Alterations in the antioxidant profile in patients with colorectal carcinoma

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Abstract
The present study was carried out in the patients diagnosed with the colorectal cancer and is about to get the treatment. Age and gender matched controls were also included in the study. The blood samples were collected and the biochemical parameters like Total antioxidant activity, lipid peroxidation, Glutathione, Vitamin E, Vitamin C and catalase were estimated. The significant decreased levels of total antioxidant activity, increased lipid peroxidation levels, decreased levels of glutathione, catalase, vitamin E and vitamin C were observed in the colorectal cancer subjects when compared with the control subjects. It is evident from the present study that oxidative stress plays an important role in the advancement of colorectal cancer and might be an additional parameter in the pathology of cancer progression.

Key words: Colorectal cancer, oxidative stress, antioxidants.

Introduction
Cancer is a leading cause of death in the world. Among different types of cancers, colorectal cancer is one of the most common carcinomas observed in humans and it is the fourth most commonly diagnosed cancer and second most common causes of cancer death. Colorectal cancer remains a leading cause of cancer death in the western as well as in the developing world among men and women although cancer therapy has benefited from promising new compounds directed to various targets. It has been reported that increased oxidative stress is associated with cancer (1). Oxidative stress occurs when there is an imbalance in free radical generation or oxidants and antioxidant defense capability (2). Unsaturated fatty acids (especially in cell membranes) are very susceptible to damage by oxidative stress and ultimately leading to lipid peroxidation. Antioxidants (especially vitamin C and vitamin E) work to prevent excessive levels of oxidative stress in cell membranes and therefore, lipid peroxidation.

There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of cancer initiation and progression (3). Damages to DNA, protein, cell membrane and mitochondria are involved in carcinogenesis, although no specific biochemical marker has been identified yet. In addition, information on the biochemical alterations in tissue and blood, particularly of antioxidant status is lacking. (4).

The present study is carried out on patients diagnosed with colorectal cancer and the emphasis is on the oxidative stress. In this study, the levels of lipid peroxidation product malondialdehyde (MDA) and the non-enzymatic antioxidants (glutathione, vitamins C and E) and the activity of antioxidative enzyme catalase in colon cancer patients were determined, to see the impact of carcinoma not only at a particular tissue
but its impact on the system by collecting the blood samples of the patients instead of the carcinoma tissue.

**Materials and Methods**

*Collection of Blood samples*

Samples of blood were obtained from thirty-four patients diagnosed with colorectal cancer and are about to start the treatment at MNJ Cancer Hospital, Hyderabad. Thirty subjects of age and gender matched controls were also included in the study. The study was conducted with prior medical ethical clearance. The blood samples were collected in EDTA and Heparin vials and were immediately transported to the laboratory and centrifuged at 2000 rpm for 15 min. The plasma was separated and the leukocyte layer was removed. The obtained erythrocyte sediment in the tubes was washed with 0.9% saline thrice. And the samples are stored at 4°C. The samples were used whenever required.

The levels of vitamin E, ascorbic acid and malonaldehyde (MDA) were quantified in plasma and Glutathione (GSH) and catalase were measured in RBC. Lipid peroxidation, ascorbic acid and catalase were quantified on the same day where as other investigation were done within a few weeks and stored at –70°C.

**Biochemical Investigations**

Lipid peroxidation (MDA) was quantified by thiobarbituric acid reactive substances by Spectrophotometric method described by Bhat KS (5). Malondialdehyde, chief representative product of lipid peroxides react with 2-thiobarbituric acid to form a pink colored complex in acidic medium which is quantitatively measured at 532nm. Malondialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid and produces MDA-TBA$_2$ adduct. Catalase activity was determined by the method of Aebi (6). In the ultraviolet range H$_2$O$_2$ shows a continual increase in absorption with decreasing wavelength. The decomposition of H$_2$O$_2$ can be followed directly by decrease in absorbance at 240nm. Reduced Glutathione (GSH) was measured fluorimetrically by O-phthalaldehyde (OPT) method. GSH reacts with fluorescent reagent OPT to yield fluorescent complex at pH - 8.0. The fluorescence was measured spectrofluorimetrically at excitation and emission wave lengths of 350nm and 420nm respectively(5). Ascorbic acid was determined in plasma by spectrophotometric method of Zannoni (7), which relies on the reduction of ferric iron by ascorbic acid followed by formation of a complex of the ferrous iron free product and dipyrindyl. Vitamin E was determined by HPLC according to the method described by Allard JP (8). Total serum antioxidant levels were determined using the method of Re (9). This improved technique involved the direct production of the blue/green ABTS+ [2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] radical chromophore through the reaction between ABTS and potassium persulphate.

**Results**

A total of sixty four patients including both the healthy (thirty) and colorectal cancer (thirty-four) subjects were selected for the study. The clinical status of patients whose samples were obtained was positive colorectal cancer patients and are about to undergo treatment. Elevated lipid peroxidation and decreased in non-enzymatic and enzymatic antioxidants status were noticed in colorectal cancer patients as compared to healthy subjects. In the colon cancer patients TBARS levels were significantly increased whereas other antioxidants such as reduced glutathione, catalase, vitamin C and vitamin E and the total antioxidant activity were significantly decreased when compared to the healthy subjects. The TBARS levels in colon
cancer patients were significantly increased at least by 4 nmol/ml when compared with the levels of healthy control. The activity of catalase was significantly decreased at least by 56% in colorectal cancer patients as compared to healthy subjects (Table I). The total antioxidant activity also been decrease significantly when compared with the controls by 1.93 μmoles/ml (Table I).

The level of reduced glutathione was significantly decreased by 12.5% in colorectal cancer patients as compared to healthy subjects (Fig 1). The level of vitamin C was significantly decreased by 39.3% in colorectal cancer patients as compared to healthy subjects (Fig 2). Similarly, the level of vitamin E was significantly decreased in colon cancer patients as compared to healthy subjects.

**Table 1:** Plasma TBARS, Erythrocyte Catalase levels and Total antioxidant levels in healthy and colorectal cancer patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Subjects (n=30) Mean ± SD</th>
<th>Colorectal Cancer Subjects (n=34) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TBARS (nmol/ml)</td>
<td>14.27 ± 1.51</td>
<td>18.72 ± 1.08*</td>
</tr>
<tr>
<td>Catalase Activity (units/mg protein/minute)</td>
<td>0.32 ± 0.01</td>
<td>0.18 ± 0.01*</td>
</tr>
<tr>
<td>Total antioxidant levels (μmoles/ml)</td>
<td>3.65 ± 0.185</td>
<td>1.72 ± 0.65*</td>
</tr>
</tbody>
</table>

The values are Mean ±SD. * Significantly different from controls at p<0.01

**Figure 1:** Comparison of the levels of non-enzymatic antioxidant reduced glutathione in healthy and colorectal cancer subjects. The values are Mean ± SD. * Significantly different from controls at p<0.05
Figure 2: Comparison of the levels of non-enzymatic antioxidant Vitamin C in healthy and colorectal subjects. The values are Mean ± SD.

* Significantly different from controls at p<0.05 (Fig 3).

Discussion

Colorectal cancer remains today an important cause of death, especially in developing countries. Improvements in screening programs and the encouraging results of surgery have prolonged the lifespan of patients with this pathology, but mortality is still very high. It has been demonstrated that the factors able to influence the prognosis are: grading, staging, nodal involvement, adjacent tissue involvement, and hepatic recurrences (10,11). Several studies have been undertaken to find new oncological markers able to identify a tumor before its macroscopic development. Evidence suggests the pathological role of free radicals in a variety of diseases, among which the most important are chronic inflammation, and cancer (12,13). Free radicals are inevitable byproducts of biological redox reactions. In fact, reactive oxygen species, such as 'OH, H₂O₂, and other chemical forms, are produced as part of many normal and essential biological processes (13,14). Plasma and other biological fluids are rich in antioxidant molecules, which can be subdivided into two major groups: those that prevent initiation and those that slow down the progression of a peroxidative chain reaction (15,16,17). The etiology of colorectal cancer, which is one of the most common carcinomas observed in humans, is still being investigated. Colon cancer is generally assumed to be initiated by environmental genotoxic agents causing cellular overproduction of reactive oxygen species (ROS). As a consequence, extensive oxiradical damage can cause genetic alterations required for neoplastic progression and lead to a cycle of cell death and regeneration (18). A growing body of evidence has suggested that oxidative stress plays an important role in the molecular mechanism of colorectal cancer (19,20,21) related studies carried out in both humans (21,22) and experimental animals (20,23) have shown lipid peroxide levels in malignant colorectal tissues to be higher than those in the normal tissues of the same patients. Our work was in accordance with the work done on the tissues (1,4) and we were interested to see the impact of carcinoma not only at the particular tissue but its global impact in the system by taking in the blood samples of the patients instead of the carcinoma.
tissue. Extent of lipid peroxidation could be determined by estimation of the final lipid peroxidation product – malondialdehyde (24) and the antioxidants status. In the present study, the levels of malondialdehyde in colorectal cancer patient were significantly increased. Byproducts of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. Lipid peroxidation byproducts formed under physiological and pathological conditions are scavenged by non enzymatic and enzymatic antioxidants (25). An imbalance between antioxidant defense mechanism and lipid peroxidation processes results in cell and tissue damage (26, 27). In this study lower levels of antioxidant vitamin E were reported which is supporting the relation as evidenced in earlier reports (28, 29) between á-tocopherol and cancer. The lower levels of antioxidant vitamin C in a patient suffering from colorectal cancer has been reported by Elzbieta Skrzydlewska et al., (4). The level of antioxidant glutathione has decreased significantly which is in accordance with previous work that reported decreased glutathione concentrations in colon cancer patients (30,31). Lower levels of antioxidants like glutathione, vitamin C and E and decrease in the activity of catalase were reported which is a positive correlation between the lipid peroxidation and the antioxidant status in colorectal cancer (4).

References


3. Zalewski, B. (2004). Levels of v5 and v6 CD44 splice variants in serum of patients with colorectal cancer are not correlated with pT stage, histopathological grade of malignancy and clinical features. World J. Gastroenterol, 10: 583-585


