MJMR, Vol. 34, No. 1, 2023, pages (289-295).

Open Access
ISSN:2682-4558

Research Article

Immunohistochemical Study of ALDH1 in Colorectal Adenocarcinoma

Nisreen Dahi Mohamed[1], Dalia Abdelhamid Hosny Abdelhafeez[1], Rehab Kamal Mohamed[1] and Manal Ismail Abd Elghany[1]
[1] Department of Pathology, Faculty of Medicine, Minia University, Egypt.

DOI: 10.21608/mjmr.2022.135834.1078

Abstract
Background: Colorectal cancer (CRC) is one of the most common tumors that has high frequency, and mortality rates, especially in the developing world. The stem cell marker ALDH1 plays an important role in initial differentiation and proliferation of stem cells. Expression of ALDH1 by cancer stem cells (CSCs) has been expressed in different types of malignant tumors. **Aim:** This work aimed to evaluate ALDH1 expression in CRC and to find the correlation between ALDH1 expression and different clinicopathological features. **Material and method:** This study included 60 formalin-fixed and paraffin-embedded different cases of CRC, in which ALDH1 immunostaining was performed for all cases. **Results:** There was a highly significant positive statistical correlation between ALDH1 expression in the studied cases, and lymph node metastasis, tumor infiltrating lymphocytes, modified dukes staging, and lymphovascular invasion (P-value ≤ 0.01). Also, there was a significant positive statistical correlation between ALDH1 expression, and both tumor site and TNM staging (P-value < 0.05). Other clinicopathological variables such as age, sex, tumor size, histopathological subtypes, and tumor grade showed no significant statistical correlation (P-value > 0.05). **Conclusion:** High expression of ALDH1 was associated with tumors of the colon more than the rectal tumors, lymph node metastasis, lympho-vascular invasion, and high tumor staging. Our results suggest that ALDH1 plays an important role in tumor invasiveness and nodal metastasis and consequently, it may work as a poor prognostic factor for CRC. Moreover, it may provide a useful clinical biomarker for tumor aggressiveness.

Keywords: Colorectal adenocarcinoma, ALDH1, stem cell marker.
Abbreviations: Colorectal carcinoma (CRC), Aldehyde dehydrogenases 1 (ALDH1), Cancer stem cells (CSCs), Tumor infiltrating lymphocytes (TILs).
All authors have no conflict of interest.

Introduction
Colorectal cancer (CRC) is considered as one of the most common tumors that has a remarkable increase in frequency, and mortality, especially in developing countries[(1)]. The global burden of CRC has been progressing with population, modifications in demographics, and westernization of daily lifestyle practices. In 2018, according to the World Health Organization (WHO) GLOBOCAN database, more than 18 million and 9 million new cancer cases and deaths that caused by CRC were documented, respectively[(2)].

In Egypt, CRC represents 35% of total gastrointestinal system malignancies and 6.49% of all malignancies [(3)]. CRC is the 7th most common cancer in Egypt, representing 3.47% of the male tumors and 3% of the female tumors. The estimated number of colon cancer patients (excluding rectal cancer) in 2015 was slightly more than three thousand [(4)]. The

median age is 24 years in Egypt compared to 37 years in US [5].

Aldehyde dehydrogenase 1 (ALDH1) is an enzyme responsible for catalyzing the synthesis of retinoic acid and is involved in the oxidation of several exogenous and endogenous aldehydes into their carboxylic acids [6]. ALDH1 is present on chromosome 12, and it has different isoforms, various cellular functions and tissue distributions [7]. ALDH1 is detected in the cytoplasm of normal non-cancerous epithelium of the large intestine and is localized in the bottom of normal crypts where stem cells are present. High epithelial expression of ALDH1 is strongly accompanied with ulcerative pathological lesions. This finding might add an evidence that ulcerative form is correlated with unfavorable prognosis. ALDH1 stromal cells might act as local guardians or protectors of tumor cells decreasing progression of tumor as they are significantly associated with smaller size of tumors. ALDH1 plays an important role as a prognostic and therapeutic factor for CRC [6,8,9].

The significance of ALDH1 protein expression in CRC remains unclear, and the present study aims to evaluate the immunoexpression of ALDH1 in CRC and to study the correlation between immunohistochemical expression of ALDH1 with variable clinicopathological parameters in patients with CRC.

Material and Methods

1. Tissue specimens

The present study comprised 60 formalin-fixed and paraffin embedded tissue blocks of cases of primary colorectal adenocarcinomas which were retrieved from the archives of histo-pathological laboratories of Minia University Hospital and Minya Oncology Center (in the period between December 2018 and January 2021). The cases included, 40 cases of conventional adenocarcinomas, 12 cases of mucinous adenocarcinoma and 8 cases of signet ring cell adenocarcinoma. The grade of differentiation in the cases with CRC was low grade in 15 cases and high grade in 45 cases. The available clinicopathological data included: patients' age, sex, tumor localization, tumor size, tumor type, tumor grade**,** stage, and lymph node status. Tumor type and grade were evaluated according to WHO criteria [10]. Tumor stage was estimated by TNM staging [11] and modified Dukes staging [12]**.**

2. Immunohistochemistry

Primary antibody against ALDH1: Polyclonal mouse antibody (100 µl, concentrated, Lab Vision Laboratories), diluted at (1:300). A positive and negative control slide were performed with each run. Positive control was normal hepatic tissue.

3. Scoring of Immunostaining

ALDH1 was expressed in the cytoplasm. Ten high power fields were counted per section in each case and the average of counted fields was calculated. The staining frequency of ALDH1 was semi-quantitatively scored based on the percentage of positive tumor cells immunoexpression using a four-score grading system. Cases with < 5% ALDH1-positive cells were given a score of (0), those with 5-20% ALDH1-positive cells were given a score as (1), those with 20-50% positive cells were scored as (2), and those with >50% positive cells were scored as (3). For statistical analysis, cases were divided into two groups: Low expression (scores of 0 & 1) and high expression (scores of 2 & 3) [13]. The median value (55%) was taken as a cut off point for ALDH1 expression, and the expression was divided into 2 categories; low expression above the median value and high expression below the median value [14-17]. For statistical purposes, negative cases are included among low expressions and considered as one category. Interestingly, we found no differences in the results between the two systems of scoring.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS software version 16). The Chi- square and Fisher's exact tests were used to compare categorical features.

Results

1. Clinicopathological Features

Data regarding different clinical and histopathological features for colorectal adenocarcinoma patients are summarized in **Table (1).**

Clinicopathological features	No. (%)
Age	
≥ 45	12 (20%)
> 45	48 (80%)
Gender	
Male	32 (53.3%)
Female	28 (46.7%)
Location	
Colon	38 (63.3%)
Rectum	22 (36.7%)
Tumor size	
>5	26 (43.3%)
≥5	34 (56.7%)
Histological subtypes	
Adenocarcinoma	40 (66.7%)
Mucinous carcinoma	12 (20%)
Signet ring cell carcinoma	8 (13.3%)
Tumor grade	
Grade I	14 (23.3%)
Grade II	24 (40%)
Grade III	22 (36.7%)
Nodal status	
Negative	40 (66.7%)
Positive	20 (33.3%)
Lymphovascular invasion	
Negative	16 (26.7%)
Positive	44 (73.3%)
TNM stage	
I	15 (25%)
II	14 (23.3%)
III	31 (51.7%)
Modified Dukes stage	
B1	16 (26.7%)
B2	15 (25%)
C1	10 (16.7%)
C2	19 (31.7%)
Tumor infiltrating lymphocytes	
Absent	2 (3.3%)
Mild	36 (60%)
Moderate	3 (5%)
Marked	19 (31.7%)

2. Immunohistochemical expression of cytoplasmic ALDH1 and its correlation with patients' clinicopathological features

Table (2): Correlation between cytoplasmic ALDH1 expression and clinicopathological features for patients with CRC (n=60)

Clinicopathological features	*Total* 60 (100%)	Cytoplasmic ALDH1 expression		*P* value
		Low expression (25%)	High expression (75%)	
Age				
≥45	12 (20)	4 (33.3)	8 (66.7)	0.456
>45	48 (80)	11 (22.9)	37 (77.1)	
Gender				
Male	32 (53.3)	5 (15.6)	27 (84.4)	0.067
Female	28 (46.7)	10 (35.7)	18 (64.3)	
Location				
Colon	38 (63.3)	6 (15.8)	32 (84.2)	0.03*
Rectum	22 (36.7)	9 (40.9)	13 (59.1)	
Tumor size				
>5	26 (43.3)	9 (34.6)	17 (65.4)	0.133
≥5	34 (56.7)	6 (17.6)	28 (82.4)	
Histological subtypes				
Adenocarcinoma	40 (66.7)	11 (27.5)	29 (72.5)	
Mucinous carcinoma	12 (20)	3 (25)	9 (75)	0.67
Signet ring cell carcinoma	8 (13.3)	1 (12.5)	7(87.5)	
Tumor's grade				
Grade I	14 (23.3)	4 (28.6)	10 (71.4)	
Grade II	24 (40)	7 (29.2)	17 (70.8)	0.65
Grade III	22 (36.7)	4 (18.2)	18 (81.8)	
Nodal status				
Negative	40 (66.7)	14 (35)	26 (65)	0.011*
Positive	20 (33.3)	1 (5)	19 (95)	
Lymphovascular invasion				
Negative	16 (26.7)	9 (56.2)	7 (43.8)	0.001*
Positive	44 (73.3)	6 (13.6)	38 (86.4)	
TNM stage				
I	15 (25)	2 (13.3)	13 (86.7)	
II	14 (23.3)	7 (50)	7 (50)	0.043*
III	31 (51.7)	6 (19.4)	25 (80.6)	
Modified Dukes stage				
B1	16 (26.7)	10 (62.5)	6 (37.5)	
B2	15 (25)	2 (13.3)	13 (86.7)	0.001*
C1	10 (16.7)	0 (0)	10 (100)	
C2	19 (31.7)	3 (15.8)	16 (84.2)	
Tumor infiltrating lymphocytes	2 (3.3)	0 (0)	2 (100)	
Absent	36 (60)	1 (2.8)	35 (97.2)	0.001*
Mild	3 (5)	2 (66.7)	1 (33.3)	
Moderate	19 (31.7)	12 (63.2)	7 (36.8)	
Marked				

**** P - value > 0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.***

In the current study, a statistically significant correlation was detected between cytoplasmic ALDH1 expression and the tumor site in the studied cases (P = 0.03). Also, a statistically significant correlation was detected with lymph node metastasis (P= 0.011), lymphovascular invasion (P = 0.001), advanced tumor stage assessed by both TNM staging (P = 0.043), modified Dukes staging (P = 0.001), and with tumor infiltrating lymphocytes (P = 0.001).

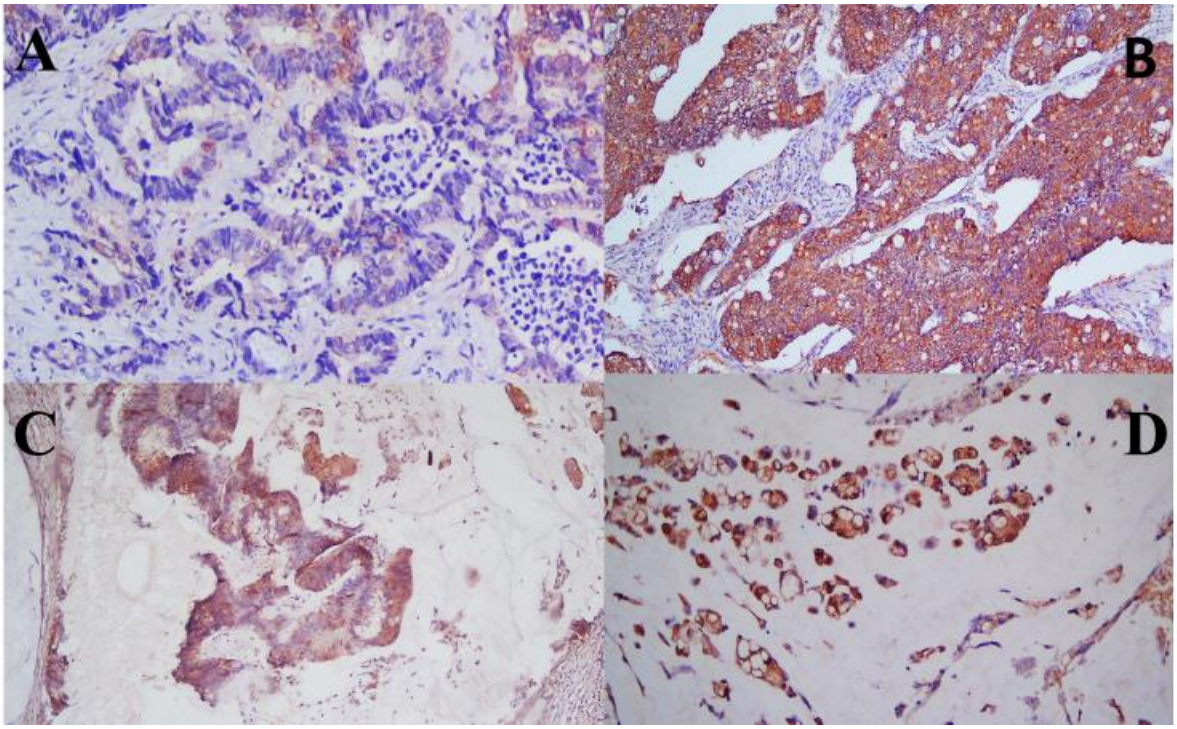

Figure (1) A: Colorectal adenocarcinoma, grade I showing low cytoplasmic expression of ALDH1 (IHC, ×400). **B**: Colorectal adenocarcinoma, grade III showing high cytoplasmic expression of ALDH1 (IHC, ×200). **C**: Mucinous adenocarcinoma showing high cytoplasmic expression of ALDH1 (IHC, ×400). **D**: Signet ring adenocarcinoma showing high cytoplasmic expression of ALDH1 (IHC, ×200).

Discussion

In the present study, 75% of CRC cases showed high cytoplasmic ALDH1 expression, while 25% showed low expression. These results were close to Yang et al. and van der Waals et al., [18,19].

In the current study, there was a statistically significant correlation between ALDH1 expression and tumor localization (P= 0.03). This was in concordance with a study done by Goossens-Beumer et al., who found that high ALDH1 expression was associated with colonic tumors rather than the rectal tumors[14].

Interestingly, the present study has showed a statistically significant correlation between ALDH1 positivity and lymphovascular invasion (P=0.001). This result was similar to a study in CRC that performed by Holah et al., who detected that there was strong statistical correlation between ALDH1 expression and vascular invasion [13].

In the present study, we found a significant statistical correlation between ALDH1 expression and lymph node metastasis, (P=0.011) and such result was in agreement with that of other studies done by Holah et al., Said et al., who found that 42.9%, and 66% of cases with nodal metastasis showed positive ALDH1 expressing cells respectively [13-20].

In our study, there was a highly significant statistical correlation between ALDH1 expression, TNM (P = 0.043), and modified

Dukes stages (P = 0.001). (80.6%) of cases with stage III TNM, (100%) of C1, and (84.2%) of C2 showed high expression. These results were in line with those of [21]. On the contrary, a previous study performed by Wangshuo et al., indicated no significant relationship between ALDH1 expression and TNM stage. Such variation in the results may have emerged from the different populations, variable diet habits, different sample size, age range, different used antibodies, variable scoring methods, as well as the possible ALDH1 gene mutations in the studies [19].

Regarding TILs, we found a strong statistical correlation between the TILs and ALDH1 expression, in which marked TILs are associated with low ALDH1 expression, (P=0.001) and this agreed with [21]. It was documented that a high level of TILs is an independent prognostic factors for favorable clinical outcome in breast cancer. These findings suggest that TILs are the key component for pathological complete response (pCR) and chemosensitivity[22].

Conclusion and recommendations

In conclusion, high ALDH1 expression was associated with colonic tumors more than the rectal ones, lymphovascular invasion, advanced tumor staging, and lymph node metastasis in CRC. ALDH1 as a stem cell marker may play an essential role in tumor invasion and lymph node metastasis and may be a valuable marker for CRC prognosis, since being associated with poor prognostic features and shorter survival outcome. Inclusion of large number of young age in the studied cases is recommended to determine the correlation between young age and tumor's aggressiveness. Larger number of studied cases, including pre malignant lesions, benign lesions and carcinoma insitu, is recommended to confirm the correlation between ALDH1 expression and different clinicopathological data and as a poor prognostic indicator. Further studies using different molecular methods on ALDH1, are suggested to detect the mechanisms by which ALDH1 may contribute to the aggressiveness, invasion, and progression of CRC.

References

1. Granados-Romero, J. J., A. I. Valderrama-Treviño, E. H. Contreras-Flores, B. Barrera-Mera, M. Herrera Enríquez, K. Uriarte-Ruíz, J. Ceballos-Villalba, A. G. Estrada-Mata, C. Alvarado Rodríguez and G. Arauz-Peña. "Colorectal cancer: a review." Int. J. Res. Med. Sci, 2017: **5**(11); 4667-4676.
2. Wong, M. C., H. Ding, J. Wang, P. S. Chan and J. Huang. "Prevalence and risk factors of colorectal cancer in Asia." Intestinal research, 2019: **17**(3); 317-325.
3. Zaghloul, A. S. and A. M. Mahmoud. "Preliminary results of robotic colorectal surgery at the National Cancer Institute, Cairo University." Journal of the Egyptian National Cancer Institute, 2016: **28**(3); 169-174.
4. Metwally, I. H., M. Shetiwy, A. F. Elalfy, A. Abouzid, S. S. Saleh and M. Hamdy. "Epidemiology and survival of colon cancer among Egyptians: a retrospective study." Journal of Coloproctology (Rio de Janeiro), 2018: **38**; 24-29.
5. Atef, N., N. Alieldin, G. Sherif, I. Loay, A. M. Mahmoud and G. Mohamed. "Microsatellite instability and lifestyle factors in sporadic colorectal cancer." Asian Pacific journal of cancer prevention: APJCP, 2020: **21**(5); 1471-1479.
6. Rezaee, M., E. Gheytanchi, Z. Madjd and M. Mehrazma. "Clinicopathological Significance of Tumor Stem Cell Markers ALDH1 and CD133 in Colorectal Carcinoma." Iranian Journal of Pathology, 2020: **16**(1); 40-50.
7. Vassalli, G.. "Aldehyde dehydrogenases: not just markers, but functional regulators of stem cells." Stem cells international, **2019:** Vol 2019, ID 3904645; 1-15.
8. Mohamed, S. Y., R. M. Kaf, M. M. Ahmed, A. Elwan, H. R. Ashour and A. Ibrahim. "The prognostic value of cancer stem cell markers (Notch1, ALDH1, and CD44) in primary colorectal carcinoma." Journal of gastrointestinal cancer, 2019: **50**(4); 824-837.
9. Vermani, L., R. Kumar, R. R. Kannan, M. K. Deka, A. Talukdar and N. S. Kumar. "Expression pattern of ALDH1, E-cadherin, Vimentin and Twist in early and late onset sporadic colorectal cancer." Biomarkers in Medicine, 2020: **14**(14); 1371-1382.
10. Brockmoeller, S. F. and N. P. West. "Predicting systemic spread in early

colorectal cancer: Can we do better?" World journal of gastroenterology, 2019: **25**(23); 2887-2895.
11. Kim, H. J. and G. S. Choi. "Clinical implications of lymph node metastasis in colorectal cancer: current status and future perspectives." Annals of Coloproctology, 2019: **35**(3); 109-117.
12. Mohd, Y., B. Balasubramanian, A. Meyyazhagan, H. Kuchi Bhotla, S. K. Shanmugam, M. K. Ramesh Kumar, M. Pappusamy, K. K. Alagamuthu, S. Keshavarao and V. A. Arumugam. "Extricating the association between the prognostic factors of colorectal cancer." Journal of gastrointestinal cancer, 2021: **52**(3); 1022-1028.
13. Holah, N. S., H. A.-E.-S. Aiad, N. Y. Asaad, E. A. Elkhouly and A. G. Lasheen. "Evaluation of the role of ALDH1 as cancer stem cell marker in colorectal carcinoma: an immunohistochemical study." Journal of clinical and diagnostic research: JCDR, 2017: **11**(1); EC17.
14. Goossens-Beumer, I., E. Zeestraten, A. Benard, T. Christen, M. Reimers, R. Keijzer, C. Sier, G. Liefers, H. Morreau and H. Putter. "Clinical prognostic value of combined analysis of Aldh1, Survivin, and EpCAM expression in colorectal cancer." British journal of cancer, 2014: **110**(12); 2935-2944.
15. Ayub, T. H., M.-D. Keyver-Paik, M. Debald, B. Rostamzadeh, T. Thiesler, L. Schroeder, W. Barchet, A. Abramian, C. Kaiser and G. Kristiansen "Accumulation of ALDH1-positive cells after neoadjuvant chemotherapy predicts treatment resistance and prognosticates poor outcome in ovarian cancer." Oncotarget, 2015: 6(18); 16437-16448.
16. Padthaisong, S., M. Thanee, N. Namwat, J. Phetcharaburanin, P. Klanrit, N. Khuntikeo, A. Titapun, S. Sungkhamanon, H. Saya and W. Loilome "Overexpression of a panel of cancer stem cell markers enhances the predictive capability of the progression and recurrence in the early stage cholangiocarcinoma." Journal of translational medicine, 2020: **18**(1); 1-17.
17. Shyshkin, M. and T. Khrystenko "Epithelial-mesenchymal transition and stem cells in colorectal cancer progression." Journal of Education, Health and Sport, 2020:10(10); 201-211.
18. van der Waals, L. M., I. H. Borel Rinkes and O. Kranenburg. "ALDH1A1 expression is associated with poor differentiation,'right-sidedness' and poor survival in human colorectal cancer." PloS one, 2018: **13**(10); e0205536.
19. Yang, W., Y. Wang, W. Wang, Z. Chen and G. Bai. "Expression of aldehyde dehydrogenase 1A1 (ALDH1A1) as a prognostic biomarker in colorectal cancer using immunohistochemistry." Medical science monitor: international medical journal of experimental and clinical research, 2018: **24**; 2864-2872.
20. Said, E. M., H. M. Abostate, N. M. Emara and H. A. Agina. "The significance of Aldehyde dehydrogenase 1A1 expression in colorectal carcinoma." Benha Medical Journal, 2021: 39;1-18.
21. Mahmood, N. A., Z. S. Abdulghany and I. M. Al-Sudani. "Expression of aldehyde dehydrogenase (ALDH1) and ATP Binding Cassette Transporter G2 (ABCG2) in Iraqi patients with colon cancer and the relation with clinicopathological features." International journal of molecular and cellular medicine, 2018: **7**(4); 234-241.
22. Kinoshita, T. and Y. Kawakami. "Interface of cancer stem cells and cancer immunity." Annals of Translational Medicine, 2020: **8**(13); 810-819.