The Evaluation of Antioxidants status in patients with bladder Cancer.

*Prof.Dr. Mufeed J. Ewad **Dr.Rafid S.Ewadh

**Dr. Imad H. Mahmood

*Kerbala University

**Babylon University

E-mail : mewadh@yahoo.com

Abstract

Sixty seven patients with prooved carcinoma of the urinary bladder in Babylon Governorate and show changes in their glutathione-S-transferase (GST) and reduced glutathione (GSH) in the blood. The studied patients were (57) males and (10) females and the control group was (36).The mean age of the patients was (55) years old,68% were exposed to chemicals,46% of patients had history of urinary stones,84% were smokers and 91% presented with negative family history of tumors.The results of the study were:both GST and GSH in the blood decrease in bladder cancer,exposure to chemicals had affected both GST and GSH,urban and rural areas had nearly the same incidence of cases,GST is affected by testosterone while GSH is affected by estrogen and the size of the tumor affected both GSH-and GST.It is concluded that bladder cancer affects GST and GSH levels in the patients' blood.Gender,smoking,exposure to chemicals and age had a signifecant effect on blood levels of GST and GSH.

الخلاصة

تمت دراسة وتقدير حالة مضادات الاكسدة في مرضى سرطان المثانة وبالتحديد لمستويات الكلوتاثايون المختزل والكلوتاثايون ترانزسفيريز وتبين بان مستويات مضادات الاكسدة تنخفض تبعا لنوعية تعرض المريض لاحد العوامل البيئية او مستويات بعض الهرمونات وكذلك الاعمار المختلفة للمرضى .

Introduction

It is one of the most common diseases treated by urologists[1].The epithelium of the bladder is transitional, therefore,it is adapted for preventing the

passage of the urinary constituents into the cells and underlying tissues.This type of epithelium is also well suited to accomodating the large scale distention which found in the bladder.The blood vessel supply of. the bladder runs in the fibrous coat and gives off branches to provide capillary networks in the muscle coat and in the mucous layer,however lymphatics are said to be present only in the muscle coat[2].Tumors arising in the urinary bladder range from small benign papillomas to large invasive cancers.The rare benign papillomas are small (0.2 to 1.0 cm) frond like structures,having a delicate fibrovascular axial core covered by multilayered well-differentiated transitional epithelium.

Table (1) : Staging systems for Bladder Cancer[3]

Findings	Jewett-Strong-Marshall staging	UICC 1987 staging
No tumor in the specimen	0	To
Carcinoma in situ	0	Tis
Non invasive papillary tumor	0	Ta
Submucosal invasion	A	T1
Superficial muscle invasion	B1	T2
Deep muscle invasion	B2	T3A
Invasion of perivesical fat	C	T3B
Invasion of contiguous organ	D1	T4
Regional lymph node metastases	D1	N (1-3)
Juxta regional lymph node metastases	D2	-
Distant metastases	D2	M1

People at Most Risk of Bladder Canecr:

a. Smokers

b.Workers with high risk industries:Textiles,Rubber,Leather, Painting and

Printing.

c.People in their late sixties of age.

d.People with chronic bladder inflammation.

e.People exposed to arsenic.

f.People with relatives who had bladder cancer.

g.Children with rare birth defects [4].

Age and Sex Related Incidence

Most cases of transitional cell carcinomas of the bladder present in the ages of 60-79 years old,wilh the average age being 67 years, but they can also occur in younger adults and children(25,26,27,28).Men are affected more often than women in two to three times, and whites are twice as likely to develop bladder cancer than other races[5].

Clinical Presentation

The most common presenting symptom of bladder cancer is painless hematuria which occurs in about 85% of patients.In reality,nearly all patients with cystoscopically detectable bladder cancer have at least microscopic hcmaturia[6].The symptom complex of bladder irritability and urinary frequency,urgency and dysuria is the second most common presentation and usually is associated with diffuse carcinoma insitu or invasive bladder cancer.These symptoms,however almost never occur without (at least) microscopic hematuria.Other signs and symptoms of bladder cancer include flank pain from ureteral obstruction,lower extremity edema,pelvic mass and rarely with advanced disease symptoms such as weight loss and abdominal or bone pain[7].

Tumor markers are molecules produced by a tumor, or by the body in response to a tumor. Tumor markers can be found in all body fluids, including blood, urine, cerebrospinal fluid (CSF) and effusions. Tumor markers are represented by small and large molecules, such as peptides, proteins, glycoproleins, enzymes, hormones, immunoglobulins, mucins, cytokeratins and low molecular weight metabolites. Most tumor markers are incidentally involved

in tumorigenesis and are byproducts of malimiant transformation . However, measurements of tumor marker levels alone are not sufficient to diagnose cancer for the following reasons:

1. Tumor marker levels can be elevated in people with benign conditions.
2. Tumor marker levels are not elevaled in every person with cancer especially in the early stages of the disease.
3. Many tumor markers are not specific to a particular type of cancer; the level of a tumor marker can be raised by more than one type of cancer..

Antioxidants Defense System and Free Radicals

Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stress or to which it invariably becomes exposed[8].The antioxidant defense system is sophisticated and adaptive,and GSH is a central constituent of this system[9].

Evaluation of the possible correlation between intracсllular glutalhione (GSH) and drug sensitivity of urothelial cancer was done in Taiwan.Tissue GSH content of surgical specimens from patients with urothelial cancer was assayed with High Performance Liquid Chromatography.GSH levels of cancer tissue were significantly higher than GSH levels of normal mucosa.All patients having measurable lesions were then treated with Methotrexate (MEC), Epirubicin and Cisplatin.

Glutathione -S-Transfcrase

Glutathione-S- transferases, (EC 2.5.1.18) are ubiquitous multifunctional enzymes[10]. Glutalhione-S-transferases are thought to play a physiological role in initiating the detoxification of potential alkylating agents, including pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with the –SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. Glulalhione conjugates are thought to be metabolized further by cleavage of the glutamate and glycine residues, followed by acetylation of the resultant free amino group of

the cysteinyl residue,to produce the final product,a mercapturic acid. The mercapturic acids, i.e. S-alkylated derivatives of N-acctyl cysleine,are then excreted[11].

Materials and Methods

Patients

Between 1stNov. ,1st , 2004 to Aug. , 15th, 2005, patients (57) of them are males and (10) are females with a mean age (55) years old,admitted to Al-Hilla Teaching Hospilal/Department of Urology,proved to have bladder cancer by histopathological study for the biopsies taken by cystoscopy.The indications of cystoscopy were:

1 .Radiologieally proved patients with bladder mass with or without haematuria.

2.Radiologieally negative patients but with haematuria:

a.Patients above 40 years old with single attack of hematuria.

b.Patients below 40 years old with two attacks of macroscopic hematuria, or three attacks of microscopic hematuria.

All patients underwent full history and physical examination including:

Age, sex, occupation, residence, smoking, family history of tumors,history of urinary stones,general examination and abdominal examination.

All patients underwent full investigations:General Urine Examination,urine for cytology,blood urea,serum creatinine,(packed cell volume)PCV , (Random blood sugar) R.B.S.,ultrasound examination for urinary bladder, (Intravenous umgraphy) IVU and scan (Computerized Tomography)CT .

Cysloscopy was done under general anasthesia using rigid cystoscope (Storz) 2IF with multiple cup biopsy and TUR (Transurethral resection) if indicated [12] .

The control group were chosen as healthy people i.e. non smokers,did not have any history of chronic disease and did not take any treatment for chronic diseases such as diabetes mellitus and hypertension as they affect antioxidants.The ages were nearly the same as those of the patients.

Chemicals

All materials used in this study have been used without any farther purification .

Determination of Erythrocyte Glutathione

Using the method described by Carl A, Edward R. TIETZ [13]

Enzymatic Assay of Glutathione-S-Transferrase (EC 2.5.1.18)1-Chloro-2,4-Dinitrobenzene as Substrate

Using the method described by Habig W.H[14]

Determination of Packed Cell Volume(PCV or Haematocrit)

Using Micro-method described by Dacie JV and Lewis SM[15]

Results and Discussion

GSH and GST in bladder cancer patients:

The mean whole blood level of reduced glutathione (GSH) had shown a decrease in its level in patients with carcinoma of the urinary bladder in comparison to that found in control group and it revealed a significant difference with whole blood GSH in control group ($p<0.01$). (table2).

Table(2): The mean whole blood GSH in bladder cancer patients in comparison to control group.

Group	MeanGSH ± S.D.(mg/dl)	P value
Patients	31.97 ± 6.9	0.0002
Control	37.0 ± 6.5	

This difference between the mean whole blood GSH for patients and control group can be related to the continuous consumption of the GSH pool found in the blood in those patients with the cancer in order to combat the oxidalive stress occurring in the tumor cell [9,16].The sensitivity of GSH in bladder cancer was 70% while the specificity was 61%.

The significant decrease is due to the consumption of GST in bladder cancer patients to overcome the activity of toxic free radicals in tumor

cells in which there is low GST concentration,so the transport of GST across cell membranes permits the enzyme to pass from the circulation i.e. erythrocytes to the entire tumor cells to conjugate with the toxic free radicals and detoxify the cell [17].

Table(3):The mean erythrocyte GST level of bladder cancer patients in comparison to control group.

Group	MeanGST±S.D. (U/L)	P value
Patients	1.37± 1.57	0.003
Control	2.42 ± 2.30	

The change in mean GST erythrocyte level in patients in relation to that in normal people is explained by the activity of the tumor itself so in grade I the tumor starts its activity with the normal GST in erythrocytes then in grade II the tumor and its toxic free radicals are controlled a little bit by the reflex activity of the antioxidant enzyme GST,but after a long period of consumption of GST it will decline reaching low levels in grade III of the tumor in which the tumor appear in its peak activity.Also these results may be explained by the patient selection according to their presentation.

Table(4):The mean erythrocyte GST level in bladder cancer patients concerning the grade of the tumor.

Group	Mean GST ± S.D. (U/L)	P value
Grade I TCC patients	1.06 ± 1.02	0.038
Grade II TCC patients	1.37± 1.62	0.007
Grade III TCC patients	0.93 ± 0.6	0.039
Control	2.42 ± 2.3	

Regarding the reduced glutathione (GSH) level in whole blood,the analysis

reflected a decrease in mean whole blood GSH in patients with grade (I,II,and III),this is referred to the activity of the tumor against its scavengers (antioxidants) so the tumor starts to fight aggressively causing very low levels of reduced GSH in whole blood with the advance in growing and development of the tumor, so the GSH pool starts to replace the consumed portion and continue to produce excessive amounts of GSH to face the development of the tumor, this way there is a gradual increnment in GSH whole blood level with the development of tumor from grade I to grade III. There was a very significant difference between whole blood GSH in patients with TCC grade II and whole blood GSH of control group ($p=0.0008$),also there was a significant difference between the whole blood GSH in patients with TCC grade I and whole blood GSH of control group ($p<0.01$).While the difference between GSH whole blood level in patients with TCC grade III and whole blood GSH of control group was not significant ($p>0.05$), (table5).

Table(5):The mean GSH whole blood level in bladder cancer patients concerning the grade of the tumor.

Group	Mean GST ± S.D. (mg/dl)	P **value**
Grade I TCC patients	29.6 ± 7.6	0.001
Grade II TCC patients	32.0 ± 7.1	0.0008
Grade III TCC patients	33.5 ± 3.5	0.07

Gender and bladder cancer

Regarding the sex of the patients with bladder cancer, 57 patients were males while the rest which is 10 patients were females see (fig.1).This male to female ratio which is about 5:1 to 6:1 differs from the international figures which refer to a ratio of 3:2 to 3:1 which can be explained by the working and productive group in Iraq which are mainly men,also this can be related to the

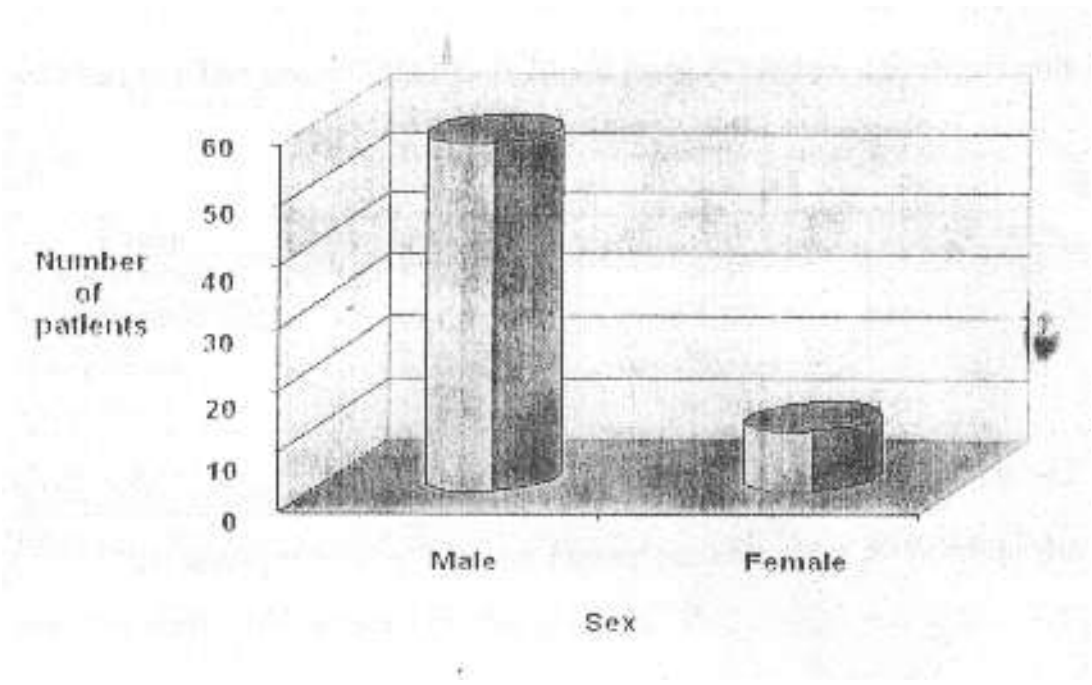
patient selection in their presentation.The mean GST erythrocyte level in female patients(n=10) had shown a mild decrease from the GST erythrocyte of controls(n=6),while the mean GST erythrocyte of male patients (n=57) had shown double fold decrease than the GST erythrocyte of the controls (n-30),the GST in erythrocytes of females had shown non significant difference in relation to GST erythrocytes of control group ($p>0.05$),While the GST in erythrocyles of male patients revealed a significant difference in comparison to the enzyme level in control group ($p< 0.05$),as in (table.6).

These results can be related to the effect of testosterone on GST,because testosterone increases oxidative stress so consumption of more GST,then females who already have less testosterone so less consumption of GST[18].

Table(6):The mean GST erythrocyte level in bladder cancer patients in relation to their sex.

Group	Mean GST ±S.D.(U/L)	P value
Femal patients	2.53 ± 2.70	0.3
Femal control	3.0 ± 4.24	
Male patients	1.17 ± 1.21	0.0003
Male control	2.31 ± 1.79	

Fig.(1)



Number of bladder cancer patients in relation to theirsex.

The mean GSH in female patients showed a marked decrease from the mean GSH for control females about (7mg/dl), while the mean GSH of male patients showed a decrease from mean GSH of male controls about (4.7mg/dl).The whole blood GSH in female patients was significantly different than GSH in whole blood of control group ($p<0.05$), while GSH in whole blood of male patients had very significant difference than GSH of whole blood in control group ($p<0.05$), (table7).

Those results of GSH difference between females and males are related to the effect of estrogen on GSH,so females were presented with higher whole blood GSH than males,because females are subjected to change in GSH during the menstrual cycle related to leuteal phase (highest estrogen secretion)then after declining and appearance of high progesterone levels GSH will show a decreamenl in this follicular phase[19,20].

TabIe(7):The mean GSH whole blood level in bladder cancer patients in relation to their sex.

Group	MeanGSH ± S.D.(mg/dl)	P value
Femal patients	31.14 ±8.37	0.04
Femal control	38.00 ±4.80	
Male patients	32.11 ± 6.73	0.001
Male control	36.81 ± 6.84	

PCV and antioxidants in bladder cancer patients

The PCV of the bladder cancer patients had shown a significant difference in relation to the whole blood GSH of them ($p<0.05$) , while the PCV of bladder cancer had a non significant difference in relation to their erythrocyte GST ($p>0.05$), (table 8).

Table(8):The mean whole blood GSH and mean erythrocyte GST in relation to Packed Cell Volume.

Parameter	Mean ± S.D.	P value
GSH	31.90(mg/dl) ± 6.93	0.004
PCV	39.90% ± 6.90	
GST	1.42 (U/L) ± 1.58	0.9

The mean PCV of smoker bladder cancer patients n=57(40.01 ±7.05)% was a little bit higher than the mean PCV of non smoker bladder cancer patients n=11 (39.7±6.57)%.While the mean PCV of the bladder cancer patients n=67 was (39.9±6.93)%.

The mean PCV of female smoker bladder cancer patients n=5 was (41.0±2)%,while the mean PCV of female non smoker bladder cancer patients n=5 was (34.4±2.8)%.

The mean PCV of male smoker bladder cancer patients n=51 was (39.9±:7.3)%,while the mean PCV of male non smoker bladder cancer patients was (44.1 ±5.26)%. These results of PCV can be explained by the effect of smoking which in turn causes secondary polycylhemia, although some results of PCV may reflect a debilitating state in which the chronic illness had lead to anemia[21]

Age and antioxiants in bladder cancer patients

The age group distribution in bladder cancer patients had shown a peak level in the age between (51-60) years old represented by (26) patients and the

least was in the group below (30) years old, as shown in (fig.2).

This age distribution is explained by the populations who differ from each other around the world, so each population has its own age group distribution depending on their environmental factors and life style habits that affect their activities of the enzymes which constitute a major part of the antioxidant defense system in the human organism[22]

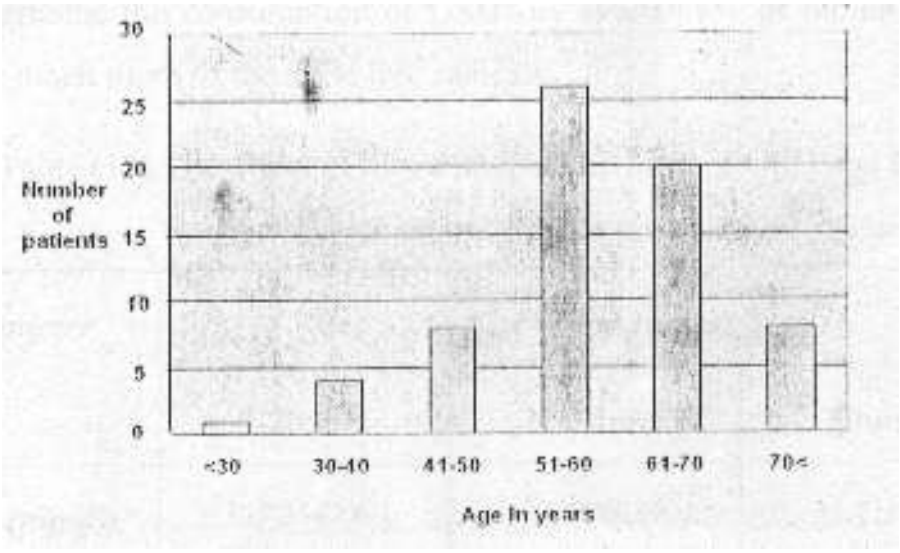


Fig (2) The age group distribution of bladder cancer patients.

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