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Glutathion-S- transferase Enzyme and Malondialdehyde (MDA) in Colorectal Cancer and in Healthy Control

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Abstract:

Oxidative stress is closely related to all aspects of cancer, from carcinogenesis to the tumor-bearing state, from treatment to prevention. The human body is constantly under oxidative stress arising from exogenous origins (e.g., ultraviolet rays) and endogenous origins (at the cellular level where mitochondria are involved). When such oxidative stress exceeds the capacity of the oxidation-reduction system of the body, gene mutations may result or intracellular signal transduction and transcription factors may be affected directly or via antioxidants, leading to carcinogenesis.

Aim: The study focuses on the plasma Glutathion S-transferase (GST) level, Malondialdehyde (MDA) level in colorectal cancer patient which result due to an imbalance between aggressive and defensive factors. Materials and Methods: the study included 40 patient and 20 healthy individuals for comparative analysis were considered for the present study. Serum (GST) and (MDA) levels of each individual were performed. Results: Statistical analysis of serum antioxidant enzymes level and antioxidant status revealed a significant increase in malondialdehyde (MDA) ($p< 0.001$ respectively. The total plasma GST exhibited nearly a 2–3-fold increase ($p < 0.001$) in the patient plasma samples as compared with the corresponding controls. The increasing preponderance of plasma MDA and GST enzyme level can be explained on the basis of alteration of enzymes activity, which may lead to disturbance in homeostasis of antioxidant/oxidant balance.

Introduction:

The frequency of colorectal cancer ranks third and second both in men and women respectively . The incidence is higher in developed countries than in developing countries (1). Alteration in the oxidant-antioxidant profile is known to occur in cancer (2, 3). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation.

There are many stages involved in the development of cancer and those involved in Colorectal cancers (CRC) development have been well studied (4,5). The main events in colon cancer development can be classified as initiation, promotion and metastasis. Under certain conditions, the overproduction of reactive oxygen species can lead to free radical induced damage to DNA (6). Left unrepeated by the endogenous DNA repair mechanisms (7), this damage could be incorporated as a permanent sequence change, potentially initiating the multi-step pathways involved in carcinogenesis (8،9). Normal cell growth and proliferation are regulated by tumor suppressor genes and proto-oncogenes, which control cell cycle and apoptosis. However, several tumor suppressor genes are known to be inactivated with the parallel activation of key oncogenes via mutation, which can result in unregulated and uncontrolled proliferation of cancer cells, allowing colon cancer to progress (5, 10).

The GSTs (glutathione S-transferases; EC 2.5.1.18) comprise a family of mostly cytosolic, dimeric enzymes that are widely distributed in all mammalian cell types. Based on their structures, the GSTs are placed into alpha, kappa, mu, omega, pi, sigma, theta and zeta classes (10–11). The GSTs catalyze

the nucleophilic addition of reduced glutathione (GSH) to electrophilic centers of heterogeneous compounds, both xenobiotics and metabolically generated, facilitating their elimination from the cell [12.13]. The GSTs are also thought to function non-enzymically by binding non-substrate ligands and reactive compounds, leading to enhanced elimination of these molecules [11,14].

Glutathione s-transferase (GST) is an enzyme involved in antioxidant defense and also involved in detoxification. It is used as a tumor marker in certain cancers such as colorectal cancer. Alterations in GST levels in tumor tissue have been reported by various studies (15, 16).

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues. The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several path physiologies [17]. Alteration in the oxidant -antioxidant profile is known to occur in cancer [18, 19]. Oxidative stress due to damage brought about by free radicals is also known to influence the response of the patients to therapy. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions [20] and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated [21]. They exist both in the aqueous and membrane compartment of cells and can be enzymes or non-enzymes.

In the present study, the following parameters were assessed in the patient's plasma to elucidate the oxidant-antioxidant status in patients with colorectal cancer. Plasma Glutathion s-transferase (GST) levels were estimated as an index of antioxidant status. Malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances (TBARS) which served as an index of extent of lipid peroxidation.

Material and Methods:

This study was conducted on patients attending the consultant department of oncology, Baghdad teaching hospital. All the patients were conducted the department to received treatment. The complete clinical and personal history of the patient and control was recorde. The patient and control were ranging in age 34 – 50 years. All the patients in the study were clinically diagnosed as patients with colorectal cancer by histophological study and they didn't reserve any treatment.

Blood sample

About 5 ml of the venous blood samples obtained from the patients and controls were used for the estimation of plasma MDA and plasma GST enzyme. Portion of blood was transferred into clean dry plain plastic tube without anticoagulant. So that, the blood was left to clot and the serum was obtained by centrifugation at 3000 rpm for 10 minutes and used for estimation of iron level. Another portion was collected in EDTA tubes and the plasma was supported to measure the plasma MDA.

Measurement of Plasma Peroxidation levels (MDA)

Measurement of plasma MDA (malonedialdehyied), a secondary product of lipid peroxidation, was based on the colorimetric reaction with thiobarbituric acid (TBA). Details of the method are described as follows below. The molar extinction coefficient of MDA is $1.56*10^{-5}$M/Cm and the results were expressed as nanomol/ml of MDA (22).

Procedure:-

	BLANK	UNKNOWN
PLASMA	----	0.5ml
D.W	0.5ml	-----
0.5% TCA	0.5ml	0.5ml
0.5% TBA	1ml	1ml

Good mixing is important after each addition. The tubes are sealed with cotton plugs and incubated at 60ºC in shaking water bath for 90 minutes. After

cooling, one ml of 70% TCA is added to each tube and swirled gently. At last (3) ml of chloroform are added to each test tube and vortex vigorously to remove the lipid. Centrifuge at 2000 rpm for at least 15 minutes and read at 532 nm.The concentration of the plasma lipid peroxide is then computed according to the following equation:-

$$\text{Plasma lipid peroxide (nanomol/ml)} = \frac{\text{A test} - \text{A blank}}{\text{E MDA}}$$

$$= \frac{\Delta A}{1.56 \times 10^5}$$

Procedure of GST enzyme assay

Centrifuge an anticoagulant blood at 4000 rpm for 10 minutes and the suppurated plasma was used in determination of enzyme.

The samples were prepared in a total 100 ul volume with GST sample buffer, including a negative control with 100 ul of GST Sample buffer only and a positive control with 2 ul of GST and 98 ul of sample buffer. 10 ul of Glutathion was added to each well containing the sample or control above.Preprared Substrate mix by adding 10 ul CDNB(1-chloro-2,4-dinitrobenzene) solution + 90 ul of GST assay buffer for each sample including the standard. Mixing well and was transferred 100 ul of the mixing into each sample (including the standard) well. Carefully shaked the plate to start the reaction. The absorbance was road once every minute at 340 nm using a plate reader to obtain at least 5 time points.

Calculation of GST Assay Results

A. Determine the change in absorbance (ΔA 340) per minutes by:

a. Plot the absorbance values as a function of the time to obtain the slop (rate) of the linear portion of the curve.

b. Select two points on the portion of the curve and determine the change in absorbance during that time, using the following equation: (ΔA 340/min = [A340 (time2)-A340 (time1)]/ [time2 (min)-time1 (min)]

B. Determine the rate of (ΔA 340/min) for the background wells and subtract the rate from that of the sample wells.

C. Use the following formula to calculate the GST activity. The reaction rate at 340 nm can be determined using the (CDNB) extinction coefficient of 0.0053uM-1

GST Activity = [(ΔA 340/min)/0.0053 uM-1] x [0.2ml/A] x sample dilution=nmol/min/ml

A= Sample Volume Per Well in Milliliter

Statistical Analysis:

Statistical analysis between controls and patients was performed by the student t-test. The data were expressed as mean + SD. $p < 0.05$ was considered as significant.

Results:

Measurement of plasma malondialdehyde (MDA) levels

The total plasma MDA in patients and healthy controls are shown in table (1). The total plasma MDA in patients shows a significant increase ($p< 0.001$) when compared with healthy controls. The mean of patient is: (mean $0.09x10^{-}5 \pm 0.004x10^{-}5$nmole), While, the mean of control is ($0.05x10\text{-}5 \pm 0.004x10\text{-}5$ nmole). Figure (1), shows significant differences between patient and control.

Table (1): plasma MDA levels in colorectal patients and Controls.

Groups	Control nanomol/ml	Colorectal cancer nanomol/ml
Mean	0.05×10^{-5}	0.09×10^{-5}
S.D.	0.007×10^{-5}	0.015×10^{-5}
S.E.	0.004×10^{-5}	0.009×10^{-5}
Probability		P<0.001

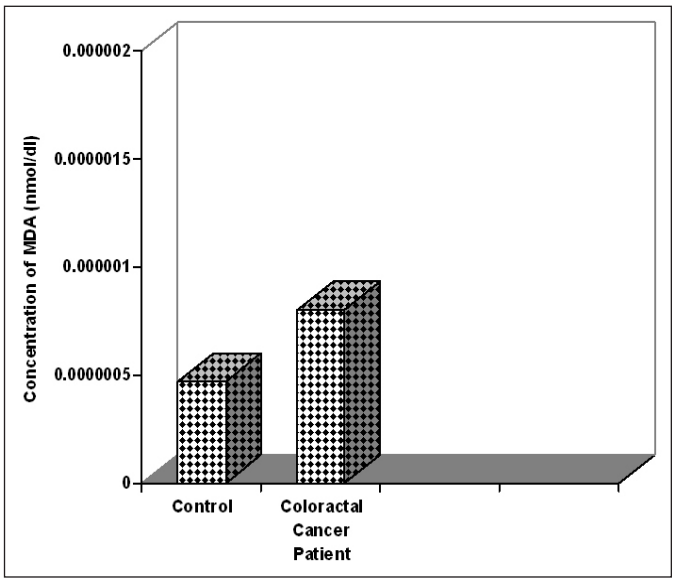


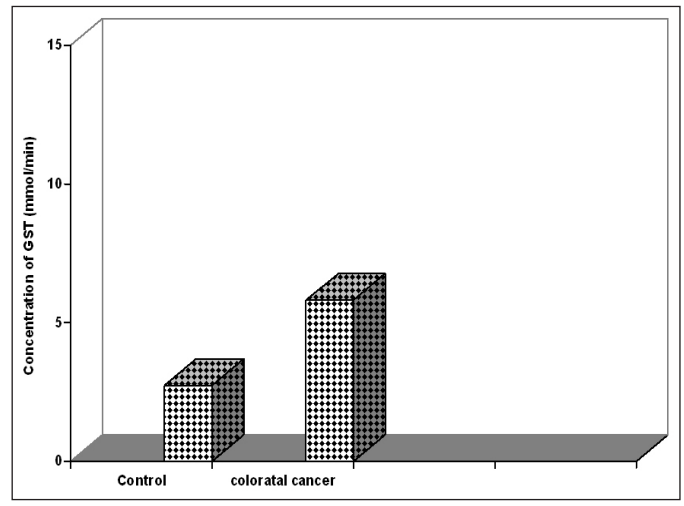
Figure (1): MDA levels in the patients and control

Measurement of plasma Glutathione S-transferase (GST)levels

The total plasma GST in patients and healthy controls are shown in table (2). The total plasma GST exhibited nearly a 2–3-fold increase ($p < 0.001$) in the patient plasma samples compared to the corresponding controls The mean of patient is: (mean 5.83 ± 0.72), While, the mean of control is (2.72 ± 0.63). Figure (2), shows significant differences between patient and control.

Table (2): plasma GST levels in colorectal patients and Controls.

Groups	Control nmol/min/ml	Colorectal cancer nmol/min/ml
Mean	0.05 x10^{-5}	0.09 x10^{-5}
S.D.	0.007 x10^{-5}	0.015 x10^{-5}
S.E.	0.004 x10^{-5}	0.009 x10^{-5}
Probability		P<0.001



Discussion:

The GST activity is involved in xenobiotics detoxification and excretion of xenobiotics and their metabolites, including MP (23). It plays an important role in protecting tissue from oxidative stress (24). Increased GST activity in tissues may indicate the development of a defensive mechanism to counteract the effects of MP and may reflect the possibility of a more efficient protection against pesticide toxicity. The increased GST activity in all tissues observed in the present study after exposure to MP suggests that the detoxification processes were increased and corroborates these ascertains. GST has been reported as a biomarker for assessing the environmental impact of organic xenobiotics generating oxidative stress (25). The GST was more active in hepatic tissue than in white muscle and gill, which indicates the effective role of liver in xenobiotics detoxification (26).

The increase in plasma lipid per oxidation in colorectal cancer seen in the present study was associated with enhanced antioxidant capacities. GST, which is involved in the detoxification of electrophilic toxins and carcinogens, is increased in most of the human tumors studied. High concentrations of GST may rapidly detoxify anticancer agents, thereby preventing their cytotoxic action. Enhanced GST activity in colorectal cancer samples in our study supports ubiquitously-reported induction of GST, especially the isoenzyme GST-P in various cancer tissues and cell lines. (27, 28)

Overproduction of OFR coupled with antioxidant depletion is recognized to result in oxidative stress. (29, 30).

Recent reports suggest that oxidative stress can cause up regulation of antioxidant enzymes that render cells more resistant to subsequent oxidative insult. (31) Prolonged exercise generates oxidative stress, which results in increased endogenous antioxidants.

Over expression of antioxidants has been documented in a wide variety of malignant tumors, including breast cancer. (32, 33, 34) Cancer cells with increased activities of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes.(35) From the results of the present study, we suggest that increased lipid per oxidation and host antioxidant defenses associated with the development of breast cancer may offer a selective growth advantage to tumor cells over their surrounding normal counterparts.

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