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Significance of Transmembrane 9 Superfamily 4 (TM9SF4) Immunohistochemical Expression and Nuclear Morphometry in Premalignant Colonic Lesions and Colorectal Carcinoma

Naglaa H. Shalan, Norhan M. Morgan, Rasha M. Abdrabh, Abd El Latif M. Elbalshy, Rana M. Abdalla

Anatomic Pathology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to: Norhan M. Morgan, Anatomic Pathology Department, Faculty of Medicine Benha University, Egypt.

Email: morgannorhan49@gmail.com

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Abstract:

Background: Although different markers are used as diagnostic methods for colorectal carcinoma (CRC) such as; carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), low sensitivity and low specificity of these markers revealed the importance of searching for other markers. Transmembrane 9 Superfamily 4 (TM9SF4) is autophagic protein involved in multiple stages of tumorigenesis and development. Nuclear morphometry is a method for digital histological analysis which has been subjected to significant technological advances in the last years. **Aim:** To evaluate the role of Transmembrane 9 Superfamily 4 (TM9SF4) and nuclear morphometry in premalignant colonic lesions and colorectal carcinoma. **Material and methods:** This is a retrospective study carried on 11 ulcerative colitis cases, 16 colonic adenoma cases and 23 colorectal adenocarcinoma cases with 6 normal colon specimens as a control. Clinicopathological characteristics of examined cases were correlated with IHC of TM9SF4 and nuclear morphometry. **Results:** There is a significant statistical correlation between TM9SF4 scoring and different histopathological types of studied cases, lymphovascular invasion, lymph node metastasis, distant metastasis and stage of CRC. There is a significant statistical correlation between nuclear morphometry and different histopathological types of studied cases. Also, there is a significant statistical correlation between area and perimeter nuclear morphometry and perineural invasion and grade of colorectal adenocarcinoma respectively. **Conclusion:** TM9SF4 may have role in tumorigenesis and development of CRC and also it has a prognostic role in CRC. Nuclear morphometry may have a diagnostic role in CRC.

Keywords: Colorectal adenocarcinoma; Transmembrane 9 Superfamily 4; nuclear morphometry.

Introduction

Colorectal cancer (CRC) is an important health problem being the third most common and second most fatal cancer worldwide [1]. About 9.4% of cancer-related deaths were attributed to CRC in 2020 [2].

In Egypt, CRC is the seventh most common cancer and the third most common male neoplasm and fifth most common female neoplasm [3].

Molecular pathways for development of colorectal cancer includes; Adenoma-carcinoma sequence (traditional pathway), serrated pathway, alternative pathway and De novo pathway [4].

Although different markers are used as diagnostic methods for CRC such as; carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), their low sensitivity and specificity necessitates the importance of searching for other markers [5].

TM9SF4 is the fourth member TM9SF family encoded by TM9SF4 gene. TM9SF is a well-defined family of proteins characterized by the presence of a large variable extracellular domain and nine putative transmembrane domains [6].

Despite its action the role of TM9SF4 protein expression in colorectal carcinoma still unclear, thus a trial to evaluate role of TM9SF4 in in colorectal carcinoma becomes a necessity.

Nuclear morphometry is the quantitative analysis of morphological changes in the nucleus which is applied by the assessment of different nuclear parameters such as nuclear area, nuclear perimeter, nuclear length, nuclear width and nuclear roundness [7].

Aim of the work: The study aims at detecting the role of nuclear morphometry in colorectal cancer and premalignant lesions.

Material and methods

Study group:

This is a retrospective study included 50 different cases of colorectal adenocarcinoma, colon polyps and ulcerative colitis designated as 23 colorectal cancer cases (14 colectomy cases and 9 endoscopic cases), 16 colonic polyp cases, 11 ulcerative colitis cases with 6 specimens of normal colonic tissue as a control processed during the years 2017-2022. Sections of normal colon are used as normal control and gastric carcinoma tissue were taken as a positive control for TM9SF4 immunohistochemical expression.

The material included archival formalin fixed paraffin embedded blocks as well as Hematoxylin and Eosin (H&E) stained slides for review. The blocks were collected from Department of Pathology and Early Cancer Detection Unit; Faculty of Medicine, Benha University, Egypt. Clinicopathological data were collected from the files of patients. Being a retrospective study, a written informed consent was not needed.

Inclusion criteria: Cases with available clinicopathological data regarding age, sex, tumor size, tumor site, lymphovascular invasion, perineural invasion, primary tumor(T), grade, lymph node status, distant metastasis and stage.

Exclusion criteria: Cases with no available paraffin blocks or clinicopathological data were excluded from the current study.

The Ethics Committee of Faculty of Medicine, Benha University, Egypt approved this study code (MSC 25/1/2022).

Histopathological studies:

Formalin fixed /Paraffin embedded blocks were cut at 5 μm thickness and stained using hematoxylin and eosin stain. The cases were re-evaluated for diagnosis and graded into well differentiated (I), moderately differentiated (II), and poorly differentiated (III) tumors [8]. Lymph node status was evaluated and TNM staging system was applied to the cases according to AJCC, 8th edition [9].

TM9SF4 immunohistochemical study:

For immunohistochemical staining, two positive slides were prepared:

Slides were immunostained according to manufacturer's instructions with TM9SF4 rabbit polyclonal antibody (Hansa BioMed OU, Tallin Estonia) at a dilution of 1:50, at 4°C overnight. Immunodetection was carried out using a standard labeled streptavidin-biotin system (Genemed, CA 94080, USA, South San Francisco). Antigen retrieval was done by using 3% hydrogen peroxide in 30% methanol for 15 minutes in the microwave. The chromogen diaminobenzene (DAB, Envision TM Flex /HRP-Dako, REF K 8000) used was freshly prepared. The counter satin was Mayer's hematoxylin. Normal colon tissue and gastric carcinoma tissue used as an external positive control [(5).] For negative control, primary antibody was omitted (Phosphate- buffered Saline) [(10).]

Interpretation of TM9SF4 expression:

Positivity was considered as brownish homogenous cytoplasmic staining of tumor cells [(5).] The immunohistochemical scores were obtained by light microscopy as the staining intensity (scored from 0–3) multiplied by the percentage area of positive immunostaining within the visual field (the percentage of positive cells within 5 high power fields in hot areas) (scored from 0–3). The intensity of TM9SF4 protein expression was scored as: 0 (no staining); 1 (weak staining); 2 (moderate staining); or 3 (strong staining). The percentage area of positive immunostaining was scored as: 0 (0%); 1(1-10%); 2(11-50%); 3(≥51%) [(5).]

Nuclear morphometry study:

The slide to be examined was placed on the stage of the microscope. The light source was set to the required level. Successful adjustment of illumination was checked for the video monitor, The morphometric analysis was carried out on hematoxylin and eosin-stained slides to measure the nuclear parameters including area, length, width, perimeter, and nuclear roundness, Slides were digitalized using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core I7® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for particle analysis and counting, 13 slides from colorectal cancer, 9 slides from adenoma, 6 slides from ulcerative colitis and 3 slides from normal colon were used, 5 random field from each slide were analyzed.

Statistical analysis:

Statistical analysis was performed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Descriptive statistics include Mean, standard deviation (± SD), median, standard error (±SE). Range was used for description of numerical data while frequency and percentage were used for description of non-numerical data. P value is considered significant if <0.05 at confidence interval 95%.

Results:

Clinicopathological results (figure 1):

The Age distribution of the studied cases ranged from 18 to 72 years with 28 (50%) were < 45 years while 28 (50%) were ≥45 years with the mean age 44.93 ± 13.55. The gender distribution of the studied cases was 34 (60.7%) male while, 22 (39.3%) were female. Type of studied adenoma cases was classified as 16 cases tubular and 6 cases villous with degree of dysplasia was 11cases were of low-grade dysplasia while 5 cases of high-grade dysplasia. Tumor size of studied colorectal adenocarcinoma colectomy cases ranged from 2 to 12 cm. The grade distribution in colorectal adenocarcinoma cases was 3 cases grade I, 16 cases grade II and cases grade III with significant statistical correlation wuth both depth of invasion and stage (P value= 0.05, 0.040 respectively). Perineural invasion in colorectal adenocarcinoma colectomy cases were

found in 4 cases. TNM stage in colorectal adenocarcinoma colectomy cases wre classified as one case stage I, 5 cases stage IIA, 5 cases stage III while 3 cases stage IVA (Table 1).

Table 1. Clinicopathological data of studied cases of colorectal adenocarcinoma.

Clinicopathological data		**Colorectal adenocarcinoma (Colectomy and endoscopic) n=23**
Site		n (%)
Right		18 (78.2%)
Left		5 (21.8%)
Grade	**I**	3(13%)
	II	16(69.5%)
	III	4(17.5%)
Colectomy cases n=14		
		n (%)
Size	<6.5	7(50%)
	≥6.5	7(50%)
Mean ± SE.		5.86 ± 0.95
Median		5.0
Min. – Max.		2.0 – 12.0
LV invasion	+ve	5 (35.7%)
	-ve	9 (64.3%)
Perineural invasion	+ve	4(28.5%)
	-ve	10 (71.5%)
Depth of invasion (T)	T2	2(14.2%)
	T3	11(78.6%)
	T4a	1(7.2%)
Lymph node metastasis(N)	N0	6 (42.8%)
	N1	5 (35.7%)
	N2b	3 (21.5%)
Distant metastasis (M)	M0	11 (28.6%)
	M1a	3 (71.4%)
TNM stage	I	1 (7.2%)
	IIA	5 (35.7%)
	III	5 (35.7%)
	IVA	3 (21.4%)

n=number, SD=standard deviation, Min=minimum, Max=maximum.

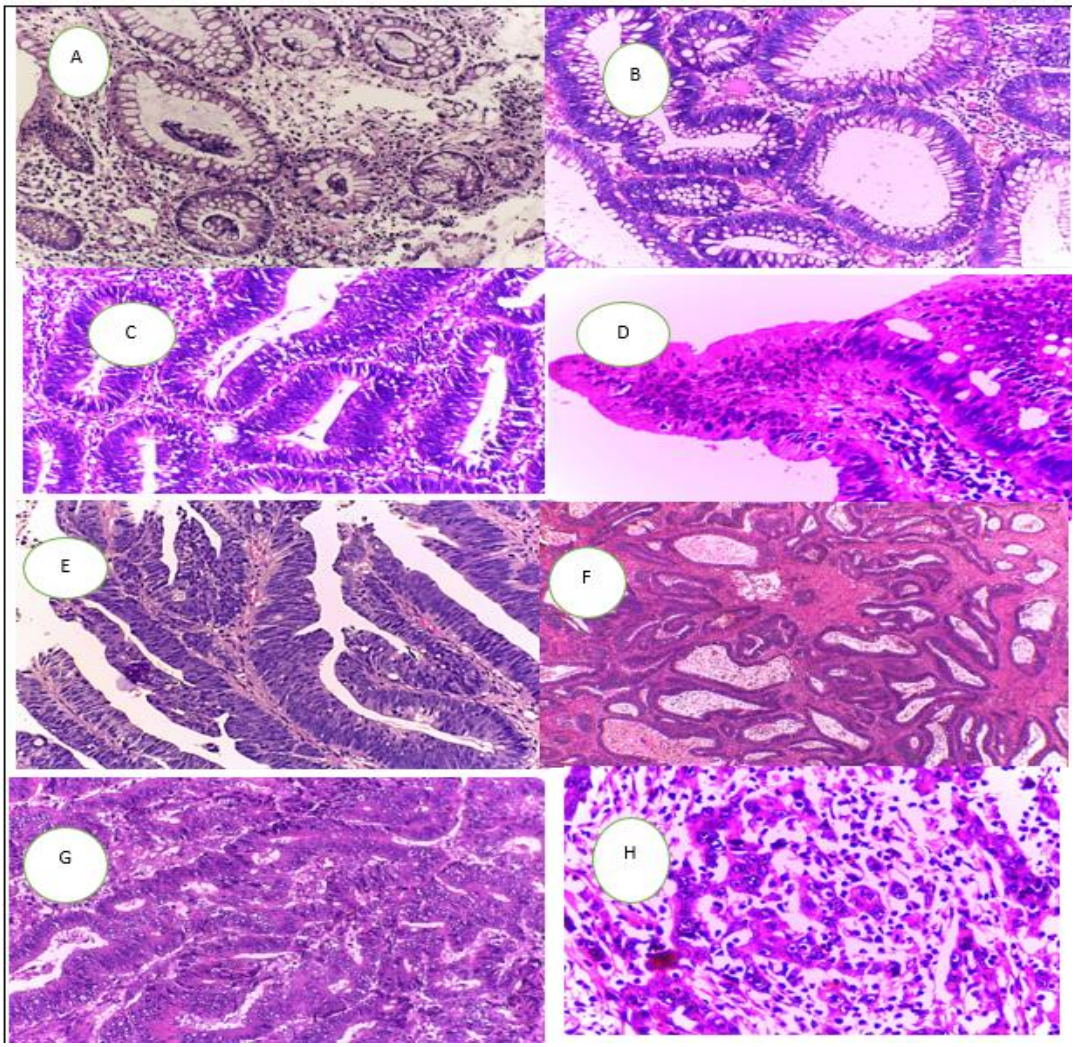

Figure 1:

A. A case of Ulcerative colitis showing cryptitis (orange arrow) and crypt abscess (black arrow) (H&Ex200)
B. A case of Tubular adenoma, low grade dysplasia in the form of pseudo stratification with crowded hyperchromatic nuclei occupying lower 1/3 of epithelial thickness (H&Ex200)
C. A case of Tubular adenoma, high grade dysplasia in the form of pseudo stratification with loss of nuclear polarity, nuclear pleomorphism and hyperchromasia occupying full epithelial thickness (H&Ex200)
D. A case of villous adenoma, low grade dysplasia in the form of pseudo stratification with crowded hyperchromatic nuclei occupying lower 1/3 of epithelial thickness (H&Ex400)
E. A case of villous adenoma, high grade dysplasia in the form of pseudo stratification with loss of nuclear polarity, nuclear pleomorphism and hyperchromasia occupying full epithelial thickness (H&Ex200)
F. A case of well differentiated adenocarcinoma (grade I) showing variable sized and shaped malignant glands occupying >95% of lesion (H&Ex40)
G. A case of moderately differentiated adenocarcinoma (grade II) showing sheets and fused variable sized and shaped malignant glands occupying 50-95% of lesion (H&Ex200)
H. A case of poorly differentiated adenocarcinoma (grade III) showing sheets and scattered variable sized and shaped malignant glands occupying <50% of lesion (H&Ex400)

Immunohistochemical results (figure 2): Positivity was considered as brownish homogenous cytoplasmic staining of tumor cells [(5)]. Score 1 of TM9SF4 was found in 66.8%, 18.1% and 43.7% of normal colonic tissue (control), ulcerative colitis and adenoma cases respectively. Score 2 of TM9SF4 was found in 16.6%, 27.2%, 18.7 and 8.6% of normal colon, ulcerative colitis adenoma and CRC cases

respectively. Score 3 of TM9SF4 was found in 54.7%, 37.6 and 91.4% of ulcerative colitis adenoma and CRC cases respectively. (Table 2)

A statistically significant correlation was found between TM9SF4 scoring and histopathological types of studied cases (P =<.001) (table 2).

A statistically significant correlation was found between TM9SF4 scoring and stage, lymphovascular invasion, LN metastasis and distant metastasis of studied colorectal adenocarcinoma colectomy cases (P value= 0.001, 0.001, 0.001, 0.005 respectively) (Table 3). While no statistically significant correlation was found between TM9SF4 scoring and site, size, grade and depth of invasion of studied colorectal cancer colectomy cases and also there is no statistically significant correlation was found between TM9SF4 scoring and clinicopathological variables of ulcerative colitis and adenoma studied cases.

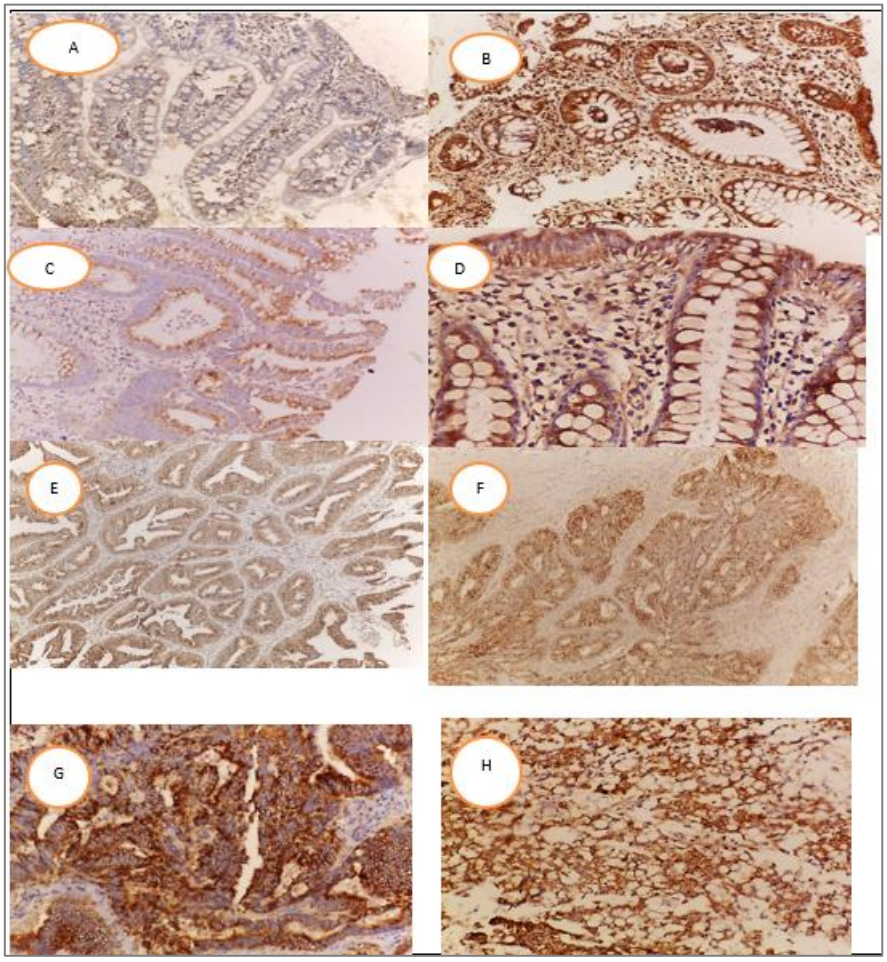

Figure 2 :

A. A case of ulcerative colitis showing score 2 IHC cytoplasmic expression of TM9SF4 (ABCx200)
B. A case of ulcerative colitis showing score 3 IHC cytoplasmic expression of TM9SF4 (ABCx200)
C. A case of tubular adenoma showing score 2 IHC cytoplasmic expression of TM9SF4 (ABCx200)
D. A case of tubular adenoma showing score 3 IHC cytoplasmic expression of TM9SF4 (ABCx400)
E. A case of adenocarcinoma, grade I showing score 2 IHC cytoplasmic expression of TM9SF4 (ABCx100)
F. A case of adenocarcinoma, grade II showing score 2 IHC cytoplasmic expression of TM9SF4 (ABCx100)
G. A case of adenocarcinoma, grade II showing score 3 IHC cytoplasmic expression of TM9SF4 (ABCx200)
H. A case of adenocarcinoma, grade III showing score 3 IHC cytoplasmic expression of TM9SF4 (ABCx200).

Table 2. Comparison between different studied groups regarding TM9SF4.

TM9SF4	Colorectal adenocarcinoma n=23	Adenoma n = 16	Ulcerative colitis n = 11	Normal colon n = 6	Test	P
Expression						
Negative	0 (0%)	0(0%)	0 (0%)	1 (16.6%)	x^2=	MC
Cytoplasmic	23 (100%)	16 (100%)	11 (100%)	5 (83.4%)	7.205	0.661
Scoring						
Score						<0.001*
0	0 (0%)	0 (0%)	0 (0%)	1 (16.6%)		
1	0 (0%)	7 (43.7)	2 (18.1%)	4 (66.8%)		
2	2 (8.6%)	3 (18.7)	3 (27.2%)	1 (16.6%)		
3	21 (91.4%)	6 (37.6%)	6 (54.7%)	0 (0%)		
Mean ± SE.	2.93 ± 0.07	1.88 ± 0.26	2.36 ± 0.24	1.33 ± 0.21	H=21.354	

SE. Standard error, Min.: Minimum, Max.: Maximum, H: Kruskal Wallis, x^2: Chi-Square,
FE: Fisher-Exact, MC: Monte-Carlo, P: Comparing between the different studied groups.
*: Significant when p value < 0.05.

Table 3. Relation of TM9SF4 scoring with clinicopathological data of colorectal adenocarcinoma colectomy cases.

colorectal adenocarcinoma colectomy cases		Score 2 n=1	Score 3 n=13	test	P
Age (years)		mean±SD (63)	(51.2±11.6)	0.982	0.346
		n (%)	**n (%)**		
Gender	Male	0 (0.00%)	9 (69.20%)	1.938	0.164
	Female	1 (100.00%)	4 (30.80%)		
Grade	I	0 (0.00%)	2 (15.40%)	0.808	0.668
	II	1 (100.00%)	7 (53.80%)		
	III	0 (0.00%)	4 (30.80%)		
Stage	I	1 (100.00%)	0 (0.00%)	1.437	0.001
	IIA	0 (0.00%)	5 (38.50%)		
	III	0 (0.00%)	5 (38.50%)		
	IVA	0 (0.00%)	3 (23%)		
Site	Right	0 (0.00%)	10 (76.90%)	2.692	0.286
	Left	1 (100.00%)	3 (23.10%)		
LV	Negative	1 (100.00%)	8 (61.5%)	0.117	0.001
	Positive	0 (0.00%)	5 (38.4%)		
PN	Negative	0 (0.00%)	10 (76.90%)	2.692	0.286
	Positive	1 (100.00%)	3 (23.10%)		
LN	N0	1 (100.00%)	5 (38.4%)	0.117	0.001
	N1	0 (0.00%)	5 (38.4%)		
	N2A	0 (0.00%)	3 (23.2%)		
M	M0	1 (100.00%)	10 (76.9%)	0.525	0.005
	M1a	0 (0.00%)	3 (23.1%)		
Size	<6.5 cm	1 (100%)	8 (61.5%)	0.598	1
	≥6.5	0 (0.00%)	5 (38.5%)		
Depth of invasion (T)	T2	0 (0.00%)	2 (15.4%)	0.294	0.778
	T3	1 (100%)	10 (76.9%)		

n=number, SD=standard deviation, LV=lymphovascular invasion, PN=perineural invasion, LN= lymph node, M= metastasis.

Nuclear morphometry results (figure 3):
A statistically significant correlation was found between nuclear morphometric parameters and histopathological types of studied cases (P =<.001) (table 4).
Besides, a statistically significant correlation was found between nuclear area morphometry and prineural invasion in colorectal adenocarcinoma cases (P value=0.008)
Also, a statistically significant correlation was found between nuclear perimeter and grade in colorectal adenocarcinoma cases (P value=0.002)
Also, a statistically significant correlation was found between nuclear roundness morphometry with age in colorectal adenocarcinoma cases (P value=0.012).

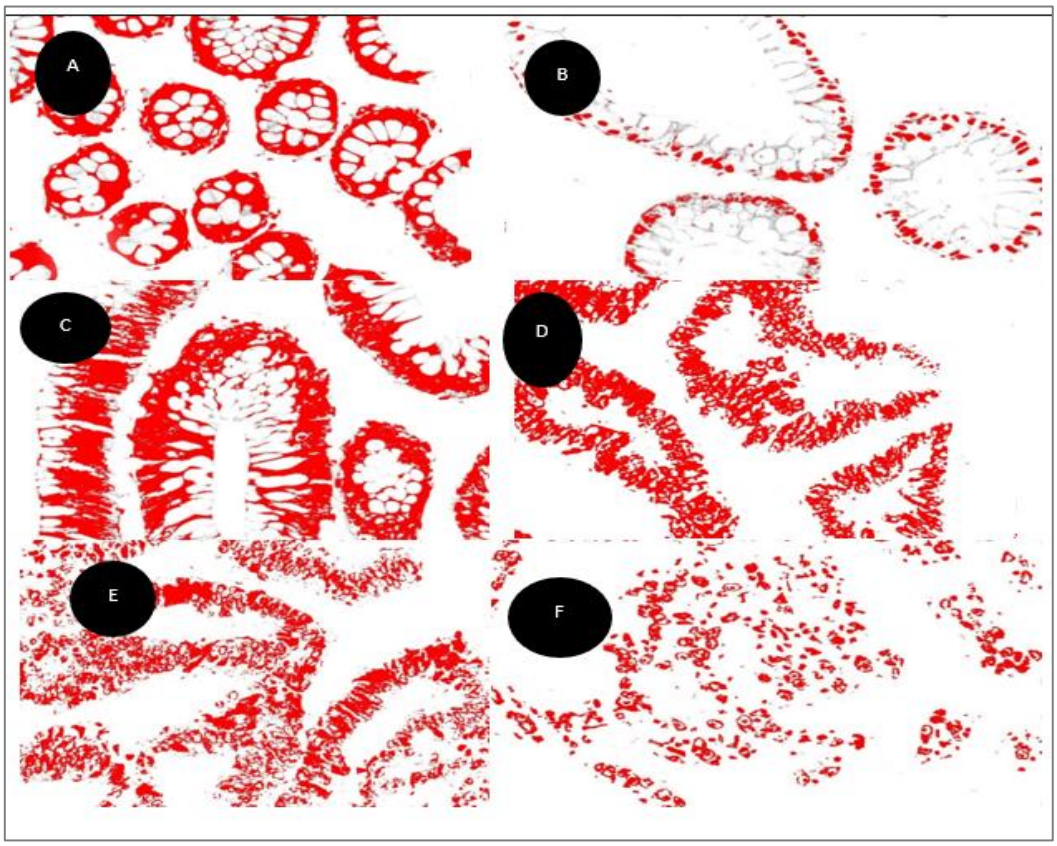

Figure 3 :
A. A case of normal colon nuclear morphometry showing
Mean area= 1262 pixel Mean perimeter= 183 pixel Mean length=44 pixel
Mean width= 27 pixel Mean roundness=0.9pixel
B. A case of ulcerative colitis nuclear morphometry showing
Mean area= 1349 pixel Mean perimeter= 211 pixel Mean length=52 pixel
Mean width= 30 pixel Mean roundness=0.8pixel
C. A case of tubular adenoma nuclear morphometry showing
Mean area= 2421 pixel Mean perimeter= 269 pixel Mean length=107 pixel
Mean width= 40 pixel Mean roundness=0.7pixel
D. A case of colorectal adenocarcinoma grade I nuclear morphometry showing
Mean area= 3330 pixel Mean perimeter= 378 pixel Mean length=129 pixel
Mean width= 47 pixel Mean roundness=0.5pixel
E. A case of colorectal adenocarcinoma grade II nuclear morphometry showing
Mean area= 3330 pixel Mean perimeter= 380 pixel Mean length=131 pixel
Mean width= 49 pixel Mean roundness=0.3pixel
F. A case of colorectal adenocarcinoma grade III nuclear morphometry showing
Mean area= 3333 pixel Mean perimeter= 384 pixel Mean length=132 pixel
Mean width= 52 pixel Mean roundness=0.2pixel

Table 4. Nuclear morphometry among the different studied groups.

Nuclear morphometry	Colorectal cancer n = 23	Adenoma n = 16	Ulcerative colitis n = 11	Normal colon n = 6	Test	P
Nuclear area						
Mean ± SD.	2439.6± 3.73	1860.3± 2.66	1684.8± 1.16	1592.7± 1.05	F=147658	<0.001*
Perimeter						
Mean ± SD.	250.7 ± 7.51	227.96± 1.10	173.3 ± 0.95	163.8 ± 0.61	F=720.8	<0.001*
Length						
Mean ± SD.	106.8 ± 0.90	89.10 ± 2.54	44.18 ± 0.71	36.20 ± 0.40	F=3550.9	<0.001*
Width						
Mean ± SD.	42.28 ± 0.66	29.86 ± 0.29	26.55 ± 1.01	24.14 ± 0.35	F=944.9	<0.001*
Roundness						
Mean ± SD.	0.36 ± 0.00	0.37 ± 0.01	0.47 ± 0.01	0.63 ± 0.04	F=184.4	<0.001*

SD. Standard deviation, Min.: Minimum, Max.: Maximum, F: One Way ANOVA
P: Comparing between the different studied groups.
*: Significant when p value < 0.05.

Discussion:

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second most common cause of cancer-related death in both men and women [(11).]

In Egypt, the estimated rate of CRC is 6.5 % of all malignant tumors, being the third most common male neoplasm and fifth most common female neoplasm with 14.0 % of all colonoscopies revealing the presence of the disease [(12).]

The occurrence of colorectal cancer is associated with non-modifiable risk factors, including age and hereditary factors, as well as with modifiable environmental and lifestyle factors [(13).]

Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are among the best available IHC markers for digestive cancers, including CRC, however, low sensitivity and low specificity of these markers affects their use as biomarkers for early diagnosis so, it is better to find other markers with more specificity and sensitivity [(5).]

Transmembrane 9 superfamily 4 (TM9SF4) is the fourth member TM9SF family encoded by TM9SF4 gene. TM9SF is a well-defined family of proteins that activates Vacuolar ATPase which is a proton pump involved in the tumor pH gradient alterations resulting in extracellular acidosis and cytosol alkalization, this function appeared to be associated with its role in drug resistance, progression and invasiveness of cancer cells [(14)] as in breast cancer and acute myeloid leukemias.

In the present study, there was a significant statistical correlation between scoring of TM9SF4 and different studied cases as 54.7% of studied ulcerative colitis cases, 37.6% of studied adenoma cases and 91.3% of studied CRC cases showed score 3 which agreed with those who reported that most cases of normal colon cases showed score 1 while most CRC cases showed score3 [(5)] suggesting role of TM9SF4 in tumor development, which can be explained by the relation between TM9SF4 and exosomes (natural lipidic extra cellular nanovesicles produced and released by virtually all cell types) [(15).]

Exosomes participate in the progression of cancer development and metastasis by transferring bioactive molecules between cancer and various cells in local and distant microenvironments and such intercellular cross-talk results in changes in multiple cellular and biological functions in cells [(16).] This action is increased with acidic PH which is achieved by TM9SF4 and this can explain the role of TM9SF4 in progression of neoplastic pathway [(17).]

Regarding TM9SF4 expression in ulcerative colitis, most cases (54.7%) were score 3 which can be explained by mild

activity of this cases concerning the relation between TM9SF4 level and degree of activity, as TM9SF4 deficiency increases endoplasmic reticulum (ER) stress, promotes inflammation and impair intestinal epithelial barrier to aggravate IBD while increased TM9SF4 level is associated with low level of activity in IBD so, high TM9SF4 level is associated with mild activity and vice versa [(14).]

Our study revealed that there was no significant statistical correlation between TM9SF4 scoring and clinico-pathological variables in studied ulcerative colitis, adenoma cases. This can be explained by difference in number of cases.

Regarding CRC cases there was a highly significant statistical correlation between TM9SF4 scoring and lymphovascular invasion, lymph node metastasis, distant metastasis and stage. This agreed with those who found that increased expression of TM9SF4 is associated with tumor invasion and metastasis [(5)] which can be explained by the role of TM9SF4 in enhancing tumor invasion through its interaction with the V-ATPase, a proton pump highly expressed in CRC cancer results in activating V-ATPase that plays a significant role in tumor invasion and metastasis via activating different types of proteases including cathepsins, metalloproteases, and gelatinases which degrade the extracellular matrix [(6).] While there was no significant statistical correlation between TM9SF4 scoring and site, size, grade, perineural invasion and depth of invasion (T).

Nuclear morphometry is the quantitative analysis of morphological changes in the nucleus which is applied by the assessment of different nuclear parameters [(18).] In this study all studied cases were analyzed for nuclear morphometric parameters including nuclear area, perimeter, length, width and roundness.

As regard nuclear morphometry, it was found that there is a significant statistical correlation (P<0.001) between nuclear area and different studied groups as it was 1592.7± 1.05 pixel, 1684.8 ± 1.16 pixel, 1860.3 ± 2.66 pixel and 2439.6 ± 3.73 pixel in normal colon, ulcerative colitis, adenoma and CRC groups respectively. This was close to those who found significant statistical correlation [(19-23).] However, those results disagreed with those who found no significant statistical correlation [(24, 25).]

Also, there is a significant statistical correlation (P<0.001) between nuclear perimeter and different studied groups as it was 163.8 ± 0.61 pixel, 173.3 ± 0.95 pixel, 227.96 ± 1.10 pixel and 250.7 ± 7.51 pixel in normal colon, ulcerative colitis, adenoma and CRC groups respectively. This was close to those who found significant statistical correlation [(22,23,26,27).] This disagreed with those who found no significant statistical correlation [(25).]

Also, there is a significant statistical correlation (P<0.001) between nuclear length and different studied groups as it was 36.20 ± 0.40 pixel, 44.18 ± 0.71 pixel, 89.10 ± 2.54 pixel and 106.8 ± 0.90 pixel in normal colon, ulcerative colitis, adenoma and CRC groups respectively. This was close to those who found significant statistical correlation [(22, 23).] While disagreed with those who found no significant statistical correlation [(25).]

The difference in nuclear area, perimeter and length can be explained by different molecular events of cancer progression as nuclear parameters are directly related to molecular events of cancer progression and are often specific for individual cancer types [(28)] and also can be attributed to different statistical analysis method.

Concerning mean nuclear width, it significantly correlates (P<0.001) with different studied groups as it was 24.14 ± 0.35 pixel, 26.55 ± 1.01 pixel, 29.86 ± 0.29 pixel and 42.28 ± 0.66 pixel in normal colon, ulcerative colitis, adenoma and CRC groups respectively. This is in agreement with those who found significant statistical correlation [(29).] This was in disagreement with those who found no significant statistical correlation [(23, 25).]

Also, there is a significant statistical correlation (P<0.001) between nuclear roundness and different studied groups as the mean was 0.63 ± 0.04 pixel, 0.47 ± 0.01 pixel, 0.37 ± 0.01 pixel and 0.36 ± 0.00 pixel in normal colon, ulcerative colitis, adenoma and CRC groups respectively. This goes with those who found significant statistical correlation [29] While was in disagreement with those who found no significant statistical correlation [23 ,25].

The difference in results in both nuclear width and roundness can be explained by that nuclear dysplasia occurring in adenomatous polyps are precedent of the nuclear changes in CRC so nuclear measurements are closer to malignant lesions and show no significant correlation when compared together.

As regarding correlation between parameters of nuclear morphometry and clinicopathological data of different studied groups, there is no statistically significant correlation between nuclear parameters and clinicopathological data of ulcerative colitis and adenoma cases. In contrast to studies carried out by [30] who found statistically significant correlation between nuclear area and degree of dysplasia of adenoma (P<0.05). The difference in results can be explained by different statistical analysis method.

However, there is statistically significant correlation between nuclear area morphometry and perineural invasion in colorectal adenocarcinoma cases (P value=0.008), while there is no statistically significant correlation with other clinicopathological data. This is in contrast with [25] who found statistically significant correlation between nuclear area and grade of CRC (p value=0.0023) and [24] who found statistically significant correlation between nuclear area and vascular invasion and lymphatic invasion of CRC (p value=0.001). The difference in results can be explained by mean nuclear area of DNA aneuploid tumors was larger and more associated with invasion than that of DNA diploid tumors.

There is statistically significant correlation between nuclear perimeter morphometry and grade in colorectal adenocarcinoma cases (P value=0.002). This is in agreement with [25] who found statistically significant correlation between nuclear perimeter and grade of CRC (p value=0.006).

There is statistically significant correlation between nuclear roundness morphometry and age in colorectal adenocarcinoma cases (P value=0.012), while there is no statistically significant correlation with other clinicopathological data. This goes with [29] who found statistically significant correlation.

Regarding the previously discussed findings, we can suggest that TM9SF4 may have a notable role in tumorigenesis and progression of CRC.

Also, we found that nuclear morphometry has a role in the differentiation between premalignant colonic lesions and CRC.

Conclusion:

- Transmembrane 9 Superfamily 4 (TM9SF4) may have a role in tumorigenesis and CRC development being expressed in normal colon, ulcerative colitis, adenoma and colorectal carcinoma cases.
- Transmembrane 9 Superfamily 4 (TM9SF4) showed a significant correlation with adenocarcinoma invasion and stage indicating it may have a prognostic role.
- Nuclear morphometry may be useful as a diagnostic tool in adenocarcinoma through the differentiation between colorectal adenocarcinoma and premalignant colonic lesions.
- Nuclear morphometry area and perimeter have a significant statistical correlation with perineural invasion and grade of colorectal adenocarcinoma respectively suggesting they may have a prognostic value.

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