J Oncol Sci 5 (2019) 60–64

Contents lists available at ScienceDirect

J Oncol Sci

journal homepage: www.journalofoncology.org

Original Article

Evaluation of dynamic serum thiol-disulphide homeostasis in colorectal cancer

Burak Bilgin [a, *], Mehmet AN. Sendur [a], Mutlu Hizal [a], Sümeyye Ulutas Kandil [b], Samet Yaman [b], Muhammed Bülent Akıncı [a], Didem Şener Dede [a, a], Salim Neselioglu [c], Özcan Erel [c], Bülent Yalçın [a]

[a] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Oncology, Ankara, Turkey
[b] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Internal Medicine, Ankara, Turkey
[c] Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

ARTICLE INFO

Article history:
Received 11 May 2019
Received in revised form
29 July 2019
Accepted 3 August 2019
Available online 9 August 2019

Keywords:
Colorectal cancer
Disulphide
Oxidative stress
Thiols

ABSTRACT

Aim: Data about the relationship between oxidative stress and cancer pathogenesis has been increased in recent years. Thiol and disulphide play an important role in cell signal mechanisms, antioxidant protection, and detoxification. In this study, we aimed to evaluate the role of Thiol-Disulphide homeostasis (TDH) in colorectal cancer (CRC) by using a new method.

Material and Method: The patients (pts) who diagnosed with CRC and healthy control subjects were included to study. Serum samples for the thiol-disulphide test were obtained at the time of diagnosis. TDH tests were measured by the automated spectrophotometric method by describing Erel and Neselioğlu. Thiol-disulphide homeostasis was also evaluated according to tumor stage and localization.

Results: Eighty-eight pts with CRC and 110 control were enrolled. Native thiol (NT), disulphide and total thiol (TT) levels were significantly lower in patients compared with the control arm (Median NT: 402 −424, $p = 0.003$; median Disulphide 18.7−21, $p = 0.011$; median TT: 437−467, $p = 0.001$). Thiol/disulphide balance was also maintained ($p = 0.149$). TT and NT levels were not differed according to tumor localization whereas disulphide level was significantly higher in left-sided tumors than right-sided (19.9 −13.07 respectively, $p = 0.007$). In addition, disulphide/NT ratio was also significantly higher in left-sided than right-sided (0.1−0.12 respectively, $p = 0.015$). Thus, the balance of dynamic TDH is disrupted in favor of disulphide between left-sided and right-sided tumor. There was also no significant difference between thiol-disulphide levels and tumor stage whereas thiol level tends to lower in stage 4 disease ($p = 0.7$).

Conclusion: This is the first trial that evaluates the relationship with dynamic TDH and CRC according to tumor stage and localization. Thiol and disulphide may play an important role in the pathogenesis of CRC.

1. Introduction

Colorectal cancer (CRC) is the third frequent cancer type worldwide and also one of the leading causes of cancer-related death. In 2019, estimated numbers of newly diagnosed with CRC and CRC-related deaths are nearly 101.420 and 51.020 at United States of America (USA), respectively.[1] Many factors are implicated for the development of colon cancer including genetic predisposition, cigarette, high amount fat diet, obesity, and also reactive oxidative stress (ROS).

Under the normal physiological condition, oxygen-free radicals and antioxidant defense systems are underbalanced. When this balance diminishes, the situation of the oxidative stress can occur. It is well known that low amounts of ROS have beneficial effects on several physiological processes whereas the high amount of ROS can cause oxidative tissue damage and the other several harmful effects.[2] Many exogenous and endogens factors including inflammation, ischemia, infection, ultraviolet radiation, drugs, alcohol, and smoking can increase the production of ROS.

Thiols are essential and potent anti-oxidant molecules. It contains organic components including hydrogen and sulfhydryl group that plays an essential role in decreasing to ROS. Thiols can undergo oxidation reaction and form disulphide bonds. Later, the formed

* Corresponding author. Ankara Ataturk Chest Disease and Chest Surgery Research and Training Hospital, Department of Medical Oncology, 06100, Kecioren, Ankara, Turkey.
E-mail address: drbbilgin@hotmail.com (B. Bilgin).
Peer review under responsibility of Turkish Society of Medical Oncology.

https://doi.org/10.1016/j.jons.2019.08.001

disulphide bonds can be reduced to thiol groups, and dynamic thiol–disulphide homeostasis is maintained, ultimately.[3–5] In physiological conditions, thiol/disulphide equilibrium is under balance. It is known that dynamic thiol-disulphide homeostasis has a crucial role for several functions including apoptosis, oxidation of proteins, antioxidant defense and cellular signal transduction mechanism.[6] There is also growing evidence demonstrating that an abnormal thiol disulphide homeostasis state is related to the pathogenesis of several benign and some malignant conditions.[5,7–10]

Historically, oxidative and antioxidant status evaluated by using various indirect methods. However, oxidant and antioxidant status can be directly measured, nowadays. Firstly, Elman and Lyskohave developed a method for measure directly thiol − disulphide homeostasis but in this methods, only one side of this two-sided balance can be measured.[11] However, Erel and Neselioglu developed a novel automated and spectrophotometric method for measurement of the two-sided of the dynamic thiol-disulphide homeostasis.[5]

In our knowledge, there is no clinical trial that evaluates the relationship between dynamic thiol-disulphide homeostasis and CRC by using a new method by describing Erel and Neselioğlu. Thus, we aimed to compare dynamic thiol/disulphide homeostasis of the patients with CRC and healthy individuals and also investigate to levels of thiol and disulphide between tumor stages and localization.

2. Material and Methods

2.1. Study subject

The patients who diagnosed with CRC and were not received any treatment in Ankara Yildirim Beyazit University between 2015 and 2017 were prospectively analyzed. Inclusion criteria for patients are followed: (1) over 18 years old, (2) the diagnosis of CRC made pathologically with colonoscopic biopsy, tru-cut needle biopsy from metastatic lesions or surgically resected specimens. Healthy subjects were also enrolled in our study as a control cohort. Patients with renal or liver disease, diabetes, and active inflammatory or infectious disease were excluded from the study.

The patient stratified according to tumor stage and localization. Cecum, ascending colon and right side of the middle of the transverse colon were accepted as right-sided colon tumor and descending colon, sigmoid colon, rectum and left the side of the middle of the transverse colon were also accepted as a left-sided tumor.

2.2. Blood samples collection and principle of the thiol-disulphide homeostasis

Blood samples for thiol-disulphide homeostasis analyses were collected at the time of diagnoses for the patients. Blood samples were collected from the control and patient groups in the morning and centrifuged at 1500 g for 10 min. Serum samples were separated and stored at −80 °C until being used for the analysis. Thiol/disulphide homeostasis tests were measured using a novel automatic and spectrophotometric method. In this method, dynamic and reducible disulphide bonds in the samples were reduced to free functional thiol groups by using sodium borohydride (NaBH4). In order to prevent the reduction of unused reduced sodium borohydride to dithionite-(2 nitrobenzoic) (DTNB). Native thiol (NT) levels and total thiol (TT) were measured after reaction with DTNB. Half of the difference of the result obtained by the subtraction of native thiol amount from total thiol content indicated the disulphide level. Disulphide/NT ratio that was the best marker for reflection of the thiol-disulphide homeostasis was also calculated.

2.3. Statistical analyses

The parameters were investigated using visual (histograms, probability plots and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Differences in categorical factors were determined with Fisher's exact test. Differences in continuous values between two groups were assessed with Student's *t*-test for normally distributed variables and non-parametric Mann-Whitney *U* tests for non-normally distributed variables as appropriate and also multiple sets of comparisons performed using ANOVA. In normally distributed parameters, the correlation coefficients and their significance were calculated using the Pearson test and in non-normally or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test.

All statistical procedures were performed with SPSS 17.0 (SPSS Inc, Chicago, Illinois). A P value < 0.05 was considered to statistically significant.

University Clinical Research Ethics Committee's approval was obtained (No: 26379996/131; 14/06/2017).

3. Results

Totally, 198 cases including 88 patients with CRC and 110 healthy subjects were enrolled in our study. The median age was 60.5 and 59.5 years in the patient and control arm, respectively. In the patient cohort, 64.8% and 35.2% of cases were male and female, respectively. In the control case, 57.3% and 43.7% of cases were male and female. There was no significant difference between sex and age in the patient and control arms ($p = 0.283$ for sex and $p = 0.291$ for age). In the patient arm; there were no significant differences between baseline characteristics of patients including sex, age and tumor stage between right and left-sided tumors. Detailed patient characteristics were shown in Table 1.

When compare TT, NT, disulphide level and disulphide/NT ratio between patient and control arms, we found that TT, NT and disulphide levels were significantly lower in the patient arm than control arm, whereas disulphide/NT ratio did not significantly differ between two arms. Detailed statistical data was shown in Table 2 and Fig. 1.

In the patient arm, we also compare NT, TT and disulphide levels between tumor stages. Although NT, TT and disulphide level tend to decreased when tumor stage progressed 1 to 4, there was no statistical difference between Stage 1, 2, 3 and 4 diseases. Detailed data were shown in Fig. 2. Disulphide/Native thiol ratio was 0.041, 0.0471, 0.0479 and 0.0456 in stage 1, 2, 3 and 4 diseases, respectively. There was also no statistically significant difference between

Table 1
Baseline characteristics of patients.

Parameters	N
Age (Median, Min-max)	60,5 (31–83)
Sex (M/F)	64.8%/35.2%
Stage	
1	11.5%
2	27.6%
3	39.1%
4	21.8%
Tumor Localisation	
Left-sided	74.8%
Right-sided	25.9%
CEA Level (Median, Min-max)	3.62 (0,6–2156)
CA 19.9 Level (Median, Min-max)	17.2 (0,6–5525)

Table 2
Level of the thiol and disulphide in patient and control arms.

Parameters	Patient, Median (min-max)	Control, Median (min-max)	p
Native Thiol (μmol/L)	402 (245–533)	424 (262–606)	0.003
Disulphide (μmol/L)	18.7 (1.6–34.65)	21 (8.4–38.4)	0.011
Total Thiol (μmol/L)	437 (303–577)	464 (286–631)	0.001
Disulphide/Native Thiol	0.047 (0.01–0.12)	0.05 (0.02–0.1)	0.149

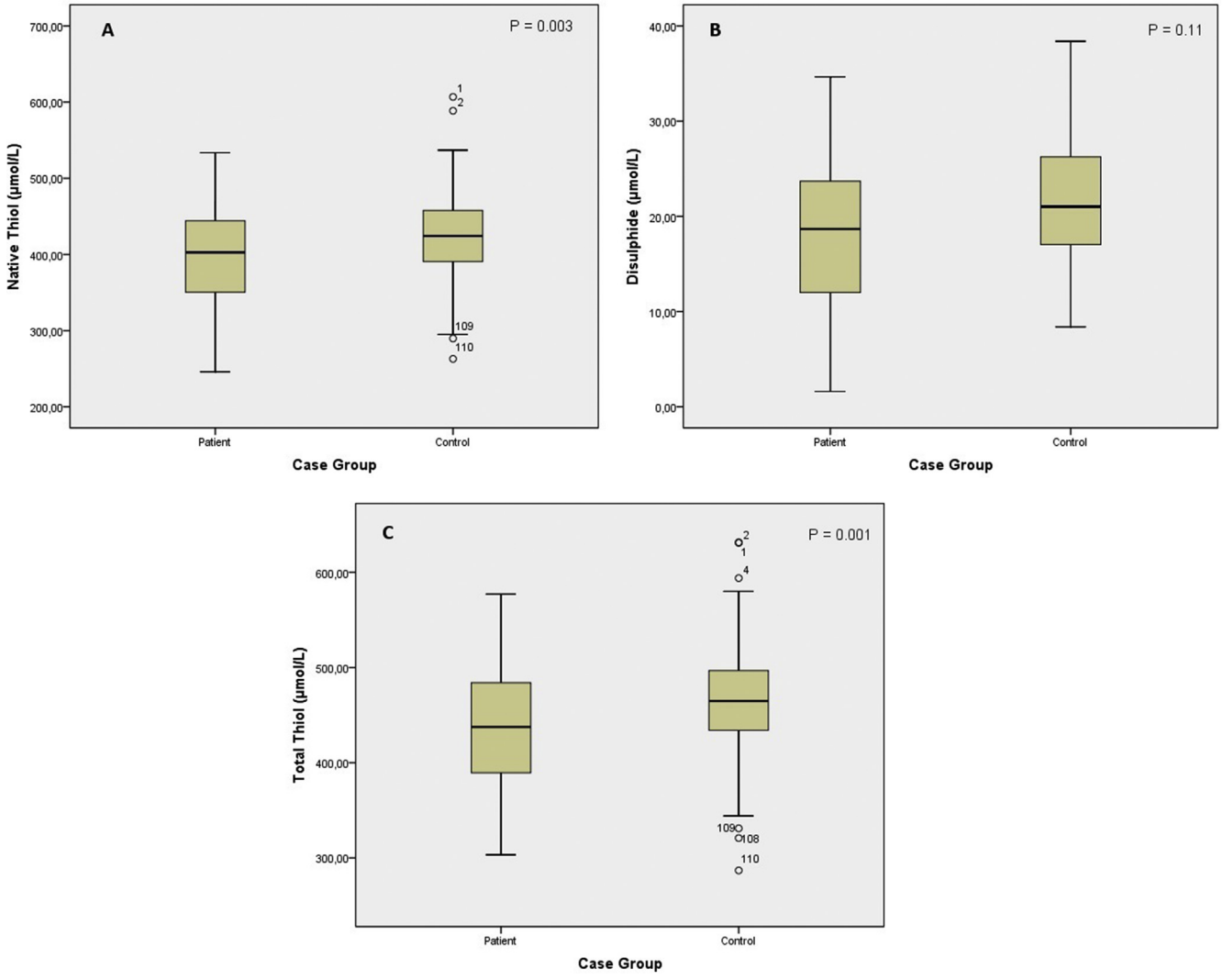

Fig. 1. Native Thiol, Total Thiol and disulphide levels in patient and control groups (A: Native thiol, B: Disulphide, C: Total thiol).

stages (p = 0.7).

When we separated groups as stage 4 disease and early stages (stage 1, 2 and 3); NT, TT, and disulphide level tend to lower in stage 4 disease compare with early stages but not statistically significant. Median NT level was 377 μmol/L for stage 4 and 403 μmol/L for the early stages (p = 0.265). TT levels was 397 μmol/L for stage 4 disease and 433 μmol/L for the early stages (p = 0.14). Disulphide level was 14.7 μmol/L for stage 4 disease and 19.8 μmol/L for the early stages (p = 0.074). NT/disulphide ratio was similar between two groups (0.039 vs. 0.053, p = 0.114).

We also compare NT, TT, disulphide levels and disulphide/NT ratio according to tumor localization in the patient arm. NT and TT level tend to lower in left-sided tumor then right-sided but this difference was not statistically significant. Median NT level was 416 μmol/L in right-sided and 402 μmol/L in left-sided tumor (p = 0.94). Median total thiol level was 442 μmol/L in right-sided and 436 μmol/L in left-sided tumor (p = 0.6). However, disulphide level was statistical significantly higher in left-sided compared with right-sided tumor (Median disulphide level; Left-sided: 19.9 μmol/L vs. Right-sided: 13.07 μmol/L; p = 0.007). Disulphide/NT ratio was also significantly higher in left-sided then right-sided tumor (0.10 vs. 0.12 in right-sided and left-sided, respectively, p = 0.015). This finding has also supported that balance of dynamic thiol-disulphide homeostasis is disrupted in favor of disulphide between left-sided and right-sided tumor.

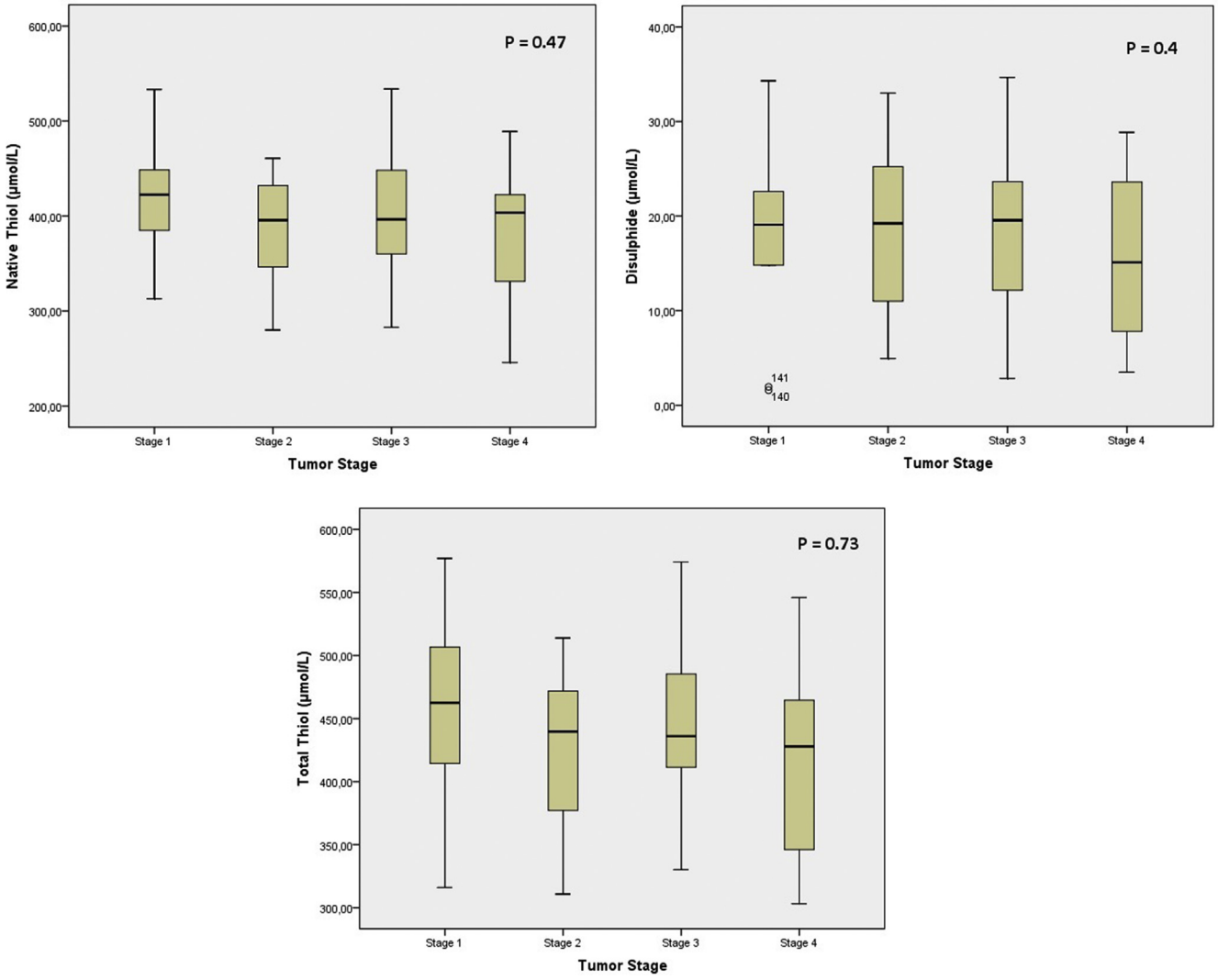

Fig. 2. Native Thiol, Disulphide and Total thiol Levels According to Disease Stage in Patient Cohort.

4. Discussion

In this study, we found that NT, TT and disulphide levels were statistically significantly lower in the patient arm compared with normal healthy population but thiol-disulphide homeostasis was continued to maintain between two arms. In the patient arm, NT, TT, and disulphide level tend to lower in advance stage disease compared with the early stage but this distance was not statistically significant. Although there was also no significant association between NT and TT level and tumor localization, disulphide level was significantly high in left-sided tumor compare with right-sided and thiol-disulphide homeostasis disrupt in favor of disulphide level.

It is known that the importance of oxidative stress in the pathogenesis of various malignant diseases including CRC. However, in previous trials, oxidative stress could be measured indirectly and also the balance of oxidant and antioxidant capacity could not be measured because of technical and methodologic problems. In the literature, several methods were used for measured oxidative stress including a measure of serum malondialdehyde (MDA), hydrogen peroxide (H_2O_2), lipid peroxidation products and protein oxidation products.

Serum MDA is an indirect marker of oxidative stress and can be high (or low) as a result of lipid peroxidation. In previous trials showed that serum MDA level was higher in various malignant diseases compared with normal population.[12–15] Hydrogen peroxide (H_2O_2) is another marker that indirectly reflects oxidative stress and also found high in melanoma, breast, lung and pancreas cancers.[16–18] In the literature, there were several studies that investigated the relationship between oxidative stress and CRC. In these studies, various parameters including serum MDA, H_2O_2, lipid peroxidation products, protein oxidation products and enzymes levels like as *superoxide dismutase, glutathione peroxidase, nitric oxide,* and *catalase* were used as the measure of oxidative stress.[19–21] These studies were also detected that oxidative stress may be related to the pathogenesis of CRC. As mentioned above, these biomarkers can measure oxidative stress, indirectly and only reflect oxidant-sided of this balance. In our trial, we used a new automated method that can directly evaluate the two-sided of the thiol-disulphide balance. Therefore, this method gives accurate information about oxidant or antioxidant status than the other methods which measure oxidant and antioxidant parameters in serum can be individual.

In our study; NT, TT and disulphide levels were significantly lower in the patient arm than healthy control arm whereas NT/disulphide level that reflects oxidant and the antioxidant balance were similar between two arms. Previous studies were shown that

NT and TT levels were lower but disulphide levels were higher in benign disease including Parkinson disease, celiac, diabetes mellitus, and psychiatric disorder compared with healthy control groups.[5,7,22] Also, the balance of thiol-disulphide was disrupted in favor of disulphide side. These findings suggested that oxidative stress was increased but total antioxidant capacity was decreased in benign disease. On the other hand, we found that all of the native thiol, total thiol, and disulphide levels were decreased, and the balance of thiol-disulphide was maintained. As similar to our study, NT, TT and disulphide level were lower in renal cell carcinoma, bladder cancer, and multiple myeloma compared with the healthy control group and balance of thiol-disulphide was also continued to maintain in previous studies that evaluate thiol-disulphide homeostasis in malignant disease.[5,23] Reduction in NT and TT levels can be related to decreased total antioxidant capacity in response to increased oxidative stress. We also found that disulphide level decreased in contrast to benign diseases, this finding can be the result of high consumption of sulphide atoms due to high cell turnover, theoretically. There is a need for further study to clarify this matter.

In this study, we found that NT, TT and disulphide levels tend to decrease when the tumor stage progressed 1 to 4. But this difference was not statistically significant. There is a limited number of studies that investigate oxidative stress and antioxidant capacity according to clinically stage in the malignant disease. Recently, Wu et al. published the trial that investigates total antioxidant status (TAS) and total oxidant status (TOS) in colorectal cancer.[24] In this trial, TOS was higher and TAS was lower in CRC patients compared with the normal healthy group. In addition, similarly to our study, TOS and TAS were not differed according to the clinical stage of the disease. The major reason for not reached to statistical significance may be the limited number of patients in our trial.

We also investigate NT, TT and disulphide level according to tumor localization. We found that NT and TT level did not differ between right-sided and left-sided tumor. However, disulphide level was significantly lower in right-sided compared with the left-sided tumor. In our knowledge, there was no study that evaluated oxidative stress parameters according to the right-sided and left-sided colorectal tumor. Wu et al. evaluated that TOS and TAS according to rectal and colon tumor and they demonstrated that there was no significant difference between rectum and colon cancer.[24] It is known that right-sided and left-sided colon tumors have distinct anatomic and molecular features.[25] Despite all of the demographics feature were similar between right-sided and the left-sided tumor decreased disulphide level in right-sided compared with left-sided tumor could be the result of a distinct feature of the right-sided tumor including more inflammatory and immunogenic feature or high tumor turnover than left-sided.

Our study had some limitations. The first was the inclusion of a relatively limited number of patients who were admitted to a single center. Second, our results were not compared with other oxidative stress parameters such as lipid hydroperoxide, total antioxidant status, total oxidant status, and oxidative stress index.

In conclusion, there are limited studies that evaluated oxidant and antioxidant system together in CRC. In our knowledge, this is the first study which investigates thiol-disulphide homeostasis that reflects two-sided of oxidant/antioxidant system in CRC. We found that thiol-disulphide homeostasis has possibly an important role in the pathogenesis of CRC. But, these results must be validated with the new studies that performed with a large number of patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA A Cancer J Clin*. 2019;69:7–34.
2. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*. 2014;94:329–354.
3. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. *J Biol Chem*. 2013;288:26489–26496.
4. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *J Free Radic Biol Med*. 2009;47:1329–1338.
5. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47:326–332.
6. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. *Biochem Pharmacol*. 2006;71:551–564.
7. Vural G, Gumusyayla S, Bektas H, Deniz O, Alisik M, Erel O. Impairment of dynamic thiol-disulphide homeostasis in patients with idiopathic Parkinson's disease and its relationship with clinical stage of disease. *Clin Neurol Neurosurg*. 2017;153:50–55.
8. Hanikoglu F, Hanikoglu A, Kucuksayan E, et al. Dynamic thiol/disulphide homeostasis before and after radical prostatectomy in patients with prostate cancer. *Free Radic Res*. 2016;50:S79–S84.
9. Korkmaz V, Kurdoglu Z, Alisik M, et al. Thiol/disulfide homeostasis in postmenopausal osteoporosis. *J Endocrinol Investig*. 2017;40:431–435.
10. Demirseren DD, Cicek C, Alisik M, Demirseren ME, Aktas A, Erel O. Dynamic thiol/disulphide homeostasis in patients with basal cell carcinoma. *Cutan Ocul Toxicol*. 2017;36:278–282.
11. Ellman G, Lysko H. A precise method for the determination of whole blood and plasma sulfhydryl groups. *Anal Biochem*. 1979;93:98–102.
12. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. *J Cancer Res Ther*. 2010;6:487–491.
13. Patel BP, Rawal UM, Dave TK, et al. Lipid peroxidation, total antioxidant status, and total thiol levels predict overall survival in patients with oral squamous cell carcinoma. *Integr Cancer Ther*. 2007;6:365–372.
14. Dursun H, Bilici M, Uyanik A, Okcu N, Akyuz M. Antioxidant enzyme activities and lipid peroxidation levels in erythrocytes of patients with oesophageal and gastric cancer. *J Int Med Res*. 2006;34:193–199.
15. Gonenc A, Ozkan Y, Torun M, Simsek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. *J Clin Pharm Ther*. 2001;26:141–144.
16. Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res*. 1991;51:794–798.
17. Aykin-Burns N, Ahmad IM, Zhu Y, Oberley LW, Spitz DR. Increased levels of superoxide and H2O2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. *Biochem J*. 2009;418:29–37.
18. Vilema-Enriquez G, Arroyo A, Grijalva M, Amador-Zafra RI, Camacho J. Molecular and cellular effects of hydrogen peroxide on human lung cancer cells: potential therapeutic implications. *Oxid Med Cell Longev*. 2016;2016:1908164.
19. Chang D, Wang F, Zhao YS, Pan HZ. Evaluation of oxidative stress in colorectal cancer patients. *Biomed Environ Sci*. 2008;21:286–289.
20. Kang KA, Kim KC, Bae SC, Hyun JW. Oxidative stress induces proliferation of colorectal cancer cells by inhibiting RUNX3 and activating the Akt signaling pathway. *Int J Oncol*. 2013;43:1511–1516.
21. Perse M. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? *BioMed Res Int*. 2013;2013:725710.
22. Kaplan M, Ates I, Yuksel M, et al. Thiol/disulphide homeostasis in celiac disease. *World J Gastrointest Pharmacol Ther*. 2017;8:120–126.
23. Guney T, Kanat ILF, Alkan A, et al. Assessment of serum thiol/disulfide homeostasis in multiple myeloma patients by a new method. *Redox Rep*. 2016:1–6.
24. Wu R, Feng J, Yang Y, et al. Significance of serum total oxidant/antioxidant status in patients with colorectal cancer. *PLoS One*. 2017;12. e0170003.
25. Shen H, Yang J, Huang Q, et al. Different treatment strategies and molecular features between right-sided and left-sided colon cancers. *World J Gastroenterol*. 2015;21:6470–6478.