Journal of Health Sciences

RESEARCH ARTICLE Open Access

Evaluation of serum levels of malondialdehyde and endogenous non-enzymatic antioxidants in relation to colorectal cancer stage and intestinal wall infiltration

Ismar Rašić[1]*, Sandin Holjan[2], Vedad Papović[3], Sanjin Glavaš[3], Adi Mulabdić[2], Azra Rašić[4]

[1]Department of Surgery, General Hospital "Prim. Dr. Abdulah Nakaš," Sarajevo, Bosnia and Herzegovina, [2]Clinic for General and Abdominal Surgery, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [3]Clinic for Gastroenterohepatology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina [4]The Oncology Clinic, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

ABSTRACT

Introduction: Oxidative stress and lipid peroxidation are pointed as possible factors in the development of colorectal cancer (CRC). The aim of this study was to assess the serum malondialdehyde (MDA) and non-enzymatic antioxidants concentration (albumin, bilirubin, uric acid, and ferritin) and their relation with the stage and histopathologic size (pT) of CRC.

Methods: One hundred and twenty patients with clinically and histopathologically confirmed CRC and the need for surgical treatment were included in a cross-sectional study. All patients were divided into groups according to the disease stage and depth of tumor invasion. The control group included 30 subjects with no signs of malignant and inflammatory bowel disease. The patients and controls did not receive vitamin supplementation. Peripheral venous blood was sampled before the surgical treatment of CRC patients and on the day of the examination of control subjects for determination of serum MDA and the concentration of the non-enzymatic antioxidants.

Results: The serum levels of MDA were progressively increased in CRC patients with the highest level in the fourth stage of disease and pT4 group. Ferritin levels increased significantly with the CRC stage and decreased with the depth of bowel wall invasion. Serum albumin concentration significantly decreased with increasing stage and increasing depth of tumor invasion of the intestinal wall, while serum bilirubin level showed no change compared to the control group. Serum uric acid concentration was significantly higher in CRC patients, but no difference was observed with CRC progression. It was confirmed that serum albumin significantly negatively correlated with the CRC stage (rho = –0.649, $p < 0.001$), while serum MDA significantly positively correlated with the CRC stage (rho = 0.750, $p < 0.001$).

Conclusion: These results indicate that serum MDA concentrations are related to the progression of CRC, to which the imbalance in non-enzymatic antioxidants also contributes.

Keywords: Colon cancer; malondialdehyde; non-enzymatic antioxidants

INTRODUCTION

Colorectal cancer (CRC) is classified as the third most common malignancy worldwide, accounting for approximately 9.4% of all causes of cancer death (1). Due to the ever-increasing incidence, CRC is becoming an increasingly important diagnostic and therapeutic problem.

Several risk factors are related to the onset and progression of CRC, such as physical inactivity, environmental factors, alcohol consumption, smoking, diet, and obesity. For the past 10 years, there is growing support for the concept that overproduction of reactive oxygen species (ROS) could result in mutations and promote oncogenic phenotypes involved in carcinogenesis, implicated in a range of diseases, including CRC (2,3). ROS oxidize structural proteins and inhibit the proteolytic system (4). ROS also have the ability to oxidize polyunsaturated fatty acids, which take part in cell membrane constitution. This reaction initiates a chain reaction of lipid peroxidation, that produces other free radicals and substances such as malondialdehyde (MDA), to which a significant role in the development of cancer has been attributed (5).

To prevent damage from ROS, the body has an antioxidant protection system, which includes enzymatic and

*Corresponding author: Ismar Rašić, Department of Surgery, General Hospital "Prim. dr. Abdulah Nakaš", Bosnia and Herzegovina.
E-mail: rasicismar@gmail.com

Submitted: 25 June 2021/Accepted: 10 September 2021

DOI: https://doi.org/10.17532/jhsci.2021.1387

UNIVERSITY OF SARAJEVO
FACULTY OF HEALTH STUDIES

non-enzymatic antioxidants. Enzymatic antioxidants belong to cellular antioxidants and are found in the cells of the arterial walls. Non-enzymatic antioxidants are present both extracellularly and intracellularly and represent the first line of defense of the body against the action of oxidizing substrates. Non-enzymatic antioxidants include exogenous antioxidants such as Vitamin E, Vitamin C and carotene, and endogenous antioxidants such as albumin, ferritin, uric acid, bilirubin, and ceruloplasmin. More than 70% of the plasma's antioxidant capacity is albumin and uric acid. Bilirubin is also a strong antioxidant, which prevents lipid peroxidation.

Several studies have documented the importance of antioxidants in slowing down and reducing adverse effects of oxidative stress and preventing colorectal carcinogenesis (6,7). However, data were performed mostly in vitro on cell cultures, or in vivo in experimental animal models (8,9).

The aim of the study was to parallel monitoring and analyzing the levels of serum MDA concentration and non-enzymatic antioxidants in patients with CRC and to determine the relationship of monitored biomarkers with the histopathologic size and stage of CRC.

METHODS

One hundred and twenty patients both genders, 68 (56.7%) male and 52 (43.3%) female, with radiologically, colonoscopically, and histopathologically confirmed CRC and with a need for surgical treatment of that cancer were included in a cross-sectional study lasting 4 years at the Clinic for General and Abdominal Surgery, Clinical Center of the University of Sarajevo. The ethical committee of the Clinical Centre of the University of Sarajevo approved this study. All patients who participated in this study gave their written informed consent. The mean age of CRC patients was 67.1 (range 49–79) years without a significant difference according to the sex of the patients (67.4-years-old in male vs. 64.8-years-old in female).

Exclusion criteria included evidence of neoplasm on an organ unrelated to colon cancer, patients undergoing oncological treatment (radiotherapy or chemotherapy) before surgery, presence of inflammatory bowel disease, history of familial adenomatous polyposis, and coexistence of other systemic or autoimmune diseases.

The control group included 30 healthy volunteers (53.3% males and 46.7% females), mean age of 59.1 (45–78) years, who underwent preventive examination at the Counseling Centre for Gastroenterohepatology, Clinical Center of the University of Sarajevo. They had no family history of cancer or clinical signs of malignant or inflammatory bowel disease, comorbid conditions such as diabetes, hypertension, coronary heart disease or autoimmune diseases, lung, thyroid, liver, kidney, and infectious diseases (HCV and HIV infection). In addition, patients and the control group did not receive vitamin supplementation for the past 3 months before inclusion in the study and they did not smoke or use medications such as antibiotics, nonsteroidal anti-inflammatory, and steroid medications.

Informed consent was obtained from all respondents included in this study. The study protocol was approved by the local Ethics Committee and was performed in accordance with the Helsinki Declaration.

Five milliliters of peripheral venous blood were sampled from fasting patients before the surgical treatment of CRC and from controls on the day of physical examination. The blood was collected in BD Vacutainer test tube and centrifuged at 5.000 rpm/min at room temperature for 10 min, with separation of serum into aliquots. All serum samples for determination of MDA concentration were stored at –80°C until analysis, while serum levels of albumin, uric acid, and bilirubin were determined on the day of the blood sampling as well as the level of ferritin in plasma sample.

Serum concentration of MDA was estimated using a commercial kit for the overall level of MDA (USCN Life Science Inc., Houston, USA). Reading of the results was carried out spectrophotometrically at 450 nm on a plate reader STAT FAX 2100 (Awareness Technology, Palm City, Florida, USA). The measured MDA concentration was expressed in nanograms per milliliter (ng/mL).

Serum albumin level was determined by electrophoresis, after spectrophotometric total protein concentration measurement on Dimension X Pand Plus system analyzer (Siemens AG, Germany), with reference value for albumin 35.0–50.0 g/L. Plasma ferritin levels were quantified on Cobas 6000 analyzer (Roche Diagnostics International Ltd, Switzerland) using an electrochemiluminescent immunoassay (reference range: 21.81–274.66 ng/mL). Quantitative determination of serum uric acid concentration was performed spectrophotometrically using a modified Kalckar method (URCA method) on Dimension RxL Max (Siemens AG, Germany; reference rank: 155–428 μmol/L). The total serum bilirubin concentration was also measured on the same analyzer by spectrophotometric method (reference range: 1.7–20.5 μmol/L).

CRC surgery was performed according to the principle of *en bloc* resection of colon cancer with associated lymph-vascular arcade under general anesthesia. After resection and macroscopic examination of surgically obtained tissue material, samples of tumor were fixed in 10% phosphate-buffered formalin for further histopathological analysis. Stage of CRC was determined according to the TNM classification of the American Association of Cancer (American Joint Committee on Cancer, AJCC guidelines) from 2010 (10), in which "T" marks the depth of tumor invasion (pT), "N" lymph node metastasis (pN), and "M" distal metastasis. Staging of the CRC was marked with numbers I–IV. All patients were divided into groups according to the disease stage and depth of tumor invasion.

Statistical analyses were performed using the MedCalc Software for Windows, version 12.6.1.0. Kolmogorov–Smirnov test or Shapiro–Wilk test was used to examine the normal distribution of data. Variables with normal distribution were presented as mean ± standard deviation and compared using the *t*-test for independent samples. Variables not displaying normal distribution were presented as median and interquartile range and compared by Mann–Whitney U-test. ANOVA and Kruskal–Wallis test was used for statistical evaluation of more than three groups. The correlation between the monitored biomarkers of oxidative and antioxidant system and the stage and histopathological depth of intestinal wall invasion was determined by Spearman correlation coefficient. Multiple

regression analysis was used to examine the impact of MDA and non-enzymatic antioxidants (albumin, bilirubin, uric acid, and ferritin) on the stage of CRC and the depth of tumor invasion. Statistical significance was established at $p < 0.05$.

RESULTS

The most common location of CRC was the rectum, in 47 (39.2%) patients, while the rarest location was cecum, in three cases (2.5%). All the CRC patients had histologically adenocarcinoma type of cancer, in two-thirds of cases moderately differentiated (G2 grade). According to the depth of the intestinal wall infiltration, 23 (19.2%) patients had tumor with invasion of muscularis propria (pT2). Tumor that spread through the muscularis propria into non-peritonealized pericolorectal tissues without involvement the other organs (pT3) was confirmed in 67 (55.8%) patients, while 30 (25.0%) patients had a tumor that penetrated the visceral peritoneum or directly affected the other organs or structures (pT4). The percentage of patients without lymph node (N0) and distant metastasis (M0) was 37.5% and 75.0%, respectively.

According to the laboratory examination, 75 (62.5%) patients had decreased serum albumin concentration, while 45 (37.5%) cases had normal serum albumin values. Normal plasma ferritin levels were confirmed in about half of the patients (63; 52.5%), decreased in 21 (17.5%) cases and increased in 36 (30.0%) cases. Most patients had normal serum uric acid concentration (95; 72.2%) and serum bilirubin concentration (117; 97.5%). The basic information about the study patients are summarized in Table 1.

Serum MDA concentration was significantly higher in patients with second stage of colorectal malignancy compared to controls (47.5 [16.5–58.3] vs. 26.9 [17.4-31.2] ng/mL; $p < 0.05$). The results of the study indicate that serum MDA concentration shows a progressive increase through the stages (II–IV stage) of CRC (47.5 [16.5–58.3] vs. 61.35 [54.6–69.5] vs. 66.3 [60.8–69.5] ng/mL; $p < 0.001$) (Figure 1).

According to the depth of tumor invasion (pT), there was no difference between the pT2 and pT3 groups in terms of serum MDA level ($p = 0.590$). The concentration of serum MDA was significantly higher in the pT4 group (66.3 [59.2–71.0] ng/mL) compared to the pT3 group of patients (58.3 [46.8–66.8] ng/mL, $p = 0.002$) and the pT2 group of patients (57.6 [46.0–60.4] ng/mL, $p = 0.012$) (Figure 2).

Parallel monitoring of non-enzymatic parameters of the antioxidant system indicated a gradual decrease of serum albumin concentrations in patients with progression of CRC (Table 1). The highest reduction in serum albumin level was observed in the fourth stage of CRC ($p < 0.001$). The serum albumin level also decreased significantly with the depth of tumor invasion. A significant difference in the serum albumin concentration was detected in the pT4 group of patients with CRC compared to pT2 and pT3 group ($p = 0.014$).

The decline in serum albumin level was accompanied by a significant increase in plasma ferritin concentration, with the highest level of ferritin in the fourth stage of colorectal

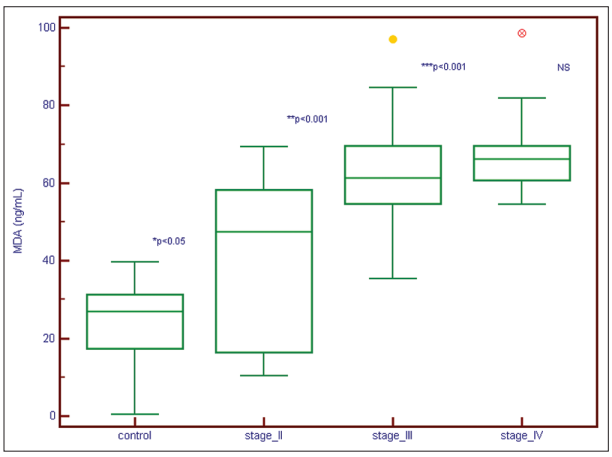

FIGURE 1. Serum malondialdehyde concentration in patients with different stages of CRC. The data are presented as median, with minimum–maximum values. *p<0.05 - difference between control and stage II of CRC, **p<0.001- difference between II and III stage of CRC, ***p<0.001 - difference between II and IV stage of CRC. NS: No significance between III and IV stage of CRC. CRC: colorectal cancer, MDA: Malondialdehyde.

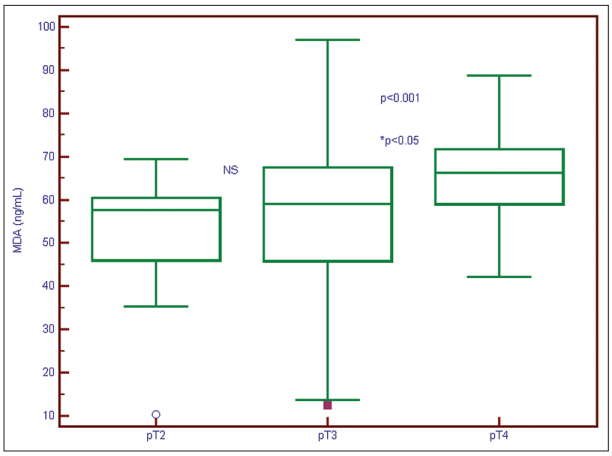

FIGURE 2. Serum malondialdehyde concentration in CRC patients with different depth of tumor invasion. The data are presented as median, with minimum–maximum values. p<0.001 - significance difference between pT3 and pT4, *p<0.05 - significance difference between pT2 and pT4, NS: No significance between pT2 and pT3. CRC: Colorectal cancer, MDA: Malondialdehyde, pT: Depth of tumor invasion.

malignancy ($p < 0.05$). While plasma ferritin levels increased with the increasing stage of CRC, it also showed a tendency to decline with depth of tumor invasion, with the lowest level observed in the pT4 group (Table 2). While plasma ferritin levels increased with the increasing stage of CRC, it also showed a tendency to decline with depth of tumor invasion, with the lowest level observed in the pT4 group (Table 2). Serum uric acid concentration did not change significantly through the CRC stages and different depth of malignant infiltration of the intestinal wall, but was significantly higher in CRC patients compared to controls. Serum bilirubin values were indistinguishable from the control group and had no specific oscillations according to the stage or depth of tumor invasion.

It was found that serum albumin had significant negative correlation with CRC stage (r = –0.649, $p < 0.001$), while the negative insignificant correlation of this biomarker with the depth of tumor invasion was confirmed (r = –0.189, $p = 0.062$). Serum MDA had a significant positive

TABLE 1. Basic characteristics of the study group

Parameter	Patient number (%)
Age (years)	
>60	31 (25.8%)
<60	89 (74.2%)
Gender	
Male	68 (56.7%)
Female	52 (43.3%)
Tumor location	
Cecum	3 (2.5%)
Ascending colon	18 (15.0%)
Transverse colon	3 (2.5%)
Descending colon	9 (7.5%)
Sigmoid colon	40 (33.3%)
Rectum	47 (39.2%)
Histological type	
Adenocarcinoma	120 (100.0%)
Grade I	14 (11.6%)
Grade II	80 (66.7%)
Grade III	20 (16.7%)
Grade IV	6 (5.0%)
Depth of tumor invasion (pT)	
pT1	0 (0.0%)
pT2	23 (19.2%)
pT3	67 (55.8%)
pT4	30 (25.0%)
Lymph node metastasis (pN)	
N0	45 (37.5%)
N1	39 (32.5%)
N2	36 (30.0%)
Distant metastasis (pM)	
M0	90 (75.0%)
M1	30 (25.0%)
Stage of CRC	
I	0 (0.0%)
II	23 (19.2%)
III	67 (55.8%)
IV	30 (25.0%)
Serum concentration of albumin	
Normal	45 (37.5%)
Decreased	75 (62.5%)
Increased	0 (0.0%)
Plasma ferritin concentration	
Normal	63 (52.5%)
Decreased	21 (17.5%)
Increased	36 (30.0%)
Serum uric acid concentration	
Normal	95 (72.2%)
Decreased	5 (4.2%)
Increased	20 (16.6%
Serum bilirubin concentration	
Normal	117 (97.5%)
Decreased	0 (0.0%)
Increased	3 (2.5%)

CRC: Colorectal cancer

correlation with CRC stage (r = 0.750, $p < 0.001$) and intestinal wall infiltration (r = 0.380, $p < 0.001$) (Table 3).

In the linear regression analysis model, an independent positive predictor of CRC stage was serum MDA concentration, while albumin was an independent negative predictor of CRC stage (Table 3). In addition, an independent positive predictor of tumor size or depth of tumor invasion (pT) was serum MDA concentration (Table 4).

DISCUSSION

The oxidative stress usually results either due to excessive ROS production or impaired antioxidant system, or a combination of these factors. The pro-oxidative/antioxidative imbalance between the ROS formation and ability of the several antioxidant defense mechanisms (including enzymes and non-enzymatic antioxidants) to eliminate that disturbances leads to various pathophysiological conditions. ROS overproduction associated with insufficient antioxidant defense lead to protein, DNA and lipid oxidation and oxidative cell damage, which can trigger cancer initiation and progression, including CRC (2).

Several published studies suggest that ROS overproduction, including also MDA as a product of lipid peroxidation, may play a significant role in all stages of carcinogenesis (11,12). Increased concentrations of MDA in plasma or urine have been observed in patients with various neoplasms, including gastric cancer (13), breast cancer (14), and bladder cancer (15). The study by Zinczuk *et al*. also indicated significantly higher plasma concentrations of MDA in CRC patients compared to healthy controls (16). In addition, the research of Janion *et al*. demonstrated differentiation in the intensity of lipid peroxidation process in relation to the location of the primary tumor on the right side of the colon (17). In this study, serum MDA levels reached the highest values in patients with stage IV CRC, but these results were not statistically significant.

Branković *et al*. proved the significant presence of oxidative stress in tumor tissue samples from resected colon preparation with a highly significant increase of MDA concentration in both tumor and adjacent tissue compared to the values in healthy tissue specimens (18).

In the study by Leung *et al*., it was shown that the patients with advanced inoperable CRC had a much higher concentration of MDA serum compared to those with primary localized CRC (19). Our research demonstrated statistically significant differences in serum MDA levels in the third and the fourth stage of the CRC compared to the second stage of CRC. Similar results were reported by Surinenaite *et al*. (20), who also found that MDA levels decreased significantly after surgical treatment compared to preoperative status.

Our study also confirmed significantly higher serum MDA concentrations in patients in whom the tumor perforated the visceral peritoneum or involved adjacent organs (pT4) compared to the group in which the tumor spread through the muscularis propria to non-peritonealized pericolorectal tissues without involvement of other organs (pT3) and to the group in which the tumor invaded muscularis propria (pT2). Serum MDA concentration significantly positively correlated with CRC stage and depth of intestinal wall infiltration. These findings indicate the carcinogenic potential of MDA and its association with the progression of CRC. In recently published study, Zinczuk *et al*. observed a link

TABLE 2. The serum concentration of non-enzymatic antioxidants in relation to the stage and histopathological features of CRC

Variabe	Control	Stage of CRC			
		II	III	IV	p
Albumin (g/L)	42.0 (39.0–44.0) [a]	38.0 (32.0–40.0) [b]	37.0 (32.0–39.0) [b]	29.5 (25.0–33.0) [c]	<0.001
Bilirubin (μmol/L)	8.7 (6.6–10.2)	8.1 (6.9–11.9)	10.7 (7.6–13.1)	9.5 (5.8–12.1)	NS
Uric acid (μmol/L)	272.0 (220.0–288.0) [a]	288.5 (237.0–362) [b]	281.5 (204.0–363.0) [b]	263.0 (215.0–340.0) [b]	<0.05
Ferritin (ng/mL)	49.54 (38.0–115.0) [a]	84.7 (39.7–189.3) [b]	80.3 (32.2–150.1) [b]	95.2 (50.9–226.6) [c]	<0.05
		pT			
		pT2	pT3	pT4	p
Albumin (g/L)		33.8±6.5[a]	34.5±6.2[a]	29.8±7.3[b]	<0.05
Bilirubin (μmol/L)		11.6 (10.5–12.8) [a]	9.2 (6.9–12.9) [a]	9.4 (6.7–11.9) [a]	NS
Uric acid (μmol/L)		281.8±105.1	309.3±111.5	275.5±88.8	NS
Ferritin (ng/mL)		99.2 (54.7–116.2) [a]	71.0 (28.6–150.9) [b]	66.1 (38.8–136.6) [b]	<0.05

Data are presented as mean±standard deviation (SD) or median and interquartile range q1-q3.[abc]Values in the same row that do not contain a same letter differ significantly. NS: Insignificantly, CRC: Colorectal cancer, pT: Depth of tumor invasion

TABLE 3. Correlation of monitored biomarkers with stage of CRC and depth of tumor invasion

Biomarkers	Stage	pT
Albumin		
rho	−0.649**	−0.189
p	0.000	0.062
Bilirubin		
rho	0.106	−0.121
p	0.247	0.236
Uric acid		
rho	0.062	−0.011
p	0.500	0.916
Ferritin		
rho	0.137	0.022
p	0.136	0.830
MDA (ng/mL)		
rho	0.750**	0.380**
p	0.000	0.000

pT: Depth of tumor invasion, rho: Correlation coefficient, *p<0.05, **p<0.01. MDA: Malondialdehyde, CRC: Colorectal cancer

between oxidative stress index, MDA and colon tumor budding, which suggest the participation of oxidative stress in the remodeling of tumor (21). In addition, this study observed significantly higher MDA level in patients with lymph node metastasis in comparison to those without metastasis.

Oxidative stress may arise from an imbalance between the production of ROS and the mechanisms of cellular antioxidant defense (7). Ozgonul *et al*. found that patients with CRC have lower levels of total antioxidative capacity compared to healthy controls (22). However, information on biochemical alterations in tissue and blood, especially antioxidant status, and their correlation with the clinical stage of the disease are lacking. Due to the increase in oxidative stress in these patients, the classification of oxidative-antioxidant specificities of different stages of CRC is of particular importance. In our study, serum albumin concentration showed a gradual decrease in higher stages of CRC, while plasma ferritin concentrations showed irregular oscillations in different stages of the disease. A significant decrease in serum albumin in the fourth stage of CRC compared to the second and third stages of this disease was accompanied by a significant increase in serum ferritin concentration. Some authors have suggested that hypoalbuminemia is a systemic inflammatory response to malignancy (23,24). However, the results of our study indicated that CRC progression was associated with a progressive decrease in serum albumin as a reflection of disorders of the antioxidant system. In a systematic review of 927 participants from seven case–control studies, Feng *et al*. found that patients with CRC had lower serum ferritin levels than healthy controls (25). Ferritin is a protein that binds to iron and belongs to the antioxidant system. Based on *in vitro* evidence, it is assumed that iron facilitates DNA mutation through augmentation of oxygen radical synthesis through the Haber-Weiss reaction, as well as suppresses tumoricidal activity of macrophages.

The results of our study suggest that the antioxidant imbalance present in patients with CRC contributes to the increase in MDA concentration and progression of colorectal carcinoma. Furthermore, serum MDA was found to be in significant positive correlation with CRC stage, whereas serum albumin was in significantly negative correlation with CRC stage, indicating the association of these parameters with CRC.

The research of Gopcevic *et al*. confirmed that lipid peroxidation is higher in all stages of CRC compared to the control group, but with no significant differences among the stages of the disease (26). At the same time, these authors found a significantly lower activity of superoxide dismutase and glutathione reductase as enzymatic indicators of antioxidant status in all stages of CRC compared to the control group, but with a significant increase in stage IV disease, concluding that CRC was associated with an increase in oxidative stress followed by increase in antioxidant imbalance. The results by Stone *et al*. point out that the oxidative status expression is caused by a reduction of the potential antioxidant defense (27), which is also consistent with our results.

Several studies have found some genetic factors that may affect susceptibility to CRC, which relate to several single-nucleotide polymorphisms in genes implicated in antioxidative protective system, such as eosinophil peroxidase, myeloperoxidase, and selenoprotein (28,29). It is considered that genetic variation of antioxidative protective proteins can be associated with CRC risk and survival after diagnosis. All these studies and their results highlight the complexity of the pathogenetic basis of CRC and the

TABLE 4. Independent predictors of the CRC progression

	B	Standarderror	Beta	*p*	95% Confidence interval
Albumin	−0.043	0.011	−0.207	0.000	−0.065–0.021
Bilirubin	0.005	0.015	0.014	0.746	−0.025–0.035
Uric acid	0.002	0.001	0.113	0.909	0.000–0.003
Ferritin	−3.329E–05	0.001	−0.002	0.958	−0.001–0.001
MDA	0.021	0.004	0.335	0.000	0.013–0.031
Dependent variable: CRC stage					
Albumin	−0.010	0.010	−0.118	0.307	–0.030–0.010
Bilirubin	−0.025	0.014	−0.188	0.081	–0.053–0.003
Uric acid	0.000	0.001	0.027	0.790	–0.001–0.001
Ferritin	0.000	0.001	0.123	0.241	0.000–0.002
MDA	0.010	0.002	0.367	0.001	0.005–0.017

Dependent variable: Depth of tumor invasion
B: Regression coefficient, Beta: Ratio probability, *p*: level of significance, MDA: Malondialdehyde, CRC: Colorectal cancer

presence of complex disharmony in the balance of oxidative stress and the antioxidant barrier.

CONCLUSION

A significant increase in serum MDA concentration relative to the CRC stage and depth of tumor invasion suggests that MDA plays a significant role in the carcinogenesis and progression of CRC. The changes found in the selected non-enzymatic parameters of the antioxidant system indicate an imbalance in the network of non-enzymatic activity of system, especially emphasizing the association of hypoalbuminemia with the progression of colorectal carcinoma.

Although our study has certain limitations regarding the lack of enzyme parameters of the antioxidant system and the relatively small sample of patients with CRC, the results of this study can be the starting point for further clinical research in a large population of colon cancer patients to assess diagnostic values of these biomarkers in CRC and for potential strengthening of the antioxidant protection system as a therapeutic approach in this disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Sung H. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2020;71(3):209-49.
https://doi.org/10.3322/caac.21609.
2. Perše M. Oxidative stress in the pathogenesis of colorectal cancer: Cause or consequence? Biomed Res Int 2013;2013:725710.
https://doi.org/10.1155/2013/725710.
3. Kruk J, Aboul-Enein HY. Reactive oxygen and nitrogen species in carcinogenesis: Implications of oxidative stress on the progression and development of several cancer types. Mini Rev Med Chem 2017;17(11):904-19.
https://doi.org/10.2174/1389557517666170228115324.
4. Shringarpure R, Davies KJ. Protein turnover by the proteasome in aging and disease. Free Radic Biol Med 2002;32(11):1084-9.
5. Cejas P, Casado E, Belda-Iniesta C, de Castro J, Espinosa E, Redondo A, et al. Implications of oxidative stress and cell membrane lipid peroxidation in human cancer. Cancer Causes Control 2004;15(7):707-19.
https://doi.org/10.1023/b: caco.0000036189.61607.52.
6. Carini F, Mazzola M, Rappa F, Jurjus A, Geagea AG, Al Kattar S, et al. Colorectal carcinogenesis: Role of oxidative stress and antioxidants. Anticancer Res 2017;37(9):4759-66.
7. Dusak A, Atasoy N, Demir H, Dogan E, Gursoy T, Sarikaya E. Investigation of levels of oxidative stress and antioxidant enzymes in colon cancers. J Clin Anal Med 2017;8(6):469-73.
https://doi.org/10.4328/jcam.5210.
8. Dolfi SC, Yang Z, Lee MJ, Guan F, Hong J, Yang CS. Inhibitory effects of different forms of tocopherols, tocopherol phosphates and tocopherol quinones on growth of colon cancer cells. J Agric Food Chem 2013;61(36):8533-40.
https://doi.org/10.1021/jf401076g.
9. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39(1):44-84.
https://doi.org/10.1016/j.biocel.2006.07.001.
10. Edge SB, Compton CC. The American joint committee on cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17(6):1471-4.
https://doi.org/10.1245/s10434-010-0985-4.
11. Mena S, Ortega A, Estrela JM. Oxidative stress in environmental-induced carcinogenesis. Mutat Res 2009;674(1-2):36-44.
12. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 2007;121(11):2381-6.
https://doi.org/10.1002/ijc.23192.
13. Borrego S, Vazquez A, Dasi F, Cerda C, Iradi A, Tormos C, et al. Oxidative stress and DNA damage in human gastric carcinoma: 8-oxo-7 '8-dihydro-2'-deoxyguanosine (8-oxo-dG) as a possible tumor marker. Int J Mol Sci 2013;14(2):3467-86.
https://doi.org/10.3390/ijms14023467.
14. Sawczuk B, Maciejczyk M, Sawczuk-Siemieniuk M, Posmyk R, Zalewska A, Car H. Salivary gland function, antioxidant defence and oxidative damage in the saliva of patients with breast cancer: Does the BRCA1 mutation disturb the salivary redox profile? Cancers (Basel) 2019;11(10):1501.
https://doi.org/10.3390/cancers11101501.
15. Lepara Z, Lepara O, Fajkić A, Rebić D, Alić J, Spahović H. Serum malondialdehyde (MDA) level as a potential biomarker of cancer progression for patients with bladder cancer. Rom J Intern Med 2020;58(3):146-52.
https://doi.org/10.2478/rjim-2020-0008.
16. Zinczuk J, Maciejczyk M, Zareba K, Romaniuk W, Markowski A, Kedra B, et al. Antioxidant barrier, redox status, and oxidative damage to biomolecules in patients with colorectal cancer. Can malondialdehyde and catalase be markers of colorectal cancer advancement? Biomolecules 2019;9(10):637.
https://doi.org/10.3390/biom9100637.
17. Janion K, Szczepanska E, Nowakowska-Zajdel E, Strzelczyk J, Copija A. Selected oxidative stress markers in colorectal cancer patients in relation to primary tumor location-a preliminary research. Medicina (Kaunas) 2020;56(2):47.
https://doi.org/10.3390/medicina56020047.
18. Brankovic B, Stanojevic G, Nestorovic M, Veljkovic A, Stojanovic I, Petrovic D, et al. Trosative stress parameters in colon cancer tumor, adjacent and healthy tissue. Acta Med Med 2016;55(1):44-50.
https://doi.org/10.5633/amm.2016.0107.
19. Leung EY, Crozier JE, Talwar D, O'Reilly DS, McKee RF, Horgan PG, et al. Vitamin antioxidants, lipid peroxidation, tumour stage, the systemic inflammatory response and survival in patients with colorectal cancer. Int J Cancer 2008;123(10):2460-4.
https://doi.org/10.1002/ijc.23811.
20. Surinenaite B, Prasmickiene G, Milasiene V, Stratilatovas E, Didziapetriene J. Influence of surgical treatment and red blood cell transfusion on changes in antioxidative and immune system parameters in colorectal cancer patients. Medicina (Kaunas) 2009;45(10):785-91.
https://doi.org/10.3390/medicina45100102.
21. Zinczuk J, Maciejczyk M, Zareba K, Pryczynicz A, Dymicka-Piekarska V, Kaminska J,

et al. Pro-oxidant enzymes, redox balance and oxidative damage to proteins, lipids and DNA in colorectal cancer tissue. Is oxidative stress dependent on tumour budding and inflammatory infiltration? Cancers (Basel) 2020;12(6):1636.

https://doi.org/10.3390/cancers12061636.

22. Ozgonul A, Aksoy N, Dilmec F, Uzunkoy A, Aksoy S. Measurement of total antioxidant response in colorectal cancer using a novel automated method. Turk J Med Sci 2009;39(4):503-6.
23. Nazha B, Moussaly E, Zaarour M, Weerasinghe C, Azab B. Hypoalbuminemia in colorectal cancer prognosis: Nutritional marker or inflammatory surrogate? World J Gastrointest Surg 2015;7(12):370-7.

https://doi.org/10.4240/wjgs.v7.i12.370.

24. Almasaudi AS, Dolan RD, Edwards CA, McMillan DC. Hypoalbuminemia reflects nutritional risk, body composition and systemic inflammation and is independently associated with survival in patients with colorectal cancer. Cancers (Basel) 2020;12(7):1986.

https://doi.org/10.3390/cancers12071986.

25. Feng Z, Chen JW, Feng JH, Shen F, Cai WS, Cao J, et al. The association between serum ferritin with colorectal cancer. Int J Clin Exp Med 2015;8(12):22293-9.
26. Gopcevic KR, Rovcanin BR, Tatic SB, Krivokapic ZV, Gajic MM, Dragutinovic VV. Activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in different stages of colorectal carcinoma. Dig Dis Sci 2013;58(9):2646-52.

https://doi.org/10.1007/s10620-013-2681-2.

27. Stone WL, Krishnan K, Campbell SE, Palau VE. The role of antioxidants and pro-oxidants in color cancer. World J Gastrointest Oncol 2014;6(3):55-66.
28. Hong Y, Wu G, Li W, Liu D, He K. A comprehensive meta-analysis of genetic associations between five key SNPs and colorectal cancer risk. Oncotarget 2016;7(45):73945-59.

https://doi.org/10.18632/oncotarget.12154.

29. Fedirko V, Jenab M, Meplan C, Jones JS, Zhu W, Schomburg L, et al. Association of selenoprotein and selenium pathway genotypes with risk of colorectal cancer and interaction with selenium status. Nutrients 2019;11(4):935.

https://doi.org/10.1201/9780429423482-77.

RELATED ARTICLES PUBLISHED IN JHSCI

1. Gubaljevic J, Srabović N, Jevrić-Čaušević A, Softić A, Rifatbegović A, Mujanović-Mustedanagić J, Dautović E, Smajlović A, Mujagić Z. Serum levels of oxidative stress marker malondialdehyde in breast cancer patients in relation to pathohistological factors, estrogen receptors, menopausal status, and age. JHSCI. 2018;8(3):154-61.