

ORIGINAL ARTICLE

Study the Effect of Caspase-3, Lactate Dehydrogenase, and Oxidative Stress Levels as Promising Biomarkers to Mediate Colon Cancer Therapy

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Abstract

Background Colon cancer is a global health concern prevalent in low-income countries. Its progression is linked to oxidative stress, apoptosis, and metabolic changes.

Aim This study aims to evaluate the diagnostic and prognostic significance of lactate dehydrogenase (LDH) and caspase-3 (CASP3) in colon cancer patients, alongside assessing oxidative and antioxidative markers such as malondialdehyde (MDA) and catalase activity (CAT).

Materials and Methods The study included 20 colon cancer patients and 20 healthy controls (9 males and 11 females). Serum levels of LDH and CASP3 were measured using the ELISA technique, while MDA and CAT levels were determined spectrophotometrically.

Results Colon cancer patients exhibited significantly higher levels of LDH, CASP3, and MDA compared to healthy controls, indicating increased metabolic activity, apoptosis, and oxidative stress. Conversely, CAT activity was significantly reduced in patients, suggesting impaired antioxidant defenses. Elevated LDH levels correlated with tumor stage, size, and cell differentiation, influencing disease-free and overall survival. CASP3 levels also showed potential as a diagnostic biomarker for colorectal cancer. MDA levels reflected oxidative damage, while CAT reduction indicated disruption in the oxidant-antioxidant balance.

Conclusion This study highlights LDH and CASP3 as valuable diagnostic and prognostic biomarkers, with MDA and CAT providing additional insights into oxidative stress in colon cancer. These biomarkers could serve as potential therapeutic targets, aiding disease management and treatment strategies.

Keywords: MDA; CAT; CASP3; LDH; Colon cancer

1 Introduction

Colon cancer is a disease that occurs in countries where the development index is high. Most of the diagnosed cases are in low-income countries [1,2]. It ranks fourth globally according to Globocan in 2018 [3] and is linked to daily foods rich in animal fats and simple sugars

[1,2]. It has been confirmed that eating large amounts of simple sugars increases the risk of right colon cancer in women, increasing consumption of fats (especially trans fats) increases the risk of proximal colon cancer (Ferrucci et al.), and eating red meat increases the risk of left colon cancer [4] and high calcium, vitamin D in the blood has a negative effect on left colon cancer

[5] Lactate dehydrogenase LDH converts pyruvate into lactate anaerobically to obtain energy. However, it did not clarify the importance of high LDH in the patient's serum as an indicator that predicts malignant potential as well as the diagnosis of colon cancer. The aim of the study is to determine the importance of (LDH) as a prognostic factor in colon cancer patients. It is an enzyme found in all places and in high concentrations in red blood cells, liver, and skeletal muscles, as well as in the kidneys and cardiac muscles [6].

Through recent studies and discoveries, LDH has an important and effective role in the behavior of tumors [7,8], and it is considered one of the many vital elements that represent chemical parameters in people with cancer. In addition to that, it is easy to measure routinely in hospitals and multiple clinical laboratories [9,10]. Furthermore, the antioxidant enzyme Catalase (CAT) is an enzyme that plays a role in the antioxidant defense process as well as cell growth and may be linked to tumor processes [11]. It also has an important role in arginine metabolism, as cancer cells use it for this purpose [11,12]. It is the cause of sudden cell death, as it is considered a biological sign and a therapeutic target for colon and rectal cancer together [13]. On the other side, the end product of lipid peroxidation, malondialdehyde, is one of the most common and also the most harmful products of lipid peroxidation as it can cause cell damage and can also react with free amine groups in nucleic acids and proteins with an activity known as targeted mutagenic activity at the guanine site in the NAD sequence [14,15]. The use of MDA determination enables the assessment of the severity of oxidative stress or damage resulting from lipid peroxidation [16].

Caspases are one of the key mediators of programmed cell death, including caspase-3, which is a frequently activated death protease that catalyzes the specific cleavage of key cellular proteins. However, the specific requirements of caspase-3 or other caspases for sudden cell death are not yet known. The activation of caspase-3 is determined to be dependent on caspase-3 function and cytochrome c release or independent of it. Caspase-3 is essential in brain development and sudden cell death, and it is particularly significant in cell type or tissue or even death triggers [17].

The aim of the study is to determine and evaluate the importance of LDH as a prognostic factor and of caspase in colon cancer patients, in addition to predicting the levels of oxidants or antioxidants by measuring catalase activity and MDA levels in colon cancer patients.

2 Materials and methods

2.1 Study population

The study was conducted in Al-Diwaniyah Governorate, Iraq, on 20 people with colon cancer and 20 healthy people. The subjects were selected during the period (October 10th, 2023, to February 18th, 2024), and the ages ranged (from 20 to 70) and identically. Age was one of the priorities of the study. Patients are still undergoing treatment.

2.2 Methods

A blood sample (4 ml) was taken from each participant and collected in a gel tube. The gel tube of blood was centrifuged at 3600 rpm for 10-15 min at 4 °C to separate the serum. The serum was then separated into three parts using Eppendorf tubes, which were kept at -20 °C for biochemical analysis. The levels of CASp3 and LDH in the serum were measured by using the sandwich ELISA method and MDA. CAT was measured by using the Spectrophotometer

2.3 Statistical analysis

Microsoft Office Excel 2013 and GraphPad Prism 9.2.0 were used to gather, analyze, and present the data. Numbers were used to represent categorical data, while mean Standard Error Mean was used to convey numerical data. An unpaired t-test and a one-way ANOVA were used to compare the mean values across the different groups for regularly distributed variables. A chi-square analysis was performed on the qualitative data. Bivariate correlation was carried out using Pearson's correlation coefficient. When the P-value was less than 0.05, it was deemed significant.

3 Results

3.1 Measurement of lactate dehydrogenase

The study's findings indicate that colon cancer patients had higher levels of lactate dehydrogenase (LDH) (336.6 ± 17.56) mU/mL than control subjects (248.5 ± 11.88) U/L. The results indicated a significant difference (p-value =0.0002) between the LDH concentrations in the control group and colon cancer patients. as seen in Figures 1-3

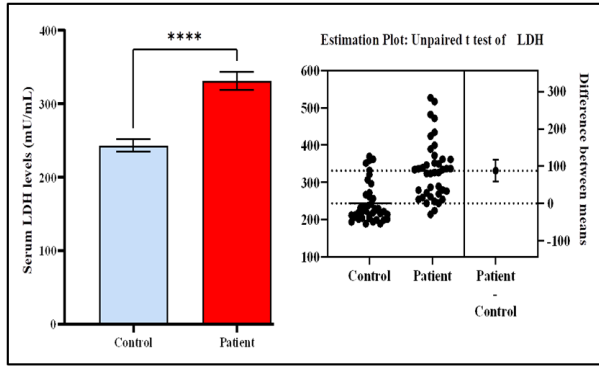


Figure 1: Comparison of mean L-lactate dehydrogenase(LDH) between the control group and patients with colon cancer group show a significant difference.

The figures show a highly significant (p-value =0.0002) difference in patients with diabetes compared with control. Data are expressed as means ± SEM. * indicates significant P≤0.05.

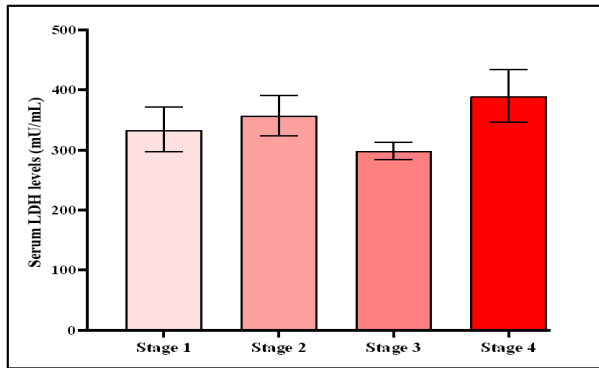


Figure 2: Comparison of concentration LDH based on differences between Stages of colon cancer.

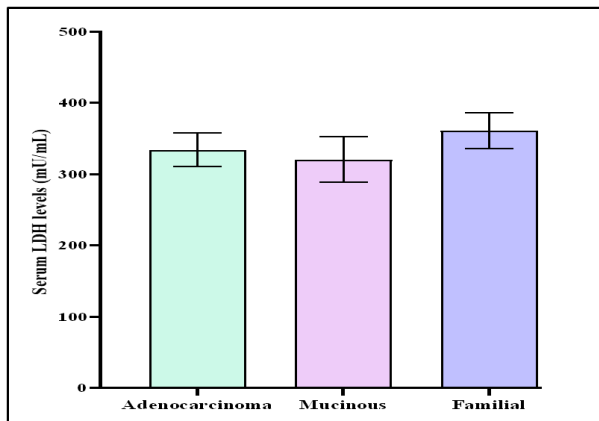


Figure 3: Comparison of concentration LDH based on differences between types of colon cancer.

3.2 Measurement of CASP3

The study’s findings indicate that colon cancer patients had higher levels of caspase 3 (CASP3) (21.44 ± 0.7045) ng/ml than control subjects (12.83 ± 0.1984) ng/ml. The results indicated a significant difference (p-value < 0.0001) between the CASP3 concentrations in the control group and colon cancer patients, as seen in Figures 4-6.

The Figures show a highly significant (p-value < 0.0001) difference in patients with diabetes as compared with control. Data are expressed as means ± SEM. * indicates significant P≤0.05.

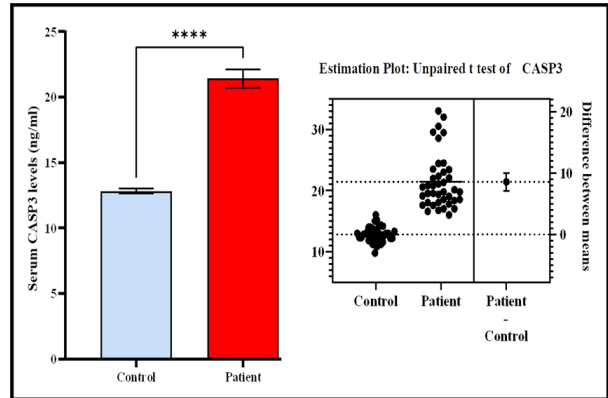


Figure 4: Comparison of concentration CASP3 based on differences between Stages of colon cancer.

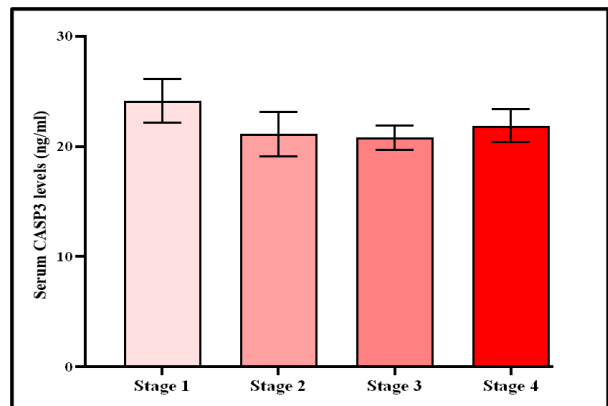


Figure 5: Comparison of concentration CASP3 based on differences between Stages of colon cancer.

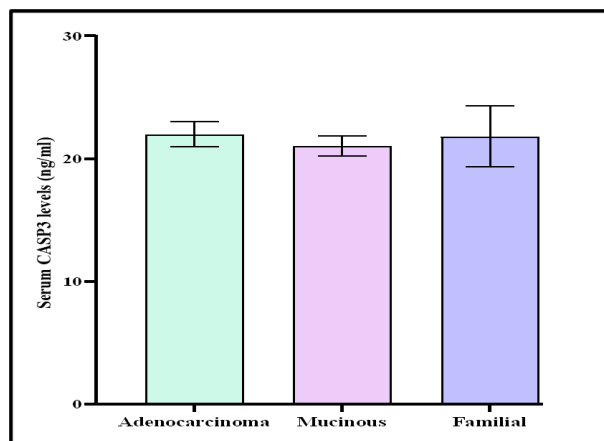


Figure 6: Comparison of concentration CASP3 based on differences between types of colon cancer.

3.3 Measurement of serum Malondialdehyde

The study's findings indicate that colon cancer patients had higher levels of malondialdehyde (MDA) (2.52 ± 0.0912) (nmol/ml) than control subjects (0.105 ± 0.0112) (nmol/ml). The results indicated a significant difference (p-value < 0.0001) between the CAT concentrations in the control group and colon cancer patients. as seen in Figures 7-9

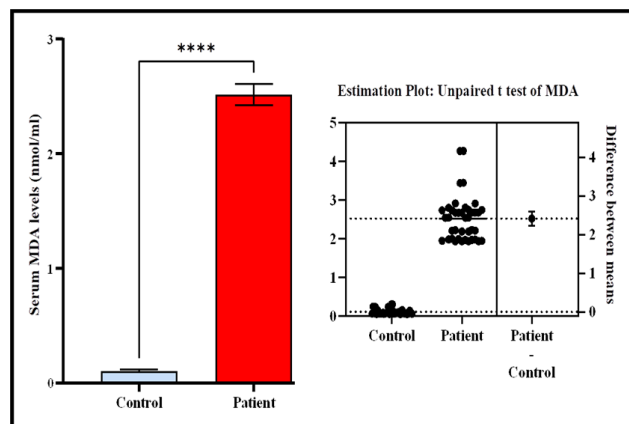


Figure 7: Comparison of mean serum malondialdehyde (MDA) between the control group and patients with colon cancer group show a significant difference.

The Figures show a highly significant (p-value < 0.0001) difference in patients with diabetes as compared with control. Data are expressed as means \pm SEM. * indicates significant $P \leq 0.05$.

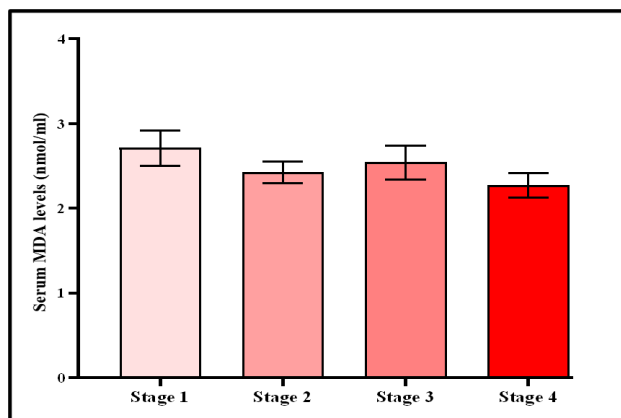


Figure 8: Comparison of concentration MDA based on differences between Stages of colon cancer.

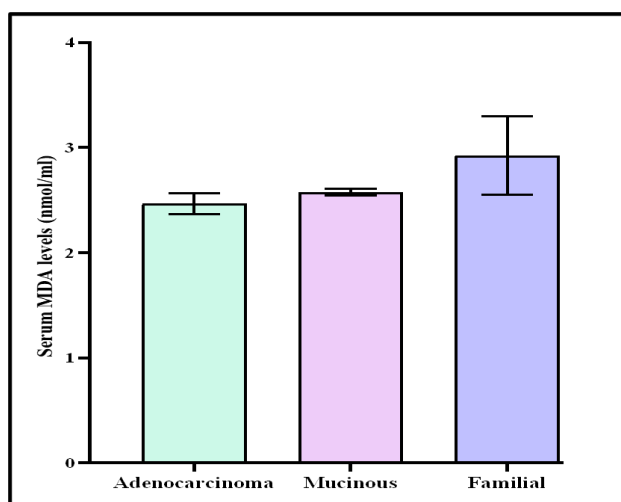


Figure 9: Comparison of concentration MDA based on differences between types of colon cancer.

3.4 Measurement of serum Catalase Activity (U/ml)

The study's findings indicate that colon cancer patients decreased levels of catalase activity (CAT) (2.242 ± 0.2853) U/mL than control subjects (10.96 ± 0.5552) U/mL. The results indicated a significant difference (p-value < 0.0001) between the CAT concentrations in the control group and colon cancer patients. as seen in Figures 10-12.

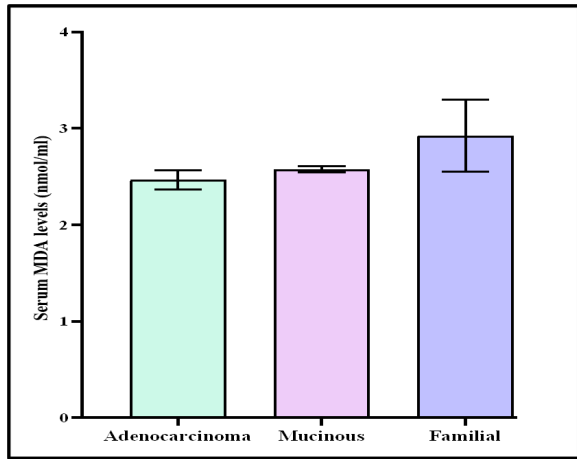


Figure 10: Comparison of mean serum catalase activity (CAT) between the control group and patients with colon cancer group show a significant difference.

The Figures show a highly significant (p -value < 0.0001) difference in patients with diabetes as compared with control. Data are expressed as means \pm SEM. * indicates significant $P \leq 0.05$.

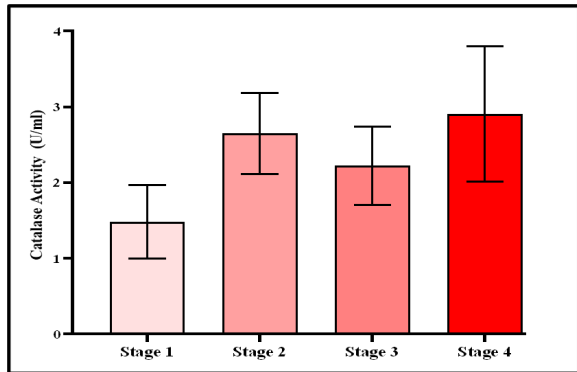


Figure 11: Comparison of concentration CAT based on differences between Stages of colon cancer.

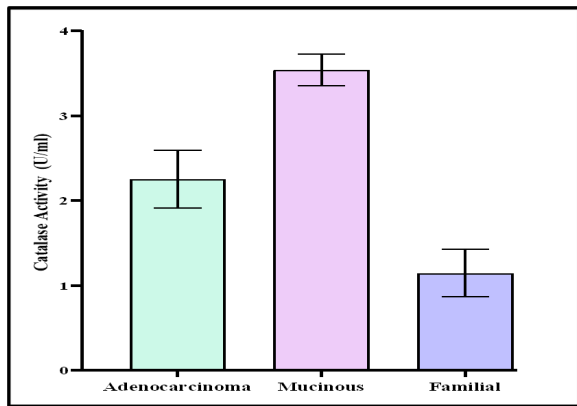


Figure 12: Correlation among different parameters in patients with colon cancer.

3.5 Correlation study

This study was also focused on the association between investigated parameter in all patient there were not significant correlation was shown in the Table 1 below between LDH,CASP-3,CAt,MDA

Table 1: Disparities in blood biomarker measurements between BC patients and the control group.

Characteristic	Correlation	BMI	LDH	CASP3	MDA	CAT
Age	Pearson r	-0.165	-0.184	0.009	-0.169	0.119
	P value	0.308	0.255	0.956	0.298	0.464
BMI	Pearson r		0.026	0.094	-0.232	-0.150
	P value		0.874	0.566	0.150	0.355
LDH	Pearson r			-0.239	0.234	0.225
	P value			0.138	0.146	0.162
CASP3	Pearson r				-0.249	-0.290
	P value				0.122	0.070
MDA	Pearson r					-0.146
	P value					0.370

R: correlation coefficient; LDH: L-lactate dehydrogenase; CASP3: Caspase 3; ; MDA: Malondialdehyd; CAT: Catalase Activity

4 Discussion

Our study showed that the presence of LDH is high in patients with colon cancer, as LDH converts pyruvate to lactic acid, as cancer cells multiply abnormally and use oxygen abundantly in their microenvironment, and they also depend on anaerobic decomposition to provide the energy needed for their reproduction and survival. These results have been supported by many researchers in different studies, as previous studies have shown that LDH is abnormally expressed in several types of cancer, including squamous cell carcinoma associated with the mouth, as well as liver cancer, breast cancer, and others. This increase is closely related to malignant development [18]. Studies have also confirmed that high expression of LDH is closely related to tumor stage, tumor size, and degree of cell differentiation and has an impact on disease-free survival (DFS) and overall survival (OS), which is an important factor in clinical diagnosis of the disease [18, 19]. Studies have also shown that inhibiting LDH in different types of cancer reduces tumor proliferation [20] Through our study, the results showed an increase in the percentage of Caspase-3 in patients with colon cancer, and in contrast, this percentage decreased in healthy people, and this result was supported by many previous studies. A previous study showed that high caspase-3 activity increases the risk of recurrence and is ultimately a prognostic factor for colon cancer as well as rectal cancer. This study agreed with two previous studies as well and showed that the frequency of apoptosis increased from the stages of colon cancer spread according to the Dukes classification from stage I, II, III to the final stage IV) (Everstson et al.,1999) as well as from the normal mucosa all the way to adenoma as well

as cancer (Baretton et al. 1999, Hawkins et al .1997) [19]. Lymphocyte infiltration in colorectal tumors has been shown to be of prognostic value (DiGiorgio et al .1992; Ropponen et al., 1997; Jass,1986; SVennivg et al ., 1984) [18].

Previous studies showed that worms and mice that were Caspase-3 deficient had a specific deficiency in apoptosis but, at the same time, were sensitive to some stress, which was related to inflammation [19]. Studies have also shown that genetic ablation. Caspase-3, present in colon cancer cells, works to increase sensitivity to DNA-damaging agents through RIFI-dependent necrosis without compromising the process of programmed cell death.

Therefore, pharmacological manipulation of Caspase-3 is considered a modern method to enhance the killing of cancer cells that are resistant to Chemicals [21]. Decreased caspase-3 activity in normal, as well as tumor mucosa, is an independent factor in predicting tumor recurrence in people with colon cancer who have adjuvant therapy based on 5-fluorouracil and has also been associated with poorer disease-free survival and increased repetition rate [18].

Previous studies have confirmed, although the reason is not clear, that cancer cells use CAT-1 primarily for Arg metabolism, but there is a lot of evidence available, including the first- Up-regulation of CAT-1 may be through several factors in the tumor microenvironment such as polyamines, Second- Rapid mitotic signals, Third- Pathological stress, Fourth- Cytokines that play an important role in cancer development and spread as they are pro-inflammatory [11,12]. Another study conducted in the laboratory showed that the destruction of CAT-1 in CRC cells is the cause of sudden cell death (apoptosis) and inhibition of cell growth, which indicates that CAT-1 may be a unique molecular biomarker as well as a therapeutic target for CRC [13]. Interestingly, it was shown that nickel, lead, and Cadmium solutions with a concentration of 0.2 mM inhibit CAT activity in colon cancer [11].

Using qRT-PCR, it was found that the expression of CAT-1 was at a high level in CRC tissues. When compared with other normal colon tissues, another study showed that changes in the level of CAT-1 mRNA do not necessarily affect the level of CAT-1 protein [22]. A study also showed that CAT-1 plays a very important role in the uptake of arginine in breast cancer cells, as well as the production of nitrogen oxides. Mercury activates the CAT enzyme in colon cancer [11]. Through our study, we found that MDA, which is an indicator of lipid oxidation and oxidative stress, is elevated in patients with colon cancer. The high presence of lipids in the cell membrane and also in the subcellular organelles of the cell membrane is the site from which oxidative damage to lipids begins. The process of lipid oxidation produces a variety of degra-

ation products, including MDA. The action of MDA targets guanine in DNA, which is a cause of mutations. It may also contribute to cancer, including colon and rectal cancer. This has been supported by many previous studies, as the results of (Skrzydewska et al.) indicated that lipid oxidation products, including MDA, increase the tissues of rectal and colon cancer [23]. As reported by (Surinenaite et al.), The concentration of MDA in the serum of patients with colorectal cancer was observed to be high in stage III disease when compared with stage II patients and was significantly decreased after surgical treatment (tumor resection) when compared with the level before surgery [24]. Gopcevic et al. found that MDA, or what is known as lipid peroxidation, was increased in all disease stages of colorectal cancer but without a significant difference in disease stages [21].

5 Conclusion

This study underscores the significant biochemical changes associated with colon cancer. Elevated levels of lactate dehydrogenase (LDH), caspase-3 (CASP3), and malondialdehyde (MDA) highlight increased metabolic activity, apoptosis, and oxidative stress in colon cancer patients. Conversely, reduced catalase (CAT) activity reflects impaired antioxidant defense mechanisms. These findings suggest that LDH and CASP3 could serve as valuable prognostic biomarkers for colon cancer, while MDA and CAT provide insights into the oxidative and antioxidative status of patients. Collectively, these biomarkers have potential clinical applications for disease monitoring, risk assessment, and guiding therapeutic strategies in colon cancer.

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Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work.

Ethical consideration: This research is part of an approved student thesis under the supervision of the Faculty of Sciences, University of Al-Qadisiyah, Iraq, and it received the approval of the Department of Chemistry, Faculty of Sciences, University of Al-Qadisiyah, Iraq.

Authors' Contribution: Ashwaq Nouri Mawat and Dr. Zainab Al-Abadi participated in the study design. Analyze the results and draft the report. The

authors gave their approval. The final version to be submitted.

References

- [1] Patel M, McSorley ST, Park JH, Roxburgh CS, Edwards J, Horgan PG, et al. The relationship between right-sided tumour location, tumour microenvironment, systemic inflammation, adjuvant therapy and survival in patients undergoing surgery for colon and rectal cancer. *British journal of cancer*. 2018;118(5):705-12. doi:10.1038/bjc.2017.441. [Backref page 1]
- [2] Arnold M, Sierra M, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017;66(4):683-91. doi:10.1136/gutjnl-2015-310912. [Backref page 1]
- [3] Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Przeegląd Gastroenterologiczny*. 2019;14(2):89-103. doi:10.5114/pg.2018.81072. [Backref page 1]
- [4] Ferrucci L, Sinha R, Huang W, Berndt S, Katki H, Schoen R, et al. Meat consumption and the risk of incident distal colon and rectal adenoma. *British journal of cancer*. 2012;106(3):608-16. doi:10.1038/bjc.2011.549. [Backref page 1]
- [5] Hjartåker A, Aagnes B, Røsbahm T, Langseth H, Bray F, Larsen I. Subsite-specific dietary risk factors for colorectal cancer: a review of cohort studies. *Journal of Oncology*. 2013;2013(1):703854. doi:10.1155/2013/703854. [Backref page 24]
- [6] Rajae N, Khaoula D, Zahra H, Samia A, Nawfel M. Prognostic Factors in Colorectal Cancer: Serum LDH Levels Predict Survival in Metastatic Disease. *Annals of Oncology*. 2014;25:ii81. doi:10.1093/annonc/mdu165.212. [Backref page 24]
- [7] DeBerardinis R, Lum J, Hatzivassiliou G, Thompson C. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metabolism*. 2008;7(1):11-20. doi:10.1016/j.cmet.2007.10.002. [Backref page 24]
- [8] Luo J, Solimini N, Elledge S. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009;136(5):823-37. doi:10.1016/j.cell.2009.02.024. [Backref page 24]
- [9] Benboubker L, Valat C, Linassier C, Cartron G, Delain M, Bout M, et al. A new serologic index for low-grade non-Hodgkin's lymphoma based on initial CA125 and LDH serum levels. *Annals of Oncology*. 2000;11(11):1485-91. doi:10.1023/A:1026789232033. [Backref page 24]
- [10] Koukourakis M, Kontomanolis E, Giatromanolaki A, Sivridis E, Liberis V. Serum and tissue LDH levels in patients with breast/gynaecological cancer and benign diseases. *Gynecologic and Obstetric Investigation*. 2009;67(3):162-8. doi:10.1159/000183250. [Backref page 24]
- [11] Closs E, Simon A, Vékony N, Rotmann A. Plasma membrane transporters for arginine. *Journal of Nutrition*. 2004;134(10):2752S-2759S. doi:10.1093/jn/134.10.2752S. [Backref page 24], [Backref page 28]
- [12] Kaneko S, Okuda-Ashitaka E, Ando A, Nishimura K, Igarashi K, Maeda M, et al. Polyamines upregulate the mRNA expression of cationic amino acid transporter-1 in human retinal pigment epithelial cells. *American Journal of Physiology-Cell Physiology*. 2007;293(2):C729-37. doi:10.1152/ajpcell.00018.2007. [Backref page 24], [Backref page 28]
- [13] Lu Y, Wang W, Wang J, Yang C, Mao H, Fu X, et al. Overexpression of arginine transporter CAT-1 is associated with accumulation of L-arginine and cell growth in human colorectal cancer tissue. *PLOS One*. 2013;8(9):e73866. doi:10.1371/journal.pone.0073866. [Backref page 24], [Backref page 28]
- [14] Štefan L, Tepšić T, Zavidic T, Urukalo M, Tota D, Domitrović R. Lipid peroxidation-causes and consequences. *Medicina Fluminensis*. 2007;43:84-93. [Backref page 24]
- [15] Salzman R, Pacal L, Tomandl J, Kaňková K, Tóthová E, Gál B, et al. Elevated malondialdehyde correlates with the extent of primary tumor and predicts poor prognosis of oropharyngeal cancer. *Anticancer research*. 2009;29(10):4227-31. [Backref page 24]
- [16] Zalewski B. Levels of v5 and v6 CD44 splice variants in serum of patients with colorectal cancer are not correlated with pT stage, histopathological grade of malignancy and clinical features. *World Journal of Gastroenterology*. 2004;10(4):583. doi:10.3748/wjg.v10.i4.583. [Backref page 24]
- [17] Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death and Differentiation*. 1999;6(2):99-104. doi:10.1038/sj.cdd.4400476. [Backref page 24]

- [18] Noble P, Vyas M, Al-Attar A, Durrant S, Scholefield J, Durrant L. High levels of cleaved caspase-3 in colorectal tumour stroma predict good survival. *British Journal of Cancer*. 2013;108(10):2097-105. doi:10.1038/bjc.2013.166. [Backref page 27], [Backref page 28]
- [19] Wang Y, Nie H, Liao Z, He X, Xu Z, Zhou J, et al. Expression and clinical significance of lactate dehydrogenase A in colon adenocarcinoma. *Frontiers in Oncology*. 2021;11:700795. doi:10.3389/fonc.2021.700795. [Backref page 27], [Backref page 28]
- [20] Sharma D, Singh M, Rani R. Role of LDH in tumor glycolysis: regulation of LDHA by small molecules for cancer therapeutics. In: *Seminars in Cancer Biology*. Elsevier; 2022. p. 184-95. doi:10.1016/j.semcancer.2022.11.007. [Backref page 27]
- [21] Gopčević KR, Rovčanin BR, Tatić SB, Krivokapić ZV, Gajić MM, Dragutinović VV. Activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in different stages of colorectal carcinoma. *Digestive diseases and sciences*. 2013;58:2646-52. doi:10.1007/s10620-013-2681-2. [Backref page 28]
- [22] Nicholson B, Manner CK, Kleeman J, MacLeod CL. Sustained nitric oxide production in macrophages requires the arginine transporter CAT2. *Journal of Biological Chemistry*. 2001;276(19):15881-5. doi:10.1074/jbc.M010030200. [Backref page 28]
- [23] Skrzydlewska E, Sulkowski S, Koda M, Zalewski B, Kanczuga-Koda L, Sulkowska M. Lipid peroxidation and antioxidant status in colorectal cancer. *World Journal of Gastroenterology*. 2005;11(3):403. doi:10.3748/wjg.v11.i3.403. [Backref page 28]
- [24] Surinėnaitė B, Prasmickienė G, Milašienė V, Stratilatovas E, Didžiapetrienė J. The influence of surgical treatment and red blood cell transfusion on changes in antioxidative and immune system parameters in colorectal cancer patients. *Medicina*. 2009;45(10):785. [Backref page 28]

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