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Original article

The Significance of Aldehyde Dehydrogenase 1A1 Expression in Colorectal Carcinoma

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Abstract:

Background: Colorectal carcinoma (CRC) is the third most common cancer in the world constituting about 10% of all cancers**.** ALDH1A1 has been used as a stem cell marker, and plays important functional roles in stem cells. Expression of ALDH1A1 by cancer stem cells (CSCs) has been demonstrated in multiple types of cancers. **Aim:** This work aimed to evaluate ALDH1A1 expression in colorectal adenocarcinoma and determine the relation between ALDH1A1 expression and different clinico-pathological variables. **Material and method:** This is a selected retrospective study which included 50 different cases of colorectal adenocarcinoma. ALDH1A1 immunostaining was performed for all cases. **Results:** There was a highly significant statistical difference between ALDH1A1 expression in studied cases according to tumor site, lymph node metastasis and TNM stage (P value <0.01). Also, there was a significant statistical difference according to lymphovascular and perineural invasion (P value <0.05). Other clinico-pathological variables such as age, sex, tumor size, histopathological subtype, tumor grade, depth of tumor invasion and distant metastasis showed no significant statistical difference (P value >0.05). **Conclusion:** High ALDH1A1 expression was associated with right sided tumors, lymphovascular invasion, perineural invasion and lymph node metastasis in colorectal carcinoma. ALDH1A1 may play an important role in tumor invasion and lymph node metastasis and may work as a useful marker for prognosis of CRC.

Keywords: Colorectal adenocarcinoma, ALDH1A1, stem cell marker.

Abbreviations: Colorectal carcinoma (CRC), Aldehyde dehydrogenases 1A1 (ALDH1A1), cancer stem cells (CSCs).

Introduction:

Colorectal carcinoma (CRC) is the third most common cancer in the world, constituting about 10% of all cancers. It is the second most common cause of cancer-related deaths throughout the world with 1.8 million new cases and almost 881,000 deaths in 2018 **(1).**

In Egypt, according to National cancer institute registry, Cairo University, colorectal carcinoma constitutes 35% of total gastrointestinal tract malignancies and 6.49% of all malignancies **(2).**

Life style factors associated with risk of CRC include smoking, alcohol intake, high red meat and processed meat consumption, high fat and protein diet intake, physical inactivity, and overweight. According to the National Institute for Clinical Excellence, about three quarters of CRC cases are associated with the population lifestyle **(3).**

Clinicopathological parameters play an important role in management of CRC. However, they are usually not reliable predictors of prognosis **(4).** Therefore, studying novel biomarkers, which have the potential value of serving as prognostic markers and new therapeutic targets, are still a clinical problem to be solved **(5).**

Cancer stem cells (CSCs) are cancer cells that are responsible for initiation, progression, metastasis, and recurrence of cancer. CSC theory in colorectal carcinoma has been investigated and several stem cell markers have been studied **(6).**

Aldehyde dehydrogenase 1A1 (ALDH1A1), a main member of the Aldehyde dehydrogenase (ALDH) superfamily, catabolizes the oxidation of intracellular aldehydes, and it has a critical role in stem cell differentiation and protection **(7).**

Colorectal cancers are molecularly and histologically heterogeneous, reflecting different pathways of carcinogenesis **(8).**

The correlation between increased enzymatic activities of Aldehyde dehydrogenase with cancer stem cells properties has been shown in a wide spectrum of malignancies. Expression of ALDH1A1 by cancer stem cells has been demonstrated in multiple types of cancers such as stomach, breast and lung cancer **(9).**

However, the significance of ALDH1A1 protein expression in colorectal carcinoma remains unclear and the study aims to

evaluate the significance of its expression in Egyptian colorectal carcinoma cases.

Material and methods:

Study Groups:

This selected retrospective study included 50 different cases of colorectal adenocarcinoma designated as; 38 cases of conventional adenocarcinoma, 6 cases of mucinous adenocarcinoma and 6 cases of signet ring cell carcinoma (all cases were right or left colectomy specimens). Six control cases were taken from viable margins in patients with intestinal infarction. Sections of normal liver tissue (adjacent liver tissue to cholecystectomy specimen) were taken as a positive control for ALDH1A1 immunohistochemical expression.

The material included archival formalin fixed paraffin embedded blocks processed during the years 2015-2020 as well as stained Hematoxylin and Eosin (H&E) slides for review. The blocks were retrieved from Pathology Department and Early Cancer Detection Unit archives; 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. The study was approved by the Research Ethics committee of Faculty of Medicine, Benha University, Egypt.

Histopathological studies:

Re-evaluation of sections from all selected cases, unaware of their diagnosis, was performed. The cases were re-evaluated for their subtype and graded into well differentiated, moderately differentiated, and poorly differentiated tumors **(10)**. Lymph node status was evaluated and TNM staging system was applied to the cases according to AJCC, 8th edition **(11, 12).**

ALDH1A1 immunohistochemical study:

Slides were immunostained according to manufacturer's instructions with ALDH1A1 rabbit polyclonal antibody (Chongqing biopsies co., Cat No YPA1390, China, conc) at a dilution of 1:50, at room temperature 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 10 mmol/L citrate monohydrate buffer (pH 6.0) and heating for 15 minutes in the microwave. Freshly prepared chromogen diaminobenzine (DAB, Envision ^{TM} Flex /HRP-Dako, REF K 8000) was used. Negative (cold Phosphate- buffered Saline)

and positive control (normal liver tissue) were enclosed in each run **(13, 14).**

Interpretation of ALDH1A1 expression:

Positivity was considered as brownish homogenous cytoplasmic staining of tumor cells **(15).** The immunohistochemical scores were obtained by light microscopy (Olympus, Tokyo, Japan) as the staining intensity (scored from 0–3) multiplied by the percentage of positive cells within 5 high power fields (in hot areas) (scored from 0–4). The intensity of ALDH1A1 protein expression was scored as: 0 (no staining); 1 (weak staining); 2 (moderate staining); or 3 (strong staining). The percentage of positive cells was scored as: 0 (<5%); 1 (5–25%); 2 (26–50%); 3 (51–75%); or 4 (>75%). The cut-off value for high versus low expression of the ALDH1A1 protein was determined using receiver-operating characteristic (ROC) curve analysis and SPSS statistical software, defining a final immunostaining score of >3.5 as high ALDH1A1 protein expression **(16) (figure 1, table 5).**

Statistical analysis:

Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation (SD). Chi square test (χ^2), or Fisher's exact test were used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05, using Student "t" test if normally distributed, or Man-Whitney *U* test and Kruskal-Wallis test if not normally distributed for analyzing the difference. Receiver-operating characteristic (ROC) curve was used to predict sensitivity, specificity and accuracy of immunohistochemical score to detect lymph node invasion. Differences were considered significant at a calculated P value of <0.05. Statistical analysis was performed using SPSS version 25 (SPSS Inc, Chicago, IL, USA).

Results:

Demographic and clinical parameters:

This study was carried upon 50 cases of colorectal adenocarcinoma, 28 cases (56%) were males and 22 cases (44%) were females. The age of studied cases ranged between 30- 80 years with the mean age 55±13.7 years. 23 cases (46%) aged < 55 years and 27 cases (54%) aged ≥ 55 years.

Histopathological results:

Thirty-six cases (72%) were located in the right colon and 14 cases (28%) were located in the left colon. Tumor size ranged from 3cm to 12cm in the largest dimension,

with mean size 6.5 cm (Mean ±SD = 6.5±2.2). Twenty six cases (52%) were < 6.5 cm, while 24 cases (48%) were ≥ 6.5 cm. As regard depth of tumor invasion, 2 cases (4%) were T2, 21 cases (42%) were T3 and 27 cases (54%) were T4. Lymphovascular invasion was detected in 39 cases (78%), while no lymphovascular invasion was detected in 11 cases (22%). Perineural invasion was detected in 17 cases (34%), while no perineural invasion was detected in 33 cases (66%). Regional lymph node metastasis was detected in 33 cases (66%), while 17 cases (34%) were free of regional lymph node metastasis (N0).

Table 1: Comparison between histopathological subtypes according to the grade of studied cases.

Histopathological subtype	Grade I (N=3)	Grade II (N=33)	Grade III (N=14)	Test of significance	P-value
Conventional adenocarcinoma (N=38)	3 (7.9%)	31 (81.6%)	4 (10.5%)		
Mucinous adenocarcinoma (N=6)	0	2 (33.3%)	4 (66.7%)		
Signet ring cell carcinoma (N=6)	0	0	6 (100%)	FET= 22.7	<0.001**

N, number; FET, fisher exact test, ** highly significant.

Table 2: Comparison between histopathological subtypes according to the stage of studied cases.

Histopathological subtype	Stage II (N=15)	Stage III (N=28)	Stage IV (N=7)	Test of significance	P-value
Conventional adenocarcinoma (N=38)	**15 (39.4%)**	**21 (55.3%)**	**2 (5.3%)**		
Mucinous adenocarcinoma (N=6)	**0**	**4 (66.7)**	**2 (33.3)**	**FET= 12.5**	**0.005****
Signet ring cell carcinoma (N=6)	**0**	**3 (50%)**	**3 (50%)**		

N, number; FET, fisher exact test, ** highly significant.

Table 3: Comparison between the grades regarding the stage of studied cases (TNM).

Tumor grade	Stage II (N=15)	Stage III (N=28)	Stage IV (N=7)	Test of significance	P-value
Grade I (N=3)	2 (66.7%)	0	1 (33.3%)		
Grade II (N=33)	11 (33.3%)	19 (57.6%)	3 (9.1%)	FET= 6.98	0.095
Grade III (N=14)	2 (14.3%)	9 (64.3%)	3 (21.4%)		

N, number; FET, fisher exact test

Immunohistochemical results:

- In apparently normal colorectal mucosa (control cases), ALDH1A1 displayed a heterogeneous pattern, ranging from no staining at all to uniform staining throughout the whole crypts **(fig. 2-a).** These findings were in agreement with other study in CRC **(17).**

- ALDH1A1 expression in studied CRC cases:

Out of 50 cases of CRC, 24 cases (48%) showed low ALDH1A1 expression and 26 cases (52%) showed high expression **(fig. 2-b, c, d, e and f).**

Statistical analysis was performed on the relation between ALDH1A1 expression in studied CRC cases and clinico-pathological variables. It revealed a highly significant statistical difference between ALDH1A1 expression in studied cases according to tumor site, lymph node metastasis and TNM stage (P value = 0.007, 0.001 and <0.001 respectively). It also revealed a significant statistical difference according to lymphovascular and perineural invasion (P value = 0.011 and 0.013 respectively). Other clinico-pathological variables showed no significant statistical difference **(table 4).**

Table (4): Comparison between ALDH1A1 expression in studied cases according to clinico-pathological features:

Clinico-pathological features		Total	ALDH1A1 expression		P-value
			Low expression	High expression	
Age (years) (mean= 55±13.7)	**<55**	23	11 (47.8%)	12 (52.2%)	0.982
	≥55	27	13 (48.1%)	14 (51.9%)	
Gender	**Male**	28	13 (46.4%)	15 (53.6%)	0.802
	Female	22	11 (50%)	11 (50%)	
Tumor site	**Right colon**	36	13 (36.1%)	23 (63.9%)	0.007**
	Left colon	14	11 (78.6%)	3 (21.4%)	
Tumor size (Mean = 6.5±2.2)	**<6.5 cm**	26	15 (57.7%)	11 (42.3%)	0.153
	≥6.5 cm	24	9 (37.5%)	15 (62.5%)	
Tumor subtype	**Conventional adenocarcinoma**	38	20 (52.6%)	18 (47.4%)	0.573
	Mucinous adenocarcinoma	6	2 (33.3%)	4 (66.7%)	
	Signet ring cell carcinoma	6	2 (33.3%)	4 (66.7%)	
Tumor grade	**Grade I**	3	3 (100%)	0	0.065
	Grade II	33	17 (51.5%)	16 (48.5%)	
	Grade III	14	4 (28.6%)	10 (71.4%)	
Depth of tumor invasion (T)	**T2**	2	1 (50%)	1 (50%)	0.194
	T3	21	13 (61.9%)	8 (38.1%)	
	T4	27	10 (37%)	17 (63%)	
Lymphovascular invasion	**Present**	39	15 (38.5%)	24 (61.5%)	0.011*
	Absent	11	9 (81.8%)	2 (18.2%)	
Perineural invasion	**Present**	17	4 (23.5%)	13 (76.5%)	0.013*
	Absent	33	20 (60.6%)	13 (39.4%)	
Lymph node metastasis (N)	**N0**	17	14 (82.4%)	3 (17.6%)	0.001**
	N1	14	6 (42.9%)	8 (57.1%)	
	N2	19	4 (21.1%)	15 (78.9%)	
Distant metastasis (M)	**M0**	43	19 (44.2%)	24 (55.8%)	0.239
	M1	7	5 (71.4%)	2 (28.6%)	
Tumor stage (TNM)	**Stage II**	15	13 (86.7%)	2 (13.3%)	<0.001**
	Stage III	28	6 (21.4%)	22 (78.6%)	
	Stage IV	7	5 (71.4%)	2 (28.6%)	

ALDH1A1, Aldehyde dehydrogenase 1A1; * significant; ** highly significant.

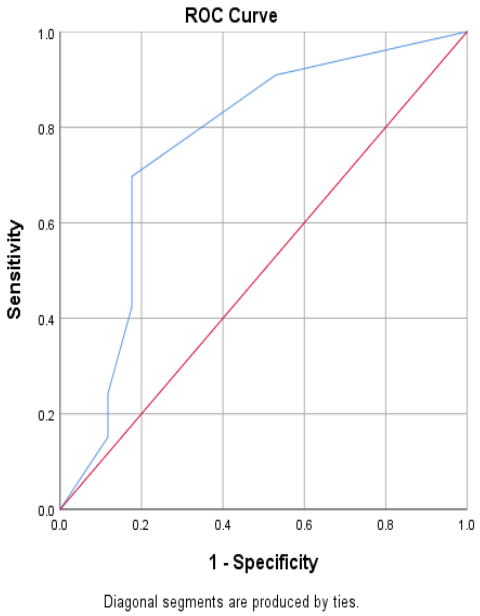

Figure (1): Receiver-operating characteristic curve analysis to determine cut-off score for ALDH1A1 expression.

Table (5): Validity of immunohistochemical score in prediction of lymph node metastasis.

Immunohistochemical score	Positive LN metastasis (33)		Negative LN metastasis (17)		Statistical test (x^2)	P value
	No	%	No	%		
Positive	**23**	**69.7**	**3**	**17.6**	**12.18**	**<0.001****
Negative	**10**	**30.3**	**14**	**82.4**		
AUC(95%CI)	**0.761 (0.604-0.918)**					
Cut-off point	**3.5**					
Sensitivity	**69.7**					
Specificity	**82.4**					
PPV	**88.5**					
NPV	**58.3**					
Accuracy	**74.0**					

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; LN, lymph node.

Receiver-operating characteristic (ROC) curve was used to predict sensitivity, specificity and accuracy of immunohistochemical score to detect lymph node metastasis. Sensitivity was 69.7%, specificity was 82.4% and accuracy was 74%.

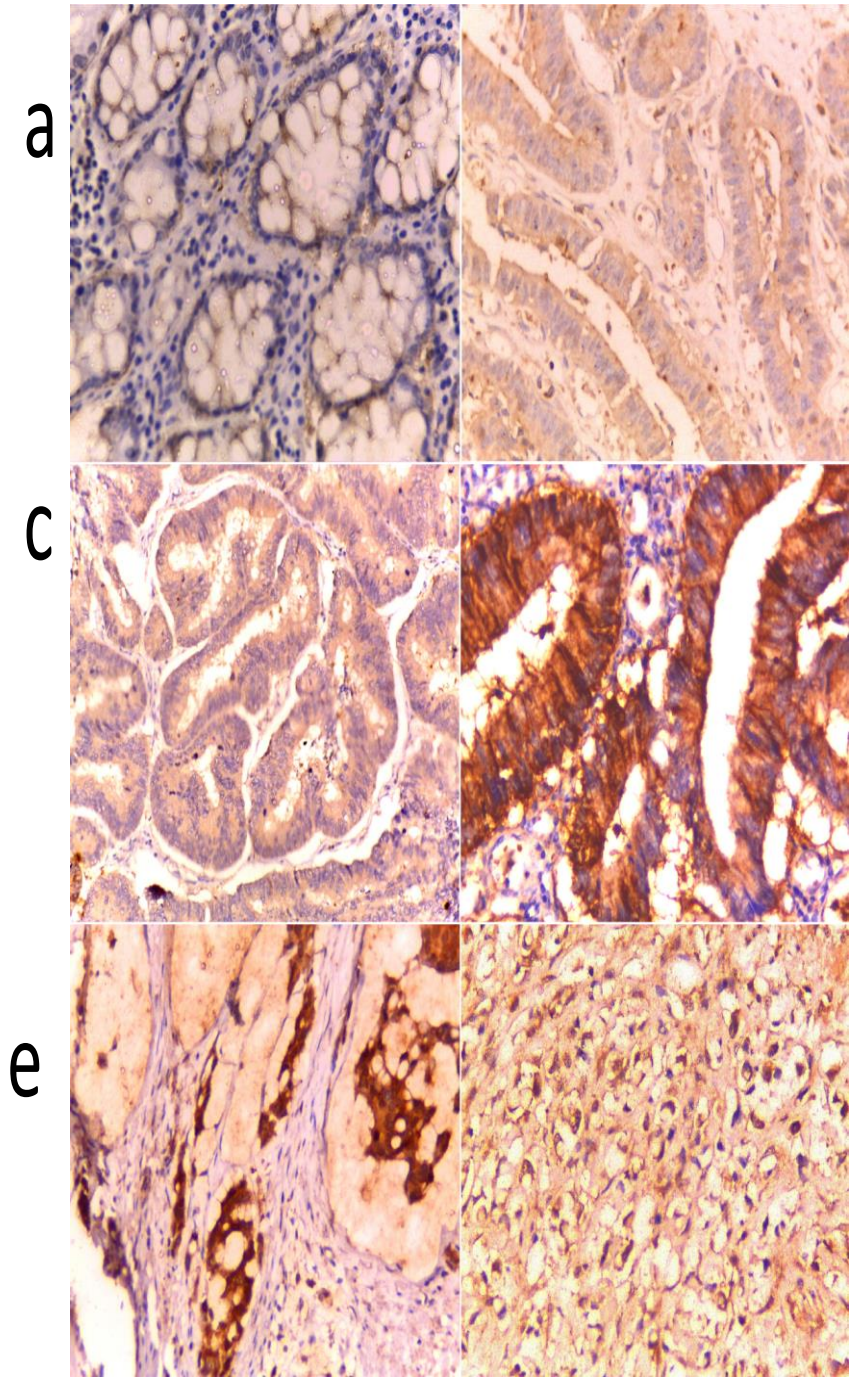

Figure (2): a- Normal colonic mucosa with scattered epithelial cells showing weak cytoplasmic staining of ALDH1A1 (score 2) (ABC X400). b- Adenocarcinoma, grade I showing weak cytoplasmic staining of ALDH1A1 (score 3) (ABC X400). c- Adenocarcinoma, grade II showing moderate cytoplasmic staining of ALDH1A1 (score 6) (ABC X200). d- Adenocarcinoma, grade II showing strong cytoplasmic staining of ALDH1A1 (score 12) (ABC X400). e- Mucinous adenocarcinoma, showing strong cytoplasmic staining of ALDH1A1 (score 9) (ABC X400). f- Signet ring cell carcinoma, showing moderate cytoplasmic staining of ALDH1A1 (score 8) (ABC X400).

Discussion:

Colorectal carcinoma is one of the most common cancers worldwide and represents one of the major causes of cancer related deaths throughout the world **(1).**

In our study, there was a highly significant statistical difference between histopathological subtypes according to the grade and stage of studied cases.

Regarding grade, 81.6% of conventional adenocarcinoma cases were grade II while 66.7% of mucinous adenocarcinoma and all signet ring cell carcinoma cases were grade III as signet ring cell carcinoma is a poorly differentiated tumor by definition **(18).** Regarding stage, conventional adenocarcinoma cases were mainly stage II and III while mucinous and signet ring carcinoma were mainly stage III and IV. These results were matched with **Nitsche et al., (19)**.

In accordance with many studies, mucinous adenocarcinoma and signet ring cell carcinoma had more advanced stages **(20, 21)** and higher grade **(21, 22).** These findings were more demonstrated with signet ring cell carcinoma than with mucinous adenocarcinoma. This may be explained by the more aggressive tumor biology of mucinous adenocarcinoma and particularly signet ring cell carcinoma when compared with conventional adenocarcinoma (**19, 23).**

Comparison between the grades regarding the stage of studied cases was also performed with no statistically significant difference. Sixty-six and seven tenths percent of grade I cases were stage II, while 57.6% of grade II and 64.3% of grade III cases were stage III. This may suggest high grade tumors are usually associated with lymph node metastases (stage III). In contrast, low-grade tumors have fewer lymph node metastases.

These results were in agreement with a study performed by **Derwinger et al., (24)** on 1239 cases of CRC, who found that high grade tumors were associated with presence of lymph node metastasis with a larger number of positive lymph nodes. The tumor grade in their study was significantly correlated with the overall TNM stage. The significant correlation may be due to the larger number of cases in their study.

ALDH1A1 has been used as a stem cell marker, and plays important functional roles in stem cells **(9).**

In the current study, 48% of studied CRC cases showed low ALDH1A1 expression

and 52% showed high expression. These results were close to those of **Yang et al., (16)** and **van der Waals et al., (15)**.

In this study, there was a highly significant statistical difference between tumor site and ALDH1A1 expression. In right colon cases, 63.9% showed high expression while 78.6% of left colon cases showed low expression.

These results were matched with **van der Waals et al., (15)** who found that ALDH1A1 expression in untreated right-sided tumors was significantly higher (3.8-fold) when compared to left-sided tumors and **Holah et al., (25)** who found a statistically significant association between ALDH1 positivity and right sided tumors. They explained that right-sided tumor location is a negative prognostic variable in CRC. Although the exact mechanisms are not clear, differences in clinical, chromosomal and molecular characteristics may play a role in the poor prognosis of these tumors. They are more likely to have high-grade histology and advanced tumor stage at initial presentation compared with left sided tumors. This may predict that increased ALDH1A1 expression in right side might be associated with poor prognosis **(26).**

In contrast, **Yang et al., (16)** found insignificant statistical correlation between ALDH1A1 expression and tumor site. This may be due to different geographic and genetic variability between races because the study was performed on Chinese patients.

Regarding tumor size, 57.7% of cases with tumor size <6.5cm showed low ALDH1A1 expression while 62.5% of cases with tumor size ≥ 6.5cm showed high expression. However, it didn`t reach a statistical significance value. This agrees with other studies carried out on CRC **(16)** and gastric carcinoma **(27).** The increased expression in larger tumor sizes may be explained by the role of ALDH1A1 in increasing tumor growth and cancer stem cell proliferation. ALDH1A1 promotes tumor growth through oxidation of aldehydes and reduction of NAD+ to NADH by using glutathione (GSH) and dihydrolipoic acid (DHLA) as electron donors **(28).**

Regarding histopathological subtype, 66.7% of both mucinous adenocarcinoma and signet ring cell carcinoma cases showed high ALDH1A1 expression while 52.6% of conventional adenocarcinoma cases showed low expression. There was statistically insignificant difference . These results were in agreement with **Holah et al., (25)** in their study on 49 cases of CRC, who found insignificant statistical association between

ALDH1 expression and histopathological subtype with 88.9 % of mucoid carcinoma cases showing positive expression. The increased expression in mucinous and signet ring carcinoma cases may be related to high grade and advanced tumor stage of these tumors.

The current study has shown that 100% of grade I and 51.5% of grade II cases showed low expression while 71.4% of grade III cases showed high expression. However, it didn`t reach a statistical significance value. These results were in line with **van der Waals et al., (15)** and **Lugli et al., (29)** in their studies in CRC cases and **Althobiti et al., (30)** who found that ALDH1A1 expression was associated with high grade in invasive breast carcinoma. The association of ALDH1A1 expression with poor differentiation may reflect the CSC-like nature of ALDH1A1-expressing tumor cells **(15)**. Other studies performed by **Penumatsa et al., (31)** on cancer ovary, **Kahlert et al., (32)** on pancreatic carcinoma and **Tanaka et al., (33)** on hepatocellular carcinoma revealed that well differentiated tumors showed higher expression of ALDH1A1 compared to poorly differentiated tumors. These variabilities of ALDH1A1 expressions among studies may be due to different tissues with different molecular signature or different tissue specificity.

There was statistically insignificant difference between depth of tumor invasion and ALDH1A1 expression. These findings are consistent with those with CRC **(16)**. Other study performed by **Li et al., (27)** on gastric carcinoma found a highly significant association between ALDH1A1 expression and depth of tumor invasion. The increased expression of ALDH1A1 with increased depth of invasion may be explained by the role of ALDH1A1 in increasing tumor growth and cancer stem cell proliferation **(28).** ALDH1A1 also promotes epithelial mesenchymal transition (EMT), an important phenomenon associated with tumor invasion and metastasis **(34).**

In the current study, there was a statistically significant difference between ALDH1A1 expression regarding lymphovascular invasion. These results were in agreement with **Holah et al., (25)** study in CRC and **Althobiti et al., (30)** study in invasive breast carcinoma who found a statistically significant association between ALDH1A1 expression and presence of lymphovascular invasion. ALDH1A1 acts as a promoter, inducing EMT in cancer cells. EMT can stimulate tumor invasion and metastasis through promoting cancer cell progression

through the basement membrane and invasion into the surrounding microenvironment such as lymphovascular spaces **(35, 25).**

The current study has shown that 76.5% of cases with positive perineural invasion showed high expression of ALDH1A1 with a statistically significant difference. This is in agreement with that reported by **Masoud et al., (36)** on prostatic carcinoma but not with that by **Liu et al., (34)** on gastric carcinoma. The increased ALDH1A1 expression in CRC can be explained as most cases with positive perineural invasion (88.2%) were also characterized by increased depth of tumor invasion (T4). Also, lymph node metastasis was detected in all cases with positive perineural invasion.

In the current study, there was a highly significant statistical difference between ALDH1A1 expression regarding lymph node metastasis (P-value = 0.001). Eighty-two and four tenth% of cases without LN metastasis (N0) showed low expression, while 57.1% of cases with N1 and 78.9% of cases with N2 showed high expression. These results were in agreement with studies performed by **Yang et al., (16)** in CRC, **Li et al., (27)** in gastric carcinoma and **Althobiti et al., (30)** in invasive breast carcinoma. Beside the role of ALDH1A1 in promoting EMT which contributes to invasive and metastatic tumor growth, ALDH1A1 was found to stimulate tumor invasion and LN metastasis via the Wnt/β-catenin signaling pathway. This pathway was found to be associated with retinoic acid signaling pathway **(37, 16).**

On the contrary, **van der Waals et al., (15)** found insignificant statistical association between ALDH1A1 expression and LN metastasis in CRC. This may be due to different interpretation of ALDH1A1 expression and different number of cases with positive and negative lymph node metastasis.

There was statistically insignificant difference between ALDH1A1 expression and distant metastasis in studied cases. Fifty-five and eight tenths percent of cases without distant metastasis (M0) showed high expression, while 71.4% of cases with distant metastasis (M1) showed low expression. These results were in agreement with studies performed by **Yang et al., (16)** in CRC and **Ye et al., (38)** in gastric neuroendocrine carcinoma**.** ALDH1A1 was found to be associated with activation of angiogenic factors, such as hypoxia inducible factor-1α (HIF-1α) and proangiogenic factors, such as vascular endothelial growth factor (VEGF). This

suggests that ALDH1A1 may promote tumor metastasis **(39)**. However, 71.4% of cases with distant metastasis showed low expression which may be due to limited number of cases with distant metastasis.

In our study, there was a highly significant statistical difference between ALDH1A1 expression in studied cases and TNM stage . Eighty six and seven tenths percent of cases with stage II and 71.4 of cases with stage IV showed low expression, while 78.6% of cases with stage III showed high expression. Many studies showed that ALDH1A1 expression was significantly associated with advanced tumor stage indicating the role of ALDH1A1 in tumor progression and advanced stage. These studies were carried out by **Li et al., (40)** on lung adenocarcinoma, **Li et al., (27)** on gastric carcinoma and **Xing et al., (41)** in papillary thyroid carcinoma. On the contrary, other studies showed no significant relation between ALDH1A1 expression and TNM stage including **Yang et al., (16)** on CRC, **Liu et al., (34)** on gastric carcinoma and **Khalifa et al., (42)** on epithelial ovarian tumors. These opposing results in CRC may have resulted from the differing populations and diets across the studies, as well as any differences in ALDH1A1 gene mutations. The differences in ALDH1A1 expression between studies could arise from variations in the tissue specificity, stage distribution, use of different clones of antibodies and different means of interpretation.

In conclusion, high ALDH1A1 expression was associated with right sided tumors, lymphovascular invasion, perineural invasion and lymph node metastasis in CRC. ALDH1A1 may play an important role in tumor invasion and lymph node metastasis and may work as a useful marker for prognosis of CRC. Further studies on ALDH1A1expression on a larger number of cases are recommended to clarify its association with advanced tumor stage. Further studies using different molecular methods on ALDH1A1 are recommended to detect the mechanisms by which ALDH1A1 may contribute to the development and progression of CRC.

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