Measuring MDA & GSH as Biomarkers for Oxidative Stress in Bladder Cancer Patients

قياس المالون داي الديهايد والكلوتاثيون المختزل كدوال حيوية للإجهاد التاكسدي لدى مرضى سرطان المثانة

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الخلاصة

إن الهدف من هذه الدراسة هو تقييم حالة الإجهاد التاكسدي في سرطان المثانة. تم دراسة 30 مريض مصاب بسرطان المثانة بمعدل أعمار (66.4±14.2) سنة فضلا عن 33 شخصا سويا كمجموعة سيطرة بمعدل أعمار (61±3.8) سنة . تم تقدير تراكيز ال (MDA, GSH) في أمصال المرضى و الأصحاء على حد سواء.

النتائج تم تحليلها إحصائيا باستخدام تحليل ANOVA ولم تظهر أي زيادة معنوية في تراكيزMDA في حين كان هناك نقصان معنوي (P<0.005) في تراكيز ال GSH لدى المصابين بسرطان المثانة عند مقارنتها مع مثيلاتها في مجموعة السيطرة. كما لوحظ أيضا زيادة مستويات ال MDA لدى المرضى المدخنين (P<0.005) في حين قلت مستويات ال GSH(P<0.005) عند مقارنتهم مع مثيلاتهم من مجموعة السيطرة.

Abstract

The aim of this study was to evaluate the oxidative stress in bladder cancer. Thirty bladder cancer patients of ages 66.4±14.2 years and, 33 healthy individuals of ages 61±3.8 years used as a control group were enrolled. The concentrations of malondialdehyde (MDA), reduced glutathione (GSH) were measured in sera of patients and the control group. The results analyzed by ANOVA analysis don't exhibit a significant elevation for MDA concentration, while seen a significant (P<0.005) decrease for GSH concentration were indicated in the group of patients when compared with those of the control group.

Smoking was found to increase significantly (P<0.005) the levels of MDA and decrease significantly (P<0.005) the levels of GSH in the group of patients when compared with healthy individuals.

MDA levels were illustrated to increase significantly (P<0.001) in patients of current smoking and significant (P<0.005) decrease for GSH levels when compared with control group.

Introduction

Oxidative stress occurs in a cellular system when the production of free radical moieties exceed the antioxidant capacity of that system. If cellular antioxidant do not remove free radicals, radicals attack and damage protein, lipid, carbohydrate, and nucleic acid [1]. Both enzymatic and non-enzymatic pathways leading to formation of reactive compound by one electron reduction or oxidation generate free radicals. The role of free radicals has been proposed in the pathogenesis of many disease involving different organs such as breast, gastric, colon, multiple myloma, ovarian, oral and bladder cancer [2-6].

Reactive oxygen species (ROS) are involved in many cellular metabolic and signaling processes and are thought to have a role in aging and smoking [7-11]. Therefore, their detoxification and elimination are necessary for normal physiologic cell activity and survival. Critical sites of ROS attack are the membrane of intracellular organelles, like phospholipids-rich lysosomal membrane. Lipid peroxidation, well correlate with oxidative stress intensity. It is a chain reaction, in which polyunsaturated fatty acids are degraded to small, more reactive particles such as conjugated dines [12]. Reactive oxygen species play an important role in carcinogenesis. It has been reported that changes in malondialdehyde (MDA) levels and reduced glutathione (GSH) levels were associated with the pathogenesis of bladder cancer, whereas increase of MDA levels, and decreased levels of GSH were linked to metastasis [13].

Antioxidant naturally occurs either endogenous such as vitamin E, coenzyme Q, vitamin C, catalase, superoxide dismutase, or exogenous that can be extracted from food such as fruits, vegetables, seeds, nuts, and oil [14-16]. Antioxidants are considered to be effective groups because they are willing to give up their own electrons to free radicals. When a free radicals gains the electron from an antioxidant, it no longer need to attack the cell and the chain reaction of oxidation is broken [14].

Almost all bladder malignancies originate on the uroepithelial surface. The majority of patients who die from bladder cancer do so from metastasis disease; treatment for metastasis bladder cancer is rarely, if ever, curative [17]. Internationally, transitional cell carcinoma account for about 95 percent of bladder neoplasm [18,19]. The remaining 5 percent consist of squamous cell carcinomas and adenocarcinomas, with squamous cell carcinoma being the more common. However, bladder cancer is associated with a number of occupations or occupational exposures [20].

Many biomarkers of oxidative damage are labor-and time intensive and many not be appropriate for use in epidemiologic studies or clinical trials. The present investigation was carried out to evaluate MDA and GSH levels in bladder cancer patients hopping to elucidate their benefits in the pathogenesis and management of this disease.

Materials and methods

Specimens Collection

This study was conducted in department of pharmaceutical chemistry over a period of July 2008 to April 2009. Patients mean age 66.4±14.2 years were obtained from the department of urology at Al-Sadder teaching hospital. Diagnosis of patient was made on clinical, and histological criteria by the specialist doctors. The control group comprised of 33 healthy individuals of age 61±3.8.

Chemicals and Apparatus

All laboratory chemicals and reagents were of pure grade purchased as below. Di-sodium hydrogen phosphate-2-hydrate, sodium citrate, trichloroacetic acid, sodium chloride, ethylene diamine tetra acetic acid di sodium (EDTA) were BDH company, England, meta-phosphoric acid, was Merck company, Germany ,thio-barbituric acid, was Merck company, England, gltathion was Fluka company, Switzerland.

The apparatus used were UV-VIS spectrophotometer Unico 1100 Germany, water bath Julabo F34 Germany, balance Satorious BL 2105 Germany, centrifuge Bestell kolb England, vortex Thermolyne 65800 U.S.A

Methods

Determination of serum Malondialdehyde concentration in sera of patients and the control group .

Lipid peroxidation was measured by estimating serum malondialdehyde (MDA) levels, expressed in µM according to Raad et al method [21]. Briefly one ml of 17.5% TCA and one ml of

0.6% TBA were mixed 150µl of serum sample. The content were shaken well by vortex, incubated in boiling water bath for 15 minutes, and allowed to cool. One ml of 70% TCA was added and the mixture was let to stand at room temperature for 20 minutes. The mixture was centrifuged at 3000 Xg for 15 minutes, and the supernatant was taken out for measuring the absorbance at 532 nm.

The level of MDA was estimated from the following equation.

*The concentration of MDA = Absorbance at 532 * D/L*E*$_{\circ}$

L: Light path (1cm), E$_{\circ}$*: Extinction coefficient* $(1.56 \times 10^{5}\ M^{-1} \cdot Cm^{-1})$*, D Dilution factor.*

Determination of reduced glutathione concentration in sera of patients and control.

Serum GSH levels were determined according to the method of Burtis [22]. The procedure achieved through the following , 100 µl of serum, 800 µl of distilled water, and100 µl of trichloroacetic acid were mixed well by vortex mixer for 10 minutes, and centrifuge for 15 minutes at 3000 xg . Four hundred microliters were withdrawn from the supernatant and mixed with 800 µl tris-EDTA buffer, then 20 µl of DTNB. The mixture was mixed well by vortex and read against blank at 412 nm within 4 minutes. Was added

Statistical Analysis

Statistical analysis was done using SPSS software program (version 10). One-way ANOVA analysis assessed the significance of oxidative stress parameters among patients and control group. Significant variation was considered when P value was less than 0.05.

Results and Discussion

The determination of MDA and GSH concentrations in 30 patients of bladder cancer and 33 healthy individuals (control group) revealed that a significant (P<0.005) decrease of GSH levels in the group of patients when compared with those of the control group. However, MDA levels failed to exhibit a significant variation (Table 1). The evaluation of the effect of smoking on MDA and GSH levels alterations indicated a significant (P<0.005) elevation of MDA in the group of smokers healthy individuals in comparison with those of the group nonsmokers healthy individuals, while GSH levels showed a significant (P<0.005) decrease during a comparable evaluation. Nonsmokers patients demonstrated a significant (P<0.005) rise of MDA levels in comparison with those of nonsmokers healthy individuals, while GSH levels stated a significant (P<0.005) decrease during the same comparison. However, smokers patients illustrate a significant (P<0.005) decrease of MDA levels in comparison with those smokers healthy subjects (Table 2).

Table.1: Malondialdehyde, and reduced glutathione levels in bladder cancer patients and the healthy group

Characteristics	*No.*	*Group*	*Mean ± SD*	*Range*	*P - Value*
MDA µM	33	Control	10.252±4.366	2.69 –19.51	
	30	Patients	14.74±3.37	5.4-22.2	N.S
GSH µM	33	Control	402.59±94.13	28.23 – 560	
	30	Patients	167.81±104.6	62.9 - 490	0.005

Lipid peroxidation which was measured as endogenous MDA levels in sera of patients and control group pointed out non significant variation in the group of patients, while GSH levels showed a significant (P<0.005) decrease. This finding may suggest that lipid peroxidation is not involved in the carcinogenesis of the bladder. Oxidative stress is found to be involved in the pathogenesis of various diseases [23,24]. Several cancers have been observed to be developed due

to oxidative stress also [25,26]. In the present study oxidative stress is still serious factor that implicated in the bladder cancer. Since GSH levels where found to be decreased in the enrolled patients. The explanation of such finding may be unclear, but the hypothesis of independency on lipid peroxidation seen to be approximately true. Further study with large number of patients may be needed to verify the exact role of lipid peroxidation in bladder cancer.

Cigarette smoking was observed a directing factor in the elevation of lipid peroxidation and oxidative stress in healthy individuals. This finding is clearly expected due to the roles of various pollutance and toxic substances in smoking. In previous works smokin have been mentioned to elevate oxidative stress [26,27]. The nice observation that obtained in the present investigation is the significant elevation of MDA levels in non smokers patients when compared with those of non smokers healthy individuals. In addition to a similar trained of GSH decrease in the group of non smokers patients when compared with those of non smokers healthy individuals. Thus, smoking is seen to elicit oxidative stress in bladder cancer. The conclusion that could be highlighted from the present study is that lipid peroxidation is not involved in bladder cancer and cigarettes smoking provoke the implication of oxidative stress in this diseased.

Table.2: Influence of smoking on MDA and GSH levels in bladder cancer patients and healthy subjects

Characteristics	***Group***		***Mean ± SD***	***P- value***
MDA μM	Control	Smokers **(14)**	14.5 ± 2.7	P<0.005*
		Nonsmokers **(19)**	7.16 ± 2.2	
	Patients	Smokers **(18)**	6.23 ± 5.3	P<0.005**
		Nonsmokers **(12)**	14.59 ± 9.2	P<0.005**
GSH μM	Control	Smokers **(14)**	504.3 ± 43.3	P<0.005*
		Non smokers **(19)**	327.7 ± 20.8	
	Patients	Smokers **(18)**	169.4 ± 66.5	P<0.005**
		Non smokers **(12)**	179.9 ± 136.9	P<0.005**

* Relative to the control group.
** Relative to patients group

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