Oxidative Stress and Antioxidant Status in Colorectal Cancer and Healthy Subject

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Abstract :

Background: Total antioxidant status(TAO), superoxide dismutase (SOD) and Catalase (CAT) are antioxidant defensive enzymes that are catalyze the reduction of reactive oxygen species (ROS) to non harmful substance. Aim: The study focuses on the serum super oxide dismutase enzyme level, CAT level and total antioxidant status in colorectal cancer patients, which result due to an imbalance between aggressive and defensive factors. Materials and Methods: the study included 40 patients and 20 healthy individuals for comparative analysis were considered for the present study. (TAO), serum (SOD) and (CAT) levels of each individual were performed. Results: Statistical analysis of serum antioxidant enzymes level and antioxidant status revealed a significant increase in SOD, CAT of patients group ($p.\geq0.05$), and ($P.\geq0.01$) respectively. The TAO was significant increase ($p.\geq0.001$). The increasing preponderance of serum SOD, CAT levels and the TAO can be explained on the basis of alteration on enzymes activity, which may lead to disturbance in homeostasis of antioxidant/oxidant balance... Conclusions: TAO, Catalase and Superoxide dismutase enzyme used a biomarker of enzymatic alteration in different diseases including cancer.

Introduction:

Colorectal cancer is one of the most frequent neoplastic diseases in human population and one of the most frequent causes of death. There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of cancer initiation and progression [1]. 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, and its correlation with the clinical staging of the disease, is lacking.

Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of the reactive oxygen species (ROS), or both, leading to cells damage (2). ROS, such as superoxide anion radical ($O_2 \bullet -$), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical ($\bullet OH$) can react with susceptible biological macromolecules and produce lipid per oxidation (LPO), DNA damage and protein oxidation, resulting in oxidative stress (3). Despite the potential danger of ROS, cells present a variety of defense mechanisms to neutralize the harmful effects of free radicals. The antioxidant defense system includes enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GST) and other low molecular weight scavengers such as glutathione (GSH) (4).

The uncontrolled production of free radicals is considered to be an important factor in the tissue damage induced by several pathophysiologies (5, 6). Alteration in the oxidant - antioxidant profile is known to occur in cancer (7, 8).

Superoxide dismutase (SOD) is not only suppresses cell proliferation, but also affects inflammation. The association between chronic inflammation and cancer is now well established (9, 10). One important mechanism of inflammation-induced cancer is due to oxidative stress (11, 12), which results from the release of free radicals from activated immune cells and cytokines.

In the present study the effects of benzene on (TAO)

status and (SOD), (CAT) levels were investigated in the serum of petrol station workers and in healthy control.
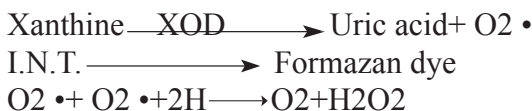
Material and Methods

Patient

This study was conducted on patients attending the consultant department of oncology, Baghdad teaching hospital. All patients was attending the department to received treatment. The complete clinical and personal history of the patient and control was recorded. 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 didn't resave any treatment.

Measurement of SOD enzyme level
Assay Principle

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical ($O_2 \cdot{-}$) produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthin and xanthin oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that causes a 50% inhibition of the rate of the reaction of INT under the condition of the assay. This assay provides a simple, reproducible and fast tool for assaying SOD activity in plasma, serum, erythrocyte lysates, tissue homogenates and cell lysates. Mitochondrial Mn-SOD can be assayed separately following the procedure outlined under sample preparation.

Xanthine $\xrightarrow{\text{XOD}}$ Uric acid+ $O_2 \cdot$
I.N.T. $\xrightarrow{\hspace{2cm}}$ Formazan dye
$O_2 \cdot + O_2 \cdot +2H \longrightarrow O_2+H_2O_2$

Superoxide dismutase assay kit.
Cat.No.574601 Calbiochem

Calculation:

The SOD enzymatic activity was expressed as the percentage of inhibition of INT reduction. Where one unit of SOD is defined as the amount of sample that causes 50% decreases in the SOD inhibition (%) caused by SOD was calculated from regression line of standard curve.
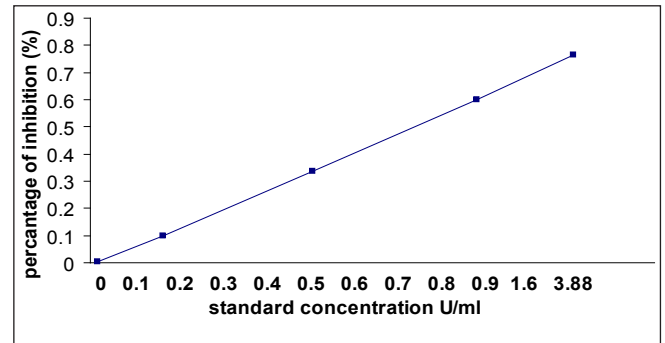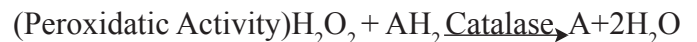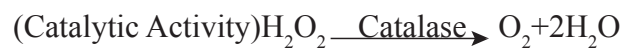

Figure (1) SOD standard curve

Measurement of Catalase Activity
Assay Principle

The Calbiochem Catalase assay kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrozino-5-mercapto-1, 2, 4-triazole(Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocyclic with aldehydes, which upon oxidation changes from colorless to purple color. The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates and cell lysate.

(Catalytic Activity)$H_2O_2 \xrightarrow{\text{Catalase}} O_2+2H_2O$

(Peroxidatic Activity)$H_2O_2 + AH_2 \xrightarrow{\text{Catalase}} A+2H_2O$

Catalase Assay Kit
Cat. No. 219265

Calculation:

Catalase activity of the sample using the following aquation. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol formaldehyde per minute at 25 c.

$$\text{CAT Activity} = \frac{\text{uM of sample}}{20 \text{ minutes}} \times \text{sample dilution} = \text{nmol/min/ml}$$
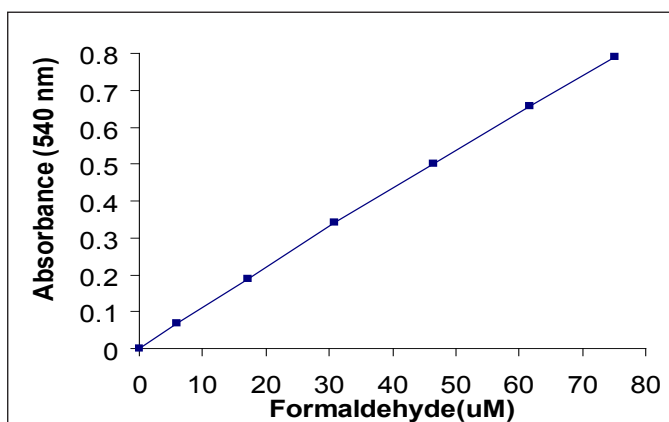
Figure (2): Typical Formaldehyde Stander Curve

Measurement of Total Antioxidant Status (TOA) Level

Assay Principle

ABTS® (2,2-Azino-di-[3-ethylbenzthiazoline sulphnate] was incubated with a peroxidase (Metmyoglobin) and H2O2 to produce the radical cat ion ABTS®+.This has a relatively stable blue-green color, which is measured at 600 nm.Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

HX-Fe + H2O2 ⟶ X-[Fe = O] + H2O
ABTS® + X-[Fe = O] ⟶ ABTS®+ + HX-Fe
HX-Fe = Metmyoglobin
X-[Fe = O] = Ferrylmyoglobin
ABTS® = 2, 2-Azino-disses-[3-ehtylbenzthiazoline sulphonate]
ABTS®+ is a registered trademark of Boehringer Mannheim
Total Antioxidant Status (RANDOX) Cat. No. NX 2332

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) following the Mann–Whitney U-test to determine the statistical difference in the enzyme studies. Student's t-test was used to compare the tumor burden between control and treated groups. $P \leq 0.05$ was considered significant.

Results

Serum super oxide dismutase (SOD) activity

The total serum SOD activity in this study is shown in table (1) and Figure (3). The results illustrated that the total serum SOD activity has a significant increase ($p < 0.01$) in patients when compared with healthy controls. The mean value in patients is (mean, 8.67 ±0.2 U/ml) While, the mean of controls is (mean 6.92±0.153 U/ml).

Table (1): level of SOD in petrol workers and in control

Colorectal Cancer Patient	Control	Groups
8.67	6.92	Mean
0.255	0.153	S.D.
0.24	0.09	S.E.
p < 0.01		Probability

Serum Catalase (CAT) activity

The total serum catalase activity is illustrated in table (2) and figure (3). The results showed a significant increase in the total catalase activity ($P < 0.05$) in patients when compared with controls. The mean of patients is :(mean 6.81± 2.2 U/ml). While, the mean of controls is (mean 4.76± 0.02 U/ml).

Table (2): level of CAT in petrol workers and in control

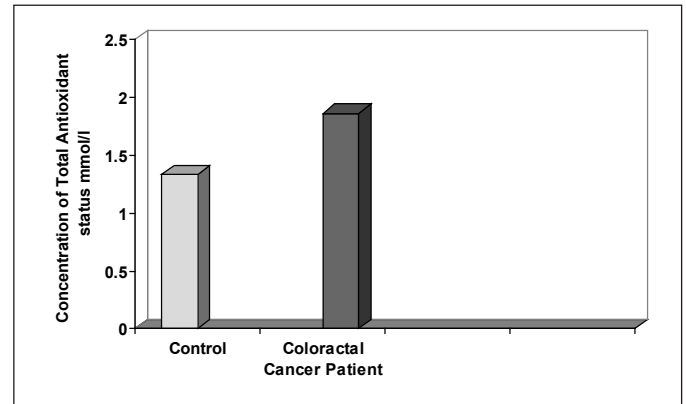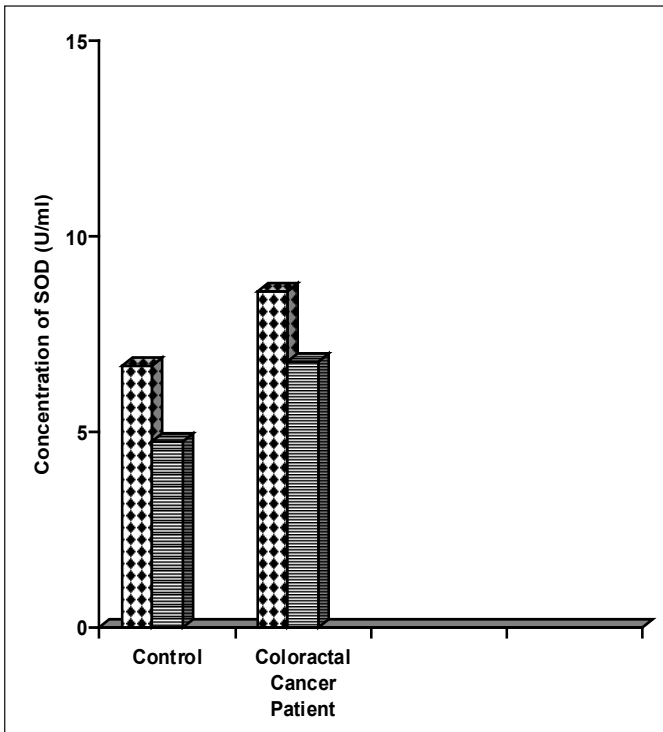| Groups	Control	Colorectal Cancer Patient
Mean	4.76	6.81
S.E	1.98	2.2
Probability		P<0.01

Figure (4): Total Antioxidant Status in colorectal cancer patients and control

DISCUSSION:

Formation of reactive oxygen species is a normal consequence of a variety of essential biochemical reactions. It is also known that oxygen radicals could be formed in excess in chronic diseases of the gastrointestinal tract [13]. The main source of oxidants in the gut is probably phagocytes, which are accumulated in the mucus of patients with bowel diseases and could generate oxidants upon activation, which might contribute to the increased risk of cancer [14]. Therefore, an adequate range of anti oxidative defenses within and outside the cells has also been considered to be very important to offer protection against oxidative damages of cell components including membrane phospholipids [15].

In recent years, several groups have focused their studies on structural protein modifications by free radicals [16, 17]. Recently it has been demonstrated that superoxide anion as well as alkoxyl peroxyl and radicals could inactivate one of the antioxidant enzymes - catalase and reduce the effectiveness of cells to defend against free radical damage [18]. In this paper, it was shown that during colorectal cancer development the activity of catalase decreased. Catalase is used by cells to defend against the toxic effects of hydrogen peroxide, which is generated by various reactions and/or environmental agents or by the action of superoxide dismutase, enzymes while detoxifying superoxide anion [19].

Another important finding of this study was a significant increase in the activity of Cu, Zn-SOD,

Serum Total Antioxidant Status (TAO) level activity

The total antioxidant status is illustrated in table (3) and figure (4); the results showed a significant increase in the total antioxidant status (P < 0.01) in patient as compared with controls. The mean of patient is :(mean 1.86 ± 0.07mmol/l). While, the mean of controls is (mean 1.33± 0.04 mmol/l).

Table (3):Total Antioxidant Status in Colorectal Cancer Patient

Groups	Control	Colorectal Cancer Patient
Mean	**1.33**	**1.86**
S.E	**0.04**	**0.06**
S.D.	**0.15**	**0.23**
Probability	**⬩ P < 0.001**	

GSH-Px and GSSRG-R in all clinical stages of cancer patients as compared with the control group, the maximum was at G3-grade adenocarcinoma and mucinous adenocarcinoma as well as in clinical IV stage of colorectal cancers. It was reported that increase in activity of Cu Zn-SOD, GSH-Px and GSSRG-R might occur on the way of induction of genetic expression [20]. Increased superoxide dismutase activity could augment superoxide radical dismutation, thus leading to intensification of hydrogen peroxide generation. Since catalase activity reduced, the level of hydrogen peroxide increased in cancer tissue. It may correspond with the report, which showed that some human cancer lines produced a large amount of hydrogen peroxide [21]. At the same time, oxygen radicals might increase secretion of the matrix metalloproteinase and collagenase as well as production of angiogenic factors (e.g. VEGF and IL-8).

Oxidants, including hydrogen peroxide, have been found to be able to induce expression of genes coding enzymes of antioxidative system. This kind of induction of antioxidative endogenic enzymes caused by hydrogen peroxide was found in human fibroblast cultures [22]. This increase might be caused by more extensive accessibility of enzymatic cofactors such as transient metal ions [23]. As a result of oxidative stress, iron and copper ions would become more accessible to antioxidative enzymes. Induction of genes coding superoxide dismutase activity has also been revealed in cases of burns and skin infections [24]. The above changes in dismutase activity, under genetic control, could make up a defensive mechanism, which enables organism adaptation to various environmental stresses.

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الأكسدة الاجهادية ومضادات الأكسدة الانزيمة عند المرضى المصابين بسرطان القولون وعند الأشخاص الإصحاء

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الخلاصة:

قيست مضادات الاكسده الكليه وانزيمي الكاتليز والسوبر اوكسايد دسميوتيز والذين يرتبطون بصوره اساسيه مع زياده الاكسده الاجهاديه والناتجة من زياده جذور الاوكسجين الحرة. حيث تم قياس مستويات الانزيمات في مصول المرضى المصابين بسرطان القولون والمستقيم وعند مقارنتها مع الأشخاص الاصحاء حيث تم قياس مستويات الانزيمات عند ٤٠ مريضا مصابا بسرطان القولون والمستقيم و ٢٠مصلا من اشخاص اصحاء. وجد ان مستوى انزيم السوبر اوكسايد دسميوتيز وانزيم الكاتليز قد ارتفع بشكل ملحوظ بالمقارنة مع الاشخاص الاصحاء.وبينت الدراسه ان مستوى مضادات الاكسده الكليه قد ارتفعت ايضا وبشكل ملحوظ عند المرضى المصابين بالسرطان وعند الاشخاص الاصحاء. بينت الدراسه ان زياده نسبه الانزيمات عند المرضى بالمقارنة مع الاشخاص الاصحاء ناتجة عن تاثر الانزيمات المؤكسده بالنسب العاليه للجذور الحرة والتي تؤثر على الموازنه بين المواد المؤكسده والانزيمات المضادة للاكسده.