Relation between E-cadherin immunohistochemical expression and clinicopathologic characteristics of studied cases:

Reduced E-cadherin expression was significantly related to larger tumor size (P = 0.007), high tumor grade (P = 0.012), presence of perineural invasion (P = 0.019), high depth of invasion (P < 0.001), presence of lymph node metastasis (P = 0.050) and distant metastasis (P = 0.013), as well as advanced TNM staging (P < 0.001) (Table 3, Fig. 3).

Relation between Slug immunohistochemical expression and clinicopathologic characteristics of studied cases:

High Slug expression was significantly related to histopathological types (P = 0.021), being more expressed in mucinous adenocarcinoma and signet ring cell carcinoma; high depth of invasion (P < 0.001); presence of lymph node and distant metastasis (P = 0.042 and 0.018, respectively); as well as advanced tumor stage (P < 0.001). Slug expression was higher in high-grade tumors, yet it did not reach the statistically significant level (P = 0.052) (Table 4, Fig. 4).

Relation between MTA3, E-cadherin, and Slug immunohistochemical expression in studied cases:

There is a significant relation between MTA3 expression and both E-cadherin and Slug expression in the studied cases (Table 5).

A significant moderate positive correlation between MTA3 and E-cadherin expression was detected (rs = 0.520, P < 0.001), as high MTA3 expression was correlated with preserved E-cadherin expression (Fig. 5). A statistically significant weak negative correlation existed between MTA3 and Slug expression (rs = -0.290, P = 0.009); high MTA3 expression was correlated with low Slug expression (Fig. 6). The correlation between Slug and E-cadherin expression was a significant moderate negative correlation (rs = -0.580, P < 0.001); reduced E-cadherin was correlated with high Slug expressions (Fig. 7).

Table 3. Relation between E-cadherin immunohistochemical expression and clinicopathologic characteristics of studied cases (N = 81)

Clinicopathologic characteristics		Reduced E-cadherin expression (n=48)	Preserved E-cadherin expression (n=33)	P Value
Age (Years)				
Mean ± SD **Min. – Max.** **Median (IQR)**		50.5 ± 12.67 30.0 – 72.0 53.5 (38.25 – 61.5)	49.3 ± 11.35 30.0 – 68.0 48.0 (40.5 – 57.0)	0.810
Sex				
Male **Female**		31 (58.5%) 17 (60.7%)	22 (41.5%) 11 (39.3%)	0.846
Tumor Size	< 5	22 (46.8%)	25 (53.2%)	0.007*
	≥ 5	26 (76.5%)	8 (23.5%)	
Tumor Site	Right side	14 (51.9%)	13 (48.1%)	0.337
	Left side	34 (63 %)	20 (37%)	
Perforation	Present	6 (75%)	2 (25%)	0.462
	Absent	42 (57.5%)	31 (42.5%)	
Histopathological Types	Adenocarcinoma NOS	29 (61.7%)	18 (38.3%)	0.298
	Mucinous adenocarcinoma	10 (66.7%)	5 (33.3%)	
	Adenocarcinoma with Mucinous differentiation	4 (40%)	6 (60%)	
	Signet ring cell carcinoma	3 (60%)	2 (40%)	
	Medullary carcinoma	0 (0%)	2 (100%)	
	Adenosquamous carcinoma	2 (100%)	0 (0%)	
Histopathological Grade	Grade 1	0 (0%)	4 (100%)	0.012*
	Grade 2	22 (53.7%)	19 (46.3%)	
	Grade 3	20 (83.3%)	4 (16.7%)	
	Grade 4	6 (50%)	6 (50%)	
Lymphovascular invasion				
Present **Absent**		19 (65.5%) 29 (55.8%)	10 (34.5%) 23 (44.2%)	0.392
Perineural invasion				
Present **Absent**		8 (100%) 40 (54.8%)	0 (0%) 33 (45.2%)	0.019*
Depth of invasion (T)	T1	0 (0 %)	8 (100%)	<0.001*
	T2	11 (44%)	14 (56%)	
	T3	31 (77.5%)	9 (22.5%)	
	T4	6 (75%)	2 (25%)	
Lymph node status (N)	N0	27 (50%)	27 (50%)	0.050*
	N1	13 (81.3%)	3 (18.8%)	
	N2	8 (72.7%)	3 (27.3%)	
Distant metastasis (M)	M0	37 (53.6%)	32 (46.4%)	0.013*
	M1	11 (91.7%)	1 (8.3%)	
TNM staging	Early stages Stage I	10 (31.3%)	22 (68.8%)	<0.001*
	Stage II Advanced stages	15 (75%)	5 (25%)	
	Stage III	12 (70.6%)	5 (29.4%)	
	Stage IV	11 (91.7%)	1 (8.3%)	

NOS: Not otherwise specified, TNM: Tumor-Node-Metastasis, E-cadherin: Epithelial-cadherin, * Significant as P-value ≤ 0.05.

Table 4. Relation between Slug immunohistochemical expression and clinicopathologic characteristics of studied cases (N = 81)

Clinicopathologic characteristics		Low Slug Expression (n=23)	High Slug Expression (n=58)	P Value
Age (Years)				
Mean ± SD **Min. – Max.** **Median (IQR)**		49.3 ± 11.17 34.0 – 70.0 48.0 (39.0 -57.0)	50.3 ± 12.52 30.0 – 72.0 53.5 (38.75-60.5)	0.842
Sex				
Male **Female**		13 (24.5%) 10 (35.7%)	40 (75.5%) 18 (64.3%)	0.288
Tumor Size	< 5	17 (36.2%)	30 (63.8%)	0.068
	≥ 5	6 (17.6%)	28 (82.4%)	
Tumor Site	Right side	7 (25.9%)	20 (74.1%)	0.727
	Left side	16 (29.6%)	38 (70.4%)	
Perforation	Present	1 (12.5%)	7 (87.5%)	0.294
	Absent	22 (30.1%)	51 (69.9%)	
Histopathological Types	Adenocarcinoma NOS	15 (31.9%)	32 (68.1%)	0.021*
	Mucinous adenocarcinoma	0 (0%)	15 (100%)	
	Adenocarcinoma with Mucinous differentiation	6 (60%)	4 (40%)	
	Signet ring cell carcinoma	1 (20%)	4 (80%)	
	Medullary carcinoma	1 (50%)	1 (50%)	
	Adenosquamous carcinoma	0 (0%)	2 (100%)	
Histopathological grade	Grade 1	1 (25%)	3 (75%)	0.052
	Grade 2	17 (41.5%)	24 (58.5%)	
	Grade 3	3 (12.5%)	21 (87.5%)	
	Grade 4	2 (16.7%)	10 (83.3%)	
Lymphovascular invasion				
Present **Absent**		7 (24.1%) 16 (30.8%)	22 (75.9%) 36 (69.2%)	0.526
Perineural invasion				
Present **Absent**		0 (0%) 23 (31.5%)	8 (100%) 50 (68.5%)	0.061
Depth of invasion (T)	T1	8 (100%)	0 (0%)	<0.001*
	T2	10 (40%)	15 (60%)	
	T3	4 (10%)	36 (90%)	
	T4	1 (12.5%)	7 (87.5%)	
Lymph node status (N)	N0	20 (37%)	34 (63%)	0.042*
	N1	2 (12.5%)	14 (87.5%)	
	N2	1 (9.1%)	10 (90.9%)	
Distant metastasis (M)	M0	23 (33.3%)	46 (66.7%)	0.018*
	M1	0 (0%)	12 (100%)	
TNM staging	Early stages Stage I	18 (56.3%)	14 (43.8%)	<0.001*
	Stage II Advanced stages	2 (10%)	18 (90%)	
	Stage III	3 (17.6)	14 (82.4%)	
	Stage IV	0 (0%)	12 (100%)	

NOS: Not otherwise specified, TNM: Tumor-Node-Metastasis, * Significant as P-value ≤ 0.05.

Table 5. Relation between MTA3, E-cadherin, and Slug immunohistochemical expression in studied cases (N= 81)

		Low MTA3 Expression (N = 25)	High MTA3 Expression (N = 56)	P Value
Slug	High expression	24 (41.4%)	34 (58.6%)	0.001*
	Low expression	1 (4.3%)	22 (95.7%)	
E –cadherin	Preserved expression	5 (15.2%)	28 (84.8%)	0.011*
	Reduced expression	20 (41.7%)	28 (58.3%)	
		Low Slug expression (N = 23)	High Slug expression (N = 58)	P Value
E –cadherin	Preserved expression	19 (57.6%)	14 (42.4%)	<0.001*
	Reduced expression	4 (8.3%)	44 (91.7%)	

E-cadherin: Epithelial-cadherin, MTA3: Metastasis-Associated Gene 3. * Significant as P-value ≤ 0.05.

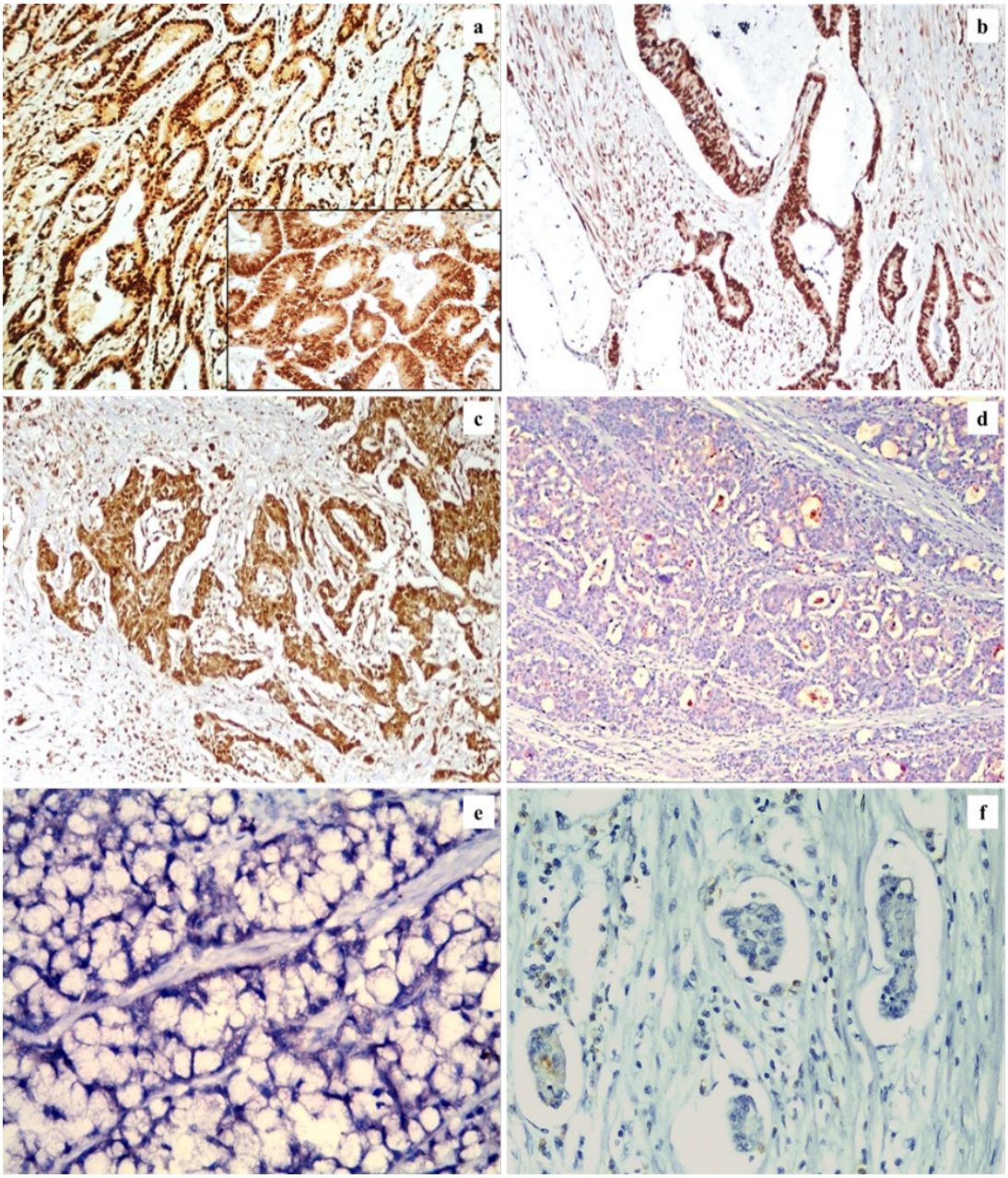

Fig. 2. Metastasis-Associated Gene 3 (MTA3) immunohistochemical nuclear expression in colorectal carcinoma specimens. (a) Adenocarcinoma, NOS, grade 2 showing high MTA3 expression (x100, inset x400), (b) Mucinous adenocarcinoma, grade 2 showing high MTA3 expression (x200), (c) Medullary carcinoma showing high MTA3 expression (x100), (d) Adenocarcinoma,

NOS, grade 3 showing low MTA3 expression (x100), (e) Signet ring carcinoma showing low MTA3 expression (x400), (f) Lymphovascular emboli showing low MTA3 expression (x400)

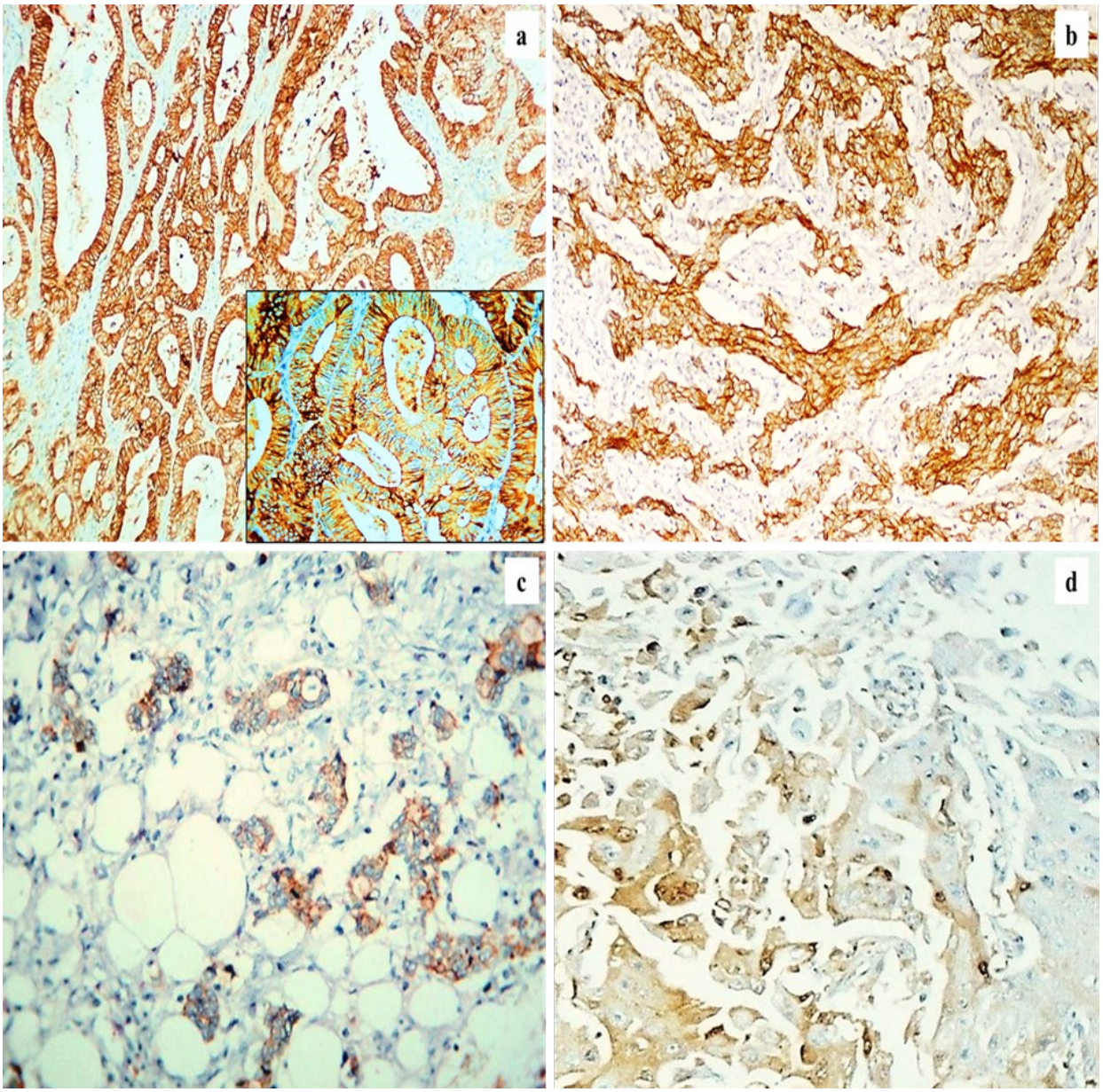

Fig. 3. E-cadherin immunohistochemical membranous expression in colorectal carcinoma specimens. (a) Adenocarcinoma, NOS, grade 2 showing preserved E-cadherin expression (x100, inset x400), (b) Medullary carcinoma showing preserved E-cadherin expression (x100), (c) Adenocarcinoma, NOS, grade 3 with serosal fat invasion showing reduced E-cadherin expression (x200), (d) Adenosquamous carcinoma showing reduced E-cadherin expression (x400)

Discussion

Colorectal carcinoma (CRC) is the most prevalent malignancy of the gastrointestinal tract. It is considered the 3rd most prevalent cancer and ranks 2nd in cancer death rates in the world (14).

Tumor metastasis is a complex process, involving tumor cell invasion into the microenvironment, entrance into the blood, migration, proliferation, and angiogenesis. About 90% of CRC-associated deaths are caused by metastatic disease rather than primary tumors (15).

MTA3 is one of the MTA family and could regulate cancer cell invasion and mediate epithelial-mesenchymal transition (16).

In this study, high MTA3 expression was related to low tumor grade, early tumor stage, absence of lymph node and distant metastasis, and absence of lymphovascular and perineural invasion.

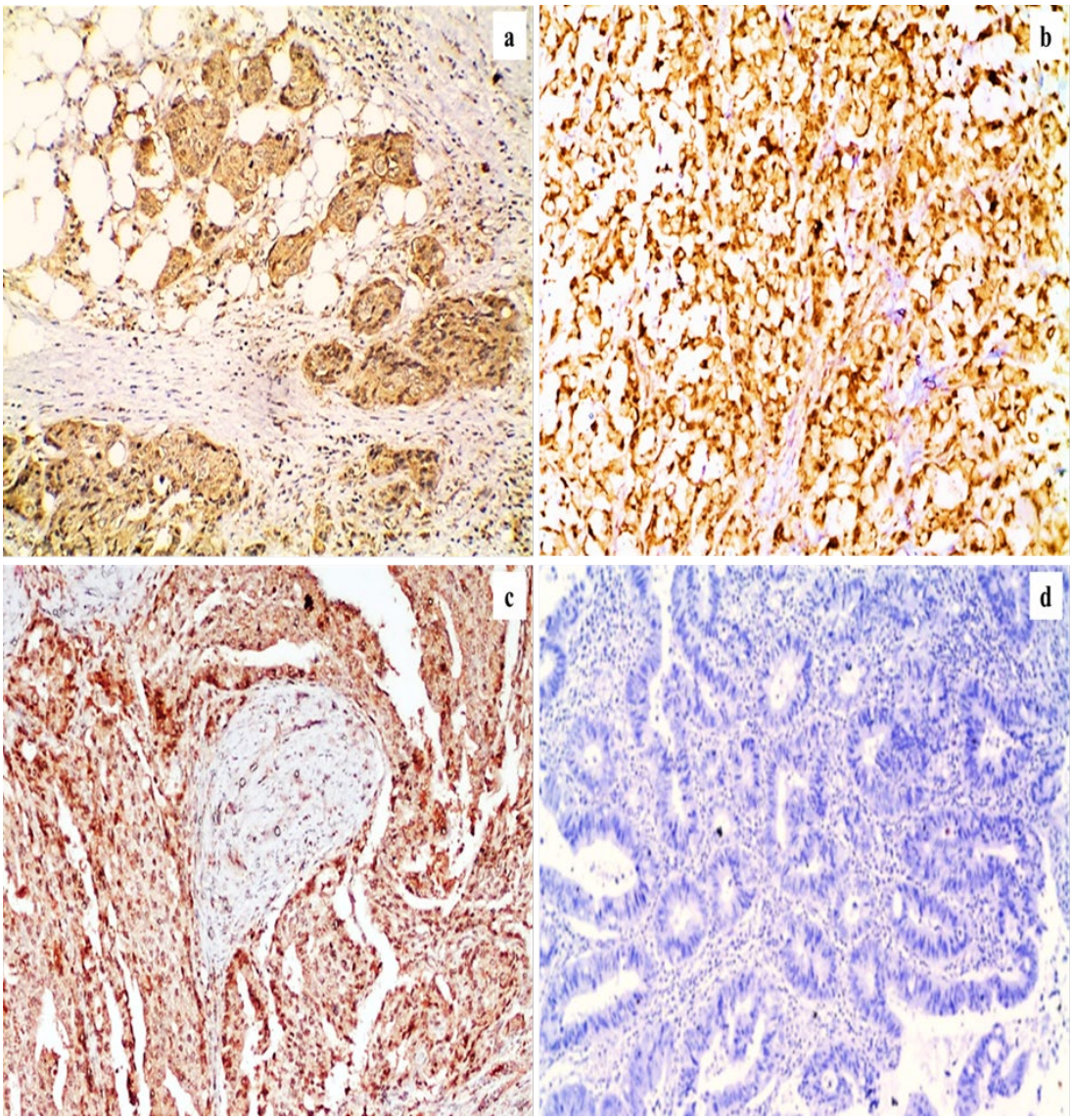

Fig. 4. Slug immunohistochemical cytoplasmic/perinuclear expression in colorectal carcinoma specimens. (a) Adenocarcinoma NOS, grade 3 with serosal fat invasion showing high Slug expression (x200), (b) Signet ring cell carcinoma showing high Slug expression (x200), (c) Perineural invasion in adenocarcinoma, NOS, grade 3 showing high Slug expression (x200), (d) Adenocarcinoma, NOS, grade 2 showing low Slug expression (x200)

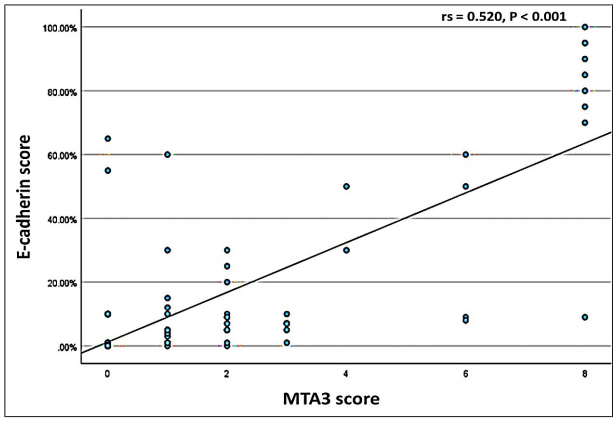

Fig. 5. Correlation between Metastasis-Associated Gene 3 (MTA3) and Epithelial- cadherin (E-cadherin) expression, rs; Spearman Correlation Test

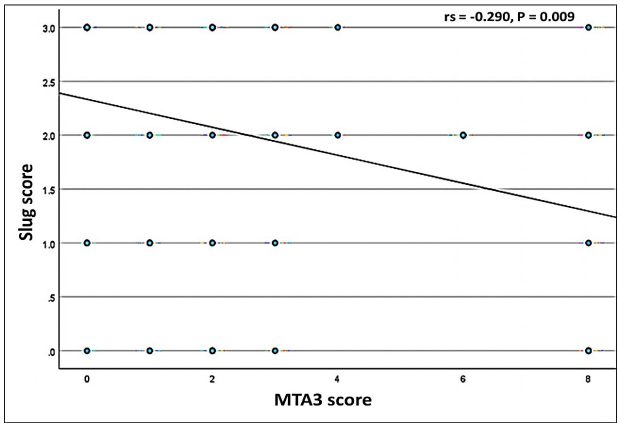

Fig. 6. Correlation between MTA3 and Slug expression, rs; Spearman Correlation Test

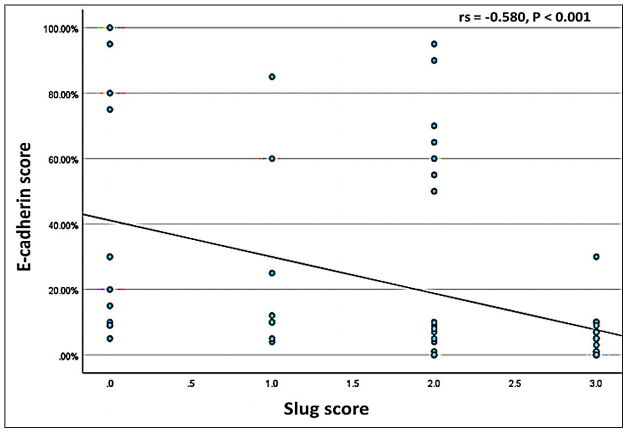

Fig. 7. Correlation between Slug and E–cadherin expression, rs; Spearman Correlation Test

This agreed with Huang et al. (16), who stated that positive MTA3 expression in CRC was significantly related to low tumor grade, absence of lymph node and distant metastasis, as well as early TNM stage. They concluded that MTA3 may act as a tumor suppressor gene in CRC. They also reported that MTA3 could inhibit Snail gene transcription and affect downstream gene expression, including E-cadherin; thus, MTA3 may play a role in regulating tumor cell invasion and metastasis.

Dong et al. (13) stated that there was a significant relation between high MTA3 expression and low grade and stage of gastroesophageal and endometrioid carcinoma.

In contrast to this study, Jiao et al. (17) stated that high MTA3 expression in CRC was associated with advanced TNM stage and high Ki-67 proliferation index. They explained that MTA3 could regulate cell cycle-related proteins, promote tumor cell invasion, and chemoresistance together with the upregulation of the Bcl2/Bax ratio, thus promoting CRC progression. These contradictory results could be explained by different immunohistochemical scoring methodology (they considered nuclear staining in < 10% of cells as "MTA3 low expression" and nuclear staining in ≥ 10% of cells as "MTA3 high expression"; also, they included molecular testing and studied other markers) and different patient populations.

Ma et al. (18) stated that MTA3 possesses both tumor-suppressing and tumor-promoting characteristics according to the tumor type. MTA3 could inhibit Wnt-target genes, which in turn suppress the Wnt/β-catenin pathway that regulates cell cycle progression; thus, MTA3 could inhibit tumor cell invasion and metastasis by dampening Wnt pro-growth metastatic signals. These findings could explain the possible tumor suppressor role of MTA3 in CRC demonstrated by our study.

As MTA3 could regulate many important signaling pathways, its targeting may act as a potential biomarker in the development of efficient cancer therapeutics (19). Liu et al. (20) studied the clinical significance of MTA3 in pan-cancer therapy and demonstrated the efficacy of MTA3 as a therapeutic target in hepatocellular carcinoma.

In the current study, preserved E-cadherin expression was associated with low tumor grade, early tumor stage, absence of perineural invasion, as well as absence of lymph node and distant metastasis.

This agreed with Christou et al. (21) and Rajkumar et al. (22), who stated that E-cadherin expression on the crypt and colonic surface epithelium basolateral aspects is responsible for mucosal integrity. Thus, reduced E-cadherin expression was indicative of poorly differentiated CRC, facilitating tumor cell invasion, metastasis, and high tumor stage. Also, Markowski et al. (23) stated that reduced E-cadherin expression could be an indicator of high cancer staging and hence progression.

Cadherin switching is a crucial process during EMT, including decreased E-cadherin and increased N-cadherin (or P-cadherin) expression, allowing cellular detachment and cancer metastasis (24).

Studies have demonstrated controversial results regarding preserved E-cadherin expression in high-stage tumors. Jurčić et al. (25) reported preserved E-cadherin expression in liver metastases. A possible explanation was that tumor cells may regain epithelial features at metastatic sites. The difference between their study and ours is that we assessed only membranous E-cadherin expression versus both cytoplasmic and membranous expression in their study. Also, they studied E-cadherin expression in both primary tumors as well as metastatic sites.

Loh et al. (26) reported preserved E-cadherin expression in various cancers, such as glioblastoma, ovarian, and prostatic carcinomas, suggesting that E-cadherin could promote metastasis in certain tumors instead of tumor suppression. The dual role of E-cadherin is attributed to the presence of 2 forms of E-cadherin: soluble and membrane-tethered E-cadherin.

In the current work, high Slug expression was related to histopathological types such as mucinous adenocarcinoma and signet ring cell carcinoma, high tumor grade, advanced stage, as well as the presence of lymph node and distant metastasis.

This agreed with Qian et al. (27), who reported that Slug expression was correlated with tumor progression in CRC, as well as with poor patient survival. They also stated that Slug expression could be an independent prognostic factor for CRC metastasis and a target for gene therapy.

Toiyama et al. (28) also stated that high Slug expression was significantly related to high T stage and liver metastasis in CRC. They also suggested that Slug expression could predict lymph node metastasis. Also, Findlay et al. (29) stated that Slug overexpression was a poor prognostic indicator, as it could decrease the sensitivity of CRC to radiotherapy.

In this study, the relation as well as the correlation between MTA3 expression and both E-cadherin and Slug expression were significant. Low MTA3 expression was related to reduced E-cadherin and high Slug expression.

These results matched those of Handaya et al. (30), who stated that SNAI1 and Slug were significantly upregulated in metastatic CRC, while MTA3 was downregulated in such cases.

Du et al. (4) concluded that MTA3 could function as a transcriptional regulator, particularly in the regulation of metastasis, via forming independent NuRD complexes that recruit histone deacetylases to various target genes, thus playing a crucial role in cancer cell progression. They stated that MTA3 could be associated with cancer-related factors such as Slug, E-cadherin, and signal transducer and activator of transcription.

Thus, loss of MTA3 expression could regulate EMT via loss of E-cadherin expression, which leads to loss of cell-to-cell adhesion and increased expression of Slug, promoting tumor progression, invasiveness, and metastasis.

This study is limited by its retrospective design, single-institution cohort, and lack of follow-up data and survival analysis. Small sample size and the lack of molecular and microsatellite (mismatch repair [MMR]) testing are also considered limitations of this work. Inadequate data specific to rectal carcinomas and the inability to separate them from colonic carcinoma should also be considered.

Conclusion

Unraveling the role of MTA3 in CRC, whether as a tumor suppressor or promoter, as well as its relation to EMT regulation, is still challenging. In this study, MTA3 is expressed in CRC and is related to favorable clinicopathological parameters such as low tumor grade, early tumor stage, absence of lymph node and distant metastasis, and absence of lymphovascular and perineural invasion.

Reduced E-cadherin and high Slug expression are related to poor clinicopathological parameters in CRC, such as high tumor grade, advanced stage, as well as the presence of lymph node and distant metastasis. MTA3 could play a role in regulating the expression of EMT markers (E-cadherin and Slug), thus may play a possible role in controlling tumor cell progression.

Therefore, we recommend further immunohistochemical and molecular analysis of MTA3 together with E-cadherin and Slug in CRC on large-scale studies to standardize the role of MTA3 as either tumor suppressor or promoter. To predict the prognostic role of MTA3, E-cadherin, and Slug in CRC patients, relation to patient overall and progression-free survival is also recommended.

Acknowledgments

None.

Authors' Contributors

Aya Salah Ali Saad Rezk: Writing – original draft, Project administration, Methodology, Investigation, Resources. Mohamed Alaa Mokhtar: Writing – review and editing, Supervision. Mohamed Ali Alrashidy: Writing – review and editing, Supervision. Assia Mahfouz Abd El-Raoof: Writing – review and editing,

Supervision. Nehal Abd El-Ghaffar Heabah: Writing – review and editing, Project administration, Visualization, Validation, Methodology, Investigation, Supervision.

Data Availability

There is no additional data separate from available in cited references.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics Approval

Approval from the research ethics committee, Faculty of Medicine, Tanta University, Egypt with approval code (36068/11/22) was taken antecedent to conducting the study.

Consent to Participate:

This retrospective study was conducted on archived formalin-fixed paraffin-embedded tissue samples. Approval was obtained from the Research Ethics Committee, Faculty of Medicine, Tanta University. The requirement for informed consent was waived due to the retrospective nature of the study.

Conflict of Interest

The authors declared no conflict of interest.

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