Research Article

Study of Immunohistochemical Expression of Ezrin and Icam-١ in Colorectal Adenocarcinoma

Shaimaa A. Sadek, Reda F. Abd El-Meguid, Heba M. Tawfik, Manal I. Abd El-Ghany and Dalia M. Abdel Rehim
Department of Pathology, El-Minia Faculty of Medicine

Abstract

Background: Colorectal cancer is one of the most common cancers worldwide. Colorectal carcinogenesis is a complex process resulting from the cumulative effect of sequential genetic alterations. Ezrin is involved in the regulation of cellular architecture, controlling cellular adhesion and motility which are essential for the entire process of tumorigenesis and distant metastasis. Intercellular adhesion molecule-١ (ICAM-١) is a member of the immunoglobulin superfamily and is expressed on many cell types including cancer cells where it can be upregulated in response to various cytokines. **Aim:** The present work was conducted to study the immunohistochemical expression of Ezrin and ICAM-١ in different types of colorectal carcinoma (CRC), to evaluate their expression patterns and examine their association with various clinicopathological features and finally to investigate the presence of a possible relationship between both markers in CRC. **Material and Methods:** The present study comprised one hundred and two (١٠٢) cases of primary colorectal carcinomas including; ٥٩ (٥٧.٨ %) cases of adenocarcinoma, ٢٨ (٢٧.٥٪) cases of mucoid carcinoma and ١٥ (١٤.٧٪) cases of signet ring cell carcinoma. Sections from ٤٥ tissue blocks of adjacent normal colorectal epithelium were also immune histocemically stained as the control group. Thirty four lymph node metastases were also evaluated for Ezrin and ICAM-١ expression. Immunohistochemistry for Ezrin and ICAM-١ antibodies was performed using the avidin biotin-peroxidase complex method. **Results:** The present study identified Ezrin expression in ٩٧.١٪ of colorectal carcinoma cases Ezrin expression was significantly higher in carcinoma cases compared to adjacent normal colorectal epithelium with different Ezrin localization reflecting different functions in both locations. Cytoplasmic Ezrin expression was significantly associated with lymph node spread (p< ٠.٠٠٠١) lymphovasular invasion (p= ٠.٠٣٨), advanced TNM (p< ٠.٠٠٠١) and Dukes stages (p< ٠.٠٠٠١). Regarding membranous Ezrin expression, statistically significant positive associations were detected between membranous expression of Ezrin and nodal metastasis (p= ٠.٠٢), advanced TNM stage (p= ٠.٠٣) and Dukes stage (p= ٠.٠٢). Regarding ICAM-١ expression, the present study identified ICAM-١ expression in ٣٥.٣٪ of carcinoma cases while it was undetectable in normal epithelium. Cytoplasmic ICAM-١ expression was significantly higher in adenocarcinoma compared to mucoid and signet ring cell carcinoma subtypes (p< ٠.٠٠٠١). Among adenocarcinoma cases, a significant inverse association was found between cytoplasmic ICAM-١ expression and tumor grade (p< ٠.٠٠٠١). Also, statistically significant inverse associations were found between cytoplasmic ICAM-١ expression and both TNM stage (p= ٠.٠٣٨), Dukes stage (p= ٠.٠٠٤) and lymphovascular invasion (p= ٠.٠٠٧). However, statistically significant positive associations were found between cytoplasmic ICAM-١ expression and both tumor infiltrating lymphocytes (p= ٠.٠٠٧) and tumor budding (p= ٠.٠٠١). A significant positive relationship was found between membranous ICAM-١ expression and tumor grade ((p= ٠.٠٢). No significant correlation was observed between Ezrin and ICAM-١ expression in the tumor cells (r= - ٠.٠٣ and p = ٠.٨ by Spearman's rho test, p = ٠.٢ by Chi-Square test). **Conclusion:** Ezrin expression is significantly associated with poor prognostic factors and thus can be used as a poor prognosticator for colorectal carcinoma patients. Cytoplasmic expression of ICAM-١ is significantly associated with good prognostic features except for tumor budding reflecting contradictory functions of ICAM-١ in CRC. In spite of their complementary functions in carcinogensis, Ezrin and ICAM-١ are not

significantly correlated in this series of colorectal carcinoma. Altogether, this study suggests the important role of Ezrin and ICAM-١ in colorectal carcinogenesis and progression
Key Words: Ezrin, ICAM-١, Colorectal Carcinoma, Adenocarcinoma, Mucoid Carcinoma, Signet Ring Cell Carcinoma.

Introduction

Colorectal cancer is the most common malignancy of gastrointestinal tract and is a common leading cause of mortality in many countries around the world. Colorectal carcinogenesis is a multifactorial process, in which genetic factors, environmental exposures (including diet), and inflammatory conditions of the digestive tract are involved[١٤].

The main prognostic factors in colorectal cancer are clinicopathological characteristics of the disease, including tumor stage, and grade. Although these parameters do reflect biological features of the tumor, they do not fully predict individual clinical outcome and thus, there is a strong need for better markers to identify patients with poor prognosis. Recent researches have focused on the potential role of new biological factors involved in the carcinogenic process as prognostic markers to aid accurate prediction of clinical outcome of patients with colorectal cancer[١٢].

Much attention has been focused on the involvement of Ezrin in tumor development and progression. Ezrin is a cytoskeleton associated protein that has traditionally been known as a molecule involved in maintaining the integrity and morphology of cells. It also has been widely reported to regulate the migration of the cells being suggested to be a candidate molecule in directional movement of cancer cells helping the processes of cancer invasion and metastasis. The main functions of Ezrin are thought to be acting as protein linker who links the cell membrane to the cytoskeletal proteins, cell surface molecules (cell adhesion molecules) to the cytoskeleton and, by doing so regulating cell adhesion and migration).

Among the many intriguing molecular features of cancer is the expression of intercellular adhesion molecule-١ (ICAM-١), which is widely known for its ability to stimulate immune activity. ICAM-١ is a member of the immunoglobulin super-family, which belongs to cell adhesion molecules. The interaction, mediated by ICAM-١, between cell-cell and between cells and extracellular matrix, is a form of communication between cells, which plays an important role in cell differentiation, movement, and immunity. ICAM-١ is also expressed in many tumor types where it is associated with good prognosis and improved survival[١١].

Material and Methods

Subject description: The present study comprised ١٠٢ randomly selected cases of primary colorectal carcinomas which were obtained from the archives of histopathological laboratories of Minia University Hospital and National Cancer Institute of Cairo (In the period between ٢٠٠٤ and ٢٠١٢). The cases included; ٥٩(٥٧.٨٪) cases of adenocarcinoma, ٢٨ (٢٧.٥٪) cases of mucoid carcinoma and ١٥ (١٤.٧٪) cases of signet ring cell carcinoma. Another ٤٥ tissue blocks of adjacent normal colorectal epithelium (available blocks) were immunostained as the control group. In addition, ٣٤ of metastatic malignant lymph nodes for lymph node positive cases were also included in the study.

Drugs and reagents: Monoclonal Mouse anti-human Ezrin and ICAM-١ Abs (Lab vision Laboratories) were used and DAB (Lab vision Laboratories) was used as a detection kit. Immunostaining was performed following the instructions of the manufacturers. PBS (٠.٠١ mmol/L) was used to replace the first antibody as a negative control, while the normal colorectal mucosa was a positive control for Ezrin and normal human tonsilar tissue was a positive control for ICAM-١.

Assessment of staining: To assess positive staining for Ezrin, immunoreactivity was

scored on a scale of ٠-٣ depending on the intensity of staining and the percentage of positive cells. Staining intensity was graded as ٠ (negative), ١(weak), ٢ (moderate) and ٣(strong). The percentage of positive cells was scored as ٠(<١٪), ١(١-٢٥٪), ٢(٢٦-٥٠٪), ٣(٥١-٧٥٪) and ٤ (٧٦-١٠٠٪). The two scores were multiplied and then the total immunohistochemical score I(H score) values (٠-١٢) were classified as follows: score ٠ as negative, score ١(multiplication values ١and ٢) as weakly positive, score ٢(multiplication values ٣, ٤ and ٦) as moderately positive and score ٣ (multiplication values ٨, ٩, ١٢) as strongly positive. Cytoplasmic and membranous staining, when observed, were evaluated separately.

For scoring of ICAM-١ staining, staining intensity was graded as ٠(negative), ١(weak), ٢(moderate) and ٣(strong). The numbers of positive cells were expressed as the percentage of the total number of epithelial. The two scores were multiplied and then immunohistochemical score (IH score) for each tumor specimen was calculated. Cytoplasmic and membranous staining, when observed, was evaluated separately. No definite cut-off point for identification of positivity and grade of ICAM-١ expression was reported in literature. Therefore, we preferred to use continuous scores to study the association of ICAM-١ in relation to different clinicopathologic features.

Statistical analysis

Data were checked, coded, entered and analyzed by using SPSS (The Statistical Package for Social Sciences) version ٢٢ software. Association between immunereactivity and different clinicopathological data were done by Chi-Square test, McNemar test, Mann-Whitney test and Kruskal-wallis test. Spearman's rho coefficient Wilcoxon test and Friedman test were used for continuous variables. Stati-stical significance was determined at p value of ≤٠.٠٥ for all tests except Friedman test where significance level is set as ٠.٠٢.

Results

Information regarding different clinical and histopathological features for all colorectal carcinoma (CRC) patients is illustrated in Table (١).

On studying the cytoplasmic expression of Ezrin (table ٢), the expression was significantly higher in colorectal carcinoma compared to normal tissues.

Cytoplasmic Ezrin expression was signify-cantly higher in lymph node positive than lymph node negative cases and in cases with lymphovasular invasion than cases without invasion. Similarly, there were significant variations in cytoplasmic Ezrin expression among different stages of colorectal carcinoma being markedly higher in tumors with advanced TNM and Dukes stages compared to lower stage tumors.

As for adenocarcinoma cases, grade III tumors showed more frequent strong cytoplasmic Ezrin expression than grade I and II however, such differences did not reach the significant statistical level (p value= ٠.٢). No significant associations were also observed between cytoplasmic Ezrin expression and patients' age, gender, tumor size, location, tumor type, perineural invasion, tumor budding or tumor infiltrating lymphocytes.

On comparing cytoplasmic and membranous Ezrin expression in primary tumors and their corresponding metastatic lymph nodes, no significant differences were observed in Ezrin expression between both locations.

Concerning cytoplasmic ICAM-١ expression (table ٣), this work identified significant differences in expression between different types of colorectal carcinoma being markedly higher in adenocarcinoma compared to mucoid and signet ring subtypes. Among adenocarcinoma cases, a significant inverse association was found between cytoplasmic ICAM-١ expression and tumor grade where ICAM-١ expression scores were significantly higher in grade I than grade II and III tumors.

Statistically significant inverse associations were found between cytoplasmic ICAM-١ expression and both TNM and Dukes staging systems and lymphovascular invasion. However, statistically significant

positive associations were found between cytoplasmic ICAM-١ expression and both and tumor infiltrating lymphocytes and tumor budding. No significant associations were found between cytoplasmic ICAM-١ expression and other clinicopathologic features.

Comparing ICAM-١ expression in primary tumors and their corresponding metastatic lymph nodes revealed no significant differences in ICAM-١ expression scores between primary tumors and their matched lymph node metastasis.

Despite having apparently complementary functions, Ezrin and ICAM-١ immuno-reactivity results did not display significant association between both markers in this study.

Table ١: Clinicopathological features for patients with CRC (n=١٠٢)

Clinicopathological features	No. (%)
Age at Surgery, y	
- ≤٤٠	٢١ (٢٠.٦٪)
- >٤٠	٨١ (٧٩.٤٪)
Gender	
-Male	٥٣ (٥٢٪)
-Female	٤٩ (٤٨٪)
Location	
١- Caecum	٢ (٢٪)
٢- Ascending colon	٣٦ (٣٥.٣٪)
٣- Transverse colon	٩ (٨.٨٪)
٤- Descending colon	١٧ (١٦.٧٪)
٥- Sigmoid colon	١٩ (١٨.٦٪)
٦- Rectum	١٩ (١٨.٦٪)
Histological subtypes	
١- Adenocarcinoma	٥٩ (٥٧.٨٪)
٢- Mucoid carcinoma	٢٨ (٢٧.٥٪)
٣- Signet ring cell carcinoma	١٥ (١٤.٧٪)
Tumor grade (for adenocarcinoma only)*	
١- Grade I	٤ (٦.٨٪)
٢- Grade II	٣٢ (٥٤.٢٪)
٣- Grade III	٢٣ (٣٩٪)
Regional lymph node involvement	
Absent	٥٨ (٥٦.٩٪)
Present	٤٤ (٤٣.١٪)
TNM stage groupings	
١-I	١٨ (١٧.٦٪)
٢-II	٣٧ (٣٦.٣٪)
٣-III	٣٦ (٣٥.٣٪))
٤-IV	١١ (١٠.٨٪)
Dukes classification	
١-A	٠ (٠٪)
٢-B١	١٣ (١٢.٧٪)
٣-B٢	٤١ (٤٠.٢٪)
٤-C١	٣ (٢.٩٪)
٥-C٢	٣٤ (٣٣.٣٪)
٦-D	١١ (١٠.٨٪)
Perineural invasion	
Absent	٨٢ (٨٠.٤٪)
Present	٢٠ (١٩.٦٪)
Lymphovascular invasion	
Absent	٧٢ (٧٠.٦٪)
Present	٣٠ (٢٩.٤٪)
Tumor budding	
Absent	٧٩ (٧٧.٥٪)
Present	٢٣ (٢٢.٥٪)
Tumor infiltrating lymphocytes	
١-Absent	٤٦ (٤٥.١٪)
٢-Mild	٢٢ (٢١.٦٪)
٣-Moderate	١٣ (١٢.٧٪)
٤-Marked	٢١ (٢٠.٦٪)

Table (٢): Association between cytoplasmic Ezrin expression and clinicopathological features for patients with CRC

Clinicopathological features	*Total* ١٠٢	Negative expression No. (%)	Weakly positive No. (%)	Moderately positive No. (%)	Strongly positive No. (%)	*P* value
Age at Surgery, y						
- ≤٤٠	٢١(٢٠.٦)	٠ (٠)	٢ (٩.٥)	٩ (٤٢.٥)	١٠ (٤٧.٦)	٠.١٦
- >٤٠	٨١(٧٩.٤)	٣ (٣.٧)	١٨ (٢٢.٢)	٢٩ (٣٥.٨)	٣١ (٣٨.٣)	
Gender						
-Male	٥٣ (٥٢)	٢ (٣.٨)	١٠ (١٨.٩)	١٩ (٣٥.٨)	٢٢ (٤١.٥)	٠.٨
-Female	٤٩ (٤٨)	١ (٢)	١٠ (٢٠.٤)	١٩ (٣٨.٨)	١٩ (٣٨.٨)	
Location						
١-Caecum	٢ (٢)	٠ (٠)	٠ (٠)	١ (٥٠)	١ (٥٠)	
٢-Ascending colon	٣٦ (٣٥.٣)	١ (٢.٨)	١٠ (٢٧.٨)	٨ (٢٢.٢)	١٧ (٤٧.٢)	
٣-Transverse colon	٩ (٨.٨)	١ (١١.١)	١ (١١.١)	٢ (٢٢.٢)	٥ (٥٥.٦)	٠.٢٣
٤-Descending colon	١٧ (١٦.٧)	٠ (٠)	١ (٥.٩)	٨ (٤٧.١)	٨ (٤٧.١)	
٥-Sigmoid colon	١٩ (١٨.٦)	١ (٥.٣)	٤ (٢١.١)	١٠ (٥٢.٦)	٤ (٢١.١)	
٦-Rectum	١٩ (١٨.٦)	٠ (٠)	٤ (٢١.١)	٩ (٤٧.٤)	٦ (٣١.٦)	
Histological subtypes	٥٩ (٥٧.٨)	٠ (٠)	٩ (١٥.٣)	٢٥ (٤٢.٤)	٢٥ (٤٢.٢)	
١-Adenocarcinoma	٢٨ (٢٧.٥)	١ (٣.٦)	٨ (٢٨.٦)	١٠ (٣٥.٧)	٩ (٣٢.١)	٠.٢٥
٢-Mucoid carcinoma	١٥ (١٤.٧)	٢ (١٣.٣)	٣ (٢٠)	٣ (٢٠)	٧ (٤٦.٧)	
٣-Signet ring cell carcinoma						
Tumor grade						
(adenocarcinoma) *	٤ (٦.٨)	٠ (٠)	١ (٢٥)	٢ (٥٠)	١ (٢٥)	
١-Grade I	٣٢ (٥٤.٢)	٠ (٠)	٦ (١٨.٨)	١٦ (٥٠)	١٠ (٣١.٢)	٠.٢
٢-Grade II	٢٣ (٣٩)	٠ (٠)	٢ (٨.٧)	٧ (٣٠.٤)	١٤ (٦٠.٩)	
٣-Grade III						
Regional lymph node involvement	٤٤ (٤٣.١)	٢ (٤.٥)	٠ (٠)	١٤ (٣١.٨)	٢٨ (٦٣.٦)	
Present	٥٨ (٥٦.٩)	١ (١.٧)	٢٠ (٣٤.٥)	٢٤ (٤١.٤)	١٣ (٢٢.٤)	< .٠٠٠١*
Absent						
TNM stage groupings	١٨ (١٧.٦)	٠ (٠)	١٢ (٦٦.٧)	٦ (٣٣.٣)	٠ (٠)	
١-I	٣٧ (٣٦.٣)	١ (٢.٧)	٨ (٢١.٦)	١٨ (٤٨.٧)	١٠ (٢٧)	< .٠٠٠١*
٢-II	٣٦ (٣٥.٣)	٢ (٥.٥)	٠ (٠)	١٤ (٣٨.٩)	٢٠ (٥٥.٦)	
٣-III	١١ (١٠.٨)	٠ (٠)	٠ (٠)	٠ (٠)	١١ (١٠٠)	
٤-IV						
Dukes classification						
١-A	٠ (٠)	٠ (٠)	٠ (٠)	٠ (٠)	٠ (٠)	
٢-B١	١٣ (١٢.٨)	٠ (٠)	١١ (٨٤.٦)	٢ (١٥.٤)	٠ (٠)	< .٠٠٠١*
٣-B٢	٤١ (٤٠.٢)	١ (٢.٤)	٩ (٢٢)	٢١ (٥١.٢)	١٠ (٢٤.٤)	
٤-C١	٣ (٢.٩)	٠ (٠)	٠ (٠)	٢ (٦٦.٧)	١ (٣٣.٣)	
٥-C٢	٣٤ (٣٣.٣)	٢ (٥.٩)	٠ (٠)	١٣ (٣٨.٢)	١٩ (٥٥.٩)	
٦-D	١١ (١٠.٨)	٠ (٠)	٠ (٠)	٠ (٠)	١١ (١٠٠)	
Perineural invasion						
Present	٢٠ (١٩.٦)	١ (٥)	١ (٥)	١١ (٥٥)	٧ (٣٥)	٠.٨٦
Absent	٨٢ (٨٠.٤)	٢ (٢.٤)	١٩ (٢٣.٢)	٢٧ (٣٢.٩)	٣٤ (٤١.٥)	
Lymphovascular invasion	٣٠ (٢٩.٤)	٠ (٠)	٣ (١٠)	١١ (٣٦.٧)	١٦ (٥٣.٣)	٠.٠٣٨*
Present	٧٢ (٧٠.٦)	٣ (٤.٢)	١٧ (٢٣.٦)	٢٧ (٣٧.٥)	٢٥ (٣٤.٧)	
Absent						
Tumor budding						
Present	٢٣ (٢٢.٥)	٠ (٠)	٢ (٨.٧)	١٢ (٥٢.٢)	٩ (٣٩.١)	٠.٦٤
Absent	٧٩ (٧٧.٥)	٣ (٣.٨)	١٨ (٢٢.٨)	٢٦ (٣٢.٩)	٣٢ (٤٠.٥)	
Tumor infiltrating lymphocytes	٤٦ (٤٥.١)	٢ (٤.٤)	٨ (١٧.٨)	١٥ (٣٣.٣)	٢٠ (٤٤.٤)	
١-Absent	٢٢ (٢١.٦)	١ (٤.٦)	٣ (١٣.٦)	٧ (٣١.٨)	١١ (٥٠)	٠.٤٧
٢-Mild	١٣ (١٢.٧)	٠ (٠)	٣ (٢٣.١)	٧ (٥٣.٨)	٣ (٢٣.١)	
٣-Moderate	٢١ (٢٠.٦)	٠ (٠)	٥ (٢٣.٨)	٩ (٤٢.٩)	٧ (٣٣.٣)	
٤-Marked						

Table (٣): Association between cytoplasmic ICAM-١ expression and clinicopathological features for patients with CRC (n=١٠٢)

Clinico-pathological features	Total (%)	Cases with no expression	cases showing expression	(Min-Max) Expression scores	Median	M ± SD	P value
Age at Surgery, y							
- ≤٤٠	٢١(٢٠.٦)	١٦ (٧٦.٢)	٥ (٢٣.٨)	(٠-١٨٠)	٠	١٤.٥٢ ±	٠.١٩
- >٤٠	٨١(٧٩.٤)	٥٠ (٦١.٧)	٣١(٣٨.٣)	(٠-٢٧٠)	٠	٤٣.٨	
						٢٧.٩ ± ٥٩.٦	
Gender							
-Male	٥٣ (٥٢)	٣٣ (٦٢.٣)	٢٠(٣٧.٣)	(٠-٢٧٠)	٠	٢٠.٨ ± ٥٦.٣	٠.٢٧
-Female	٤٩ (٤٨)	٣٣ (٦٧.٣)	١٦(٣٣.٧)	(٠-٢١٠)	٠	٢٩.٩ ± ٥٧.٦	
Location							
١-Caecum	٢ (٢)	٢ (١٠٠)	٠ (٠)	(٠-٠)	٠	٠ ± ٠	٠.٩٤
٢-Ascending colon	٣٦(٣٥.٣)	٢٧ (٧٥)	٩ (٢٥)	(٠-١٨٠)	٠	١٣.٣ ± ٣٩.٧	
٣-Transverse colon	٩ (٨.٨)	٦ (٦٦.٧)	٣ (٣٣.٣)	(٠-٢١٠)	٠	٢٦.١ ± ٦٩.٣	
٤-Descending colon	١٧(١٦.٧)	١٠ (٥٨.٨)	٧ (٤١.٢)	(٠-١٠٠)	٠	٢٢.٦ ± ٣٨.٣	
٥-Sigmoid colon	١٩(١٨.٦)	٩ (٤٧.٤)	١٠(٥٢.٦)	(٠-٢٧٠)	٥	٥٣.٤ ± ٨٩	
٦-Rectum	١٩(١٨.٦)	١٢ (٦٣.٢)	٧ (٣٦.٨)	(٠-١٦٠)	٠	٢٤ ± ٤٩.٧	
subtypes							
١-Adenocarcinoma	٥٩(٥٧.٨)	٢٧ (٤٥.٨)	٣٢ (٥٤.٢)	(٠-٢٧٠)	٠	٤٣.٣ ± ٦٩.٤	<٠.٠٠٠١*
٢-Mucoid	٢٨(٢٧.٥)	٢٥ (٨٩.٣)	٣ (١٠.٧)	(٠-٥)	٠	٠.٣٢± ١	
٣-Signet ring	١٥(١٤.٧)	١٤ (٩٣.٣)	١ (٦.٧)	(٠-٣)	٠	٠.٢ ± ٠.٧٧	
Tumor grade *(adenocarcinoma)*							
١-Grade I	٤ (٦.٨)	٠ (٠)	٤ (١٠٠)	(٨٠-٢٧٠)	١٨٠	١٧٧.٥ ±٨١.٤	<٠.٠٠٠١*
٢-Grade II	٣٢(٥٤.٢)	٨ (٢٥)	٢٤ (٧٥)	(٠-٢٤٠)	٢٠	٥٦.٧ ± ٧٦.٥	
٣-Grade III	٢٣(٣٩)	١٩ (٨٢.٦)	٤ (١٧.٤)	(٠-٢٠)	٠	١.٣ ± ٤.٣	
lymph node involvement							
Present	٤٤(٤٣.١)	٣٢ (٧٢.٧)	١٢(٢٧.٣)	(٠-٢٧٠)	٠	٢٣.٦ ± ٥٩.٧	٠.٢
Absent	٥٨(٥٦.٩)	٣٤ (٥٨.٦)	٢٤(٤١.٤)	(٠-٢٤٠)	٠	٢٦.٣ ± ٥٥.١	
TNM stage							
١-I	١٨(١٧.٦)	١١ (٦١.١)	٧ (٣٨.٩)	(٠-٢٤٠)	٠	٤٣.٤ ±٧٤.٨	
٢-II	٣٧(٣٦.٣)	٢٠ (٥٤.١)	١٧(٤٥.٩)	(٠-٢١٠)	٠	٢٠.٢ ± ٤٣.٨	٠.٠٣٨*
٣-III	٣٦(٣٥.٣)	٢٦ (٧٢.٢)	١٠(٢٧.٨)	(٠-٢٧٠)	٠	٢٢.٥ ± ٥٧.٢	
٤-IV	١١(١٠.٨)	٩ (٨١.٨)	٢ (١٨.٢)	(٠-٢١٠)	٠	٢٠.٩ ± ٦٣	
Dukes classification							
١-A	٠ (٠)	٠ (٠)	٠(٠)	٠-٠	٠	٠ ± ٠	
٢-B١	١٣(١٢.٨)	١١ (٨٤.٦)	٢ (١٥.٤)	(٠-٢٤٠)	٠	٢٤.٦ ± ٦٨.٤	
٣-B٢	٤١(٤٠.٢)	٢٠ (٤٨.٨)	٢١(٥١.٢)	(٠-٢١٠)	١	٢٨ ± ٥٣.٣	
٤-C١	٣ (٢.٩)	٠ (٠)	٣ (١٠٠)	(٦٠-٢٧٠)	٦٠	١٣٠ ± ١٢١	٠.٠٠٤*
٥-C٢	٣٤(٣٣.٣)	٢٦ (٧٦.٥)	٨ (٢٣.٥)	(٠-١٥٠)	٠	١٤.١ ± ٣٨.٧	
٦-D	١١(١٠.٨)	٩ (٨١.١)	٢ (١٨.٩)	(٠-٢١٠)	٠	٢٠.٩ ± ٦٣	
Perineural invasion							
Present	٢٠(١٩.٦)	١٠ (٥٠)	١٠ (٥٠)	(٠-١٦٠)	٢.٥	٣٩ ± ٥٤.٧	
Absent	٨٢(٨٠.٤)	٥٦ (٦٨.٣)	٢٦(١٩.٦)	(٠-٢٧٠)	٠	٢١.٨ ± ٥٧.١	٠.٠٨
Lymphovascular invasion							
Present	٣٠(٢٩.٤)	٢٥ (٨٣.٣)	٥ (١٦.٧)	(٠-١٦٠)	٠	٧.٥ ± ٣٠.٢	٠.٠٠٧*
Absent	٧٢(٧٠.٦)	٤١ (٥٦.٩)	٣١(٤٣.١)	(٠-٢٧٠)	٠	٣٢.٦ ± ٦٣.٥	
Tumor budding							
Present	٢٣(٢٢.٥)	٨ (٣٤.٨)	١٥(٦٥.٢)	(٠-٢٧٠)	١٠	٤٤.٦ ± ٦٨.٩	٠.٠٠١*
Absent	٧٩(٧٧.٥)	٥٨ (٧٣.٤)	٢١(٢٦.٦)	(٠-٢٤٠)	٠	١٩.٥ ± ٥١.٩	
TILs							
١-Absent	٤٦(٤٥.١)	٣٧ (٨٠.٤)	٩ (١٩.٦)	(٠-١٥٠)	٠	٩.٧ ± ٣٠.٩	٠.٠٠٧*
٢-Mild	٢٢(٢١.٦)	١٤ (٦٣.٦)	٨ (١٦.٤)	(٠-١٨٠)	٠	٢١.٦ ± ٥٠	
٣-Moderate	١٣(١٢.٧)	٤ (٣٠.٨)	٩ (٦٩.٢)	(٠-٢١٠)	٢٠	٤١.٣± ٦٠.١	
٤-Marked	٢١(٢٠.٦)	١٢ (٥٧.١)	٩ (٤٢.٩)	(٠-٢٧٠)	٠	٤٢ ±٧٧.٩	

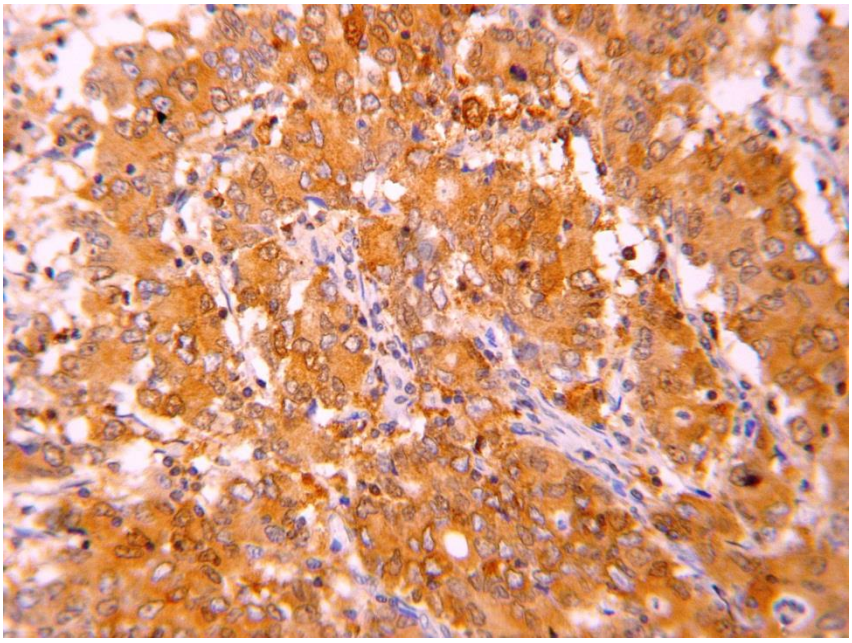

Figure ١: Immunohistochemical appearance of grade III colorectal adenocarcinoma stained with Ezrin, showing positive cytoplasmic staining of strong intensity (original magnification ٤٠٠).

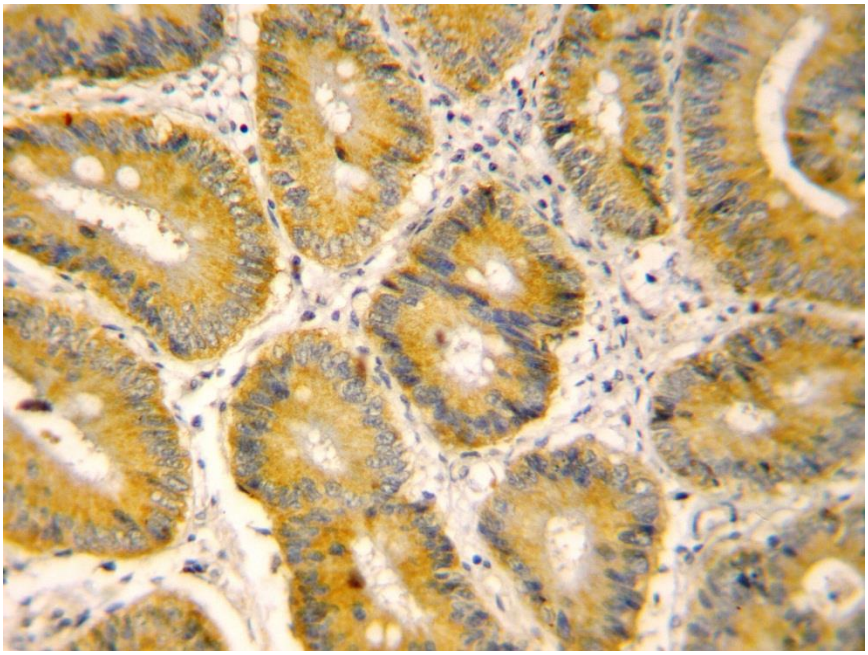

Figure٢: Immunohistochemical appearance of grade I colorectal adenocarcinoma with ICAM-١ immunostaining, showing positive cytoplasmic staining of strong intensity (original magnification ٤٠٠).

Discussion

Colorectal cancer is one of the most common cancers worldwide. It affects men and women of all racial and ethnic groups, and is most often found in people aged ٥٠ years or older in developed countries[٤].

Ezrin plays specific roles in the adherent junction between trans-membranous proteins and cytoskeleton within cell and between cells, and acts as a signal transducer. In this way, Ezrin is involved in the regulation of phenotypical change of cellular architecture, controlling cellular motility, tethering action between cell to cell and cell to matrix, recognizing and conducting internal and/or external signal. All of these cellular functions are essential for maintaining homeostasis of normal cell as well as entire process of tumorigenesis and distant metastasis[٨,١٣]. Intercellular adhesion molecule (ICAM)-١ (CD٥٤) is a member of the immunoglobulin superfamily and expressed on many cell types including endothelial cells, leukocytes and cancer cells where it can be upregulated in response to various cytokines[٧].

Regarding subcellular localization of Ezrin, the current study detected Ezrin expression in the intercellular junctions and cytoplasm of normal colorectal epithelial cells which explains an important function of Ezrin in normal cells, i.e. maintaining intercellular adhesion. In tumor cells, Ezrin was found either in the cytoplasm or in both cytoplasm and membrane. This finding was reported by several studies performed on Ezrin expression in normal colorectal mucosa and colorectal carcinoma [٢]. In the current study, a statistically significant difference in expression of Ezrin was detected between normal colorectal mucosa and colorectal carcinoma and this was in agreement with other studies performed by[١٤,٥,١٣]. ٦٣.٦٪ of cases positive for lymph node metastases showed strongly positive cytoplasmic Ezrin expression as compared to those negative for lymph node metastases which showed only ٢٢.٤٪ strongly positive expression. This was in accordance with[٢,١٤] who demonstrated significant association between Ezrin expression and nodal metastases. A significant association was observed between cytoplasmic Ezrin expression and advanced TNM stage. The frequency of cytoplasmic Ezrin expression was much higher in both stage IV and stage III when compared to stage II and stage I. This was in line with findings of [٩]. Our findings together with others suggest that Ezrin appears to be an important prognostic factor for patients with colorectal cancer as it is more frequently expressed in carcinoma than in normal epithelium and its expression correlates with aggressive tumor features such as lymph node involvement, lympho-vascular invasion and advanced tumor stage. It may also allow therapeutic options such as miRNA and siRNA to be developed. No drugs are yet reported to specifically inhibit Ezrin; however, some of the epigenetic drugs and estrogen have been shown to upregulate Ezrin. Rational designed compounds that have impact on the activity of Ezrin have also been recently reported[١٣].

The broad expression of Ezrin together with the association of Ezrin expression with poor prognostic features in colorectal carcinoma confirms the important role of Ezrin in pathogenesis of colorectal carcinogenesis and suggests its role as an adjuvant therapy for high risk patients.

ICAM-١ expression was totally absent in normal colorectal epithelium while in colorectal carcinoma, the positive expression rate was ٣٥.٣٪. ICAM-١ was found in the cytoplasm, membrane or in both cytoplasm and membrane. This was consistent with several studies that mentioned absent ICAM-١ expression in normal mucosa and reported positive expression rates ranging from ٣٣٪ up to ٥٠٪ in colorectal carcinoma[١٠,١].

Concerning the cytoplasmic expression of ICAM-١ in different subtypes of CRC, the current study showed highly significant differences in expression between adenocarcinoma and other histopathological subtypes. ICAM-١ positive expression rates were significantly much higher in adenocarcinoma cases compared to mucoid and signet ring cell carcinomas, a finding that was not previously reported in literature as

ICAM-١ expression was not previously investigated in mucoid or signet ring colorectal carcinoma cases. In a study performed on gastric carcinoma,[٣,١٢] found significant differences in ICAM-١ expression between gastric adenocarcinoma and other subtypes including mucoid and signet ring cell carcinomas. The current study demonstrated a significant inverse association between ICAM-١expression and grade of colorectal adenocarcinoma, where positive expression rates were much higher in grades I and II compared to grade III tumors and this was consistent with previous studies[١٠,٦&٥]**.** Likewise, a significant inverse association between ICAM-١ expression and tumor stage was noticed in this study. The rate of ICAM-١ expression was significantly higher in stages I and II tumors compared to stage III and IV tumors. This was in line with[١] who reported significant association between loss of ICAM-١ expression and advanced stage. ICAM-١ positive expression was significantly lower in tumors showing lymphovascular invasion. Interestingly, ICAM-١ expression was significantly associated with tumor budding where the positive expression rates were markedly higher in tumors with budding than those without budding**.** Actually, multiple examples for adhesion molecules are known to display seemingly controversial roles, such as EpCAM or Maspin. In this respect, in breast cancer cells, silencing of ICAM-١ expression by siRNA resulted in decreased tumorigenicity in vitro[١١]**.** Another study performed on hepatocellular carcinoma suggested that, ICAM-١ can trigger the calcium influx and take a part in disintegration of the cells' local adhesion, contraction of cells and the reconstruction of cytoskeleton, so as to promote the transfer of cancer cells and that the high expression of ICAM-١ helps cancer cells to separate from each other becoming detached and transfer to other sites[١٥], a mechanism that could explain the significant association between ICAM-١ expression and tumor budding. Infiltration of TILs was more frequently observed in the ICAM-١-positive tumors than in the ICAM-١-negative tumors**,** a finding that was also supported by previous studies performed on colorectal carcinoma[١٠;٦]**.** Taken together, our previous findings suggest that positive cytoplasmic ICAM-١ expression in colorectal carcinoma is a useful important biologic marker predicting good prognosis.

Reference

١. Dippold W, Wittig B, Schwaeble W, Mayet W and Myer KH. (٢٠٠٣): Expression of intercellular adhesion molecule ١ (ICAM-١, CD٥٤) in colonic epithelial cells. *Gut*; ٣٤:١٥٩٣-١٥٩٧.

٢. Elzagheid A, Korkeila E, Bendardaf R, Buhmeida A, Heikkilä S, Vaheri A, Syrjänen K, Pyrhönen S and Carpen O (٢٠٠٨): Intense cytoplasmic Ezrin immunoreactivity predicts poor survival in colorectal cancer. Human Pathology; ٣٩:١٧٣٧-٤٣.

٣. Fujihara T, Yashiro M, Inoui T, Sawada T, Kato Y, Ohira M and Nishiguchi Y. (٢٠٠٥): Decrease in ICAM-١ expression on gastric cancer cells is correlated with lymph node metastasis. Gastric Cancer; ٢:٢٢١-٢٢٥.

٤. Gado A, Ebeid B, Abdelmohsen A and Axon A. (٢٠١٣): Colorectal cancer in Egypt is commoner in young people: Is this cause for alarm? Alexandria Journal of Medicine; ١١:٦٥-٧٧.

٥. Jinshan L, Mingcai Z and Pujian D. (٢٠١١): Expression of Ezrin and intercellular adhesion molecule ١ in colon cancer and its clinical significance. Journal of Third Military Medical University; ١٧:٦٥٤-٦٦٢.

٦. Kelly CP, O'Keane JC, Orellana J, Schroy PC, Yang S, LaMont JT and Brady HR. (٢٠٠٦): Human colon cancer cells express ICAM-١ in vivo and support LFA-١-dependent lymphocyte adhesion in vitro. American Journal of Physiology; ٢٦٣: ٨٦٤-٧٠.

٧. Ksiazek K and Mikula-Pietrasik J. (٢٠١٠): Oxidative stress-dependent increase in ICAM-١ expression promotes adhesion of colorectal and pancreatic cancers to the senescent peritoneal mesothelium. International Journal of Cancer; ١٢٧: ٢٩٣–٣٠٣

٨. Li Y, Harada T, Juang YT, Kyttaris VC, Wang Y, Zidanic M, Tung K and Tsokos GC. (٢٠٠٨): Phosphorylated ERM is responsible for increased T cell

polarization, adhesion, and migration in patients with systemic lupus erythematosus. Journal of Immunology; ١٧٨: ١٩٣٨–١٩٤٧.

٩. Lin LJ and Chen LT (٢٠١٣): Association between Ezrin protein expression and the prognosis of colorectal adenocarcinoma. Molecular Medicine Reports; ٢٢: ٦١-٦٦.

١٠. Maeda K and Kang SM. (٢٠٠٢): Expression of intercellular adhesion molecule-١ and prognosis in colorectal cancer. Oncology Reports; ٩:٥١١-٤.

١١. Marloes L., Kazemier HG, Huisman C and Marijke M. (٢٠١٣): Upregulation of endogenous ICAM-١ reduces ovarian cancer cell growth in the absence of immune cells. International Journal of Cancer; ٦٧: ١٤٥٦-١٤٦٤.

١٢. Nasu R, Mizuno M, Kiso T, Shimo K, esu T, Nasu J, Tomoda J, Okada H and Tsuji T. (٢٠٠٧): Immunohistochemical analysis of intercellular adhesion molecule-١ expression in human gastric adenoma and adenocarcinoma. Cancer Research; ٤٣٠: ٢٧٩-٢٨٣

١٣. Toms AM, Davies ML, Hargest R, Hiscox SE and Jiang WG. (٢٠١٢): Distribution and expression of the ERM family members, Ezrin, Radixin, Moesin and EHM٢ in human colon cancer and the clinical relevance. Gastrointestinal Cancer; ١:٢٠٥-٢١٤.

١٤. Wang LM, Kevans D and Mulcahy H. (٢٠٠٩): Tumor budding is a strong and reproducible prognostic marker in T٣ N٠ colorectal cancer. American Journal of Surgical Pathology; ٣٣:١٣٤-١٤١.

١٥. Zhu X and Gong J. (٢٠١٣): Expression and Role of ICAM-١ in the Occurrence and Development of Hepatocellular Carcinoma. Asian Pacific Journal for Cancer Prevention; ١٤ (٣):١٥٧٩-١٥٨٣.

.