Oxidative Stress and Erythrocyte Glutathione Defense System in Smokers and Non-smokers Gastric Carcinoma Patients

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Abstract :

The oxidative stress and the status of erythrocyte glutathione defense system were studied in male patients with gastric carcinoma in order to elucidate their clinical importance in the diagnosis. The study included 60 male patients with gastric cancer (30 non-smokers and 30 smokers) and an equal number of age-matched healthy control subjects.

The incidence of oxidative stress was determined by measurement of the oxidative stress marker-malondialdehyde (MDA) in the plasma. The status of glutathione defense system was determined by measuring the level of reduced glutathione, as well as the activity of the enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) in the erythrocytes. After data analysis the following observations were obtained: plasma MDA level was significantly higher in smokers than non-smokers gastric cancer patients.

The activities of erythrocyte SOD, CAT, GSH-Px, GST and reduced GSH levels were decreased in gastric cancer patients who were smokers as compared to non-smokers gastric cancer patients. In conclusion, smokers with gastric carcinoma are at high risk of oxidative stress, which in turn may result in initiating the promotion phase of carcinogenesis and generation of more cancerous cells. Moreover; plasma MDA and erythrocyte glutathione defense system may serve as a good auxiliary biochemical index in the diagnosis of patients with gastric carcinoma.

keyword: Oxidative stress, Gastric carcinoma, Glutathione, superoxide dismutase.

Introduction:

Oxidative damage to DNA, protein and other macro-molecules has been implicated in the pathogenesis of a wide variety of diseases, most notably cancer [1].

Growing bodies of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidant may play a pivotal role in preventing or slowing the progression of cancer [2-4].

Epidemiological evidence consistently related low antioxidant intake or low blood levels of antioxidants with increased cancer risk [3].

Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn

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is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division [5].

Tumor cells generate superoxide and other reactive oxygen species ROS, and this generation, if it also occurs in vivo, might have effects on tumor cell proliferation and invasion [6-8].

It has been hypothesized that the production of ROS combined to a decreased antioxidant enzyme level may be characteristic of tumor cells [4, 9, and 10].

Cigarette smoke is known to contain a large number of oxidants, which are capable of causing an increase in the generation of various ROS like superoxide ($O^{\cdot}2$), hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals. These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation [11].

Tobacco smoke contains a variety of carcinogens including N-nitroso compounds and nitrogen oxides that may promote endogenous formation of N-nitroso compounds,

which have been linked to gastric carcinogenesis [11].

The aim of the present study is to determine the oxidative stress and erythrocyte glutathione defense system in smokers and non-smokers gastric carcinoma patients.

Materials and methods

1. Subjects: Sixty male patients with gastric cancer (30 non-smokers and 30 smokers) from the hospital of Specialized Surgeries and their illness were approved by the consultant medical staff.

Sixty normal healthy subjects (30 non-smokers and 30 smokers) of comparable age and gender were considered as normal controls. Smoker patients or healthy subjects who smoked were included if they smoked >30 cigarettes per day for a minimum of 2 years.

Patients who were receiving chemotherapy or radiotherapy at the time of the study and those with other gastric illnesses that might affect free radical status were excluded from the study. Controls were free of any medication for at least one week before and during the study.

2. Blood sampling: Blood samples (5 ml) were transferred into plain tubes containing acid citrate dextrose (ACD) as anticoagulant.

Tubes were mixed and placed immediately in crushed ice, then assayed within 1-2 hours after blood collection. Blood samples were centrifuged at 3000 rpm for 10 min., then; plasma and buffy coat were removed by aspiration. Erythrocyte were washed three times with phosphate buffered saline (PBS) pH=7.4 (0.02M phosphate; 0.123M NaCl). The packed cell volume (PCV) after the final wash was used for the assays of SOD, CAT, GSH-Px and GST. The remainder of PCV was resuspended in an equal volume of PBS, and used for the assay of GSH-conc. Plasma was stored at -20°C and used for the assay of MDA.

3. Chemicals: The chemicals and reagents used in this investigation were of analar grade unless otherwise specified and were obtained from BDH chemicals Ltd., England; Hopkins and Williams, England; Sigma chemicals USA and Fluka, A.G., Germany.

4. Biochemical tests: Biochemical tests included plasma MDA level and erythrocyte superoxide dismutase SOD (EC 1.15.1.1), Catalase CAT (EC 1.11.1.6), Glutathione peroxidase GSH-Px (EC 1.11.1.9), Glutathione-S-transferase GST (EC 2.5.1.18) activities, as well as erythrocyte GSH-conc. were determined in controls and gastric carcinoma patients.

5. Methods:

A- Plasma malondialdehyde (MDA) assay:

MDA was assayed according to the method of Ohkawa et al. [12] with a minor modification from Hirayama et al. [13]. The acid-reactive substances (TBA-RS) depend on the condensation of two molecules of TBA with one molecule of MDA to generate a reddish chromogen that absorbs light at 532nm wave length.

B- Erythrocyte superoxide dismutase (SOD) assay:

SOD was investigated according to the method described by Kakkar et al. [14]. The method is based on the inhibition of reduction of nitro blue tetrazolium (NBT) by O_2-produced via photo reduction of riboflavin. Fifty percent inhibition was defined as one unites of SOD activity.

C- Erythrocyte catalase (CTA) assay:

The determination of CAT activity was measured according to the Beutler method [15].Catalase catalyses the breakdown of H_2O_2 to H_2O and O_2. The rate of decomposition of H_2O_2 by catalase is measured spectrometrically at 230nm, since H_2O_2 absorbs light at this wavelength. Ethanol is added to stabilize the haemolysate by breaking down (complex II) of catalase and H_2O_2. After the addition of 50µl tris buffer, 900µl of H_2O_2 and 30µl of H_2O to the cuvettes, the system is incubated at 37°C for 10min., the haemolysate is added, and, in the following 10min. the decrease of optical density is measured against blank at 412nm.

D- Erythrocyte glutathione peroxidase (GSH-Px) assay:

GSH-Px was assayed according Paglia and Valantine method [16], with some modifications from Hopkins and Tudhope [17] and plebam et al. [18]. The recycling procedure for determination of GSH-Px activity depends on the oxidation of GSH to GSSG by the enzyme in the presence of NADPH and exogenous glutathione reductase which regenerates GSH for GSSG [18].

The rate of enzyme activity was monitored by following the decrease in absorbance at 340nm as a function of NADPH exhaustion [18].

E- Erythrocyte glutathione S-transferase (GST) assay: GST was assayed by the procedure of Haing et al. [19] with some modifications from Carmagrol et al. [20]. The enzyme activity was estimated by monitoring the change in absorbance at 340nm. A complete assay mixture without glutathione was used as a reference.

F- Erythrocyte glutathione level (GSH):

Determination of erythrocyte glutathione level was performed according to the method of Virgil [21] which is a modified version of that of Beulter [22]. Virtually, all of the non-protein sulfhydryl groups of erythrocyte are in the form of reduced (GSH).

5, 5-Dithio bis (2-nitrobenzzoic acid) DTNB is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412nm and is directly proportional to the GSH level [22].

G- Hemoglobin concentration (Hb):

Hb was followed using hemoglobin kit (Randox) procedure no. 540-uv 2009. In the presence of alkaline potassium, ferricyanate hemoglobin is oxidized to methemoglobin. This then reacts with potassium cyanide to form cyanomethemoglobin which absorbs at 540nm. The intensity of this absorbance is directly related to total hemoglobin concentration.

The results were analyzed by student's "t" test to find

out level of significance. P value ≤ 0.05 was considered significant. Analysis of data was performed using the software SPSS package.

Results

Table (1) demonstrates the mean ± SD of plasma MDA level (μmol/L Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). Plasma MDA level was significantly higher in patients with gastric cancer compared with that in healthy subjects ($p ≤ 0.0001$). Amongst gastric cancer patients, the plasma MDA levels were higher in smokers.

Table (1): Biostatistical calculations and students (t-test) of plasma MDA level for normal healthy control (Group 1) and gastric cancer patients (Group 2).

Plasma MDA level (μmol/L Hb)	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	4.54 ± 1.60	5.6 ± 1.30	7.89 ± 2.5	8.8 ± 1.5
Probability		P < 0.001*	P < 0.0001**	P < 0.0001*** P < 0.0001****

* Smokers versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1
***Smokers in group 2 versus smokers in group 1
****Smokers in group 2 versus non-smokers in group 2

Table (2) demonstrates the mean ± SD of erythrocyte SOD activity (U/mg Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). Erythrocyte SOD activity was decreased in group 2 as compared with controls (group 1) ($p≤0.0001$)

Table (2): Biostatistical calculations and students (t-test) of erythrocyte SOD activity for normal healthy control (Group 1) and gastric cancer patients (Group 2).

Erythrocyte SOD activity U/mg Hb	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	4.0 ± 0.5	2.9 ± 0.4	1.8 ± 0.9	1.2 ± 0.4
Probability		P < 0.001*	P < 0.0001**	P < 0.001*** P < 0.0001****

* Smokers in group 1 versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1
***Smokers in group 2 versus smokers in group 1
****Smokers in group 2 versus non-smokers in group 2

Table (3) demonstrates the mean ± SD of erythrocyte CAT activity (U/mg Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). The activity of erythrocyte CAT in gastric cancer patients (group 2) was lower than those in healthy controls (p ≤ 0.0001).

Table (3): Biostatistical calculations and students (t-test) of erythrocyte CAT activity for normal healthy control (Group 1) and gastric cancer patients (Group 2)

Erythrocyte CAT activity U/mg Hb	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	68.9 ± 7.1	53.3 ± 6.6	40.9 ± 6.9	31.4 ± 5.8
Probability		P < 0.001*	P < 0.0001**	P < 0.0001*** P < 0.0001****

Smokers in group 1 versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1*
***Smokers in group 2 versus smokers in group 1*
****Smokers in group 2 versus non-smokers in group 2*

Table (3) demonstrates the mean ± SD of erythrocyte CAT activity (U/mg Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). The activity of erythrocyte CAT in gastric cancer patients (group 2) was lower than those in healthy controls (p ≤ 0.0001).

Table (4) represents the mean ± SD of erythrocyte GSH-Px activity expressed U/g Hb of normal healthy controls (group 1) and gastric cancer patients (group 2). Erythrocyte GSH-Px activity was significantly lowers in group 2 of gastric cancer patients as compared with controls p ≤ 0.00001.

Erythrocyte glutathione peroxidase activity GSH-Px U/g Hb	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	32.7 ± 2.1	28.1 ± 3.2	24.5 ± 4.5	22 ± 0.9
Probability		P < 0.001*	P < 0.0001**	P < 0.0001*** P < 0.00001****

Smokers in group 1 versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1*
***Smokers in group 2 versus smokers in group 1*
****Smokers in group 2 versus non-smokers in group 2*

Table (5) demonstrates the mean ± SD of erythrocyte GST activity (U/g Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). Erythrocyte GST activity was significantly lower in group 2 as compared with group 1 (p ≤0.0001).

Table (5): Biostatistical calculations and students (t-test) of erythrocyte GST activity for normal healthy control (Group 1) and gastric cancer patients (Group 2).

Erythrocyte GST activity U/g Hb	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	1.8 ± 0.4	1.0 ± 0.2	0.8 ± 0.4	0.6 ± 0.2
Probability		P < 0.01*	P < 0.001**	P < 0.001*** P < 0.0001****

Smokers versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1*
***Smokers in group 2 versus smokers in group 1*
****Smokers in group 2 versus non-smokers in group 2*

Table (6) demonstrates the mean ± SD of erythrocyte GSH-conc. (μmol/g Hb) of normal healthy controls (group 1) and gastric cancer patients (group 2). The level of GSH-conc. in gastric cancer patients was lower than those in controls (p ≤ 0.00001)

Table (6): Biostatistical calculations and students (t-test) of erythrocyte GSH-conc. for normal healthy control (Group 1) and gastric cancer patients (Group 2)

Erythrocyte GSH-conc. μmol/g Hb	Normal healthy control (Group 1)		Gastric cancer patients (Group 2)	
	Non-smokers	Smokers	Non-smokers	Smokers
Sample size (n)	30	30	30	30
Mean ± SD	6.9 ± 1.3	5.9 ± 0.9	4.5 ± 1.5	3.2 ± 0.9
Probability		P < 0.001*	P < 0.0001**	P < 0.0001*** P < 0.00001****

Smokers versus non-smokers in group 1
**Non-smokers in group 2 versus non-smokers in group 1*
***Smokers in group 2 versus smokers in group 1*
****Smokers in group 2 versus non-smokers in group 2*

Discussion:

Plasma MDA level is the most convenient marker used to detect oxidative stress and lipid peroxidation [23], although there are some factors that contribute to limit its utility [12, 24-27].

Elevated level of plasma MDA has been reported in several disorders associated with oxidative stress such as cancer [28]. In this study, we found increased MDA level in plasma from patients with gastric cancer as compared with normal healthy controls (table 1).

The extent of plasma MDA was higher in smokers gastric cancer patients as compared to non-smokers gastric cancer patients. The elevated MDA level in plasma of gastric cancer patients demonstrates the high incidence of these patients to oxidative stress.

This elevation could be ascribed to an increase in the production of ROS such as superoxide anion from the cancer cells. ROS then released from these cells to the extracellular compartment to cause lipid peroxidation in other tissues with the concomitant accumulation of lipid peroxidation end-product (MDA).

Tobacco smoke has been reported to stimulate H_2O_2 and hydroxyl radicals, aromatic amines, nitrogen oxide, heavy metal ions like Cd^{+2} and other potential carcinogenic found in smoke can induce lipid peroxidation. Nicotine has been demonstrated to inhibit apoptosis, thereby facilitating cancer development [29]. Superoxide dismutase is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. In the present study, a significant decrease in the SOD activity was observed in gastric cancer patients as compared with controls (table 2).

Decreased activity of SOD has been reported in malignancies. Superoxide, a highly diffusible radical, can transverse membranes and cause deleterious effects at sites far from the tumor. It is possible that lipid peroxidation of erythrocyte in the patients with gastric cancer is due to $O_2^{\cdot-}$ produced by the tumor as well as to the low activity of SOD within the red cells [30].

In tables (3, 4, 5 and 6) our results have shown a significant decrease in activity of erythrocyte CAT, GSH-Px, GST and GSH-conc. in gastric cancer patients as compared with that of control. MDA may react with amino acid residues of proteins and lead to their oxidative modification. It can also increase oxidative stress by promoting cellular consumption of glutathione and by activating GSH-Px. GSH can act directly as a free radical scavenger by neutralizing $HO^{\cdot}$, or indirectly by repairing initial damage to macromolecules inflicted by HO.

A decrease in GSH-conc. in circulation has been reported in malignancies. The formation of GSSG during reduction of peroxides, or as a consequence of free radical scavenging, is potentially cytotoxic [31]. We found that gastric cancer patients, smokers had lower levels of activities of GSH-Px, CAT and GST.

In conclusion, smokers with gastric carcinoma are at high risk of oxidative stress, which in turn may result in initiating of the promotion phase of carcinogenesis and generation of more cancerous cells. Moreover; plasma MDA and erythrocyte glutathione defense system may serve as a good auxiliary biochemical index in the diagnosis of patients with gastric carcinoma.

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الاجهاد التأكسدي ونظام الجلوتاثايون الدفاعي في كريات الدم الحمر لدى المرضى المدخنين وغير المدخنين المصابين بسرطان المعدة

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الخلاصة:

تم دراسة مستوى الاجهاد التأكسدي ومستويات نظام الجلوتاثايون الدفاعي في كريات الدم الحمر لدى الذكور المصابين بسرطان المعدة (المدخنين وغير المدخنين) لبيان اهميتها السريرية في التشخيص. شملت الدراسة (60) ذكراً مصاباً بسرطان المعدة (30 مدخن و30 غير مدخن) وكذلك (60) شخصاً سوياً لغرض المقارنة (كمجموعة حكم سوية). أجري الكشف عن وجود الاجهاد التأكسدي بواسطة قياس مستوى دليل الإجهاد التأكسدي المالون ثنائي الالدهايد (MDA) في البلازما. كما تم تحديد نظام الجلوتاثايون الدفاعي عن طريق قياس مستوى الجلوتاثايون المختزل (GSH) وفعاليات كل من الانزيمات التالية: Glutathione peroxidase (GSH-Px، Catalase (CAT)، Superoxidase dismutase (SOD) و GST (Glutathione-S-transferase) في كريات الدم الحمر. بعد معالجة النتائج إحصائياً تم ملاحظة وجود زيادة دالة في مستوى الـMDA في البلازما عند المدخنين المصابين بسرطان المعدة مقارنة بالأصحاء. كما ان مستوى الـMDA في البلازما عند المصابين بسرطان المعدة أعلى من مستواه عند المرضى غير المدخنين المصابين بنفس المرض. إن فعاليات الانزيمات (GSH-Px، CAT، SOD، GST) في كريات الدم الحمر إنخفضت إنخفاض دال عند المدخنين المصابين بسرطان المعدة مقارنة بغير المدخنين.

نستنتج من ذلك إن المدخنين المصابين بسرطان المعدة على محك خطورة الارتفاع في في الإجهاد التأكسدي والذي بالتالي قد يؤدي الى تحفيز الطور الثاني في عملية السرطنة ومن ثم توليد خلايا سرطانية جديدة. من ناحية أخرى يمكن استخدام المالون ثنائي الالدهايد في البلازما و نظام الجلوتاثايون الدفاعي في كريات الدم الحمر كدلائل كيمياحياتية مساعدة في تشخيص ومتابعة المرضى المصابين بسرطان المعدة.