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Immunohistochemical expression of clusterin in colorectal carcinoma

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Jalal Ali Jalal[1] Zheen Othman[1]* Payman Anwar[1]

Abstract

Background and objective: Colorectal cancer is a heterogeneous malignancy characterized by a wide range of genetic and epigenetic alterations. Clusterin is a heterodimeric glycoprotein widely expressed in a variety of tissues and secreted in many body fluids. Increased clusterin expression has been reported in the normal colonic mucosa, benign polyps, and colorectal carcinoma. This study aimed to detect the frequency of the clusterin immunoexpression in colorectal carcinoma and determine its association with some clinicopathological parameters.

Methods: Sixty formalin-fixed paraffin-embedded sections of colorectal adenocarcinoma were obtained and randomly selected from the histopathology laboratory at Rizgary Teaching Hospital and some private histopathology laboratories in Erbil city over two years between December 2016 and December 2018. All patients had been diagnosed to have primary colorectal adenocarcinoma and had undergone surgery. The clinicopathological characteristics of the tumors were revised, and the specimens were analyzed immunohistochemically using anticlusterin mouse monoclonal antibody.

Results: Twenty eight cases (46.6%) were labeled as clusterin positive, while 32 cases (53.4%) were negative for clusterin expression. Clusterin expression was significantly associated with the tumor type (Non-mucinous) (P = 0.01) and tumor grade (well to moderately differentiated) (P = 0.03). At the same time, no significant association was found between clusterin immunoexpression and other clinicopathological characteristics like age, gender, tumor site, and tumor stage.

Conclusion: Our study indicated that clusterin is overexpressed in some colorectal carcinomas and is significantly associated with histological type and grade. These results suggest that clusterin may play a role in colorectal carcinogenesis. Further studies are required to understand the possible mechanism of clusterin association with carcinogenesis and cancer progression.

Keywords: Colorectal cancer; Clusterin; Immunohistochemistry.

Introduction

Colorectal cancer is the third most common cancer and the second leading cause of cancer-related mortality worldwide, with an estimated more than one million new colorectal cancer cases each year.[1] Colorectal cancer is ranked the 7th most common cancer in Iraq while it represents the 4th commonest cancer in the Kurdistan region for both genders.[2,3] Colorectal cancer is a heterogeneous malignancy characterized by a wide range of genetic and epigenetic alterations.[4] The majority of colorectal cancer cases are sporadic, while the remaining cases are familial with an inherited genetic predisposition. The carcinogenesis of colorectal cancer is a multistep process that begins with the conversion of the normal colorectal epithelium to adenoma. This is followed by consecutive accumulation of molecular alterations that eventually result in invasive carcinoma.[5] Recent advances in our knowledge regarding the molecular

[1] Department of Basic Sciences, College of Medicine, Hawler Medical University, Erbil, Iraq.
* Correspondence: zheenothman@gmail.com

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aspects of colorectal cancer may, therefore, hold a considerable promise for the early detection and future development of new prognostic markers and therapeutic targets for colorectal cancer.[6] Clusterinis a heterodimeric glycoprotein that is widely expressed in a variety of tissues and is secreted in many body fluids. Its expression has been related to numerous physiological processes that confer distinct properties crucial for carcinogenesis and tumor progression, including cellular response to stress, cell apoptosis, DNA repair, cellular senescence, cell adhesion, tissue remodeling, and immune system modulation.[7,8] Clusterin exists in two isoforms, secretory clusterin, and nuclear clusterin. The secretory form is cytoprotective, conferring the cell prosurvival properties, while the nuclear clusterin is proapoptotic migrating to the nucleus on cytotoxic stress resulting in cell death. The transition from a normal cell to a neoplastic one begins with the overexpression of secretory clusterin and the loss of nuclear clusterin expression. The ratio of secretory/ nuclear isoform correlates with tumor cell survival and with its metastatic potential.[9,10] Increased clusterin expression has been reported in the normal colonic mucosa, benign polyps, and colorectal carcinoma. Also, abnormal clusterin expression has been noted in different cancers such as prostatic, breast, ovarian, pancreatic, renal, and hepatocellular carcinomas.[11,12] This study aimed to detect the frequency of the clusterin immunoexpression in colorectal carcinoma and its association with some clinicopathological parameters like age, gender, tumor site, tumor type, tumor grade, nodal involvement, and tumor stage.

Methods

After obtaining approval of the study protocol from the research ethics committee at the College of Medicine in Hawler Medical University, this retrospective study was carried out. Sixty formalin-fixed paraffin-embedded sections of colorectal adenocarcinoma were obtained and randomly selected from the histopathology laboratory at Rizgary Teaching Hospital and some private histopathology laboratories in Erbil city during two years between December 2016 and December 2018. All patients who had been diagnosed to have primary colorectal adenocarcinoma and had undergone surgery had been selected. None of them had received chemotherapy or radiotherapy before surgery. All the slides were examined, and the most representative tumor block (no necrosis, no much mesenchymal tissue) was selected for the study. Two sections of 4 micrometers thickness were taken from each paraffin embedded tissue block. The first section was stained with Haematoxylin and Eosin (H&E) staining for histopathological evaluation. The second section was used for immunohistochemical staining. Tumors were divided according to anatomical location into two categories, namely, right and left colon with the rectum.[13] The histological type was classified into non-mucinous or mucinous while grading was coded as well-moderately differentiated and poorly differentiated.[14] Nodal involvement was labeled as positive or negative for involvement. The staging was performed according to American Joint Committee on Cancer (AJCC) and the Union International Contre Le Cancer (UICC).[15]

Immunohistochemical staining:

Thin sections (four µm) were cut, mounted on salinized slides, and dried at 60 °C for about one hour. After the slides had been deparaffinized and rehydrated at room temperature (20-25 °C), they were placed in a xylene bath and incubated for five minutes. Then, they were put in absolute ethanol for 3 minutes and followed by 95% ethanol for 3 minutes. At last, the slides were immersed in distilled water for a minimum of 30 seconds. A specific epitope retrieval method was used using 10 mmol/L citrate buffer with distilled water in 1:10 ratio. For clusterin staining, we use

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davidine-biotin-peroxidase complex procedure in the IHC analysis (DakoCytomation, Copenhagen, Denmark). Then the tissue was stained by RDEFNab 01782 (Anticlusterin mouse monoclonal antibody) at dilution of 1:50. Appropriate positive and negative controls were included in each run of IHC. Negative controls were prepared by replacing the primary antibody with distilled water. Positive control for clusterin included sections of clusterin positive ovarian adenocarcinoma.

Clusterin scoring:

Both staining intensity and the percentage of positive cells were recorded. The immunohistochemical score was calculated by multiplying the proportion of immunopositive tumor cells and staining intensity score. The intensity of clusterin staining was coded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The proportion of immunopositive tumor cells was calculated as 0 (0%), 1 (1-25%), 2 (25-50%), 3 (50-75%), and 4 (>75%), indicating the percentage of positive staining tumor cells. A staining index (values 0-12) was obtained, and only samples with a final immuoexpression score of 6 were defined as 'overexpression.[12,16,17]

Statistical analysis:

The collected data were analyzed using the computerized software statistical package for the social sciences program (version 23). Using the Pearson Chi-square test, the association between clusterin expression and clinicopathological parameters was assessed. The significance level was set at ($P \leq 0.05$). All patients diagnosed with primary colorectal adenocarcinoma and who had undergone surgery had been selected; none of them had received chemotherapy or radiotherapy before surgery.

Results

In this study, 60 cases of colorectal cancer have been included. The patient's age ranged from 19-85 years with a mean age of 52.59 years ± 15.670 years, and the median age was 52 years. There were 24 males and 36 females with a female to male ratio of 1.5:1. The clinicopathological characteristics are summarized in Table 1.

Table 1: Numbers and percentages of different clinicopathological characteristics of the studied cases.

Variables	Categories	No. (%)
Age	< 50 years	37 (61.7)
	≥ 50 years	23 (38.3)
Gender	Male	24 (40)
	Female	36 (60)
Tumor site	Right	15 (25)
	Left	45 (75)
Tumor type	Non-mucinous	53 (88.3)
	Mucinous	7 (11.7)
Tumor grade	Well - moderate	50 (83.3)
	Poor	10 (16.7)
Nodal status	Positive	35 (58.3)
	Negative	25 (41.7)
Tumor stage	I - II	25 (41.7)
	III - IV	35 (58.3)
Total		60 (100)

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Twenty eight cases (46.7%) were labeled as clusterin positive, while 32 cases (53.3%) were negative, as shown in Figure 1. Clusterin expression was significantly associated with the tumor type being more expressed in non-mucinous type (P = 0.013) and tumor grade with more expression in well and moderately differentiated tumors (P = 0.033). Although clusterin expression was higher when the lymph nodes were positive and with higher stages, the association was not significant. No significant association was found between clusterin expression and other clinicopathological characteristics like age, gender, and tumor site, as shown in Table 2.

Table 2: Association of clusterin immunoexpression with clinicopathological characteristics.

Clinicopathological characteristic		Total No.	Clusterin positive (%)	Clusterin negative (%)	*P* value
Age	<50 years	37	19 (51.4%)	18(48.6)	0.793*
	≥50 years	23	10 (43.5)	13 (56.5)	
Gender	Male	24	12 (50)	12 (50)	0.793*
	Female	36	16 (44.4)	20 (55.6)	
Tumor site	Right colon	15	6 (40)	9 (60)	0.756*
	Left colon	45	22 (48.9)	23 (51.1)	
Tumor type	Non-mucinous	53	27 (51)	26 (49)	0.013*
	Mucinous	7	0 (0)	7 (100)	
Tumor grade	Well-moderately differentiated	50	25 (50)	25 (50)	0.033*
	Poorly differentiated	10	1 (10)	9 (90)	
Nodal status	Positive	35	18 (51.4)	17 (48.6)	0.439*
	Negative	25	10 (40)	15 (60)	
Tumor stage	Stage I and II	25	10 (40)	15 (60)	0.439*
	Stage III and IV	35	18 (51.4)	17 (48.6)	

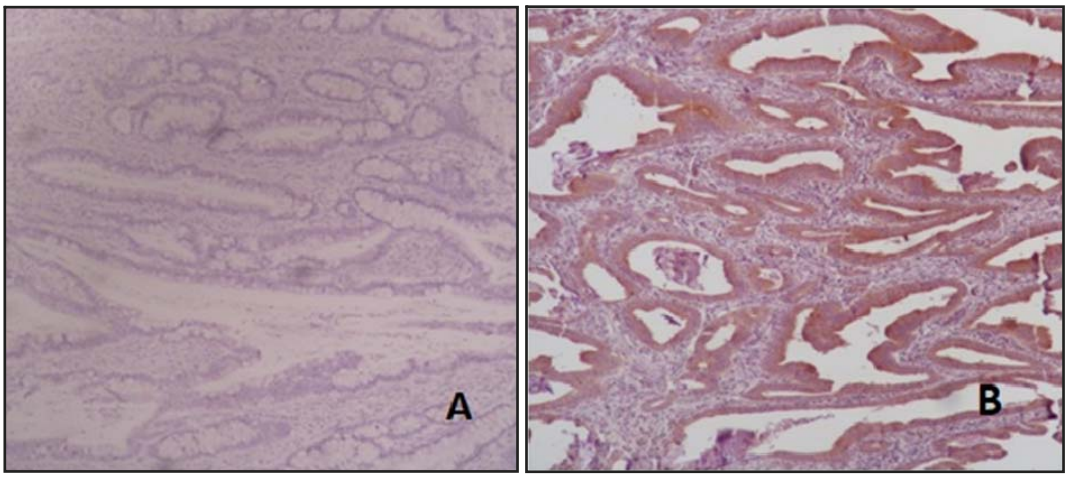

Figure 1: Clusterin immunoexpression. A. Negative clusterin immunoexpression (IHCx100). B. Postive clusterin immunoexpression (IHCx100).

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Discussion

Most colorectal cancer patients present with advanced stage disease at the time of diagnosis. However, the prognosis and the response to treatment are not the same among patients with the same stage.[18] New molecular markers are needed for early detection and diagnosis of colorectal cancer to improve therapeutic outcomes and predict prognosis. Clusterin has been regarded as a promising diagnostic and possibly a prognostic indicator of human colorectal cancer.[19] In the present study, 46.7% were labeled as positive for clusterin immunoexpression, while 53.3% were labeled as negative for clusterin immunoexpression. Our results were comparable to results from other studies.[12,20,21] In this study, the mean age of the patients was 52.59 ± 15.670 years. The highest frequency of cases was observed within patients ≥ 50 years (61.7% of patients were 50 years or above). Our results were similar to those obtained by Ali et al. from Iraq.[22-24] There was no significant association between clusterin immunoexperssion and age of the patient, which was similar to other studies.[12,20,21] There were 24 males, and 36 females with a female to male ratio of 1.5:1 with a slight female predominance which was similar to that obtained by Mahmood and Alrubaie from Iraq.[23,24] This slight female predominance was unlike other previous studies.[17,22,25] This may be attributed to age related difference in sex predominance as the male predominance may be age dependent as most of our patients were above 50 (61.7%). It might also be due to genetic and environmental factors or could be just by chance.[26] There was no significant correlation between gender and clusterin immunoexpression, which was similar to what had been observed by others.[12,17,20,21,25] Forty five (75%) cases had primary tumor of the left colon and rectum, while 15 (25%) cases had tumor in the right colon, which is compatible to the results obtained by other studies,[17,21-24] with no statistically significant association between clusterin immunoexpression and tumor location as in other studies.[12,16,17,21,25] Fifty three cases were non-mucinous adenocarcinoma (88.3%) while only 7 (11.7%) cases being diagnosed with mucinous adenocarcinoma, and the majority being well- moderately differentiated carcinoma (83.3%), while only 16.7 % had poorly differentiated tumor, this was in concordance with other studies.[17,20,21,27,28] There was a significant association between clusterin immmunoexpression and the histological type and the grade of colorectal cancer. None of the other similar studies correlated clusterin expression with histological type; however, this significant association in our study may be attributed to that most of our cases were of non-mucinous type, which was mostly well-moderately differentiated, and we stated that there was a significant correlation between histological grade and clusterin being mainly in well-moderately differentiated tumors. Gikas in his published article, showed a similar significant association between clusterin immunoexpression and gradeof the tumor. Similarly, the studies conducted by Kevans and Goma showed a higher expression of clusterin with well-moderately differentiated tumors without a statistical significance.[16,17,21] The current study indicated that the clusterin immunoexpression in the patients who had presented with lymph node metastasis were slightly more than those who did not present with lymph node metastasis with no statistically significant association with clusterin immunoexpression, our results were in alignment with the work done by Goma that showed no significant association.[21] Concerning the association of clusterin immunoexpression with the stage of the disease, it was slightly more expressed in stages III and IV of the disease than stages I and II. However, this association was statistically not significant. This result was in agreement with other studies that claimed that the immunoclusterin immunoexpression was

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associated with more advanced stages, indicating that clusterin is involved in colorectal cancer progression as several other types of carcinoma, including breast, prostate, kidney, pancreatic and ovarian cancers.[12,16,25,29,30] The possible discrepancy in associations among different studies may be due to differences in the sample size, the methods of antigen retrieval, the type of antibody used, or the methods of clusterin immunohistochemical scoring. This in addition to discrepancies among the pathologists in the evaluation of clusterin immunoexpression scoring.

Conclusion

Our study indicated that clusterin is expressed in colorectal carcinoma and is significantly associated with histological type and grade. These results suggest that this marker may play a role in colorectal carcinogenesis. Further studies are required to understand the possible mechanism of clusterin association with carcinogenesis and cancer progression.

Competing interests

The authors declare no competing interests.

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