The Egyptian Journal of Hospital Medicine (April 2025) Vol. 99, Page 1386-1395

Expression and Potential Prognostic Significance of CXCL12 and Aldolase A (ALDOA) in Colorectal Carcinoma: An Immunohistochemical Study

Zeinab I. Elshawarby*[1], Sara G. Masoud[1], Ibrahim A. Fakhreldeen[2], Hanan Ahmed[1]
Departments of [1]Pathology and [2]General Surgery, Faculty of Medicine at Benha University, Qalioubia, Egypt
***Corresponding author:** Zeinab I. Elshawarby, **Mobile:** (+20) 01007918338, **E-mail:** elshawarbyz@yahoo.com

ABSTRACT

Background: Colorectal cancer (CRC) ranks as the third most prevalent malignancy globally and represents a leading cause of cancer-related mortality worldwide. CXCL12, an essential chemokine, has been demonstrated to be critically involved in cancer metastasis. Aldolase A (ALDOA) is a crucial glycolytic enzyme that contributes to cellular energy production and has been implicated in tumor metabolism and tumor progression.

Objective: This study aimed to identify of CXCL12 and ALDOA immunohistochemical expression in CRC cases and correlation of their expression with clinicopathological data and patient's survival of the studied cases to detect their potential prognostic role.

Material and methods: This retrospective study included 40 cases CRC immunohistochemically stained with CXCL12 & ALDOA and the results to be correlated with different clinicopathological variables and patient's survival.

Results: High CXCL12 expression was positively associated with positive lympho-vascular invasion (P=0.001), tumor budding (P=0.027), deeper tumor invasion (P=0.016), positive lymph node metastasis (P=0.000), presence of distant metastasis (P=0.036) and greater tumor stage (P=0.001). High ALDOA expression was positively associated with high tumor grade (P=0.030), positive lympho-vascular invasion (P=0.005), positive perineural invasion (P= 0.020), high tumor budding (P=0.030), higher tumor stage (P=0.002) and positive lymph node metastasis (P=0.001). Elevated CXCL12 and ALDOA expression levels were indicative of poor five-year overall survival (P=0.017 and 0.044 respectively) as well as poorer disease-free survival (P=0.016 and 0.002 respectively).

Conclusion: CXCL12 and ALDOA overexpression was correlated with poor prognosis and poor patient's survival in CRC. Therefore, their expression could predict prognosis and survival of the patient, and blocking their pathways may provide new promising treatment strategies in CRC.

Keywords: CXCL12, ALDOA, CRC.

INTRODUCTION

Colorectal cancer (CRC) stands as the third most common malignancy across the globe, affecting both men and women, and represents a major cause of cancer-associated mortality. The substantial mortality rate of colorectal cancer is primarily driven by delayed detection, with most cases being diagnosed at an advanced or metastatic stage [(1)]. In Egypt, colorectal carcinoma ranks the 7th, and constitutes 3.9% of all cancer diagnoses. It ranks fifth in women and seventh in men according to GLOBOCAN, 2022. Colorectal carcinoma has a high mortality rate in Egypt as the mortality risk is 3.3% in this cancer [(2)].

Stromal cell-derived factor-1, formally recognized as C-X-C motif chemokine ligand 12 (CXCL12), was originally discovered due to its essential contribution to tumor metastasis. Originally cloned from a bone marrow-derived stromal cell line, the CXCL12 gene, mapped to the long arm of chromosome 10, was later found to enhance pre-B cell proliferation. It is expressed in six splice variants (CXCL12α to φ), which maintain the first three exons but diverge in the fourth [(3)]. Extensively studied for its metastatic role, CXCL12 facilitates cancer progression by promoting cell migration, inducing epithelial-mesenchymal transition (EMT), increasing invasiveness, and stimulating angiogenesis [(4)].

Aldolase A (ALDOA), an essential enzyme of the glycolytic cascade, plays a pivotal role in the reversible conversion of fructose-1,6-bisphosphate into two triose phosphates—dihydroxyacetone phosphate and glyceraldehyde-3-phosphate —primarily in muscle tissue. Accumulating evidence suggests that ALDOA participates in both glycolysis and gluconeogenesis, processes that sustain energy demands for tumor migration, proliferation, and drug resistance, highlighting its crucial role in tumor metabolism and progression [(5)]. By modulating tumor metabolism, ALDOA expression in different tumor cells contributes to EMT, stimulates proliferation, and accelerates cell cycle progression from G1 to G2 phase. It is expressed in different malignancies as liver, lung and stomach. While, the significance of ALDOA in malignancies is well-documented and the mechanisms by which it operates remain to be clarified [(6)].

This study aimed to assess the immunohistochemical expression of CXCL12 and ALDOA in CRC and examine their correlation with clinicopathological parameters and patient survival. By elucidating their potential prognostic significance, these findings may contribute to identify novel prognostic biomarkers and advancing targeted therapeutic strategies for CRC.

MATERIALS AND METHODS

This retrospective selected study included 40 cases diagnosed as CRC, treated with surgical resection either by total, partial or segmental colectomy for colonic carcinomas or low anterior resection or

Received: 01/01/2025
Accepted: 17/02/2025

abdominoperineal resection for rectal carcinomas at Surgery Department-Benha University Hospitals, during the period from 2015 to 2019 with a follow-up data of 5-years, in addition to para-cancer tissues (taken at more than 5 cm away from the tumor). The material included archival formalin-fixed paraffin-embedded tissue blocks containing representative samples of the tumor, processed at Pathology Department-Benha Faculty of Medicine. Clinicopathological features were collected by revising patient's files.

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

Exclusion criteria: Cases with no available paraffin blocks or clinicopathological data or cases with preoperative chemotherapy and cases with a diagnosis other than adenocarcinoma.

1. **Histological evaluation:** Upon pathologic re-evaluation of the hematoxylin and eosin-stained slides, additional slides were prepared from paraffin-embedded blocks when necessary. In alignment with the most recent WHO classification, tumors were categorized based on their least differentiated component as low-grade (Well to moderately differentiated) or high-grade (Poorly differentiated) [(7)]. Tumor staging was performed following the TNM classification system, as specified in the 8th edition of the American Joint Committee on Cancer (AJCC) [(8)].

Assessment of tumor budding: In compliance with ITBCC 2016 criteria, tumor budding (TB) was evaluated on scanned hematoxylin and eosin (H & E)-stained slides at medium power magnification (10× to 20×), using a single hotspot field area standardized to 0.785 mm^2 at the invasive front. As per its definition, TB comprised a single tumor cell or a cluster of up to four tumor cells present at the invasive margin. Total number of tumor buds was recorded alongside a three-tier classification system, applied to a 0.785 mm^2 field area, with categories defined as low (0–4 buds), intermediate (5–9 buds), and high (≥10 buds) [(9)].

2. **Immunohistochemical evaluation:** Immunohistochemical staining was conducted on 3–4 μm sections derived from paraffin-embedded tissue blocks, employing a primary rabbit polyclonal antibody against human CXCL12 **(Cat# 17402-1-AP, 100 μg, diluted 1:100; Proteintech, Rosemont, IL, USA)** and a primary rabbit polyclonal antibody against human ALDOA **(Cat# 14884-1-AP, 100 μL, diluted 1:100; Proteintech, Rosemont, IL, USA)**. The Avidin-Biotin Complex (ABC) method was utilized to apply anti-CXCL12 and anti-ALDOA antibodies to each slide, in accordance with manufacturer's guidelines. Antigen retrieval was achieved for both using a 10 mmol/L citrate buffer (pH 6.0). Incubation with primary antibodies was carried out on tissue sections at room temperature for a total duration of two hours. Immunoreaction was visualized by adding DAB as a chromogen. The slides of positive and negative controls were included in each run. Representative sections of human breast carcinoma were used as positive controls for CXCL12 and normal cells of skeletal muscle for ALDOA. Negative controls were performed by replacing the primary antibody with nonimmune IgG.

Immunohistochemical interpretation: CXCL12 was predominantly expressed in cytoplasm of tumor cells, and the percentage of positively stained cells was categorized as follows: 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (>75%). Staining intensity was assessed on a scale of 0 to 3, where 0 indicated negative staining, 1 represented weak staining, 2 denoted moderate staining, and 3 corresponded to strong staining. The final score, ranging from 0 to 12, was determined by multiplying percentage score of positive tumor cells by staining intensity score. Patients were stratified into high- and low-expression groups using the median marker score as the defining threshold [(10)]. Using median IHC score as a reference, CXCL12 expression was deemed low for final scores of 4 or below, whereas scores above 4 were classified as high.

ALDOA expression, identified as brownish cytoplasmic staining, was quantified using an immunohistochemical scoring system based on product of staining intensity (0: negative, 1: weak, 2: moderate, 3: strong) and staining extent (0: 0–5%, 1: 6–25%, 2: 26–50%, 3: 51–75% & 4: >75%). The final classification was: 0 (negative, -), 1–4 (+1), 5–8 (+2), and 9–12 (+3), with scores of +2 or +3 indicating high expression and - or +1 denoting low expression [(6)].

Ethical approval: Approval for study was granted by The Research Ethics Committee (REC) of Benha Faculty of Medicine, Benha University, with the Code Number (RC 6-12-2024). Following receipt of all information, signed consent was provided by each participant. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

Statistical package for the social sciences (SPSS) software version 22.0 was used. Quantitative data were reported as numbers and percentages, with Fisher's exact test and Chi-Square (χ^2) test applied to assess

differences in categorical variables. Kaplan–Meier survival curves were drawn and compared using the Log-rank test for statistical significance. Disease-free survival (DFS) was determined as the time from surgical resection to the first documented recurrence or metastasis, or the last follow-up assessment. Overall survival (OS) was measured from the time of primary surgical resection to death from any cause. In cases where death, metastasis, or recurrence had not been confirmed, OS and DFS calculations relied on the patient's most recent known survival date. Statistical significance was defined as $P \leq 0.05$. Based on Spearman's correlation analysis, correlations were interpreted as relatively strong if above 0.35, moderate between 0.15 and 0.35, and weak if below 0.15.

RESULTS

Clinicopathological characteristics

The study population comprised 40 CRC cases of them 20 (50%) cases were located at right colon, 15 (37.5%) cases were located at left colon & 5 (12.5%) were rectal. Age distribution of studied cases spanned from 30 to 80 years, with a median of 60 years. Out of the total number of cases, 24 (60%) were male, and 16 (40%) were female. The size of tumor ranged from 4- to 11 cm. with a median size of 5 cm. Within the follow-up period, OS was 60% with a median OS of 60 months & the DFS was 47.5% with a median DFS of 27 months. Other clinicopathologic features are illustrated in **Table (1).**

Immunohistochemical expression results:

A- CXCL12 expression in the studied CRC cases & its relation to clinicopathological variables:

CXCL12 showed high immunohistochemical expression in 29 (72.5%) of CRC cases and low expression in 11 (27.5%). While mucosa of para-cancer tissues showed low CXCL12 expression with a statistically significant difference (P <0.001) (Figure 1). A significant association was observed between high CXCL12 expression and lympho-vascular invasion (P = 0.001), increased tumor budding (P = 0.027), deeper tumor invasion (P = 0.016), lymph node metastasis (P = 0.000), distant metastasis (P = 0.036), and higher tumor stage (P = 0.001). These positive associations as well as additional findings are illustrated in Table (1).

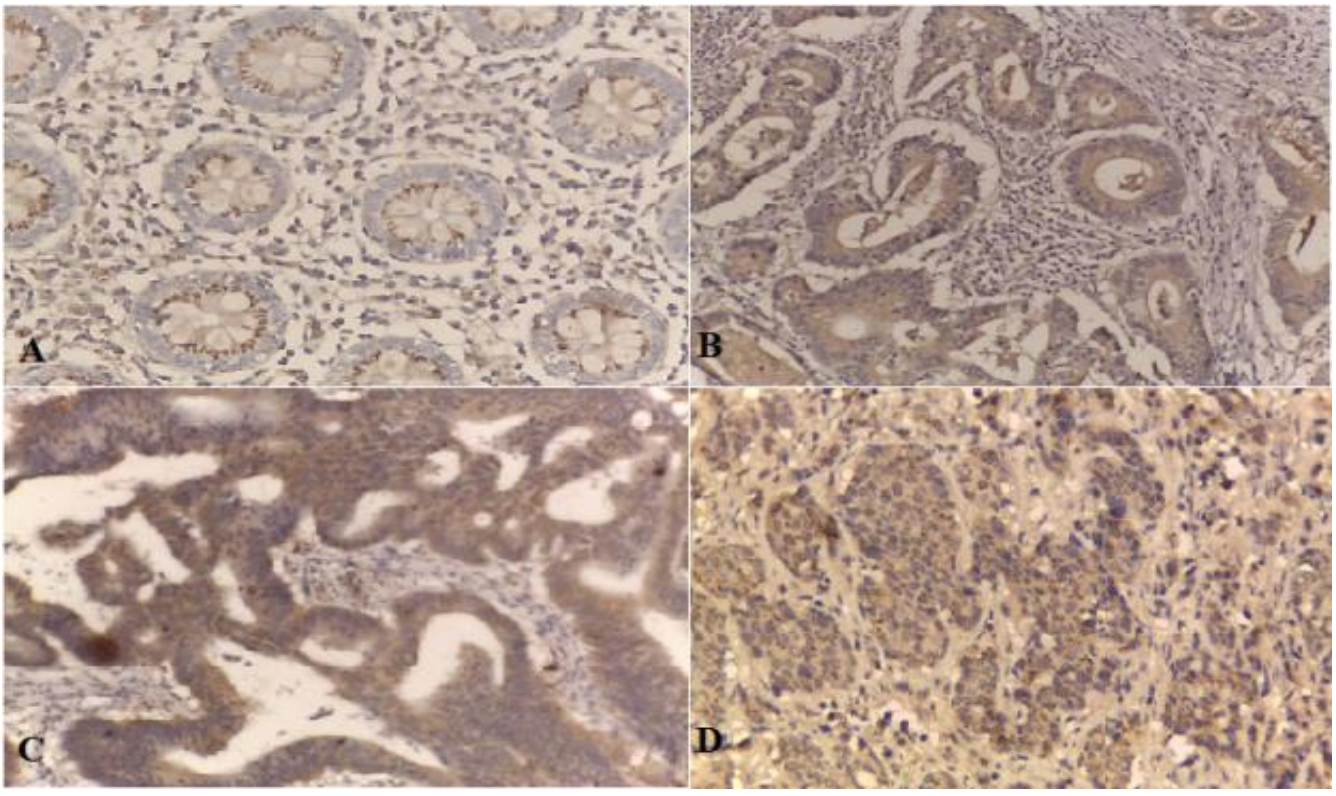

Figure (1): A photomicrograph showed CXCL12 IHC cytoplasmic expression in CRC and adjacent normal para-cancer tissue: (A) Low CXCL12 cytoplasmic expression in adjacent normal colonic mucosa (IHC, X200), (B) High CXCL12 cytoplasmic expression in well differentiated CRC (IHC, X200), (C) High CXCL12 cytoplasmic expression in moderately differentiated CRC (IHC, X200) and (D) High cytoplasmic CXCL12 immunohistochemical expression in poorly differentiated CRC (IHC, X400).

B- Aldolase A (ALDOA) expression in the studied CRC cases & its relation to clinicopathological variables: Aldolase A showed high immunohistochemical expression in 22 (55%) of CRC cases and low expression in 18 (45%). While, mucosa of para- cancer tissues showed low ALDOA expression with a statistically significant difference (P

<0.001) (Figure 2). ALDOA high expression was positively related with high tumor grade (P= 0.030), positive lympho-vascular invasion (*P*= 0.005), positive perineural invasion (P= 0.020), higher tumor stage (P= 0.002), high tumor budding (*P*= 0.030), and positive lymph node metastasis (*P*= 0.001). These positive associations as well as additional findings were illustrated in Table (1).

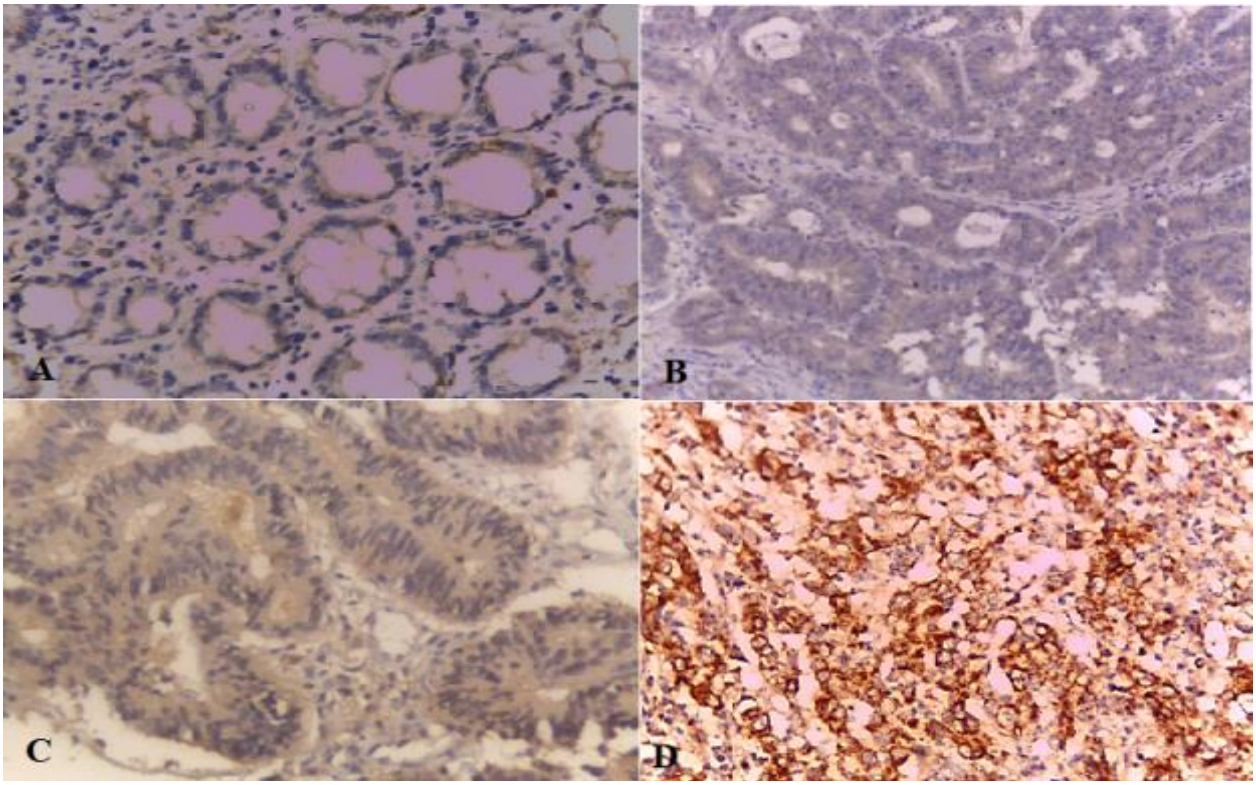

Figure (2): A photomicrograph showed ALDOA IHC cytoplasmic expression in CRC and adjacent para-cancer tissue: (A) Low ALDOA cytoplasmic expression in adjacent normal colonic mucosa (IHC, X200), (B) Low ALDOA cytoplasmic expression in well differentiated CRC (IHC, X200), (C) Low ALDOA cytoplasmic expression in moderately differentiated CRC (IHC, X400) and (D) high ALDOA cytoplasmic expression in poorly differentiated CRC (IHC, X200).

C- Correlation between CXCL12 and ALDOA expression in the studied CRC cases: The expression of CXCL12 in CRC displayed a moderate positive correlation with ALDOA expression (r = 0.34, P = 0.030).

Table (1): Clinicopathological features and relation between CXCL12 and Aldolase A expression and the clinicopathological variables of the studied CRC cases (N=40)

	Parameter NO (%)	**CXCL12 expression**		**P value**	**ALDOA expression**		**P value**
		Low	**High**		**Low**	**High**	

		(n=11)	(n=29)	(Chi-square test)	(n=18)	(n=22)	(Chi-square test)
Age (%) Median 60 (30-80)	**≤60 years** 22 (55%) **>60 years** 18 (45%)	5 (22.7%) 6 (33.3%)	17 (77.3%) 12 (66.7%)	0.456 (0.557 FET)	11 (50%) 7 (38.9%)	11 (50%) 11 (61.1%)	0.481 (0.496 FET)
Sex **Male** **Female**	24 (60%) 16 (40%)	9 (37.5%) 2 (12.5%)	15 (62.5%) 14 (87.5%)	0.072 (3.242 FET)	12 (50%) 6 (37.5%)	12 (50%) 10 (62.5%)	0.435 (0.606 FET)
Location	**Rt. Colon** 20 (50%) **Lt. Colon** 15 (37.5%) **Rectum** 5 (12.5%)	6 (30%) 5 (33.3%) 0	14 (70%) 10 (66.7%) 5 (100%)	0.330 (2.215)	11 (55%) 7 (46.7%) 0	9 (45%) 8 (53.3%) 5 (100%)	0.086 (4.916)
Size **Median (range)** **5(4-11)**	**≤5 cm** 21(52.5%) **>5 cm** 19 (32.5%)	7 (33.3%) 4 (21.1%)	14 (66.7%) 15 (78.9%)	0.382 (0.763 FET)	10 (47.6%) 8 (42.1%)	11 (52.4%) 11 (57.9%)	0.726 (0.123 FET)
Grade (G) **G1** **G2** **G3**	6 (15%) 21(52.5%) 13(32.5%)	2 (33.3%) 5 (23.8%) 4 (30.8%)	4 (66.7%) 16 (76.2%) 9 (69.2%)	0.854 (0.316)	4 (66.7%) 12 (57.1%) 2 (15.4%)	2 (33.3%) 9 (42.9%) 11 (84.6%)	**0.030*** (6.996)
LVI **Absent** **Present**	13(32.5%) 27 (67.5%)	8 (61.5%) 3 (11.1%)	5 (38.5%) 24 (88.9%)	**0.001**** (11.192)	10 (76.9%) 8 (29.6%)	3 (23.1%) 19 (70.4%)	**0.005**** (7.930)
PNI	**Absent** 31(77.5%) **Present** 9(22.5%)	9 (29%) 2 (22.2%)	22 (71%) 7 (77.8%)	0.682 (0.167 FET)	17 (54.8%) 1 (11.1%)	14 (45.2%) 8 (88.9%)	**0.020*** (5.389)
Tumor budding	**Low** 8 (20%) **Intermediate** 20(50%) **High** 12(30%)	5 (62.5%) 5 (25%) 1 (8.3%)	3 (37.5%) 15 (75%) 11 (91.7%)	**0.027*** (7.189)	6 (75%) 10 (50%) 2 (16.7%)	2 (25%) 10 (50%) 10 (83.3%)	**0.030*** (7.003)
Depth of Invasion (T) **T2** **T3** **T4**	4 (10%) 22(55%) 14 (35%)	2 (50%) 9 (40.9%) 0	2 (50%) 13 (59.1%) 14 (100%)	**0.016*** (8.310)	3 (75%) 11 (50%) 4 (28.6%)	1 (25%) 11 (50%) 10 (71.4%)	0.202 (3.203)
Lymph node metastasis	**Negative** 15(37.5%) **Positive** 25(62.5%)	9 (60%) 2 (8%)	6 (40%) 23 (92%)	**0.000**** (12.715)	12 (80%) 6 (24%)	3 (20%) 19 (76%)	**0.001**** (11.879)
Distant Metastasis	**Negative** 31(77.5%) **Positive** 9(22.5%)	11 (35.5%) 0	20 (64.5%) 9 (100%)	**0.036*** (4.405)	15 (48.4%) 3 (33.3%)	16 (51.6%) 6 (66.7%)	0.420 (0.651)
Stage **I** **II** **III** **IV**	4(10%) 12(30%) 15(37.5%) 9(22.5%)	2 (50%) 8 (66.7%) 1 (6.7%) 0	2 (50%) 4 (33.3%) 14 (93.3%) 9 (100%)	**0.001**** (16.928)	3 (75%) 10 (83.3%) 2 (13.3%) 3 (33.3%)	1 (25%) 2 (16.7%) 13 (86.7%) 6 (66.7%)	**0.002**** (15.152)

N: Number, *FET*: Fisher Exact test, *LVI*: Lympho-vascular Invasion, *PNI*: Perineural Invasion.

D- Survival analysis: The survival of the studied cases showed 24 cases (60%) were alive and 16 cases (40%) died. Kaplan-Meier analysis demonstrated that cases with high CXCL12 expression showed worse OS (Log rank=5.647; *P*=0.017) with a median OS of 34.82 vs. 59.9 months and worse DFS (Log rank=5.754, *P*=0.016) with a median DFS of 30.55 vs. 49.63 months.

Also, high ALDO expression was interrelated with worse OS (Log rank=4.041; *P*=0.044) with a median OS of 33.63 vs. 49.77 months and worse DFS (Log rank=9.754, *P*=0.002), with a median DFS of 24.88 vs. 48.67 months (Figure 3).

Combined CXCL12 and ALDOA expression in relation to patients' survival: To detect the prognostic relevance of CXCL12 and ALDOA co-expression in CRC and to determine which combination is linked to enhanced survival, the studied CRC cases were stratified into four groups as follows: Group 1: High CXCL12/high ALDOA expression, group 2: High CXCL12/low ALDOA expression, group 3: Low CXCL12/high ALDOA expression, and Group 4: Low CXCL12/low ALDOA expression. Survival analysis using Kaplan-Meier method identified that high CXCL12/high ALDOA expression was linked to the least OS, but no substantial variation was found among the four groups (Log rank=7.327, P=0.062) and was markedly related with the least DFS (Log rank=12.054, P=0.007).

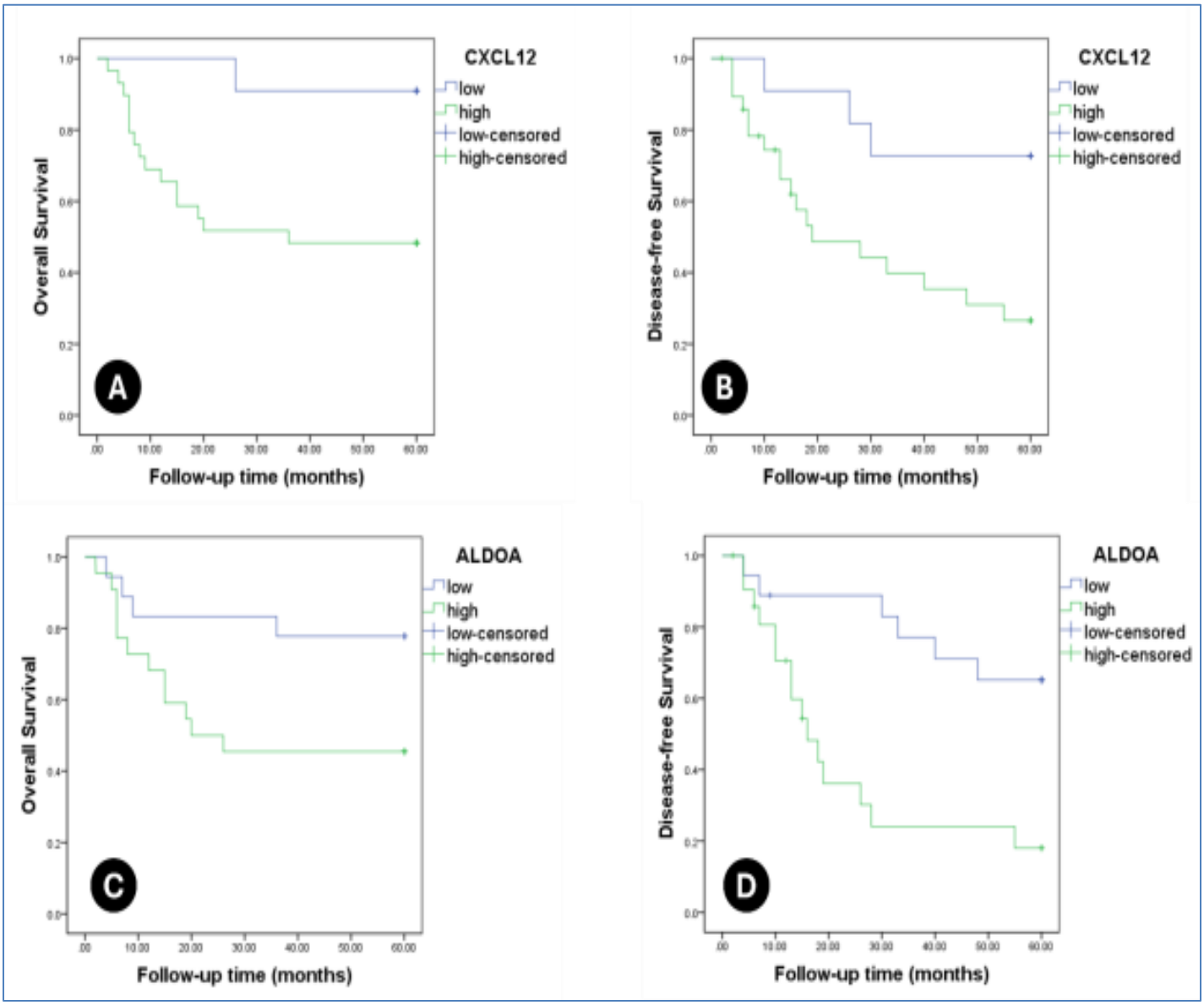

Figure (3): Kaplan-Meier survival analysis for 5-year overall survival & disease-free survival of the colorectal carcinoma cases studied in association with CXCL12 expression (A & B) and ALDOA expression (C & D).

DISCUSSION

Colorectal cancer is a leading malignancy worldwide, with late-stage metastatic diagnosis serving as the principal contributor to disease-related mortality. From a pathological perspective, chemokines and their receptors play a crucial role in pathogenesis of infectious diseases. More recently, their involvement in tumor biology has garnered significant attention in the field of oncology [(11)].

Chemokines regulate the tumor tissue microenvironment via a mixed signalling system. In normal tissues, they orchestrate immune cell activity and facilitate lymphoid organ development. However, within the tumor microenvironment, chemokines mediate cell migration and intercellular interactions through their expression by immune and stromal cells. To put it another way, chemokines may modulate key oncogenic processes, including tumor cell proliferation, invasion, angiogenesis, and metastasis. Moreover, tumor cells themselves can secrete chemokines that enhance their own proliferation and contribute to tumor expansion. Within the tumor microenvironment, both malignant and host cells have the capacity to stimulate the release of a diverse array of chemokines, which, in turn, activate and recruit various cell types, orchestrating both pro-tumorigenic and anti-tumorigenic responses [(12)].

The present study revealed that CXCL12 high immunohistochemical expression was in 72.5% of CRC cases and low expression was in 27.5%. While, mucosa of para-cancer tissues showed low CXCL12 expression with a statistically significant difference ($P < 0.001$). These findings are in agreement of **Greijer *et al.* [(13)] and Amara *et al.* [(14)]** studies that discovered high positive CXCL12 expression was greater in the carcinoma tissues when compared to the healthy tissues. In contrast, **Wendt *et al.* [(15)]** study showed that CXCL12 expression was absent in CRC epithelium while **Mousavi *et al.* [(16)]** study revealed that there was no significance in immunohistochemical expression between tumor and healthy mucosa and **Romain *et al.* [(17)]** study, which revealed the expression of CXCL12 was decreasing from adenomas (94%) to (85%) in microsatellite instability (MSI) carcinoma and (75%) in microsatellite stable (MSS) carcinoma. Unraveling the reasons behind the diverse expression of a factor in the same cancer across multiple investigations remains a persistent challenge.

One potential explanation for these discrepancies is the inclusion of both rectal and colon tumors in various studies. Additionally, a subset of rectal tumors undergoes chemotherapeutic and/or radiation treatment prior to resection, which may alter CXCL12 expression levels. In addition, immunohistochemical variability may be influenced by discrepancies in antibody selection, dilution concentrations, and antigen retrieval methodologies, which can be based on enzymatic treatment or immunofluorescence techniques [(9)].

The current research revealed that CXCL12 high expression was positively associated with positive lympho-vascular invasion ($P= 0.001$), high tumor budding ($P= 0.027$), high depth of tumor invasion ($P= 0.016$), presence of distant metastasis ($P= 0.036$), higher tumor stage ($P= 0.001$) and positive lymph node metastasis ($P= 0.000$). These findings come in parallel with **Zengin *et al.* [(11)]** study, which showed CXCL12 high positive immunohistochemical expression was notably linked to lymphatic invasion ($p = 0.009$), advanced stage ($p = 0.028$) high tumour budding ($p < 0.002$). Also, **Greijer *et al.* [(13)], Yoshitake *et al.* [(18)] and Akishima-Fukasawa *et al.* [(19)]** studies, which revealed high CXCL12 expression was substantially related to lymphatic invasion, venous invasion, high tumor stage, lymph node and distant metastases. Contrary to our findings, **Ingold *et al.* [(20)]** did not observe a significant statistical association between CXCL12 upregulation and clinicopathological variables.

Our findings on CXCL12 can be understood in light of the intricate interactions between tumor cells and its microenvironment, which comprises key cellular components such as endothelial cells, immune cells, and fibroblasts. These interactions participate to development and progression of tumor malignancy. Cancer cells' ability to secrete specific factors promotes fibroblast-driven production of tumor-associated chemokines, which subsequently influence malignant cells enhancing their proliferative, migratory and invasive capabilities. With regard to this mechanism, the CXCL12-CXCR4 signaling pathway was critically involved in various malignancies. Furthermore, infiltration of mesenchymal stromal cells (MSCs) into the tumor microenvironment promotes metastasis by releasing growth factors, particularly CXCL12, which supports tumor proliferation and neovascularization [(21)].

Mesenchymal stromal cells (MSCs), through the upregulation of CXCR4 expression, have the capacity to differentiate into cancer-associated fibroblasts (CAFs) and activate the TGF signaling pathway, ultimately enhancing tumor proliferation and metastatic potential by releasing pro-tumorigenic factors. Within tumor stroma, MSCs may indirectly promote tumors by boosting angiogenesis via recruitment of endothelial progenitor cells, which subsequently facilitate tumor development and progression by aiding in the establishment and maturation of tumor vasculature. Moreover, MSCs contribute to tumor budding in CRC, a phenomenon enhanced by an increased tumor-stromal ratio. This microenvironmental alteration promotes EMT, allowing tumor cells to invade adjacent normal tissues and facilitating tumor budding [(22)].

Accordingly, CXCL12 enhances cellular communication within the tumor microenvironment by engaging its receptors CXCR4 and CXCR7, thereby promoting interactions between cancer cells, fibroblasts, and endothelial cells. In addition, tumor hypoxia enhances the expression of CXCR4 and

CXCL12 in endothelial and cancer cells by inducing accumulation of hypoxia-inducible factor 1-alpha (HIF-1α), which stimulates VEGF-mediated angiogenesis, thereby facilitating vascular invasion by tumor cells. CXCL12 also modulates these processes by promoting migration of leukocytes that may produce angiogenic factors into tumor microenvironment, hence facilitating neovascularization and tumor proliferation [(1)]. These findings support the notion that chemokines play a role in enhancing invasive and metastatic capabilities of tumors. Furthermore, they suggest that targeting these pathways may offer new opportunities for therapeutic development and precision oncology. Growing evidence highlights metabolic reprogramming as a fundamental mechanism underlying tumorigenesis, particularly in relation to ALDOA. Through the glycolysis pathway, elevated lactic acid production and enhanced energy output create a microenvironment that facilitates cancer cell proliferation and sustains tumor growth. ALDOA, a key glycolytic enzyme, has been implicated in pathogenesis of various cancers. However, studies investigating its role in CRC remain limited, with few correlation analyses conducted, and its precise function and underlying mechanisms in CRC are not yet fully understood [(15)]. Our research provided strong evidence for the increased expression of ALDOA in CRC specimens in contrast to their adjacent normal colonic tissues.

The current study revealed that ALDOA showed high positive immunohistochemical expression in (55%) of CRC cases and low expression in 45%. While, mucosa of para-cancer tissues showed low ALDOA expression with a statistically substantial variation (P <0.001). In addition, the present work showed that ALDOA high expression was positively related with high tumor grade (P= 0.030), positive lymph node metastasis (P= 0.001), higher tumor stage (P= 0.002) positive perineural invasion (P= 0.020), high tumor budding (*P*= 0.030), and positive lympho-vascular invasion (P= 0.005). In line with our results, **Xu *et al.*** [(6)] reported a strong association between ALDOA expression and lymph node metastasis, perineural invasion, differentiation grade, and TNM stage, while no relationship was observed between ALDOA expression and tumor depth and size, venous invasion and gender, or age. Results of **Lu *et al.*** [(23)] study was partly in accordance with our results as there was a notable correlation between ALDOA expression levels and depth of tumor invasion (P= 0.011), positive lymph node metastasis (P=0.009) and TNM stage (P=0.033) but there was no substantial correlation with age, gender, degree of differentiation and location or distant metastasis. This partly different results may be due to the use of PCR technique in ALDO assessment. In contrast, **Huang *et al.*** [(24)] reported that ALDOA expression was notably linked to tumor location (P < .001) and gender (P < .001), whereas no significant association was observed with tumor differentiation. These different findings may be due to different number of cases and variability of locations of the tumor included in the study.

Our finding concerning ALDOA could be explained by the fact that ALDOA is a glycolytic enzyme and is involved in the process of glycolysis and gluconeogenesis. Both glycolysis and gluconeogenesis can provide energy required for tumor proliferation by producing ATP, which enhance tumor cell growth and provide precursors for macromolecule synthesis that promote tumor cell proliferation. Beyond its enzymatic function, its expression contributes to metastasis by promoting the EMT process. This transition is marked by the loss of epithelial characteristics through downregulation of epithelial junction proteins, such as E-cadherin, alongside the upregulation of mesenchymal markers, including vimentin and N-cadherin, ultimately enhancing tumor proliferation, invasion, and metastatic potential [(23)].

The present study displayed a notable relation between CXCL12 and ALDOA expression in CRC (0.34; *P*= 0.030). Both of them were associated with bad prognostic factors as they had the ability to stimulate and facilitate EMT, therefore invasion and metastasis.

On survival analysis, Kaplan-Meier analysis demonstrated that cases with high CXCL12 expression showed worse OS (Log rank=5.647; *P*=0.017) with a median OS of 34.82 vs. 59.9 months, and worse DFS (Log rank=5.754, *P*=0.016), with a median DFS of 30.55 vs. 49.63 months.

In general, leukocyte presence is considered indicative of a robust immune response, and infiltration of inflammatory cells has been correlated with more favorable survival outcomes in CRC tumors. A pronounced infiltration of T cells in CRC, whether in the tumor stroma or within malignant cell nests, has consistently been associated with a favorable prognosis, regardless of disease stage or nodal status. A robust T-cell infiltrate is widely regarded as a major determinant of patient prognosis following CRC resection, as it serves as a hallmark of an effective and coordinated adaptive immune response [(25)]. **Zengin *et al.*** [(11)] elucidated a significant reduction in local inflammatory response (LIR) levels within tumors characterized by elevated CXCL12 and CXCR4 expression. This phenomenon underscores the potential mechanistic interplay between these chemokines and tumor aggressiveness, implicating them in the orchestration of malignant progression, metastatic potential, and adverse prognostic implications.

As a key component of the tumor microenvironment, stromal cells actively mediate the metastatic cascade, fostering tumor cell dissociation, stromal penetration, vascular transgression, and systemic dissemination, thereby driving the formation of secondary metastatic deposits. The tumor stroma ratio serves as an indicator of the relative proportion of stromal and tumoral cells within the microenvironment. Existing literature suggests that an increase in stromal components fosters epithelial-mesenchymal transition,

a critical process that promotes tumor migration into adjacent normal tissues, thereby facilitating invasion. Moreover, a higher proportion of immature stroma has been linked to tumor budding and an enhanced resistance to chemotherapeutic agent. Collectively, these pathological characteristics contribute to heightened tumor aggressiveness and diminished survival outcomes, particularly in patients exhibiting elevated CXCL12 expression [(11)].

Regarding survival analysis of ALDOA, the current study revealed high ALDO expression that was associated with worse OS (Log rank=4.041; P=0.044) with a median OS of 33.63 vs. 49.77 months and worse DFS (Log rank=9.754, P=0.002) with a median DFS of 24.88 vs. 48.67 months. Elevated expression of ALDOA has been shown to enhance the metastatic potential and proliferation of CRC cells through multiple oncogenic pathways. It facilitates EMT, triggers the mitogen-activated protein kinase (MAPK) signaling cascade, and interacts with COPS6, a pivotal regulator of cellular dynamics. As a subunit of the COP9 signalosome (CSN6), COPS6 belongs to the translation initiation factor 3 (eIF3) superfamily and is extensively involved in modulating cell cycle progression and oncogenic signaling. MAPKs, serine-threonine protein kinases, are crucial in a range of cellular processes, and the MAPK signaling pathway is fundamental to tumorigenesis, influencing cancer cell proliferation and metastasis [(25, 26)]. In mammals, the MAPK signaling pathway comprises key components such as p38 MAPK and ERK. This evidence supports the critical role of the MAPK signaling cascade in ALDOA-driven CRC development and progression, with COPS6 serving as a key intermediary. Additionally, it reinforces the association between ALDOA overexpression and poor patient survival.

CONCLUSION

The current study concluded that CXCL12 and ALDOA overexpression was associated with poor prognosis and poor patient's survival in CRC. Therefore, their expression could predict prognosis and survival of the patient, and blocking their pathways may provide new promising treatment strategies in CRC. Larger scale studies are recommended to be done on CXCL12 and ALDOA immunohistochemical expression in CRC to understand their roles in tumour development and in tumour microenvironment that may help to modify treatment strategies and may provide new effective targeted therapies for CRC.

Conflict of interest: None.
Financial disclosures: None.

REFERENCES

1. **Goïta A, Guenot D (2022):** Colorectal Cancer: The Contribution of CXCL12 and Its Receptors CXCR4 and CXCR7. Cancers, 14 (7): 1810. doi: 10.3390/cancers14071810.
2. **Bray F, Laversanne M, Sung H *et al.* (2024):** Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin., 74 (3): 229-263.
3. **Britton C, Poznansky M, Reeves P (2021):** Polyfunctionality of the CXCR4/CXCL12 axis in health and disease: Implications for therapeutic interventions in cancer and immune-mediated diseases. FASEB J., 35: e212602021. doi: 10.1096/fj.202001273R.
4. **Zhang S, Li G (2022):** Tumor expression of CXCL12 and survival of patients with colorectal cancer. A meta-analysis. Oncol Lett., 24: 436. doi: 10.3892/ol.2022.13556.
5. **Lin J, Xia L, Oyang L *et al.* (2022):** The POU2F1-ALDOA axis promotes the proliferation and chemoresistance of colon cancer cells by enhancing glycolysis and the pentose phosphate pathway activity. Oncogene, 41 (7): 1024–1039.
6. **Xu M, Xi S, Li H *et al.* (2024):** Prognosis significance and potential association between ALDOA and AKT expression in colorectal cancer. Sci Rep., 14: 6488. https://doi.org/10.1038/s41598-024-57209-5
7. **Nagtegaal I, Arends M, Salto-Tellez M (2019):** Colorectal adenocarcinoma. In: Digestive System Tumours. WHO Classification of Tumours, (5th edit.). Lokuhetty D, White V, Watanabe R, Cree I (eds.), IARC Press, Lyon, France. Pp: 177-187. https://www.research.ed.ac.uk/en/publications/colorectal-adenocarcinoma-tumours-of-the-colon-and-rectum
8. **Amin M, Edge S, Greene F *et al.* (2017):** The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin.,67 (2): 93-99.
9. **Lugli A, Kirsch R, Ajioka Y *et al.* (2017):** Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol., 30: 1299–1311.
10. **Shim B, Jin M, Moon J *et al.* (2018):** High Cytoplasmic CXCR4 Expression Predicts Prolonged Survival in Triple-Negative Breast Cancer Patients Treated with Adjuvant Chemotherapy. J Pathol Transl Med., 52 (6): 369-377.
11. **Zengin M, Dursun N, Behzatoğlu K *et al.* (2020):** Evaluation of CXCL12 and CXCR4 to predict poor survival in lymph node-positive colorectal cancer patients. Polish Journal of Pathology, 71 (4): 328-338.
12. **Yue M, Chen M, Zhang B *et al.* (2024):** The functional roles of chemokines and chemokine receptors in colorectal cancer progression. Biomedicine & Pharmacotherapy, 170: 116040. https://doi.org/10.1016/j.biopha.2023.116040.
13. **Greijer A, Diemen P, Fijneman R *et al.* (2008):** Presence of HIF-1 and Related Genes in Normal Mucosa, Adenomas and Carcinomas of the Colorectum. Virchows Arch., 452: 535–544.
14. **Amara S, Chaar I, Khiari M *et al.* (2015):** Stromal Cell Derived Factor-1 and CXCR4 Expression in Colorectal Cancer Promote Liver Metastasis. Cancer Biomark., 15: 869–879.
15. **Wendt M, Johanesen P, Kang-Decker N *et al.* (2006):** Silencing of Epithelial CXCL12 Expression by DNA Hypermethylation Promotes Colonic Carcinoma Metastasis. Oncogene, 25: 4986–4997.

16. **Mousavi A, Hashemzadeh S, Bahrami T *et al.* (2018):** Expression Patterns of CXCL12 and Its Receptor in Colorectal Carcinoma. Clin Lab., 64: 871–876.
17. **Romain B, Benbrika-Nehmar R, Marisa L *et al.* (2017):** Histone Hypoacetylation Contributes to CXCL12 Downregulation in Colon Cancer: Impact on Tumor Growth and Cell Migration. Oncotarget., 8: 38351–38366.
18. **Yoshitake N, Fukui H, Yamagishi H *et al.* (2008):** Expression of SDF-1 Alpha and Nuclear CXCR4 Predicts Lymph Node Metastasis in Colorectal Cancer. Br J Cancer, 98: 1682–1689.
19. **Akishima-Fukasawa Y, Nakanishi Y, Ino Y *et al.* (2009):** Prognostic Significance of CXCL12 Expression in Patients with Colorectal Carcinoma. Am J Clin Pathol., 132: 202–210.
20. **Ingold B, Schulz S, Budczies J *et al.* (2009):** The Role of Vascular CXCR4 Expression in Colorectal Carcinoma. Histopathology, 55: 576–586.
21. **Ma J, Sun X, Su H *et al.* (2017):** Fibroblast-Derived CXCL12/SDF-1α Promotes CXCL6 Secretion and Co-Operatively Enhances Metastatic Potential through the PI3K/Akt/MTOR Pathway in Colon Cancer. World J Gastroenterol., 23: 5167–5178.
22. **Cuiffo B, Karnoub A (2012):** Mesenchymal Stem Cells in Tumor Development. Cell Adh Migr., 6: 220–230.
23. **Lu Y, Zhang Y, Wang X *et al.* (2023):** Aldolase A Promotes Colorectal Cancer Progression through Targeting COPS6 and Regulating MAPK Signaling Pathway. Dis Markers, 23: 1702125. doi: 10.1155/2023/1702125.
24. **Huang P, Wang S, Wu Z *et al.* (2022):** Correlations of ALD, Keap-1, and FoxO4 expression with traditional tumor markers and clinicopathological characteristics in colorectal carcinoma. Medicine (Baltimore), 101 (34): e30222. doi: 10.1097/MD.0000000000030222.
25. **Zengin M (2019):** Prognostic role of tumor-infiltrating T lymphocytes in stage IIA (T3N0) colon cancer. A broad methodological study in a fairly homogeneous population. Ann Diagn Pathol., 41: 69-78.
26. **Kim E, Choi E (2015):** Compromised MAPK signaling in human diseases: an update. Archives of Toxicology, 89 (6): 867-882.