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SHORT COMMUNICATION

LIPID PEROXIDATION AND ANTIOXIDANT VITAMIN STATUS IN COLORECTAL CANCER PATIENTS

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Abstract : Reactive Oxygen Species (ROS) can induce carcinogenesis via DMA injury. Both enzymatic and non-enzymatic antioxidants participate in cell protection against harmful influence of oxidative stress. The aim of the present study was to assess the level of end product of lipid peroxidation such as malondialdehyde (MDA) as an oxidant in colorectal cancer. Moreover, we analyzed the activity of main non-enzymatic antioxidants, vitamin E and vitamin C in colorectal cancer patients.

In the present study, total 48 samples were analyzed which includes 24 age matched healthy controls irrespective of sex (Group I) and 24 cases of colorectal cancer (Group II). The serum level of lipid peroxide, vitamin E and vitamin C were estimated in both healthy control Group I (n=24) and colorectal cancer Group II (n=24). A significant increase in the level of serum lipid peroxide ($P<0.001$), with concomitant decrease in the levels of serum vitamin E and vitamin C, ($P<0.001$) in Group II patients were noticed as compared to the healthy control Group I. Our findings suggest that increased oxidative stress and reduced antioxidants defense mechanism may play an important role in progression and pathogenesis of colorectal cancer.

Key words : antioxidant vitamins, colorectal cancer, lipid peroxidation, oxidative stress

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INTRODUCTION

Colorectal cancer is one of the most frequent neoplastic disease in human population and most common causes of death (1). On the world wide basis each year about 1 million cases are diagnosed with colorectal cancer and of these more than 50,00,000 dies from its complications (2). Colorectal cancer arises from a series of histopathological and molecular changes caused by complex interaction between genetic susceptibility and environmental factors. Gastrointestinal blood loss is the most common sign and may include a positive fecal occult blood test resulting in iron deficiency anemia or hematochezia. In case of advanced tumors, patients may suffer from symptoms like anorexia, weight loss and abdominal pain (3).

Earlier studies have shown that reactive oxygen species are formed in excess in colorectal cancer (1, 4). Along with pathological factors, reactive oxygen species (ROS), such as Superoxide anion (O^{2-}), hydroxyl radical (OH^{-}), singlet oxygen (O^{-}) and hydrogen peroxide (H_2O_2) are involved in process of cancer initiation and progression. These ROS damage the DMA, destabilize cell membrane, mitochondria and leads to carcinogenesis (1). The ROS is associated with inflammatory response and frequent cell damage which induces apoptosis where at high concentration it causes necrotic cell death (4). Oxidative stress is associated with a disturbance in pro-oxidant/ antioxidant balance.

Malondialdehyde (MDA), a product of lipid peroxidation is generated during peroxidation of polyunsaturated fatty acid in cell membranes by free radicals plays an important role in colorectal cancer (5). The vitamins E and vitamin C are important antioxidants substantially reduces the risk of colorectal cancer by neutralizing reactive oxygen species and other free radicals that can damage DMA (6).

In the present study, attempt has made to assess the level of oxidative stress by measuring plasma level of malondialdehyde and non-enzymatic antioxidants vitamins such as vitamin E and vitamin C in colorectal cancer patients.

MATERIAL AND METHODS

This study was carried out in the Department of Biochemistry, Dr. V.M. Govt. Medical College Solapur, Shree Chattrapati Shivaji Maharaj General Hospital and Shree Siddheshwar Cancer Hospital and Research Center, Solapur (Maharashtra). On the basis of area of study and prevalence of statistics we have selected 48 subjects, out of which 24 subjects were regarded as age and sex matched healthy controls and 24 were patients with colorectal cancer. The patients were diagnosed to have colon cancer and treated at the Department of Surgery, Dr. V.M. Govt. Medical College, Solapur. Blood samples were obtained from these patients after informed written consent. The study was approved by the Institute “Ethics Committee” and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1964. All the control subjects were clinically diagnosed for the diseases which induce oxidative stress and only those were selected having no such concurrent or past history of disease. All are

apparently healthy belongs to same socio-economic status and non-alcoholic. All the subjects having history of smoking, alcoholism and other diseases which induce oxidative stress such as diabetes mellitus, pulmonary diseases, respiratory diseases etc. were excluded from the study. After obtaining informed consent, 6 ml of venous blood was collected from the subject under aseptic conditions in the plain bulb. The collected blood samples were stored at 4°C and all the biochemical parameters were performed on the same day. Serum was separated by centrifugation at 3000 rpm for 10 min. at room temperature. Analysis of all the biochemical parameters was done manually using the chemicals of Qualigens fine chemicals Co., Mumbai. The parameters were run on UV Visible Spectrophotometer (Systronics). Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid and boiling with thiobarbituric acid which reacts with malondialdehyde to form pink colour as per the method of Kei Satho (7). For estimation of serum vitamin C, the serum ascorbic acid is oxidized to diketogulonic acid in presence of sulphuric acid which reacts with 2, 4 dinitrophynyl hydrazine to form red diphenylhydrazone compound as per method of Caraway et al (8). Serum vitamin E was determined by Baker and Frank method which is based on reduction of ferrous ions which forms a red colored complex with α-V dipyridyl (9).

All the results were expressed as means±SD. Unpaired student T test were applied for the comparison of data and $P<0.001$ was considered as the level of significance.

RESULTS AND DISCUSSION

Table II shows a significant elevation of serum MDA with concomitant decrease of serum vitamin E and vitamin C in colorectal cancer patients in comparison to their normal healthy control counter part.

The reason for increased lipid peroxidation could be due to increased generation of reactive oxygen species or suppression of the antioxidants defence mechanism in the metabolically active tissues. Recently it was reported the antioxidant defence has suppressed in various colorectal cancer tumors (10).

Our findings strongly supported by Elzbieta Skezydlewska et al (1), who had found that, significantly increased lipid peroxidation level in colorectal carcinoma. The reactive oxygen species is main source of oxidants present in the gut. Perhaps due to phagocytes, which are accumulated in the mucus of patients with bowel disease, could generate oxidant upon activation, which might contribute to the increased risk of colorectal cancer.

TABLE I: Depicts median age, age range, male and female ratio of both controls and colorectal cancer patients.

Patient baseline characteristics	*Data*	
	Patient	*Control*
Median Age	67	62
Age Range (years)	(31–80)	(31–80)
Sex M: F Ratio	15:9	15:9
Clinical Stages of Colorectal cancer		
Stage II	12	
Stage III	07	
Stage IV	05	

TABLE II: Showing levels of oxidants and antioxidants in controls and Colorectal cancer patients. (Values are expressed in Means±SD).

Parameter	*Group 1 (n=24)*	*Group II (n=24)*
Serum MDA(nmol/ml)	1.83±0.52 #(1.31–2.35)	3.99±0.58* #(3.41–4.57)
Serum vitamin C mg/dl	1.49±0.16 #(1.33–1.65)	1.02±0.14* #(0.88–1.16)
Serum vitamin E mg/L	10.02±0.90 #(9.12–10.92)	7.51±0.59* #(6.92–8.10)

Statistical Comparison was done between Group I and Group II.
Group I – healthy controls; Group II – Colorectal cancer patients.
n=24 number of Subjects.
*P<0.001 – highly significant.
– reference ranges.

Formation of reactive oxygen species is a normal consequence of a variety of essential biochemical reactions. It is well known that reactive oxygen species could be formed in excess in chronic diseases of the gastro intestinal tract. Production of oxygen radicals increases with clinical progression of disease is involved in increased lipid peroxidation resulting in cell membrane degeneration. Extent of lipid peroxidation product like malondialdehyde compound known to produce protein cross linking through Schiffs base with DMA and causes DMA damage. Lipid peroxidation is initiated by free radical attack on cell membrane PUFA which generates large amount of toxic radical products that are implicated in tumor initiation and promotion of colorectal cancer (5). Highly significant depletion in plasma vitamin E level was observed in the present study. Vitamin E is powerful chain breaking antioxidant and reduces the risk of cancer by neutralizing reactive oxygen species that can damage the ONA (12).

Our findings corroborate with earlier reports (11, 13, 14), where similar observation on decrease vitamin E concentration in colorectal cancer patients were noticed. The antioxidant role of vitamin E can be attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen molecule and this prevents abstraction of hydrogen molecule from polyunsaturated fatty acid. It is also possible that low level of vitamin E may be due to impaired absorption or transport across the gastrointestinal tract due to impaired formation of cilliary wall.

It is known that vitamin C plays an important role in the synthesis of connective tissue protein such as collagen, and deficiency of it, therefore affects the integrity of intracellular matrix and has a permissive effect on tumor growth. Deficiency of vitamin C could hinder tumor encapsulation. Hence low level of serum vitamin E and vitamin C from our study attribute that such non-enzymatic antioxidants are not able to prevent oxidative modifications of cell components and their levels decreased gradually with progression of colorectal cancer (1).

Jocobs et al (12) observed that supplementation of vitamin E and vitamin C inhibits colorectal cancer in rodent models and decreases fecal mutagenicity in human. Antioxidants play an important role in defense mechanism that protects the body from free radical or promoting their decomposition. Lowered level of antioxidant capacity might be due to its utilization for nullifying the action of reactive oxygen species.

Hence it may be postulated from our study, that colorectal carcinogenesis may be

associated with oxidative stress which may be reflected by greater elevation of MDA and decrease level of vitamin E and vitamin C in the serum.

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