Evaluation of oxidative stress and its impact on the antioxidant activities in patients with colorectal Carcinoma

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ABSTRACT   *The present study mainly highlights the occurrence of lipid peroxidation and possible breakdown of antioxidants in colorectal carcinoma patients as compared to healthy control subjects. Enzymatic and non-enzymatic parameters play an important role in cell protection against harmful influence of oxidative stress. Our aim of the present study was to investigate the levels of final lipid peroxidation products like (MDA) malondialdehyde and activity of antioxidative enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) in colorectal carcinoma. The study consisted of 30 diagnosed gastrointestinal carcinoma patients and an equal number of age and sex matched healthy control subjects. The Activity of MDA, glutathione peroxidase and superoxide dismutase were determined by spectrophotometric method. From this study, we observed that the MDA were increased significantly & antioxidants were altered highly significantly. Therefore, for the diagnosis of human colorectal carcinoma or recurrence, the study of lipid peroxidation & antioxidant defense mechanism may be useful tool along with the biopsy, biochemical & Pathological investigations.*

Manuscript -
Colorectal cancer is one of the most frequent neoplastic disease in human population and most common causes of death ranks third in frequency in men and second in women [1]. On the world wide basis each year about 1 million cases are diagnosed with colorectal carcinoma [2]. Colorectal cancer arises from a series of histopathological and molecular changes caused by complex interaction between genetic susceptibility and environmental factors [3].

The risk factors for colorectal cancer are hereditary genetic predisposition (i.e. familial adenomatous polyposis), age, ulcerative colitis and other colon inflammatory diseases, diets high in meat and fat or low in selenium, Lynch syndrome, smoking, and others [4]. Gastrointestinal blood loss is the most common sign and may include a positive fecal occult blood test resulting in iron deficiency anemia. In case of advanced tumors, patients may suffer from symptoms like anorexia, weight loss and abdominal pain [3].

There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of colorectal cancer initiation and progression [5]. ROS are continuously produced in aerobic organisms as biproducts of normal energy metabolism. These reactive species may react with biomolecules, including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue, thereby interfering with cell function [6, 7]. It is known that when oxidative stress increases, damage may occur in the DNA sequence, leading to GI cancer and other diseases like atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases, as a result of the deterioration in the balance between free radicals and antioxidants [8]. Antioxidant potential in all cases of gastrointestinal tract cancer has been imbalanced which has lead to increase in reactive oxygen species action and enhancement of lipid peroxidation [9, 10,11].

The aim of the present study was to estimation the levels of lipid peroxidation products like malondialdehyde (MDA) and enzymatic antioxidants (glutathione peroxidase, superoxide dismutase) in colorectal cancer.

Patients and Methods-
Details of study, Sample collection and Processing:-
The study was carried out in Department of Biochemistry and Department of Radiation Oncology, J. A. group of hospitals, G.R. Medical College, Gwalior. The study was conducted in 60 human subjects. Out of which 30 age matched normal healthy volunteers were considered as control **Group-I** and 30 were colorectal Carcinoma patients (Male & Female) **Group-II**. A detailed history was collected from the patients before starting analysis, the written consent from all subjects were taken. The study was approved by institutional ethical committee and was carried out by keeping all norms in mind.

Biochemical assays -
The analysis of Plasma MDA was done by the method of **Jean CD** et al.(1983) [12]. 1 ml of plasma was taken in a clean centrifuge tube, added 1.5 ml TBA reagent, (1 ml TBA reagent or stock + 0.5 ml 7% perchloric acid). Mixture was heated in a boiling water bath for 30 minutes. After cooling, 3 ml of n-butanol was added. Mixed by shaking and centrifuged at 3000 rpm for 15 min. Absorption of supernatant was read at 531 nm. Glutathione peroxidase (GPx) was done by **Hafeman D.G.** et al. method (1974) [13]. Glutathione peroxidase catalyzes the decomposition of hydrogen peroxide in presence of reduced glutathione forming oxidized glutathione and water. The final absorbance of the test solution and standard were read against blank at 412nm within 2 minutes and superoxide dismutase (SOD) was assayed utilizing the technique of **Misra & Fridovich**, et al. (1972)[14] epinephrine method based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome.

Statistical analysis –

Statistical evaluation was performed using the statistical Package for the Social Sciences 16.0 (SPSS) software. Data obtained from the study groups were compared use the Student-t test. The results were expressed as mean ± standard deviation and p < 0.001 value was considered statistically significant.

Table – 1. Defining characteristics of individuals participating in the study.

Parameters	Group-I	Group-II
Total number of subjects	30	30
Mean age (years)	33.41	48
Sex (Male)	20 (66.67%)	22(73.33%)
Sex (Female)	10 (33.33%)	08(26.67%)
Body mass index (kg/m2)	22.58	21.24
Smokers	07	18
Alcohols intake	09	19

Values are given as mean from 30 subjects in each group.

Table – 2 Values were shown as mean and (±) standard deviation. ***Shows the statistical significance between the patient group-II and control group-I (p < 0.001 highly significant).

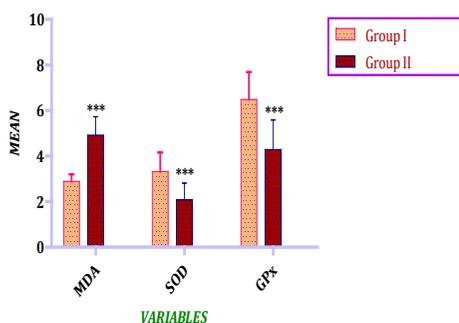| Parameters	Groups	
	Group-I Healthy control (Mean ± SD) n=30	**Group-II** colorectal Cancer patients (Mean ± SD) n=30
MDA (μ mol/L)	2.89 ± 0.31	4.92±0.81***
SOD (Unit/mg protein/ml)	3.32 ±0.84	2.09 ±0.72***
GPx (enzyme unit/mg%of Hb)	6.49 ± 1.2	4.29± 1.3***

FIGURE NO.- 1

Figure 1. Levels of lipid peroxide(MDA), SOD and GPx in Group-I (control) and Group-II . Values are expressed as mean ± SD compared with group I with group II statistical significance - ***p< 0.001significant.

RESULTS & DISCUSSION-

The result of the present study, showed significantly increased concentration of lipid peroxidation products MDA in group-II (colorectal carcinoma patients) as compared to the group –I (control), (**Figure-I and Table –II)**. Gerber *et al.* [15] and Saintot *et al.* [16] have reported similar findings in patients with breast cancer. They have also observed a decrease in plasma MDA with tumor size and progression. A similar decrease in tissue lipid peroxide product MDA was observed in oral squamous cell carcinoma [17]. The process of lipid peroxidation is the oxidative conversion of polyunsaturated fatty acids to products known as malondialdehyde (MDA) or lipid peroxides, which is the most studied, biologically relevant, free radical reaction. It is suggested that MDA itself, because of its high cytotoxicity and inhibitory action on protective enzymes, acts as a tumour promoter and a co-carcinogenic agent [18, 19]. In addition to the deleterious effects of ROS on human cells, oxidative injury can lead to apoptosis. Dysregulation of apoptosis has a role in gastrointestinal diseases, including cancer. Oxidative stress can modulate the apoptotic programme and could cause gastrointestinal cancer [20]. Our findings were strongly supported by Elzbieta S. et al.[21] who had also found significantly increased lipid peroxidation level in colorectal carcinoma.

To avoid redox imbalance and oxidative DNA damage, a wide array of enzymatic and nonenzymatic antioxidant defences exist. Primary defence mechanisms prevent oxidative damage by scavenging reactive species directly. The primary defence system includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and thioredoxin reductase.[22] Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Cells have developed a comprehensive array of antioxidants that act co-operatively *in vivo* to combat the deleterious effects of free radicals. Superoxide dismutase (SOD) and catalase (CAT) are considered to be primary antioxidant enzymes since they are involved in the direct elimination of ROS.[23] SOD scavenges the superoxide radical (O_2-) by converting it to hydrogen peroxide (H_2O_2) and hence reduces the toxic effects of this radical or other free radicals derived from secondary reactions. CAT subsequently reacts with H_2O_2 and decomposes it into water and molecular oxygen [6,24]. Glutathione peroxidase (GPx) catalyses the reduction of H_2O_2 and organic hydroperoxides with the simultaneous oxidation of GSH [25, 26].

Another important finding of the SOD and Gpx antioxidant enzymes activities were significantly decreased. from this study, we finely concluded that oxidative injury had happened to colorectal cancer patients (group-II) as compare to control (Group-I) **figure-I and Table –II**. The increase level of MDA indicates an enhanced lipid peroxidation leading to cell injury and failure of the antioxidant defense mechanisms to prevent the formation free radicals [27]. There for the study of lipid peroxidation & antioxidants defense mechanism may be useful for the diagnosis and prognosis of colorectal carcinoma *patients*.

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