Levels of Thiol–Disulfide in Colorectal Cancer*

Kolorektal Kanserde Tiyol–Disülfit Düzeyleri

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Abstract

Aim: This study aimed to evaluate total thiol (TT), disulfide (-S-S), and native thiol (-SH) concentrations as serum biomarkers in patients with colorectal cancer (CRC).

Materials and Methods: A total of 46 participants (23 patients with colorectal cancer and 23 healthy individuals) were included. Thiol/disulfide homeostasis tests (total thiol [TT], native thiol [-SH], and disulfide [-S-S]) were performed by a novel automated method. Ischemia modified albumin (IMA), albumin, carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA-19-9), TT, -SH, and -S-S levels as well as disulfide/native thiol and disulfide/total thiol ratios were compared between the groups.

Results: In the colorectal cancer group, statistically significant difference was found in IMA, CEA, and CA-19-9, compared to the control group ($p<0.05$). There was no relationship between the thiol–disulfide parameters and tumor markers in the control group ($p>0.05$). There was no relationship between the thiol–disulfide parameters and IMA, albumin, CEA, and CA-19-9 levels in the colorectal cancer group ($p>0.05$).

Discussion and Conclusion: Our study shows that the serum concentrations of native -SH, -S-S, and TT do not link to colorectal cancer as a noninvasive biomarker.

Keywords: biochemical marker; colorectal neoplasms; disulfide; thiol

Öz

Amaç: Bu çalışmada kolorektal kanserli hastalarda natif tiyol, disülfit ve total tiyol konsantrasyonları ile CEA ve CA-19-9 serum biyobelirteçlerini değerlendirmek ve sonuçları sağlıklı bireylerle karşılaştırmak amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya 23 kolorektal kanserli hasta ve 23 sağlıklı birey olmak üzere toplam 46 kişi dahil edildi. Tiyol/disülfit homeostaz testleri (natif tiyol [-SH], disülfit [-S-S] ve total tiyol [TT]) yeni bir otomatize metotla gerçekleştirildi. İskemi modifiye albümin (IMA), albümin, karsinoembriyojenik antijen (CEA), karbonhidrat antijen 19-9 (CA-19-9), TT, -SH ve -S-S seviyeleri ile disülfit/natif tiyol ve disülfit/total tiyol oranları gruplar arasında karşılaştırıldı.

Bulgular: Kolorektal kanserli grupta IMA, CEA ve CA-19-9'da kontrol grubuna kıyasla istatistiksel olarak anlamlı farklılık bulundu ($p<0,05$). Kontrol grubunda tiyol–disülfit parametreleri ve tümör biyobelirteçleri arasında bir ilişki saptanmadı ($p>0,05$). Kolorektal kanserli hastalarda tiyol–disülfit parametreleri ile IMA, albümin, CEA ve CA-19-9 düzeyleri arasında bir ilişki saptanmadı ($p>0,05$).

Tartışma ve Sonuç: Çalışmamız noninvaziv bir biyobelirteç olarak natif tiyol (-SH), disülfit (-S-S) ve total tiyol (TT) konsantrasyonları ile kolorektal kanser arasında bir ilişki olmadığını göstermektedir.

Anahtar Sözcükler: biyokimyasal belirteç; disülfit; kolorektal neoplazmlar; tiyol

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INTRODUCTION

Cancer is a serious disease still investigated in Turkey and the world. Deaths from cancer that occur frequently are estimated to continue to increase in both sexes until 2030. Colorectal cancer ranks second among death causes in Turkey. While in the US 600,920 death cases occur every year, 81,527 lives were lost in Turkey due to benign and malignant tumors according to the 2017 statistical data (1–7). Colorectal cancer, one of the most common diseases in Turkey and the world, is the leading cancer-related cause of death in the Western world. Various factors are suggested to be effective in cancer pathology and development. Although the correlation between the clinical and biochemical characteristics of the disease is not exactly known, it is observed that oxygen radicals have an impact on cancer formation in the gastrointestinal tract, which is quite sensitive to these species (2,8,9). Although colorectal cancer is closely associated with cologenetic mutations such as hereditary nonpolyposis coli and adenomatous polyposis coli, the correlation between the genetic and intestinal factors in carcinogenesis is not known adequately (10,11). Oxidative stress characterized by the generation of reactive oxygen species (ROS) is an important event in tumor generation. It is considered that oxidative stress causes a disturbance in the reduction/oxidation (redox) balance and increased oncogenic activity (2,8–11). The low-molecular-weight molecules that constitute almost the entire plasma thiol pool are organic compounds derived from the combination of alcohols with sulfur and containing the sulfhydryl (-SH) group that maintains the structural integrity of the cells. A great number of major thiols are albumin thiols (12,13).

Thiols containing free sulfhydryl groups neutralize the ROS formation by enzymatic and nonenzymatic processes and are effective molecules in antioxidant response with an important role in preventing the oxidative stress formation. While the thiol/disulfide balance is maintained during events such as antioxidant defense, a disturbance of balance is observed in various diseases such as cancer, diabetes, rheumatism, and Alzheimer's disease (14–16). Some noninvasive methods such as fecal occult blood and colonoscopy are in use as early diagnosis is important in terms of prognosis in colorectal cancers. However, these practices may lead to some medical complications. Also, although the CEA and CA 19-9 that are used as tumor markers in pancreatic, gastric, hepatic and colorectal cancers increase depending on the stage of the disease, they have a very low sensitivity especially in early stages (17,18).

In this study, we tried to find a correlation between the thiol/disulfide homeostasis and CA 19-9 and CEA that is an important marker in postoperative response assessment in colorectal cancer. Our aim was to detect a biomarker that might support the CA 19-9 measurement that is recommended along with radiological examinations during the postoperative follow-up.

MATERIALS AND METHODS

Study design and population

This prospective case-control study was conducted in the general surgery and oncology department between August 2016 and January 2017. A total of 46 individuals were included. Of these, 23 were patients with colorectal cancer. The control group consisted of 23 healthy volunteers. The patients and volunteers were over 18 years old and nonsmokers. Those using antioxidant supplements and drugs for chronic conditions and those with any chronic disease (chronic liver or renal failure, cancer, diabetes mellitus, Alzheimer's disease, Parkinson's disease) were excluded from the control group.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (2013) and approved by the Dumlupınar University Ethics Research Committee, Turkey (Protocol number: 2015-KAEK-86/08-158). Written informed consent was obtained from all participants.

Blood samples

Venous blood samples (5 ml) were collected from both groups in plain tubes containing EDTA. The serum samples were stored in -80°C in deep freezer until the analysis.

Biochemical analysis

Serum CEA and CA-19-9 were determined using an automated analyzer (Architect CEA 7K68/Architect CA-19-9 2K44). The thiol/disulfide homeostasis tests

(native thiol, total thiol, and disulfide levels) were performed using a spectrophotometric method developed by Erel and Neselioglu (19). In this method, disulfide bonds in venous blood samples are reduced to free thiol groups by sodium borohydride ($NaBH_4$). The sodium borohydride residues are removed by formaldehyde. The total thiol of the sample is measured using modified Ellman reagent. Native thiol is subtracted from the total thiol. After determining the concentrations of native thiols, total thiols and disulfide and the ratios of disulfide to total thiol (SS/[SH+SS]) and disulfide to native thiol (SS/SH), the ratios of native thiol to total thiol (SH/[SH+SS]) were calculated by using the new spectrophotometric method described by Erel and Neselioglu (19). Measurements were performed by a clinical chemistry autoanalyser. Total IMA (ABSU [absorbance units]) and albumin (g/dl) levels were measured in automatic Roche-Hitachi Cobas c501 analyzer by a calorimetric method with commercially available assay kits (20).

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences 17 software package. Normal distribution was evaluated using the Shapiro-Wilk test. Normally distributed numerical variables were expressed as mean±standard deviation and those not normally distributed as median.

RESULTS

Of the 23 patients with colorectal cancer, 14 (60.86%) were males and 9 (39.13%) females. Of the 23 healthy controls, 12 (52.17%) were males and 11 (47.82%) females. The median age was 61.56 (range: 33–89) and 50.82 (range: 27–68) years for the patients and controls, respectively. The demographic characteristics are summarized in Table 1. There was no significant difference between the patients and controls in terms of age and sex.

The IMA, albumin, CEA, CA-19-9, native thiol, total thiol, and disulfide levels as well as disulfide/native thiol and disulfide/total thiol ratios were compared between the two groups. The native thiol levels were 265.85±44.41 $\mu mol\,l^{-1}$ and 266.58±84.87 $\mu mol\,l^{-1}$ in the colorectal cancer and control groups, respectively. The total thiol level was 301.96±50.77 $\mu mol\,l^{-1}$ in the colorectal cancer group and 299.90±87.77 $\mu mol\,l^{-1}$ in the control group. The serum disulfide level was 18.05±7.81 $\mu mol\,l^{-1}$ in the colorectal cancer group and 16.66±7.81 $\mu mol\,l^{-1}$ in the controls. In the colorectal cancer group IMA, CEA, and CA-19-9 values were obtained to be statistically significantly different, compared to the control group ($p<0.05$). The thiol/disulfide homeostasis parameters (native thiol, disulfide, total thiol, and native thiol/disulfide) of both groups are summarized in Table 2. The thiol–disulfide values (native thiol [SH] [$\mu mol\,l^{-1}$], total thiol [TT] [$\mu mol\,l^{-1}$], disulfide [-S-S] [$\mu mol\,l^{-1}$], SS/SH [%], SS/total thiol [%], and SH/total thiol [%]) of the patients with colorectal cancer showed no statistical significance.

DISCUSSION AND CONCLUSION

Colorectal cancer is the second leading cause of death in Turkey. The aim of this study was to investigate the thiol/disulfide homeostasis with albumin, IMA, CEA, and CA-19-9 levels in patients with colorectal cancer and compare the results with healthy controls for the first time in the literature.

Colon mucosa is highly exposed to carcinogenic factors such as drugs, chemicals and food additives. It is known that oxygen radicals and cologenetic mutations are effective in the pathogenesis of colorectal cancer (1,2,4,7–10) and that many factors may coexist in a case. Some aspects of these factors are still not entirely understood. In this study, we aimed to determine whether there was a correlation between cancer biomarkers and thiol/disulfide parameters in colorectal cancer. The thiol/disulfide homeostasis parameters were measured by a new method developed by Erel and Neselioglu. In cases where oxidative stress increases, such as cancer, the disulfide level is expected to increase since the thiol level decreases. However, the thiol–disulfide balance may be affected by many factors (14,15,19,21).

Thiols are reversibly modified into disulfides due to oxidants. While thiol groups are modified into disulfides in such diseases as diabetes, pneumonia, and obesity, disulfide levels were reported to be low in proliferative diseases such as bladder, renal and colorectal cancers (22,23). In a study, a weak correlation was found between lung cancer and thiol/disulfide parameters (22). In another study, thiol levels markedly decreased in gas-

Table 1. Demographic characteristics of both groups

	Control group (n=23)	Colorectal CA group (Adenocarcinoma) (n=23)
Age (mean±SD)	50.82±10.01	61.56±12.64
Sex		
Female	11 (47.82%)	9 (39.13%)
Male 12 (52.17%)		14 (60.86%)

CA: cancer

Table 2. Serum biomarkers in colorectal cancer

	Control group	Colorectal CA group (Adenocarcinoma)
Native thiol (SH) (µmol/l)	266.58±84.87	265.85±44.41
Total thiol (TT) (µmol/l)	299.90±87.77	301.96±50.77
Disulfide (-S-S) (µmol/l)	16.66±7.81	18.05±7.81
SS/SH (%)	6.81±3.95	6.83±2.82
SS/Total thiol (%)	5.80±2.83	5.90±2.20
SH/Total thiol %)	88.40±5.67	88.19±4.40
IMA ABSU	1.00±0.22	0.89±0.12*
Albumin g/dL	3.98±0.34	4.04±0.12
CA19-9 U/mL	12.85±18.39	318.96±972.02*
CEA ng/mL	2.51±1.52	28.62±59.84*

*p<0.05 was considered significant.

tric adenocarcinoma patients compared with a control group (23). Contrary to this study consistent with the literature, no statistically significant decrease was observed in the thiol levels in our study. The lack of any change in the thiol levels may be due to the small sample size. It is also thought that the change in the thiol levels of cancer patients may vary depending on the stage of the disease. In our study, patients had adenocarcinoma-type colorectal cancer, with no data concerning their stage of diagnosis and treatment initiation.

It is considered that the balance between oxidants and antioxidants may change in patients undergoing intensive chemotherapy and it may even be related with resistance to chemotherapy (22). It was reported that antioxidant levels decreased in patients with large-cell and advanced-stage lung cancer and, in another study, the contents of chemotherapy caused an increase in antioxidant levels in patients with lung cancer. The lack of any change in the thiol levels in our study may have also resulted from the fact that almost all of our patients underwent chemotherapy and that it might have suppressed the oxidative stress. In our colorectal cancer group IMA, CEA, and CA19-9 levels were obtained to be statistically significantly different, compared to the control group (p<0.05 for each comparison). There was also no relationship between the thiol-disulfide parameters and IMA, albumin, CEA, and CA-19-9 levels in the colorectal cancer patients.

In our study, thiol-disulfide parameters were measured by a newly developed method for colorectal cancer. It was thought that this method could offer a fast and reliable technique and be used as a routine biochemical measurement tool in early diagnosis, along with the CA 19-9 used in the follow-up of colorectal cancer patients. To our knowledge, this is a first study in the literature. We may not have detected a correlation between the thiol–disulfide parameters and CA19-9 levels because it was conducted with a small sample in a single center. A reassessment can be provided by a multicenter study to be conducted with a larger sample.

Statement of Conflict of Interest

The authors have no conflict of interest to declare.

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