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Measurement of total antioxidant response in colorectal cancer using a novel automated method

Aim: The aim of this study was to compare the antioxidative status in patients with colorectal cancer and healthy controls, based on measurements made using a novel method.

Materials and Methods: The study was conducted between 2005 and 2007, and included 30 patients with colorectal cancer and 30 healthy controls. To determine the antioxidative status of the patients and healthy controls, serum total antioxidant capacity was measured using a novel automated method. Blood samples were taken from the healthy controls and the patients.

Results: Total serum antioxidant capacity was significantly lower in colorectal cancer patients than in healthy persons ($P < 0.023$).

Conclusions: Patients with colorectal cancer are exposed to oxidative stress, which may have a role in the pathogenesis of the disease. The novel automated method used in this study was developed in our laboratory, and is feasible and fully automated; therefore, it may be useful for evaluating oxidative-antioxidative balance in patients with colorectal cancer.

Key words: Oxidative stress, Colorectal neoplasm, Total antioxidant capacity

Kolorektal kanserli hastalarda total antioksidan yanıtın yeni bir metodla incelenmesi

Amaç: Bu çalışmanın amacı, kolorektal kanserli hastalar ile sağlıklı grup arasında yeni bir metotla anti-oksidatif durumu değerlendirmektir.

Yöntem ve Gereç: 2005 ile 2007 yılları arasında, 30 kolorektal kanserli hasta ile 30 sağlıklı birey çalışmaya dahil edildi. Hasta ve sağlıklı bireylerin anti-oksidatif durumları yeni bir metod kullanılarak ölçüldü. Hasta ve kontrol grubundan kan örnekleri alındı.

Bulgular: Total antioksidan kapasite, kolorektal kanserli hastalarda sağlıklı kişilere göre istatiksel olarak anlamlı derecede düşük idi ($P < 0.023$).

Sonuç: Kolorektal kanser hastaları, hastalığın patogenezinde rol oynayan oksidatif strese maruz kalmaktadır. Bu yeni metod ile, total anti-oksidan kapasitenin değerlendirilmesi mümkündür ve tam otomatiktir. Bu nedenle, kolorektal kanserli hastalarda oksidatif-anti-oksidatif dengenin değerlendirilmesinde kullanışlıdır.

Anahtar sözcükler: Oksidatif stres, Kolorektal kanser, Total antioksidan kapasite

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Introduction

Colorectal cancer is one of the most common neoplastic diseases in humans. In all, 50% of the Western population develops a colorectal cancer by the age of 70, and in about 1 in 10 of these individuals. There are many factors related to reactive oxygen species (ROS) and free radicals involved in the initiation and progression of cancer (1). The role of oxidative stress in the pathogenesis and development of human colorectal cancer has been suggested in various reports. The gastrointestinal tract is particularly susceptible to attack by reactive oxygen species, which leads to carcinogenesis. Antioxidants play an important role in the defense against reactive oxygen species (2); however, information on biochemical

alterations in tissue and blood, in particular antioxidative status, and their correlation with clinical staging of the disease is lacking (1,3).

A recent study reported that oxidative stress is a risk factor for colorectal carcinogenesis. In inflammatory reactions activated leucocytes produce mutagenic and mitogenic free radicals, thereby promoting tumor formation (4). It is known that ROS are formed in excess in chronic diseases of the gastrointestinal tract (5), but the precise mechanisms of oxidative stress induced in cancer cells and the role of ROS in colorectal cancer progression are not precisely known (6). Changes in some parameters of the oxidative-antioxidative system in colorectal cancer were reported in earlier studies (5,6). The oxidative effects of ROS are controlled by exogenous antioxidants such as vitamins E and C, and by endogenous antioxidants such as scavenger enzymes (i.e. superoxide dismutase and glutathione peroxidase), bilirubin, and uric acid. Under some conditions, increases in oxidants and decreases in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts towards an oxidative status (7).

Antioxidant activity is known to reflect the altered redox balance of affected fluids, tissues, or organs in several pathologic states (8,9). Plasma is an important vehicle that can either protect against oxidative damage to different blood components or distribute dietary antioxidants to the remainder of the body. Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive, and costly. As the antioxidative effects of antioxidant components in plasma are additive, measurement of total antioxidant response (TAR) reflects the antioxidative status of plasma (10). In the present study we measured and evaluated total antioxidative status in colorectal cancer patients using a recently developed measurement method (7).

Materials and methods

Subjects

The study was conducted at the Harran University Medical Faculty, Department of General Surgery and Department of Clinical Biochemistry. The study population, treated in our hospital, consisted of 30 patients with colorectal cancer and 30 healthy control volunteers. All patients and volunteers gave informed consent to participate in the study, which was approved by the local ethics committee. Patient and control demographic data were not statistically different ($P > 0.05$) (Table 1).

The study included 30 patients with clinical stage III primary colorectal cancer. Patients with stage IV cancer were excluded from the study. The diagnosis of colorectal cancer was made based on physical examination, histopathological evaluation, routine clinical biochemical parameters, and abdominal ultrasound and tomography findings. Conventional histopathological parameters, such as AJCC/UICC TNM stage, tumor type, and grade of differentiation, were evaluated by our experienced pathologist. Differentiation and histological cancer type were evaluated according to World Health Organization (WHO) guidelines (11).

Blood samples

Blood samples were collected from the patients and controls into tubes containing EDTA after overnight fasting. Plasma was separated from cells via centrifugation at 1500 g for 10 min. The samples were stored at −80 °C until use.

Measurement of plasma total antioxidative status

Plasma total antioxidative status (TAS) was measured using a novel automated colorimetric version of the total antioxidant response (TAR) method (7). With this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate *o*-dianisidine to produce the dansyl radical, which is bright yellowish brown. Upon addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction medium are suppressed by the antioxidant components of the plasma, preventing color change and, thereby, providing an effective measure of the total antioxidative status of plasma. The assay results are expressed as mol Trolox Eq/L, and the precision of this assay is excellent, the variation being less than 3% (TAS).

Statistical analysis

All data are expressed as mean ± standard deviation (SD). The significance of difference was determined using parametric statistical methods. The Student's t-test was used for pair-wise comparisons. Statistical significance was defined as $P < 0.05$. Data were analyzed using SPSS v.11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Ethical issues

This study was performed according to institutional guidelines for clinical research with human volunteers, and according to national and international regulations under the supervision of a bioethics consultant.

Results

Participant demographic data are presented in Table 1. There were no statistically differences in age, sex, or body mass index (BMI) between the patients and healthy controls ($P > 0.05$).

Measurement of the antioxidative system matched the analyzed clinical features of colorectal cancer in a statistically significant manner. There were significant differences between the patients and controls regarding plasma TAS level (Table 2). The capacity of the antioxidative system decreased ($P < 0.023$) as colorectal cancer progressed.

Table 1. Patient and control demographic data.

Parameters	Controls (n = 30)	Patients (n = 30)
Age (years)	49 ± 12	51 ± 13^{ns}
Sex (female/male)	14/16	17/13
Body mass index (kg/m^2)	22.5 ± 3.8	23.8 ± 2.1^{n}

Values are mean ± SD. ^{ns}Not significant.

Table 2. Patient and control antioxidative status (TAS).

TAS (mmol Trolox Eq/L)	Controls (n = 30)	Patients (n = 30)	P
	1.59 ± 1.13	0.62 ± 1	< 0.023

Values are mean ± SD.

Discussion

It is well known that formation of ROS is a normal consequence of a variety of essential biochemical reactions. It is also known that oxygen radicals could be formed in excess in chronic diseases of the gastrointestinal tract (5). The main source of oxidants in the gut is probably phagocytes, which accumulate in the mucus of patients with bowel disease and could generate oxidants upon activation, and potentially contribute to an increased risk of cancer (12). Therefore, an adequate range of antioxidative defense within and outside cells has also been considered very important in protecting against oxidative damage to cell components, including membrane phospholipids, proteins, and DNA (13,14). Malondialdehyde is elevated in colon cancer patients, which is most probably associated with oxidative reactions and tissue damage involved in the pathology of colon cancer (15).

Multiple factors are involved in the etiology of colorectal cancer, just as multiple steps are implicated in its pathogenesis. It is most probable that both environmental and constitutional factors are interwoven, but the process varies from one individual to another. Thus, neither one factor nor one set of factors will underlie the etiology in all cases of colorectal carcinoma, as reported by Aksoy and Akinci (16). Free radicals and lipid peroxides are considered to be very important in carcinogenesis (12,14). Colorectal tissue is constantly exposed to a variety of hazardous chemicals that may be potential carcinogens, such as drugs, food additives, and food constituents; therefore, free radicals formed during the metabolic activation of these compounds are considered an important factor in carcinogenesis (10,12,14,17). Indeed, some investigators have reported elevated lipid peroxidation in experimental (11) and human colorectal cancer (18,19,20).

Plasma contains various antioxidant molecules; proteins, particularly albumin, constitute the main antioxidant component of serum. Free sulfhydryl groups of proteins are mainly responsible for antioxidant response. Others are uric acid and bilirubin, which serve as potent antioxidants via radical scavenging and reducing activities. Because the effects of the antioxidant components in plasma are additive, measurement of total antioxidative

response accurately reflects the redox status of plasma. All of these antioxidants were measured as TAS using a novel method (7) in the present study and were significantly lower in the colorectal cancer patients than in the healthy controls. This method provided useful measurement for the rapid evaluation of total antioxidant capacity (TAC), a parameter valuable not only in the diagnosis of colorectal cancer, but also for other disorders involving oxidative stress. In terms of colorectal cancer, routine measurement of TAC may be a useful aid in the assessment of oxidative stress status, and in the determination of an appropriate treatment plan. Routine screening of TAC in colorectal cancer patients may also prove useful for early recognition of colorectal cancer oxidative stress.

In conclusion, our findings suggest that some components of antioxidative status are deficient and oxidative injury occurs in patients with colorectal cancer. We determined that colorectal cancer patients had lower TAC levels than healthy controls. It may be inferred that the patients were exposed to oxidative stress and consumed significant amounts of plasma antioxidants, which may have played a role in the etiopathogenesis of the disease. The novel assay used in this study is rapid, easy, stable, reliable, sensitive, inexpensive, and fully automated; therefore, it may be useful in evaluating oxidative-antioxidative balance in patients and may be useful for other measurements, such as antioxidant supplementation, and can increase the effectiveness of treatment.

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