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QUANTITATIVE ESTIMATION OF LIPID PEROXIDATION AND ANTIOXIDANTS ENZYMES IN THE DIAGNOSIS AND PROGNOSIS OF GASTROINTESTINAL CARCINOMA

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HOW TO CITE THIS ARTICLE:

Dinesh Maraiya, Akshay Kumar Nigam, Sanjeev Kumar Singh, Neelima Singh, Nivedita Singh. "Quantitative Estimation of Lipid Peroxidation and Antioxidants Enzymes in the Diagnosis and Prognosis of Gastrointestinal Carcinoma". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 48, June 15; Page: 8313-8318, DOI: 10.14260/jemds/2015/1207

ABSTRACT: The present study mainly highlights the occurrence of lipid peroxidation and possible breakdown of antioxidants in gastrointestinal 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 gastrointestinal carcinoma. The study consisted of 40 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 gastric cancer or recurrence, the study of lipid peroxidation & antioxidant defense mechanism may be useful tool along with the biopsy, endoscopy & Pathological investigations.

KEYWORDS: Gastric carcinoma; lipid peroxidation; antioxidants; glutathione; SOD.

INTRODUCTION: Cancer is currently the second leading cause of death in the world behind cardiovascular diseases. It is estimated that more than 1.6 million new cases of cancer were diagnosed in every year.^[1] Cancer is characterized by the proliferation of abnormal cells that fail to respond correctly to normal regulatory mechanisms. Carcinogenesis, a term used to describe cancer development, is a multiple-step process consisting of initiation, promotion, and progression of uncontrolled cells. At the initiation step, damage to DNA occurs. Finally, cells begin to proliferate and expand into abnormal cells during the promotion step and during the progression step, further changes occur to these abnormal cells leading to formation of malignant cells.^[2]

Gastric cancer is second leading cause of death from cancer, with a million of new cases diagnosed each year. Indeed, it is the fourth most common cancer worldwide. The incidence of gastric cancer is different throughout the world and 60% of deaths from gastric cancer occur in developing countries.^[3,4] Cancers of the gastrointestinal tract, including esophageal, stomach, liver, colon, and pancreas are responsible for approximately 3 million new cases and over 2 million deaths each year.^[5] Malignancies of the G.I. tract are relatively resistant to radiation therapy while chemotherapy has modest benefit so an effective therapy still remains elusive in the treatment of gastroduodenal ulceration.^[6] Early diagnosis of human gastric cancer or tumor recurrence is primarily based on endoscopy, biopsy and pathological examination. Endoscopy is a widely used method for detecting early stages of gastric cancer.^[7,8]

ROS are continuously produced in aerobic organisms as biproducts of normal energy metabolism. These reactive species may react with biomolecules, including lipids, carbohydrates,

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proteins, nucleic acids and macromolecules of connective tissue, thereby interfering with cell function.^[9,10] 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.^[11] 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.^[12,13,14]

MATERIAL 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 80 human subjects. Out of which 40 are matched normal healthy volunteers were considered as control Group-I and 40 were Gastrointestinal 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 Analysis: The analysis of Plasma MDA was done by the method of Jean CD et al.(1983).^[15] 1ml of plasma was taken in a clean centrifuge tube, added 1.5ml TBA reagent, (1 ml TBA reagent or stock + 0.5ml 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 3000rpm for 15min. Absorption of supernatant was read at 531 nm. Glutathione peroxidase (GPx) was done by Hafeman D.G. et al. method (1974).^[16] 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, etal. (1972)^[17] epinephrine method based on the capacity of SOD to inhibit auto oxidation of adrenaline to adrenochrome.

Statistics: Student's t-test (Paired & unpaired) were used in the statistical evaluation of the result using Statistical Package for the Social Sciences 16.0 (SPSS) software.

Controls/ patients Baseline characteristics	Healthy controls (n=40)			Gastric cancer patients (n=40)		
	Age	Weight	BMI	Age	Weight	BMI
Min	18	46	17.87	39	35	17.20
Max	75	85	32.14	79	67	24.29
Mean	33.41	60.40	22.70	45	56	21.58
Smoker	07			22		
Non-smoker	33			18		
Alcoholic	10			21		
Non-alcoholic	30			19		
Vegetarian	22			17		
Non-vegetarian	18			23		

Table 1: Demographic characteristic of gastrointestinal cancer and healthy control

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Groups	Biochemical Parameters		
	MDA (μ mol/L)	SOD (Unit/mg protein/ml)	GPx (enzyme unit /mg% of Hb)
Group-I Healthy control (Mean $\pm$ SD) n=40	2.92 $\pm$ 0.38	3.32 $\pm$ 0.96	6.58 $\pm$ 1.1
Group-II GI Cancer patients (Mean $\pm$ SD) n=40	5.12 $\pm$ 0.73***	2.15 $\pm$ 0.82***	4.36 $\pm$ 1.08***

Table 2: Showing the significant changes of biochemical parameters in group I and group II subjects (***P<0.001 highly significant)

There is significant changes in MDA, SOD and Gpx in GI cancer subjects as compared to the control group (***p <0.001).

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- ***p<0.001 significant.

Fig. 1

RESULTS & DISCUSSION: The result of the present study, showed significantly increased concentration of lipid peroxidation products MDA in group-II (Gastrointestinal carcinoma patients) as compared to the group -I (control), (Figure-I and Table -II). 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.

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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 (O₂⁻) by converting it to hydrogen peroxide (H₂O₂) and hence reduces the toxic effects of this radical or other free radicals derived from secondary reactions. CAT subsequently reacts with H₂O₂ and decomposes it into water and molecular oxygen.^[9,24] Glutathione peroxidase (GPx) catalyses the reduction of H₂O₂ and organic hydroperoxides with the simultaneous oxidation of GSH.^[25,26]

The SOD and Gpx antioxidant enzymes activities were significantly decreased. In this study the results also indicates that oxidative injury had happened to gastric 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] Therefore, for the diagnosis of human gastric cancer or recurrence, the study of lipid peroxidation & antioxidant defense mechanism may be useful tool along with the biopsy, endoscopy & Pathological examinations.

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FINANCIAL OR OTHER

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Date of Submission: 03/06/2015.

Date of Peer Review: 04/06/2015.

Date of Acceptance: 08/06/2015.

Date of Publishing: 13/06/2015.