Levels of Plasma Vitamin E, Vitamin C, TBARS, and Cholesterol in Male Patients with Colorectal Tumors

E. I. Saygili1*, D. Konukoglu1, C. Papila2, and T. Akcay1

Department of 1Biochemistry and 2Oncology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey;
E-mail: ilksaygili@mynet.com

Received January 16, 2002
Revision received May 20, 2002

Abstract—Vitamin E and vitamin C are involved in the defense of the body against free radical and reactive oxygen molecule induced damage. The best characterized biological damage caused by radicals is known as lipid peroxidation. Free radical formation is known to play a major role in the development of cancer. In this study, we measured plasma levels of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation, cholesterol, and vitamins E and C as antioxidants in male patients with colorectal tumors (n = 20, 54.5 ± 8.3 years). The patients had significantly higher plasma TBARS levels than age-matched healthy subjects (p < 0.001). Plasma vitamin C levels were significantly lower in the patients compared to the healthy subjects (p < 0.001). On the other hand, plasma vitamin E levels in the patients were similar to those of healthy subjects. Plasma cholesterol levels were also found to be significantly elevated in patients with colorectal tumors (p < 0.001). Our results suggest that there is an imbalance between oxidant and antioxidant status in tumor genesis.

Key words: lipid peroxidation, thiobarbituric acid reactive substances (TBARS), colorectal tumors, vitamin E, vitamin C

Free radicals and lipid peroxidation are involved in the initiation and promotion of cancer. Lipid peroxidation increases in the course of carcinogenesis. It has been suggested that the hydroperoxide dependent oxidation process is one of the mechanisms of conversion of procarcinogens to carcinogens [1]. The main plasma antioxidant vitamins are vitamin C and vitamin E. Vitamin C (ascorbic acid) is a water-soluble compound and has a broad spectrum of antioxidant activities due to its ability to react with numerous aqueous free radicals and reactive oxygen species [2]. Vitamin E (α-tocopherol) is a lipidsoluble compound present in biological membranes and lipoproteins. Vitamin E is particularly effective as a chain-breaking antioxidant, thus inhibiting lipid peroxidation [3].

Lipid peroxidation occurs when hydroxyl radicals are generated close to or within membranes and attack fatty acid side chains of membrane phospholipids [4]. As a result of severe damage in cell structures by free radical generation, some chromosomal aberrations and carcinogenesis may develop [5]. An inverse association between cancer and cholesterol has been observed in several studies [6, 7], primarily in men and for cancers of the colon [7]. Some studies have found no relation between cholesterol levels and cancer risk [8, 9]. Thus the relation of serum cholesterol to the risk of cancer remains controversial.

The aim of this study was to compare the oxidation and antioxidant potential of patients with colorectal tumors. Because plasma values have shown sex-dependent differences, we planned the study in male patients and we determined plasma thiobarbituric acid reactive substances (TBARS) levels as a marker of lipid peroxidation, plasma cholesterol levels, and plasma vitamin E and vitamin C levels as antioxidants in male patients with colorectal tumors.

MATERIALS AND METHODS

The study was carried out with 20 male patients with colorectal tumors of age 54.3 ± 8.3 years. For comparison blood samples were also obtained from 20 age-matched healthy male subjects (n = 20, 56.5 ± 7.0 years). Clinical grading of the cancer patients was as follows: seven patients had Duke B and thirteen patients had Duke C stage. Subjects selected for investigations were not administered salicylates, antioxidant drug therapy, hypolipemizing agents, vitamin E, or vitamin C drugs during the last two weeks before the study. They had normal liver and renal functions. All the subjects gave their informed written consent.
Blood samples were collected from all patients preoperatively and centrifuged at 1500g for 10 min and the plasma was separated. Plasma TBARS levels were determined according to the method of Buege and Aust [10] by a spectrophotometric method using thiobarbituric acid. TBARS concentrations were calculated using 1.56·10^5 M⁻¹·cm⁻¹ as the molar absorption coefficient. Plasma vitamin E levels were determined according to the method of Quaife et al. [11]. After the proteins in plasma were precipitated by an equal volume of absolute ethanol, the whole mixture was subjected to extraction by an equal volume of xylene. α,α'-Dipyridyl was added to an aliquot of the upper layer to estimate the principal interfering substance, β carotene, at 460 nm. At this point, ferric chloride reagent was added to the system to produce color that was measured at 520 nm. By subtracting the absorbance at 520 nm from the absorbance at 460 nm plasma vitamin E levels were calculated; α tocopherol was used as the standard of vitamin E.

Plasma ascorbic acid levels were determined by an enzymatic colorimetric method (Boehringer Mannheim, Cat. No. 409677, Germany). L-Ascorbic acid and some other substances reduce the tetrazolium salt in the presence of an electron carrier at pH 3.5 to a formosone. For the specific determination of ascorbate in a sample blank determination only the ascorbate fraction as part of all reducing substances present in the sample is oxidatively removed by ascorbate oxidase in the presence of oxygen. The dehydroascorbate formed does not react with the tetrazolium salt. The absorbance difference of the sample minus the absorbance difference of the sample blank is equivalent to the quantity of L-ascorbate in the sample.

Plasma cholesterol levels were determined enzymatically using commercial kits (Biosystems, Cat. No. 11506, USA).

Statistical comparisons were made using the unpaired Student’s t-test. \( p < 0.05 \) was considered statistically significant. All data were expressed as means ± standard deviation.

**RESULTS**

The plasma levels of vitamin E, vitamin C, TBARS, and cholesterol in the subjects are shown Table 1. Vitamin C levels were found to be significantly lower in patients with colorectal tumors than in the healthy subjects (4.4 ± 0.9 and 7.5 ± 0.7 mg/dl, respectively, \( p < 0.001 \)). The plasma levels of TBARS and cholesterol were significantly higher in the patients (6.42 ± 0.8 nmol/ml and 217.6 ± 50.6 mg/dl, respectively) than in healthy subjects (both \( p < 0.001 \)).

When the patients were classified according to their clinical grade, the levels of plasma vitamin E, vitamin C, TBARS and cholesterol in Duke B were not significantly different from those in Duke C (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Vitamin E*, mg/dl</th>
<th>Vitamin C**, mg/dl</th>
<th>MDA**, nmol/ml</th>
<th>Cholesterol**, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>1.11 ± 0.3</td>
<td>7.5 ± 0.7</td>
<td>3.88 ± 0.3</td>
<td>117.5 ± 32.5</td>
</tr>
<tr>
<td>Patients with colorectal tumors</td>
<td>1.28 ± 0.47</td>
<td>4.4 ± 0.9</td>
<td>6.42 ± 0.8</td>
<td>217.6 ± 50.6</td>
</tr>
</tbody>
</table>

* Not significant.
** \( p < 0.001 \).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Vitamin E, mg/dl</th>
<th>Vitamin C, mg/dl</th>
<th>MDA, nmol/ml</th>
<th>Cholesterol, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duke B</td>
<td>1.30 ± 0.5</td>
<td>4.32 ± 0.8</td>
<td>6.35 ± 0.75</td>
<td>213.7 ± 52.4</td>
</tr>
<tr>
<td>Duke C</td>
<td>0.95 ± 0.25</td>
<td>4.52 ± 1.1</td>
<td>6.56 ± 0.93</td>
<td>222.1 ± 48.5</td>
</tr>
</tbody>
</table>

Note: No statistically significant difference between Duke B and Duke C.