GLUTATHIONE-S-TRANSFERASE AND REDUCED GLUTATHIONE AS TUMOR MARKERS IN CARCINOMA OF THE URINARY BLADDER

A THESIS SUBMITTED TO THE COMMITTEE OF THE COLLEGE OF MEDICINE-BABYLON UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL BIOCHEMISTRY

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A.D.

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

و من النّاس و الدّواب و الانعام مختلفٌ الوانُهُ كذلكَ

انّما يَخشى اللهَ من عباده العُلَمَؤا إنّ اللهَ عزيزٌ غفور

صدق الله العظيم

سورة فاطر-

الاية(٢٨)

We certify that this thesis was prepared under our supervision at the College of Medicine, Babylon University as a partial requirement for the degree of Master of Science in Clinical Biochemistry.

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DEDICATION

TO

MY FAMILY,

MY PARENTS

&

DEVOTING WIFE

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Abstract

Sixty seven patients with prooved carcinoma of the urinary bladder were studied for the changes in their GSTGlutathione -S-Transferase and GSHreduced Glutathione) in the blood.The studied patients were 57 males and 10 females and the control group was 36.Those patients were complaining from hematuria and their condition indicated the need for cystoscopy according to clinical and radiological examination.The diagnosis of bladder cancer was made by histopathological study to the biopsy taken from the bladeer.

The study revealed presence of high number of cases with Transitional cell carcinoma(Tcc stage II) and only one case with Squamous cell carcinoma(Scc).

The study had studied the effect of :smoking,sex,chemical exposure,family history of tumor and residency area) on GST and GSH. 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 male to female ratio was 5:1. The patients had a relatively high mean PCV level related to smoking which causes secondary polycythemia.The highest percentage of the patients were from the center of Babylon Governorate.

The results of the study were both GST and GSH in the blood decreased in bladder cancer,exposure to chemicals had affected both GST and GSH,urban and rural areas had nearly the same percentage of cases,sex affected both GST and GSH and the size of the tumor also had an effect on 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 significant effect on blood levels of GST and GSH.

Abbreviations

Abbreviation	*Details*
AA	Amino acid
BCG	Bacillus Calmette-Guerin
BCON	Bladder cancer,Carbon dioxide,Oxygen,Nicotinamide
BC2001	Bladder Cancer 2001
BS06	A randomised trial of radical radiotherapy in bladder cancer
BTA	Bladder tumor associated antigen
BTF	Bladder tumor fibronectin
CA125,19 -9	Cancer Antigen 125,19 -9
CEA	Carcino Embryonic Antigen
CK 18	Cytokeratin
CT	Computerized Tomography
DTNB	5,5' Dithiobis nitrobenzoate
EC	Enzyme Classification
Glu	Glutamine
Gly	Glycine
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-Transferase
GSX	Glutathione S-conjugate
HPLC	High Performance Liquid Chromatography
i.e.	That's to say
IVU	Intravenous Urography
MCM	Minichromosomal Maintenance protein
MEC	Methotrexate Epirubicine Cisplatin
MRI	Magnetic Resonance Imaging
NAC	N-acetyl cysteine
NADPH	Nicotinamide Adenine Dinucleotide Phosphate,reduced
NMP	Nuclear Matrix Protein
OxyRad	Oxygen Radicals
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
RBS	Random Blood Sugar

Abbreviation	***Details***
Redox	Reduction Oxidation
SCC	Squamous Cell Carcinoma
-SH	Thiol group
TCC	Transitional Cell Carcinoma
TPA	Tissue polypeptide antigen
TUR	Transurethral resection
UICC	International Union Against Cancer

Contents

Figures Index

Tables Index

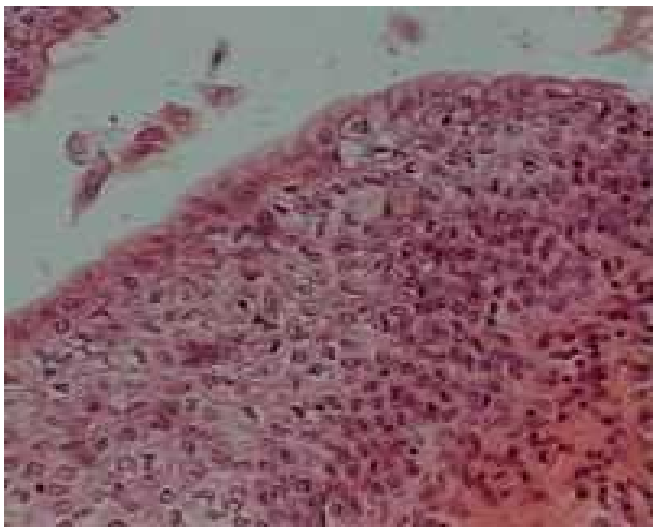
CHAPTER ONE

INTRODUCTION and Literature Review

Section I/Bladder Cancer

1-Normal Histology of Urinary Bladder

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,it gives off branches to provide capillary networks in the muscle coat and in the mucous layer.Lymphatics are said to be present only in the muscle coat 1 .



Normal transitional epithelium of urinary bladder 2

2-Pathology

Bladder cancer is one of the most common diseases treated by urologists3.Ninty percent 90% of bladder malignancies are transitional cell carcinomas TCC) reflecting their origin from the transitional cells that line the bladder.Squamous cell carcinoma accounts for 6-8% of bladder tumors,with only 2% being adenocarcinomas.

Tumors are graded on a scale of I-IV,dependent on the degree of cellular atypia and nuclear abnormalities in association with the number of mitotic figures.In practice,however,transitional cell carcinomas tend to occur in two principal forms:low-grade superficial and high-grade invasive

cancers4 .The anatomic disribution of the disease may be identified by one of several staging systemsJewett -strong-Marshal stage and UICC stage)5. The entire urinary collecting system from renal pelvis to urethra is lined with transitional epithelium,so its epithelial tumors in the collecting system above the bladder are relatively uncommon,while those in the bladder are an even more frequent cause of death than are kidney tumors.

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 epithelium6 .

Staging systems for Bladder Cancer 5

Findings	***Jewett-Strong-Marshall staging***	***UICC 1987 staging***
No tumor in the specimen	0	T0
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	N1 -3)
Juxta regional lymph node metastases	D2	-
Distant metastases	D2	M1

Transitional cell carcinomas range from atypia to carcinoma in situ,and from extremely well differentiated grade I) to highly anaplasti c aggressive cancers grade III). Grade I carcinomas are rarely invasive but may recur after removal.Whether the regrowth is a true recurrence or a

second primary growth is uncertain.Progressive degrees of cellular atypia and anaplasia are encountered in papillary exophytic growth,accompanied by increase in size of the lesion and evidence of invasion of the submucosal or muscular layers.These tumors are unequivocally transitional cell carcinomas,grade II or grade III.As these cancers approach the grade III pattern they tend to be flatter than the less aggressive forms,to cover larger areas of the mucosal surface ,to invade deeper,and to have a shaggier necrotic surface.Occasionally these cancers show foci of squamous cell differentiation,but only 5% of bladder cancers are true squamous cell carcinomas.Carcinomas of grades II and III infiltrate surrounding structures,spread to regional nodes,and on occasion metastasize widely6 .
Recently the suggestion has been made to divide all transitional cell tumors into two grades:

A -Low grade transitional cell carcinomas:are papillary tumors with normal to slightly atypical appearing transitinal epithelium.These tumors retain blood group antigens,normal chromosomes,and have normal proliferative indexes.Patients may have multiple tumors,and almost non are invasive.

B)-High grade transitional cell carcinomas:may have a papillary component but are usually flat with downward growth.They are comprised of pleomorphic cells with abnormal ploidy and loss of blood group antigen.Some 85-95% of patients who die from transitional cell carcinoma present with high grade invasive tumors as the first,and usually only,tumor.In addition to overt carcinoma,an insitu stage of bladder carcinoma can be recognized most frequently in patients with previous or simultaneous papillary or invasive tumors6 .

Indeed,wide areas of atypical hyperplasia and dysplasia are often present.It is now thought that these epithelial changes and lesions in situ are caused by the generalized influence of a putative carcinogen or urothelium

and that they may be the precursors of invasive carcinomas in some patients.The extent of the restless epithelium prov ides a plausible source for multiple and recurrent lesions6.

3-Development of Transitional Cell Carcinoma

Transitional cell carcinomas as for most other carcinomas seems to be dependent in its development on a combination of genetic and environmental factors 7.Among the environmental factors is the chemical factors which are of great importance 8.Bladder tumors are more common in industrial areas and their incidence is increased with exposure to cigarette smoke and arylamines 9-13.

There is a sharp correlation between smoking habits and the occurance of nuclear atypia in the transitional epithelium complementing the epidemilogic evidence of a dose response of cigarette smoking and urinary bladder carcinoma.Fifty percent of bladder cancers caused by cigarette smoking ,people who smoke are at least twice as likely to be diagnosed with bladder cancer as nonsmokers from cigarettes,pipes and cigars.Smoke is absorbed into the lungs and blood and then filtered by the kidneys into the bladder.The urine remains in contact with the bladder for a long time and those toxins then get absorbed in to the lining of the bladder.

Schistosoma hematobium is also pathogenetically related to transitional cell and squamous cell carcinoma of the bladder,being the greatest concentration of carcinoma of this organ occurs in areas of the world infected by this parasite14,15 .

Other environmental factors aniline dyes: particularly benzidine and β-nephthylamine)1 6,17, drugs such as:auramines,phenacetin,ifosfamide and cyclophosphamide(14,18,19.

It has been postulated that urinary tryptophan metabolites may be the endogenous counterparts of the carcinogenic dyes 20.High levels of urinary tryptophan have been reported to correlate with tumor recurrence

rates21,22 .Pyridoxine administration normalizes urinary tryptophan metabolite levels in some patients.Moreover,a controlled clinical trial showed that pyridoxine significantly reduced early tumor recurrence rates in patients with superficial bladder cancer23 .

In the above mentioned clinical trial,however tryptophan metabolite levels were not measured.In contrast,current studies suggest that endogenous tryptophan metabolites do not contribute significantly to the development of bladder cancer24. Thus the role,if any,of endogenous metabolites in the etiology of bladder cancer remains controversial.

4- People at Most Risk of Bladder Cancer:

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 such as Ectopia Vesicae(25.

5-Age and Sex Related Incidence

Most cases of transitional cell carcinomas of the bladder present in the ages of 60-79 years old,with the mean age being 67 years, but they can also occur in younger adults and children 26-29.

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 30.

6-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 hematuria(31.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 32.

7-Management

Management is started from the diagnosis by clinical presentation and examination passing through investigations ending by treatment.

7.1 -*Tumor Markers*

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, glycoproteins, enzymes, hormones, immunoglobulins, mucins, cytokeratins and low molecular weight metabolites. Most tumor markers are incidentally involved in tumorigenesis and are byproducts of malignant transformation.However, measurements of tumor marker levels alone are not sufficient to diagnose cancer for the following reasons33 :

1.Tumor marker levels can be elevated in people with benign conditions.

2.Tumor marker levels are not elevated 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.

Applications of Tumor Markers:

1. They have an important role in diagnosis of cancer.
2. Some tumor marker levels are measured before treatment to help for planning for appropriate therapy.
3. In some types of cancer ,tumor marker levels reflect the extent stage) of the disease and can be useful in predicting how well the disease will respond to treatment.
4. Tumor marker levels may also be measured during treatment to monitor a patient′s response to treatment.A decrease or return to normal in the level of a tumor marker may indicate that the cancer has responded favorably to therapy,if the tumor marker level rises,it may indicate that the cancer is growing.
5. Measurements of tumor marker levels may be used after treatment has ended as a part of follow up care to check for recurrenc(33.

There are two new urinary tumor markers, cytokeratin 18 CK18 and bladder tumor fibronectin BTF), for the detection and monitoring of bladder cancer. False positive rates are higher for BTF, CK18 and BTF in urine may eventually prove to be of benefit for specific patients with bladder carcinoma given its higher sensitivity compared with cytology 34 .

Tissue-polypeptide antigen TPA and Ca19 -9 urinary levels in patients with grade III bladder tumors were significantly higher than in those with lower graded neoplasms3 5.Urinary tumor markers are being used to follow some patients after their first treatment and any further treatment. These are the bladder tumor antigen BTA and the NMP22. Other tests are being studied. None of these are recommended for screening. It is too early to tell if these tests will take the place of urine cytology l ooking for cancer cells in the urine) and cystoscopy looking in the bladder for cancer for

diagnosis and follow-up. Periodic cystoscopy augmented by cytology is still recommended as the standard test for diagnosis and follow-up. For advanced cancer some of the markers used for other cancers such as CEA, CA 125, CA 19-9 may be elevated 3 6.

Increased CA 125 was seen in approximately 11% of patients with high grade or invasive TCC preoperatively. It was more commonly found in patients with locally advanced and lymph node positive disease, and it was associated with overall survival. However, recurrence-free survival was not associated with CA 125. Further studies are required to define the exact role of CA 125 in bladder cancer(37.

7.2 -*Urine Tests*

Malignant transitional cells can be observed on microscopic examination of the urinary sediment or bladder washings.The limitation of microscopic cytology are due to the cytologically normal appearance of cells from well-differentiated tumors,and,because well differentiated cancer cells are more cohesive,they are not readily shed into the urine,therefore microscopic cytology is more sensitive in patients with high grade tumors,however,urinary cytology may be falsely negative in 20%38.

False positive cytology may occur in 1% to 12% of patients and is usually due to urothelial atypia,inflammation,or changes caused by radiation therapy or chemotherapy38 .These changes are frequently observed after several months of therapy and may persist for more than one year after the initiation of therapy 34.

The Food and Drug Administration FDA have approved new urine tests.A new urine test that appears to be an accurate way of diagnosing bladder cancer is MCM5.BTA test stands for Bladder Tumor Associated Antigen,this te st uses monoclonal antibodies to pick up particular proteins in the urine.NMP22 is another way of diagnosis in urine.It stands

for(Nuclear Matrix Protein, which is a type of protein found in the nucleus of cells.Some NMPs have been found in some types of cancer cells ,including the cells of the commonest type bladder cancer called TCC).These cells release NMP22 into the urine(39 .

7.3 -*Radiological Investigations*

Transabdominal ultrasonography is the initial radiological investigation for detection of bladder carcinomas.Ultrasonography is safe and easily available and provides images of both upper and lower renal tract.

Intravenous Urogram is a general X-ray examination indicated to check the etiology in the upper urinary tract. Furthermore CT scan compute rized tomography can be done in order to check cancer spread to other parts of the body.MRI(magnetic resonance imaging is a scan that uses magnetism to build up a picture of the inside of the whole body to look for cancer spread.MRI scans are particularly good for looking at the soft tissues of the body.

In addition to these techniques Bone scan may be done to check cancer spread to the bones 40 .

7.4 -*Flowcytometry*

It measures the DNA content of cells.Therefore it can quantitate the aneuploid cell populations and proliferative activity in a tumor.With flow cytometry, the isolated cells,whose nuclei are stained with a DNA-binding flourescent dye,flow through a small tube in which flourescence is excited by a LASER beam.DNA content is measured from flourescent intensity 33.For diagnostic purposes,bladder wash specimen s are usually required for satisfactory results 41.

Arbitrary limits are required to define normality.For example,if more than 15% of the cells are aneuploid,the cytologic findings are defined as being positive for cancer(42.

7.5 -*Cystoscopy*

Cystoscopy is the most important test for diagnosing cancer of the bladder.It means putting a thin tube with a light inside the bladder of the patient.This tube which contains optic fibres inside allows doctors to see inside the bladder.

This test can be done under local or general anasthesia,so if tissue sample needed to be taken,there must be done under general anasthesia.

If the patient diagnosed to have invasive bladder cancer,the specialist will advise for more tests to make sure that the cancer has not spread 43.

8-Treatment of Bladder Cancer

Management of transitional cell carcinoma is directed to remove the offending malignancy ,with treatment based on the anatomic distribution of the disease.Tumors that have not invaded the muscle may be controlled by either transurethral resection or fulguration,or by intravesical drugschemoth erapy tha t promote removal of malignancy.These intravesical agents affect the tumor with varying effectiveness .

Once the tumor penetrates through the lamina propria,the patient has a 30% chance of succumbing to the malignancy.The chance of death from the submucosally invasive tumor is a function of the pathologic grade of the malignancy which seemingly reflects the biologic aggressiveness of the disease.Disease that penetrates into the muscle of the bladder is best managed by either partial or total removal of the bladder.Segmental cystectomy has fallen into disfavour because of the high intravesical recurrence rates in the residual bladder.

Patients who have no evidence of atypia or carcinoma insitu at other sites within the bladder either adjacent to or distant to the offending invasive malignancy may be appropriate candidates for partial cystectomy.

Radical cystecomy is advised for patients whose tumors demonstrate muscle invasion specially those who have-high-grade disease that penetrates

the lamina propria.The survival rates appear dependent more on the grade,stage,and cell type of the tumor rather than maneuvers that occur at the time of removal 44.

Radiotherapy is another way of treatment,BS06 is a trial that has been investigating the use of radiotherapy to stop early bladder cancer stage T1 from recurrence after treatment.There is a risk of this early bladder cancer coming back because it has just started to grow into the tissue underneath the bladder lining.This trial has been looking at high grade grade III aggressive stage T1 bladder cancer,which at the highest risk of coming back as an invasive cancer.BC2001 is another radiotherapy trial for that stage of bladder cancer-when it becomes invasive,but is still localized.The trial is looking into giving higher doses of radiotherapy,but just to the area of the bladder that contains the cancer.Another bladder radiotherapy trial is BCON.Cancer cells are more likely to be killed by radiotherapy if they have plenty of oxygen.The researchers hope that this treatment will stop the cancer from coming back after treatment.There are many clinical trials underway testing combinations of new chemothrapy drugs.The aim of this type of research is to find better ways of treating bladder cancer with chemotherapyGemcitabine ,paclitaxel,mitomycin C). Researchers hope that combined treatment chemothrapy and radiotherapy ,which is known as chemoradiation,may be successful enough to prevent most people needing surgery to remove their bladder(45.

Photodynamic therapy is a relatively new treatment that is used for a few types of cancers.In this type of treatment the drug is put into the bladder that makes the body cells very sensitive to light.Then a strong light shone directly onto the cancer.The light activates the drug inside cancer cells and they are killed,leaving nearby normal cells unharmed.One disadvantage of this type of treatment is that the drug needs time to make it work,so it is only useful for treating cancers near the surface of the bladder,where a light can

shine through a cystoscope.The light can not reach cancers that are deep in the bladder wall or have spread to other organs in the body.This is not an easy treatment to have.The patient has to stay in dim light some time after having the treatment.Other side effects include redness .The side effects are severe when the drug has to be taken by mouth or injected intravenously46 .

Gene therapy is one of the newer approaches to cancer treatment and is in the very early stages of clinical trials.There is a gene called the p53 gene which is abnormal in many different types of cancer ,including bladder cancer.The normal p53 gene codes a cell to self destruct if it is already damaged.Cancer cells do not do this because they have an abnormal p53 gene.If a normal p53 gene can be put back into bladder cancer cells,it will be able to stop the growth of the cancer,by signalling to the cancerous cell to die off naturally as normal cells would.47

Immunotherapy is treatment with substances that the body uses to clear infection and disease.Immunotherapy works by encouraging the natural defence system-the immune system-to attack cancer cells.Biological therapy is another name for immunotherapy.Different types of immunotherapy used in bladder cancer:

1.BCG is the most studied and commonly used immunotherapeutic agent to treat bladder cancer.It is still being investigated in terms of different doses,length of time of treatment and combining it with other treatments.BCG treatment helps to stop early bladder cancer from developing into invasive carcinoma.

2.Interferon is used in combination with low doses of the commonly used bladder cancer treatment.Interferon can help to lessen or stop cancer from recurrence in patients whose cancer has already recurred after previous BCG treatment45.

9-Prognosis

Prognosis5 year survival is a term used by personnel working in the medical field.It does not mean the patient will only live 5 years,but it relates the proportion of people in research studies who were still alive 5 years after diagnosis.This is because there is only a small chance that the cancer will come back more than 5 years after treatment.Doctors do like to say these people are cured because there is a small chance,so the term5 year survival is instead used.

As with many other types of cancer,the outcome of bladder cancer depends on how advanced it is when it is diagnosed4 8.

Most bladder cancers are diagnosed while they are still only in the bladder lining.About 3 out of 4 75% bladder cancers are of this type when they are diagnosed.The early bladder cancers can all be cured or controlled with surgey or immunotherapy or chemotherapy into the bladder.Low grade tumors,grade I early bladder tumors are more likely to be cured.Moderate to high grade(grade II or III early bladder tumors are more likely to come back and need further treatment.Taking all superficial bladder tumors together,about 65 out of every 100 65% will come back,most of these will recur as early non-invasive bladder cancer.

Most Ta non invasive papillary tumors are low grade.About 7 out of 10 stage Ta early bladder cancers 70%will come back.But most of these will come back again as superficial tumors that can be nipped out again.1% will come back as an invasive cancer and need serious treatment.

About 1 in 5 bladder cancers20% have grown into the muscle layer of the bladder when they are diagnosed.These are called invasive bladder cancers.About 1 in 2 of those diagnosed with T2 invasive bladder cancer 50% are alive and well 3 years after their diagnosis.Unfortunately,treatment is less successful for tumors that have

gone further into the bladder.About 1 in 4 of those with a T3 tumor(25%will be alive and well 3 years after diagnosis and treatment49.

Surgery or radiotherapy will cure many of these cancers.But these will come back and need more treatment.One of the most important factors is how far the cancer has grown in the wall of the bladder.

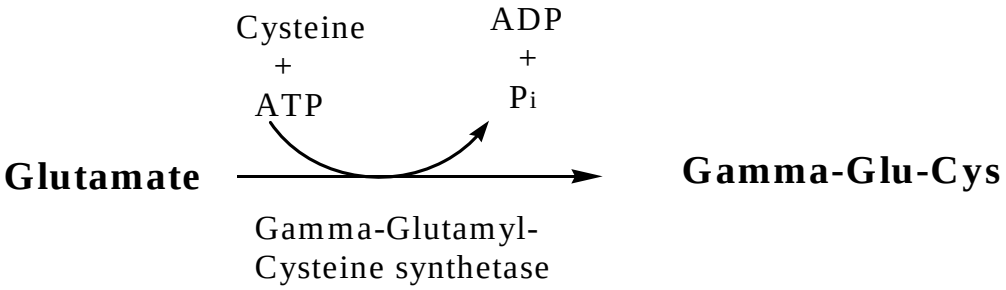
Advanced metastatic)bladder cancer is bladder cancer that has spread to another part of the body.About 1 in 20 bladder cancers 5% has already spread by the time it was diagnosed.Only about 1 in 10 people with bladder cancer that have spread to lymph nodes will live for more than 5 years after the diagnosis.

Unfortunately,if the cancer has spread to another body organ, it is not going to be curable.Yet, the patient can still have the treatment to keep it under control for a while.Once a cancer has spread in this way ,the average life expectancy is between a year and 18 months48.

Section II/Glutathione

1-Definition and Synthesis

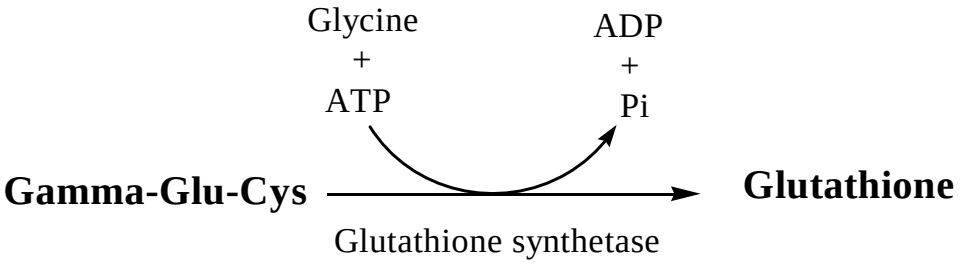
It is a tripeptide containing a sulfhydryl group.It is a highly distinctive amino acid derivative with several important roles.The first step in the synthesis of glutathione is the formation of a peptide linkage between the γ-carboxyl group of glutamate and the amino group of cysteine,in a reaction catalyzed by {γ-glutamyl cysteine synthetase}.Formation of this peptide bond requires activation of the γ-carboxyl group,which is achieved by ATP.



The first step in glutathione synthesis50

The resulting acyl-phosphate intermediate is then attacked by the amino group of cysteine.This reaction is feed back-inhibited by glutathione.

In the second step,which is catalyzed by glutathione synthetase,ATP activates the carboxyl group of cysteine to enable it to condense with the amino group of glycine.The reduced form of glutathione serves as sulfhydryl buffer that maintains the cysteine residues of hemoglobin and other red cell proteins in the reduced state(50.



The second step in glutathione synthesis50

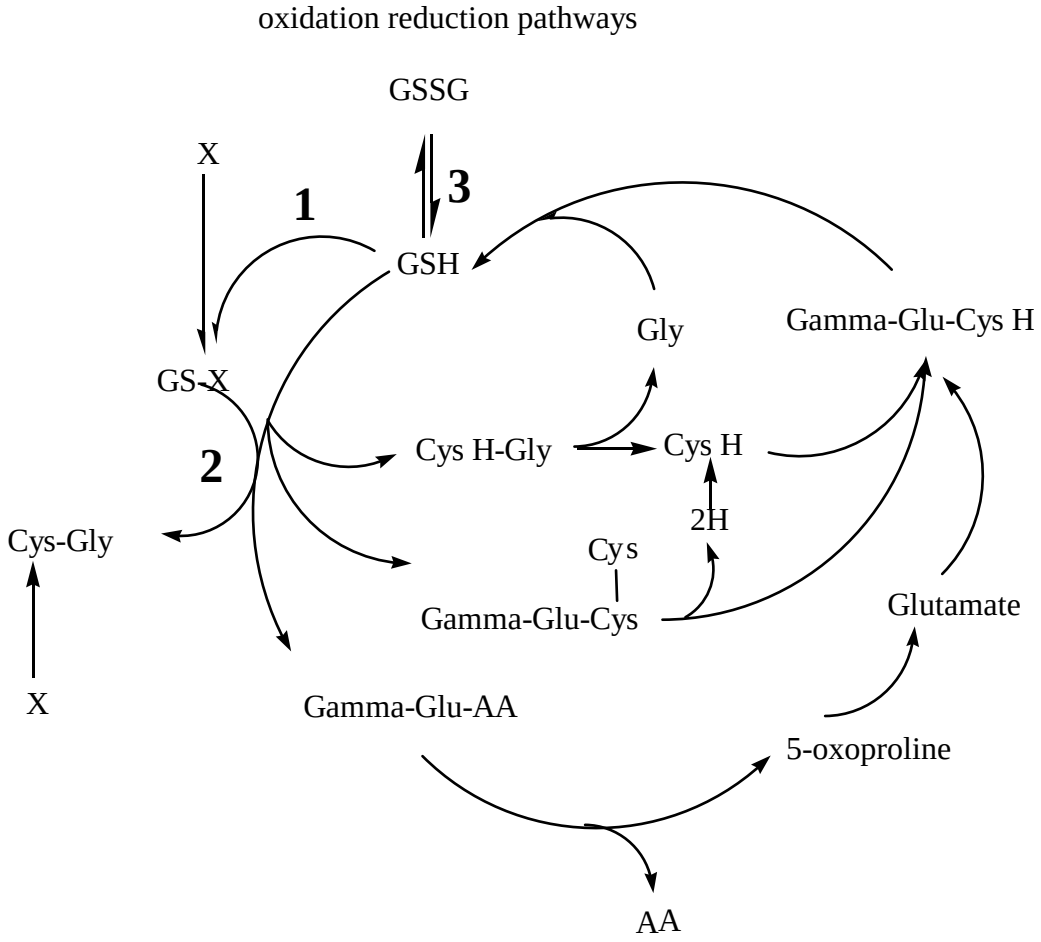
2-Metabolic Role

The GSH pool is drawn on for three major applications as shown in the scheme page-17-:a as a cofactor for the GSG -S-transferases in the detoxicative pathwaysReaction 1 ;b as substrate for the gamma -glutamyl transpeptidase ,enzymes which are located on the outer cell surface and which transfere the glutamine moiety from GSH to other amino acids for subsequent uptake into the cellReaction 2;and c) for direct free radical scavenging51 and as an antioxida nt enzyme cofactor(Reaction 3.The GSH transferases are a large group of isoenzymes that conjugate GSH with fat-soluble substances as the major feature of liver detoxification.52 -54.

Enzymes collectively known as GSH transhydrogenases use GSH as a cofactor to reconvert dehydroascorbate to ascorbate,ribonucleotides to deoxyribonucleotides, and for a variety of —S-S— ⇌ —SH interconversions.After GSH has been oxidized to GSSG,the recycling of GSSG to GSH is accomplished mainly by the enzyme glutathione reductase.This enzyme uses as its source of electrons the coenzyme NADPHnicotinam ide adenine dinucleotide phosphate,reduced.Therefore NADPH,coming mainly from the pentose phosphate shunt,is the predominant source of GSH reducing power55 .

Through its significant reducing power,GSH also makes major contributions to the recycling of other antioxidants that have become oxidized.This could be the basis by which GSH helps to conserve lipid-phase antioxidants such as alpha-tocopherol vitamin E,and perhaps also the carotenoids56 .The liver seems to have two pools of GSH;one has a fast turnover(half-life of 2-4 hours,while the other is avidly retained with a half -life of about 30 hours57 .The first corresponds to cytosolic GSH,the second

mainly to mitochondrial GSH which is known to be more tightly held.Though this pool represents a minor portion of the total GSH,the mitochondria are normally under high oxidative stress and thus conserve their GSH58 .

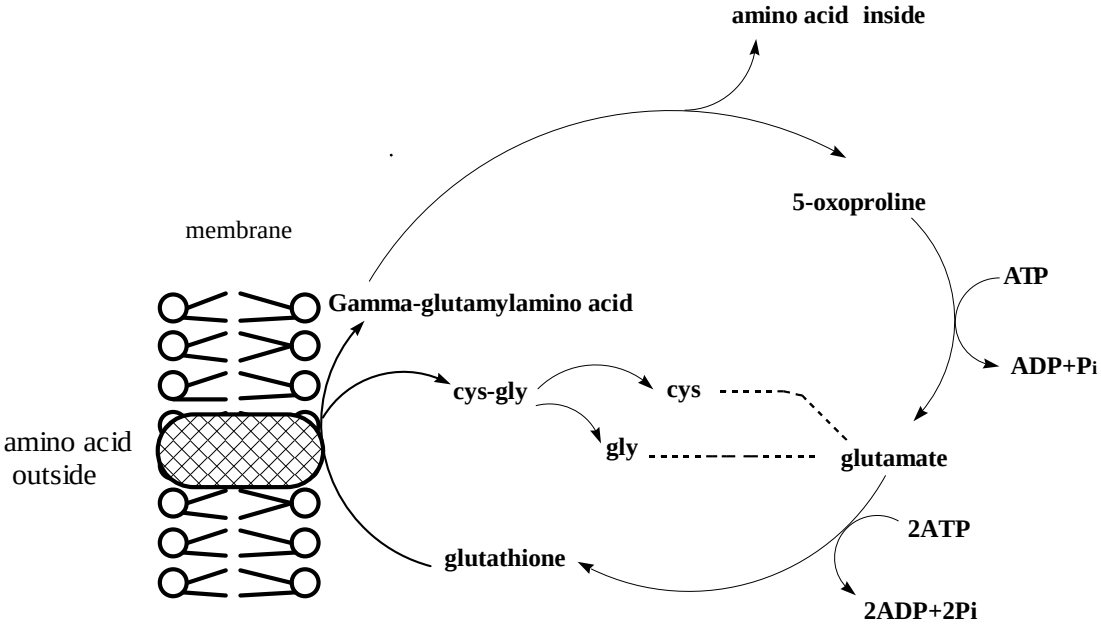


Scheme of GSH metabolism 56.

3-Transport of Amino acids

There are several machanisms for transport of amino acids across cell membranes.The Gamma-Glutamyl cycle is an example of "group transfere" transport.It is more energy-requiring than other machanisms,but is rapid and has high capacity,and functions in kidney and some other tissues.It is

particularly important in renal epithelial cells.Gamma-Glutamyl transpeptidase is located in the cell membrane.It shuttles GSH to the cell surface to interact with an amino acid.Gamma-Glutamyl amino acid is transported into the cell,and the complex is hydrolyzed to liberate the amino acid.Glutamate is released as 5-oxoproline,and cysteinyl-glycine is cleaved to its component amino acids.To regenerate GSH glutamate is reformed from oxoproline in an ATP-requiring reaction, and GSH is resynthesized from its three component parts.Three ATP molecules are used in the regeneration of glutathione,one in formation of glutamate from oxoproline and two in formation of the peptide bonds59.



γ-Glutamyl cycle for transporting amino acids59

4-Antioxidants Defense System and Free Radicals

Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stressors to which it invariably becomes exposed 60.The antioxidant defense system is sophisticated and adaptive,and GSH is a central constituent of this system61 .

Originating within the mitochondria of aerobic cells is a steady flux of oxygen free radicals,unavoidably generated from the processes that utilize oxygen to make ATP.This complex system of enzyme pathways by which the mitochondria use oxygen to break carbon-carbon bonds and produce ATP is called oxidative phosphorylationOxphos.As Oxphos substrates are processed in the mitochondria,invariably single electrons escape,leaking out of the Oxphos complex to react with ambient oxygen and generate oxygen free radicals.This oxygen radical leakage,a type of "metabolic friction"in the aerobic system,both wastes and energy poses a potential toxic risk to the organism.An estimated 2-5 percent of the electrons that pass through the Oxphos system are converted into superoxide and other oxygen radicals 62.

Since Oxphos processes at least 95 percent of all the oxygen used by the body,this flux of wayward oxygen free radicals is metabolically significant 61,62.

Evaluation of the possible correlation between intracellular glutathione GSH and drug sensitivity of urothelial cancer was done in Taiwan63.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 MEC) Methotrexate ,Epirubicin and Cisplatin.

The patients gave three different clinical responses;complete response,partial response and non response.Those cancer cells from patients with partial response and non-response contained a significantly higher level of GSH than cancer cells from patients with complete response.Intracellular GSH content may play an important role in intrinsic resistance of urothelial cancer to MEC chemotherapy.It might be potentially used to predict drug sensitivity in urothelial cancer patients before starting chemotherapy63.

The continual flux of single electrons to oxygen generates an endogenous oxidative stress in human tissues.Superoxide,peroxide,hydroxyl radical,and other free radicals derived from oxygen are highly reactive and therefore threatening to the integrity of the essential biomolecules such as DNA and RNA,enzymes and other proteins,and the phospholipids responsible for membrane integrity.The aerobic cell is continually challenged to neutralize these OxyRad time bombs before they can initiate propagative free radical reactions that could cause its disintegration.Healthy cells homeostatically oppose free radicals through the use of antioxidants.In the face of this endogenous oxidative burden,the formidable reducing power of the GSH/GSSG couple is a profound physicochemical asset for the aerobic organism.Perhaps equally as significant for life span is that GSH also helps to protect against exogenous oxidative insults,which are or ought to be) potentially more controllable64 .

In USA Department of Investigational Therapeutics,New York65,GSH levels were measured in human tumor cell lines derived from carcinomas of the bladder,ovary,colon and from melanoma and glioblastoma.High levels were found in four of five bladder cell lines.The average GSH concentration in the bladder cell lines was approximately six folds higher than in the non-bladder cell lines.GSH was also measured in two types of control tissues:a)no tumor bladder tissue from patients with TCC or a history of TCC of the bladder and bbladder tissue from patients without bladder cancer.No correlation was found between GSH levels and the proportion of tumor cells in the tissue.The results indicates:
a)significantly higher levels of GSH in TCC compared to tumor -free bladder tissue;and bhigher GSH levels in non tumor bladder tissue from patients with bladder cancer than from patients without TCC.The clinical implication of this work includes the possibility that GSH may play a role in

the resistance of bladder cancer to chemotherapy and may be associated with bladder carcinogenesis65.

5-Hereditary Deficiency

With regard to the essentiality of GSH for the survival of the whole organism,substantial information is available from studies on hereditary GSH depletion in the human,and from experimental depletion and repletion of GSH in animal models and cell cultures 52,66.

Inherited deficiency of the enzyme gamma-glutamylcysteine synthetase,the first of the two enzymes necessary for GSH synthesis,has been described in two human siblings.They exhibited generalized GSH deficiency,hemolytic anemia,spinocerebellar degeneration,peripheral neuropathy,myopathy,aminoaciduria and severe neurological complications as they moved into their fourth decade of life51 .Their red cell GSH was less than 3% of normal,their muscle GSH less than 25%,and their white cell GSH less than 50% normal.One of them may have been hypersensitive to antibiotics,having developed psychosis after a single dose of sulfonamide for a urinary tract infection.

Deficiency in GSH synthetase,the second enzyme of GSH synthesis,also is associated with hemolytic tendency and defective central nervous system function.This condition is complicated by the metabolic consequences of an excess of 5-oxoproline,formed as a "spill over" from the accumulation of gamma-glutamyl cysteine after its normal synthesis by the first enzyme and its lack of conversion to GSH by the second enzyme(52,66.

Human hereditary GSH deficiency states are not necessarily lethal,probably because some GSH is obtained directly from the diet.With laboratory animals it is possible to precisely control GSH in the diet.

The investigators found that dietary ascorbate can protect against the tissue damage that typically results from depletion of GSH54,57.

Redox phenomena are intrinsic to life processes,and GSH is a major pro-homeostatic modulator of intracellular sulfhydryl —SH groups on proteins 67,68.Many important enzymes e.g.,adenylate cyclase,glucose -6-phosphatase,pyruvate kinase,the transport Ca-ATPases,and at least eight participating in glucose metabolism,are regulatable by redox balance as largely defined by the balance of 2 —SH $\rightleftharpoons$ —S-S— 69 .

6-Exogenous Causes of GSH Depletion

Cigarette smoke contains thousands of different chemical species,and a single puff of cigarette smoke contains trillions of free radicals67 .Cigarette smoke literally burns away the antioxidant vitamins C and E,as well as other nutrients.The cigarette tars are long-lived free radical generators and potent carcinogens 70.

Many phrmaceutical products are oxidants capable of depleting GSH from the liver,kidneys,heart,and other tissues71 .

The popular over-the-counter drug acetaminophen is a potent oxidant.It depletes GSH from the cells of the liver,and by so doing renders the liver more vulnerable to toxic damage.

The halogenated hydrocarbons halocarbons are pot ent oxidants.Halocarbons are ubiquitous,being used in the plastics industry,as industrial and dry cleaning solvents,as pesticides and herbicides,and as refrigerants.The chlorofluorocarbons that currently threaten the ozone layer are one type of halocarbon 72 .

Strenuous aerobic exercise can deplete antioxidants from the skeletal muscles,and sometimes also from the other organs.Exercise increases the body’s oxidative burden by calling on the tissues to generate more energy.Making more ATP requires using more oxygen,and this in turn results in greater production of oxygen free radicals73 .

Some of the other exogenous factors known to deplete tissue GSH include:

1.Dietary deficiency of methionine,an essential amino acid and GSH precursor.The liver uses 70 percent of the total dietary intake(74.
2.Ionizing radiation,whether as X-rays or ultraviolet from sunlight 75.
3.Tissue injury,as from burns76, ischemia and reperfusion77surgery78, septic shock 79, or trauma(80.
4.Iron overload,as in hemochromatosis and transfusional iron excess 60.
5.Bacterial or viral infections,including HIV-1 61,74.
6.Alcohol intake is toxic through a number of differing pathways,some of which are free radical/oxidative in character81.

Studies on GSH status with advancing age have been few,but appears to have a correlation between age-associated GSH depletion and poor health.Lang and collaborators 82compared blood GSH concentrations between the healthy young and healthy elderly subjects.The 40 young subjects2 0-39 years of age) had a blood GSH level 17% higher on average than the 60 elderly subjects60 -79 years.Julius 83 measured GSH in 33 persons of ages 60-79 years,higher GSH concentrations were associated with good health,regardless of age;subjects with chronic diseases had lower mean GSH concentrations than those free of disease.

Combinations of antioxidants given as dietary supplements seem to offer the most promise for achieving clinical breakthroughs.At times,the administration of ascorbate orally or in travenously or of sulfhydryls GSH and NAC {N-Acetyl Cysteine} orally and intravenously will be life saving84 .

Section III/Glutathione -S-Transferase

1-Definition

Glutathione-S-transferases, EC 2.5.1.18 are ubiquitous multifunctional enzymes85 . Glutathione-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.Glutathione 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-acetyl cysteine,are then excreted 86.

An overexpression of GST has been documented in a study done in Italy,in the erythrocytes of patients with chronic renal failure.In fact,it could serve as a marker of uremic toxicity overall,which can contribute to impair the function and survival of the erythrocytes.However,the biochemical details of this phenomenon are poorly understood87.

2-Classification

Attempts have been made to classify such enzyme activities on the basis of the carbon skeleton of the electrophilic molecule or the specific leaving group involved,hence the formerly common use of the terms aryl-,alkyl,alkene, and epoxide transferase.

Transferases B and C were found to be homogenous by the criterion of sodium dodecyl sulfate-gel electrophoresis;a single band with a molecular weight of about 25 000 dalton was found for each enzyme.In mixing experiments,,the subunits of transferase A and transferase C migrated together and appeared to be identical by this criterion.Transferase B was also

examined by sedimentation equilibrium analysis;a straight line was obtained when the log of protein concentration was plotted.Sedimentation equilibrium analysis at two concentrations of transferase B,50 and 100 microgram per ml,yielded a molecular weight of 47000 dalton.

Based on their sequence homology,substrate specificity and immunological cross reactivity,Glutathione-S-transferases have been grouped into five species-independent classes of isoenzymes 88-92.Four of these classes alpha,pi,mu,and theta) comprise cytosolic enzymes,a fifth rather distinct form is microsomal.

All cytosolic Glutathione-S-transferases are found to be homo- or hetero-dimeric enzymesfrom within the same classwith a relative molecular weight of Ca 50 000 dalton 85.

The primary structure of the human microsomal glutathione S-transferase gene GST12 was determined by genomic cloning.The gene structure of GST12 spans 12.8 kb and consists of four exons and three introns.The coding sequence resides on exons 2,3 and 4.All introns commenced with nucleotides GTAA at the 5{prime} boundary and ended with nucleotides AG at the 3{prime} boundary,in agreement with the proposed consensus sequence for intron spliced donor and acceptance sites.the presence of an in-phase stop codon and an upstream false start codon in the 5{prime}-untranslated region was confirmed93.

In Italy at the general hospital of Venice,a study aiming to select a validated panel of tests to assess the GST/GSH system in a clinical setting was implicated.Matched blood and tissue samples normal and malignantfrom cancer patientsnon -urological cancers were investigated.GSH levels and GSH activity were higher in cancer tissues than in matched normal tissues in both malignancies.Moreover a strong correlation was found between the GSH level in whole blood and GST activity in cancer tissue.

The finding regarding the GSH level in blood indicates that circulating GSH could have a clinical relevance as a surrogate marker of GST activity in tumor tissue94.

3-Role in Carcinogen Detoxification

It is an important enzyme family in carcinogen detoxification,particularly the Glutathione-S-transferase M1,encoded for by the polymorphic gene,GST M1,which occurs in about 50% of the white population.Cigarette smokers who are homozygous for lacking this gene have 1.8 fold greater risk of developing bladder cancer than smokers who have one or two copies-non smokers who lack this gene,however,have a similar risk of developing bladder cancer as non smokers who have it,thus supporting the notion that this gene plays a role in developing smoking-induced bladder cancer(95.

The GSH content and GST activity have been studied in human bladder specimens obtained from controls and from patients with superficial transitional cell carcinoma at Barcelona University in Spain.

The tumor samples and peri-tumor normal tissues were taken from the same patient.It was observed that GST activity was significantly greater in tumor than in peri-tumor normal tissue patients,or in normal mucosa.GSH content was significantly greater in tumor than in peri-tumor normal tissue patients,or in normal mucosa,with this increase being evident only in smokers.When comparing normal mucosa and peritumor samples no significant differences were found either for GST activity or for GSH content.Results demonstrate the relationship between the GST/GSH system and development of Transitional Cell Carcinoma,as well as its role in cellular resistance to chemotherapy96.

A study was done in Urology Department,Assiut University in Egypt,designed to describe the expression of the GST/GSH system in squamous cell carcinoma of the bladder in certain population.The GST

activity was significantly higher in bladder cancer specimens in comparison with schistomiasis cystitis tissue and with healthy control samples.The GSH content was also significantly higher in bladder cancer than in cystitis tissue and in control samples.When control mucosa and cystitis samples were compared,two fold increased values were obtained for GST and GSH in schistosomiasis bladder tissue.Results describe an over expression of GST and GSH levels in squamous cell carcinoma of the bladder,and indicate a possible role in chemoresistance to pharmacological therapy97.

Objectives of The Study

The research main guidelines were directed to:

1.Show the relationship of Carcinoma of the urinary bladder to oxidative stress process consideringReduced Glutathione and Glutathione - S-Transferase as Biomarkers for this tumor.

2.Present the effects of age,sex,family history of tumors on GST and GSH.

3.Study the changes in GST and GSH in relation to the tumor size,tumor site,stage of the tumor and grade of the tumor.

4.Study the effect of smoking,exposure to chemicals,history of urinary stone and residency area considering rural and urban areas.

5.Clarify the changes in the level of PCV Packed Cell Volume) in relation to changes in GST and GSH simultaneously.

CHAPTER TWO

MATERIALS and METHODS

Section I/Materials

1.*Patients*

The project govern theperiod from 1st of November 2004 till 15th of August 2005.Sixty seven patients 57 male and 10 female of mean age of 55 years oldwere introduced to Al-Hilla Teaching Hospital,department of Urology,they were proved to have bladder cancer as diagnosed by histopathologist cosultant for biopsies taken by cystoscopy.The indications of cystoscopy were:

1. Radiologically proved patients with bladder mass with or without haematuria.
2. Radiologically negative patients but with haematuria:

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

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

All patients underwent full history and physical examination including: age,sex,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,PCV packed cell volume),R.B.S.Random blood sugar),ultrasound examination for urinary bladder,IVUIntravenous urography and CT scan Computer ized Tomography.

Cystoscopy was done under general anasthesia using rigid cystoscope Storz) 21F with multiple cup biopsy and TUR Transurethral resection if indicated.

The control group were 36 ,they 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.The data were analysed statistically with *t*-test.

2.*Chemicals* Table(1: Chemical compounds used in the research.

No.	***Chemical compound***	***Production***
1.	Ammonium oxalate	BDH AnalaR Poole,BH 151 TP made in England
2.	1-Chloro2,4-Dinitrobenzen	SIGMA-ALDRICH CHEMIE GmbH,made in Germany/4225
3.	Chloroform	Fluka Chemika 02870 made in USA
4.	Disodium phosphate	BDH AnalaR Poole,BH 1051 made in England
5.	5,5' Dithiobis nitrobenzoate	Fluka Biochemica 24004023 made in USA
6.	Distilled water	Laboratory Distillator
7.	EDTA Disodium	GAINLAND chemical company ,CLWYD Made in UK
8.	Ethanol96%	Fluka Chemika 25690 made in USA
9.	Glacial metaphosphoric acid	Merck Darmstdt, 2955555 made in Germany
10.	Glutathione standard GSH	SIGMA-ALDRICH CHEMIE GmbH,Steinheim, Germany /G 4251-1G
11.	Hydrochloric acid	Analar Poole,101256J made in England
12.	Normal saline, 0.9% Sodium Chloride	Pharmaceutical Solutions Industry Ltd,Jeddah 21484 Kingdom of Saudi Arabia
13.	Pottasium diphosphate	CHEM-Supply 4326410005598 GILLMAN,South Australia
14.	Pottasium monophosphate	CHEM-Supply 4326410005013 GILLMAN,South Australia
15.	Pottasium oxalate	Fluka BioChemika 60424 made in Switzerland
16.	Sodium chloride	Fisher Scientific LE115RG made in UK
17.	Sodium citrate	BDH AnalaR BH 15 1TD,301285D,made in England

3.Instruments and Materials

Table2: Instruments and Materials used in the research.

No.	***Apparatus or Material***	***Production***
1.	Balancer	Sartorius AG GÖTTINGEN BL2105 Made in Germany
2.	Centrifuge	Griffin and George BS 4402-D Made in Britain
3.	Centrifuge(hematocrit	CENTRIFUGA-MILANO,MOD.4223, 1984,Made in ITALY
4.	Distillator	Bibby Science Products Limited ST15OSA,made in England
5.	Filterpapers	Whatmann 42mm pore size,9m diameter made in England
6.	Incubator	Fisher Scientific,model 5370, CAT.11-690-538D,made in USA
7.	Magnetic stirrer with Hot plate	Classico made in India
8.	Micropipettes-automatic 0.2,1ml	SLAMED Made in Germany
9.	pH-meter	HANNA HI -9321, made in Portugal
10.	Spectrophotometer Digital ultraviolet and visible)	Spectronic 21,MILTON ROY COMPANY,Bouch and Lamp, made in USA
11.	Vortex Electronic)	VIBROFIX,VF-1 JANKE and KUNKEL IKA-Labortechnik,made in Germany
12.	Water bath	Schutzart DIN 40050-IP 20 Memmert GmbH,Schwabach FRG,made in Germany

Section II/Methods

1. ***Method of Determination of Erythrocyte Glutathione concentration.*** **98**

Principle

Virtually all of the non protein sulfhydryl groups of RBCs are in the form of reduced GSH.5,5'-Dithiobis2 -nitrobenzoic 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 412 nm. and is directly proportional to the GSH concentration.

Sample collection

The assay was conducted on whole blood,3 ml,anticoagulated with Ammonium oxalate -Potassium oxalate).GSH concentration declines in blood anticoagulated with heparin or EDTA.

Reagents

1. Precipitating solution.In a 100-ml. volumetric flask, 1.67gm. of glacial metaphosphoric acid was placed,0.2 gm. of di -sodium-EDTA,and 30.0 gm. of NaCl,and brought to volume with distilled water.This solution was stable for three weeks at 4°C.A fine precipitate was formed owing to EDTA,but this does not interfere with the test.
2. Disodium phosphate solution,0.3 mol/L.In a 1-L. volumetric flask,42.59 gm. of Na_2HPO_4 was put and brought to volume with distilled water.The solution was stable indefinitely at 4°C.If crystals formed,they were dissolved by heating.
3. DTNB reagent.In a 100-ml. volumetric flask,I placed 40.0 mg. of DTNB.I used a solution of Sodium citrate 1 gm/dl to bring it to volume.This solution was stable for at least 13 weeks at 4°C.
4. GSH calibrators.100 mg. GSH was placed in a 100-ml. volumetric flask and was brought to volume with reagent grade water.Inversion repeatedly

until GSH is completely dissolved.Preparation of 50 - and 10-mg /dl calibrators by diluting 5 ml. of the 100-mg/dl calibrator with 5 and 45 ml.,respectively,of reagent grade water.The GSH calibrators were not stable and must be freshly prepared on the day of the assay.

Procedure

1. 0.2 ml. of whole blood was placed into a 10 -ml. test tube then 1.8 ml. of distilled water was added.Mixing should be done to hemolyze the RBCs.
2. Promptly 3.0 ml. of precipitating solution was added,then mixed.
3. I Allowed it to stand 5 min. at room temperature and then filteration was done through coarse-grade filter paper.

4. Cuvets were prepared as in table.3:

Table(3:Volumes of the reagents used in the GSH estimation test.

Reagent ml	**Blank**	**Assay**
Filtrate	–	2.0
Precipitating reagent	1.2	–
Water	0.8	–
Na_2HPO_4	8.0	8.0
DTNB solution	1.0	1.0

5. Cuvets were capped and then inverted three times to mix.
6. Absorbance then is read at 412 nm. within 4 minutes of preparing cuvets.
7. Hematocrit of original whole-blood specimen were obtained.
8. Assay of the GSH calibrators,and the filtration step was omitted.
9. I prepared a calibrator curve to determine the GSH concentration of the blood specimen from the graph.
10. Calculation of the GSH concentration:

GSH mg/dl = GSH conc.calibration curve) fig.1

The following formula has been considered from the standard curve:

$$Y=0.0032\ X-0.0005$$

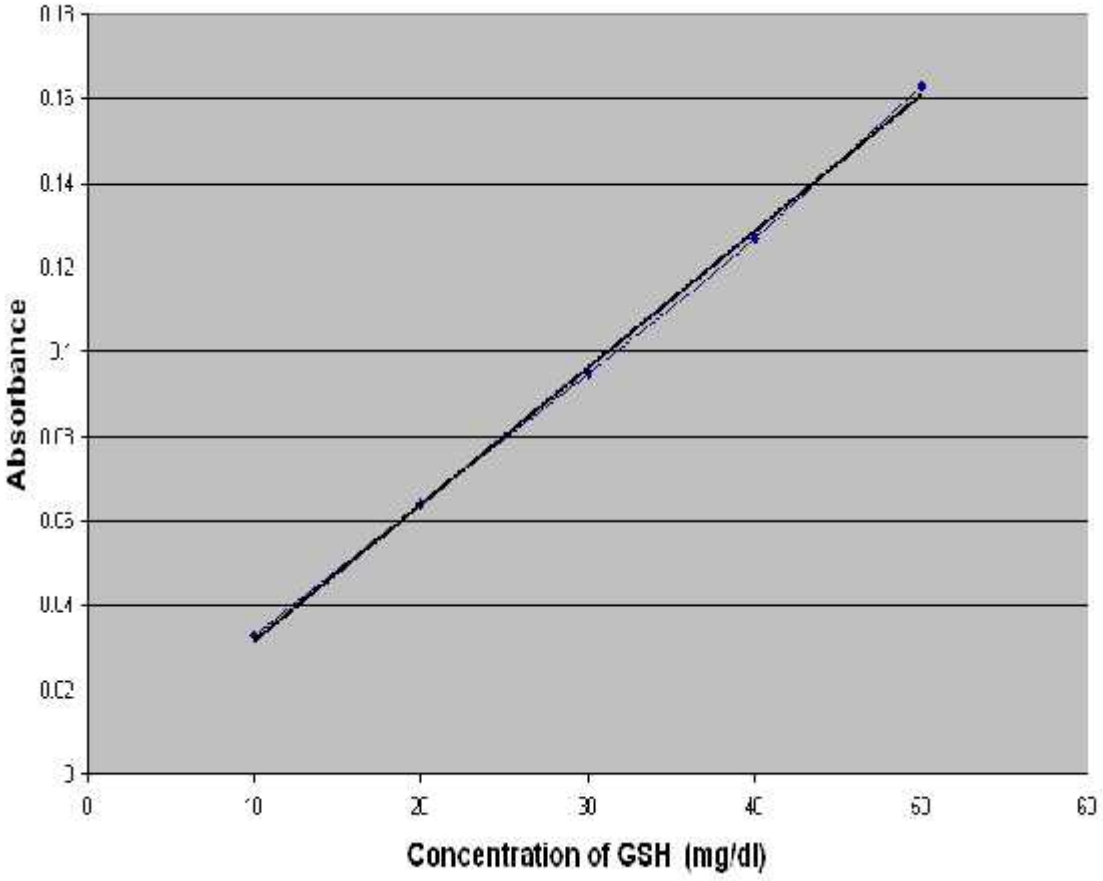


Fig.1The standard curve for GSH concentration against the absorbance.

2. *Enzymatic Assay of Glutathione-S-Transferase EC 2.5.1.18,1 - Chloro-2,4-Dinitrobenzen as Substrate* 92

Principle: GSH+CDNB $\xrightarrow{\text{Glutathione-S-Transferase}}$ G-SDNB Conjugate + HCl

Conditions: T = 25°C, pH = 6.5, $A_{340nm.}$, Light path = 1 cm**.**

Method: Continous spectrophotometric Rate Determination

Reagents:

1. Glutathione solution ,0.092 gm. of reduced standard glutathione was dissolved in 10 ml. distilled water.
2. CDNB solution1 -Chloro-2,4-Dinitrobenzene),this was formed by dissolving 0.455 gm. of CDNB in 100 ml. ethanol.
3. Phosphate buffer, this was prepared by dissolving 0.4355 gm. of K_2HPO_4 with 3.06 gm. of KH_2PO_4 in 250 ml. of distilled water pH was set to 6.5.

Procedure:

1. Washing of the RBCs:

.Sample was centrifuged 3000/min.for 15 min.

.Serum and buffy coat were removed.

.RBCs washed for three times with normal saline ,centrifugation with each time for 5 min.

. RBCs were lysed by addition of 1.5volume distilled water to 1 volume of the washed RBCs.

.Then mixing0.5ml. RBCs +3.5ml. distilled water+1ml. ethanol+ 0.6ml. Chloroform for one mi nute.

.Centrifugation of the mixture 3000/min.was done for 10 min.

.Then the supernatant was taken.

2. 0.4 ml. of distilled water was added to 0.1 ml. of the supernatant

3. 0.1 ml. of the mixture in the last step was taken and mixed with 0.4 ml. of distilled water.

4.

Table(4: Volumes of the reagents used in GST testing.

NO.	***COMPOUNDS***	***TEST***	***BLANK***
1.	Phosphate buffer	2.7 ml	2.7 ml
2.	Sample	100 µl	-
3.	Distilled water	-	100 µl
4.	CDNB solution	100 µl	100 µl
	After 3 min.		
5.	Glutathione solution	100 µl	100 µl
	Total volume	3 ml	3 ml

The absorbance was measured after each minute and for 10 minutes at a wave length of 340 nm..

CALCULATIONS:

GST U/L= **ΔA/t * *Vt* *1000 / ε *** ***Vs***

= **ΔA/10 * 3 *1000 / 9.6 * 0.1**

ΔA = change in absorbance
t = time in minutes
*V*t = total volume in milliliters
ε = extinction coefficient
Vs = volume of the sample

3. *Determination Of Packed Cell VolumePCV or Haematocrit using Micro-method.* 99

Capillary tubes 75mm. in length and having an internal diameter of about 1mm were required.They can be obtained plain or coated inside with 2 IU Internati onal Unit of heparin.The latter type is suitable for the direct collection of capillary blood.

The blood was allowed to enter the tube by capillarity,leaving at least 15mm. unfilled.Then the tube was sealed by wax. Centrifugation for 5 minutes,then measuring the PCV using a reading device.

4. *Method for preparation of anticoagulant*. 100

This anticoagulant consists of a mixture of two parts of Potassium Oxalate) and three parts of Ammonium Oxalate).Mixing 1% solution of Potassium Oxalate 0.4 ml . and 1% solution of Ammonium Oxalate 0.6 ml. in a test tube.Evaporation until dryness in the incubator.This amount of oxalates was sufficient to prevent coagulation of 5 ml. of blood.

5.Histopathological examination.10 1

The examination started from handling of the biopsy,fixation,wax embedding,microtome slicing,staining and examination under light microscope.

CHAPTER THREE

RESULTS and DISCUSSION

Results and Discussion

1.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 of the control group and it revealed a significant difference with whole blood GSH in control group $p<0.01$ table.5.

Table5:The mean whole blood GSH in bladder cancer patients in comparison to control group.

Group	Mean GSH mg/dl	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 oxidative stress occurring in the tumor cell 61,70.

On the other hand the GSTGlutathione -S-Transferase) level in erythrocytes of bladder cancer patients had shown a noticeable decrease in comparison to the enzyme level of erythrocytes in control group,also there was a significant difference between the erythrocyte enzyme level in bladder cancer patients with the enzyme level of control group $p<0.01$ table.6.

The significant decrease in GST level is due to its consumption 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 cell96.

Table6:The mean erythrocyte GST level of bladder cancer patients in comparison to control group.

Group	Mean GST (U/L	S.D. U/L	P value
Patients	1.37	1.57	0.003
Control	2.42	2.30	

2.Types and grades of tumors in patients with bladder cancer:

The research revealed presence of different grades and two types of bladder cancer among the patients{n=67}as shown in fig.2, {48}patien ts with grade II TCC, papillary formation with increase number of layers and moderate atypia fig.3 .{10}patients with grade I TCC, finger like projections with vascular core ,low number of layers and mild atypia of the transitional cells fig.4 .{8}patients with grade III TCC, no papillary formation but solid growth and moderate atypia,large nuclei some with clear cytoplasm,still the slide shows vascular core(fig.5 .Single case of SCC, ,malignant squamous cells with keratin formation and keratin nest pear ls formation,cells show large nuclei and prominent nucleoli in the outer layer with flat inner layer(fig.6 .The low incidence of SCC presented in the study can be related to the infection with Bilharziasis which is found in our country mostly in the southern parts while in the middle Euphrates area it is less likely to occur. Bilharziasis causes chronic inflammation and frequent metaplasia which changes the epithelium from transitional to squamous.While the transitional type of carcinoma is the dominant one in our country due to the effect of smoke,benzene derivatives and organophosphorus agents102.

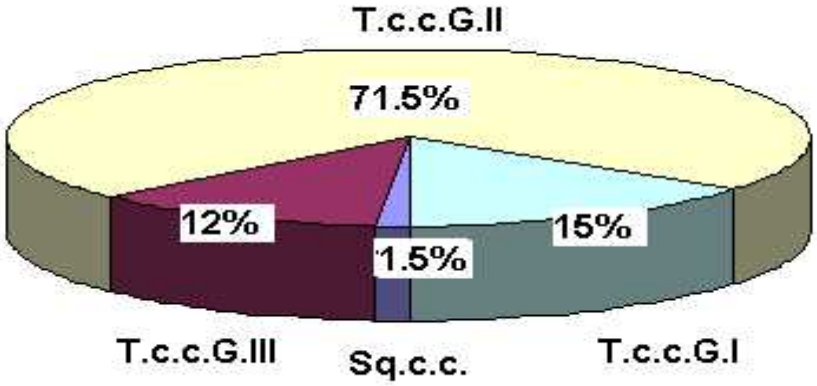


Fig.2 The grading distribution of bladder cancer patients in relation to number of patients.

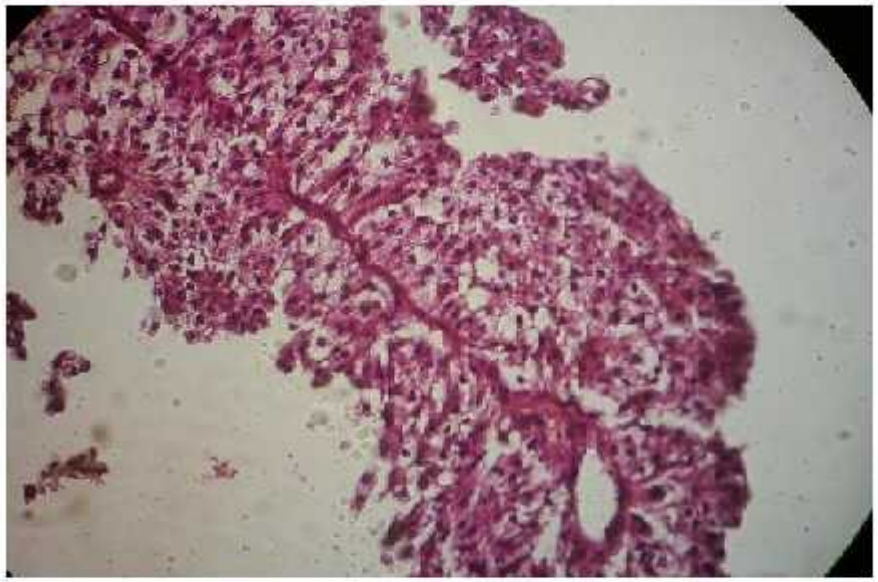


Fig.3:Histopathological section of gr ade II TCC(400 X enlargement factor)

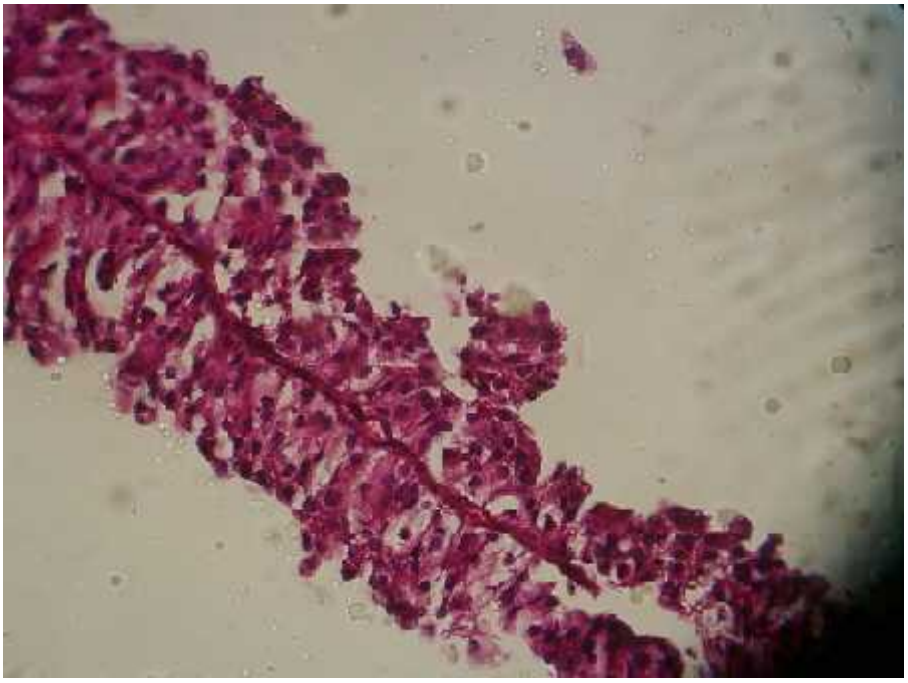


Fig.4:Histopathological section of grade I TCC(400 X enlargement factor)

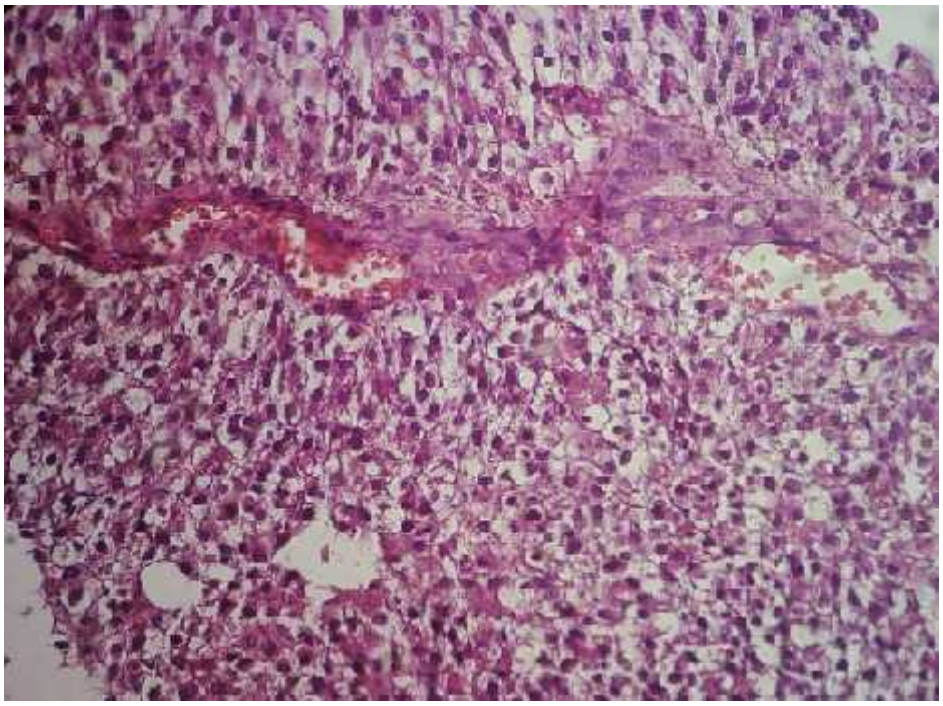


Fig.5:Histopathological section of grade III TCC(400 X enlargement factor)

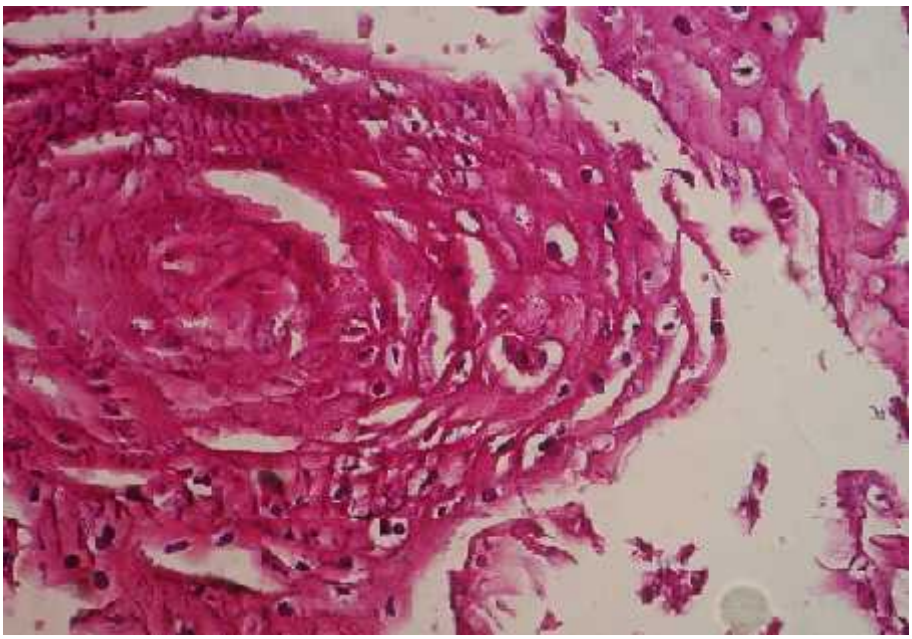


Fig.6:Histopathological section o f SCC(400 X enlargement factor

The mean GST erythrocyte level for bladder cancer patients revealed a decrease in all grades but the marked decrease was in grade III followed by grade I and then grade II.There is a significant difference between the level of GST in erythrocytes in patients with grade I TCC and the control

groupp=0.038,which is nearly equal to the significant difference of the enzyme in erythrocyte between patients with grade III TCC and the control groupp=0.039,while the significant di fference between the enzyme level in patients with grade II TCC and control group was much more higher than that in grades I and III($p<0.01$ table.7.

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.

Table7:The mean erythrocyte GST level in bladder cancer patients concerning the grade of the tumor.

Group	Mean GSTU/L	S.D.	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 tum or starts to fight aggressively causing very low levels of reduced GSH in whole blood with the advance in growing and development of the tumor the GSH pool starts to replace the consumed portion and continue to produce excessive amounts of GSH to face the development of the tumor,in this way there is a gradual increament 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 table.8.

Table8:The mean GSH whole blood level in bladder cancer patients concerning the grade of the tumor.

Group	Mean GSH mg/dl	S.D.	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
Control	37.0	6.5	

3. Exposure to chemicals and antioxidants:

Bladder cancer patients with positive exposure to chemicalspeople who were exposed to chemicals due to their occupation or residency area near factories or refineries were 46 while those with negative exposure were 21 as in fig.7.The former patientspositively exposed to chemicals had shown a GST erythrocyte level of significant difference with the GST erythrocyte level of controls p<0.01.On the other hand bladder cancer patients with negative exposure to chemicals presented a GST erythrocyte level of significant difference with that enzyme level in controls p<0.05, but less significant than positively exposed patients,as shown in table.9.These results for exposure of patients to chemicals are explained by the effects of anyline dyes,tobacco,aromatic hydrocarbons and organophosphorus agents on the antioxidant defense body system.

Table9:The mean erythrocyt e GST level of bladder cancer patients in relation to chemical exposure.

Group	Mean GST (U/L	S.D.	P value
Patients (+ve exposure	1.37	1.47	0.006
Patients (-ve exposure	1.39	1.8	0.04
Control	2.42	2.3	

The reduced glutathione GSH whole blood level i n patients positively exposed to chemicals presented with significant difference in relation to whole blood GSH in control group p=0.0005,the negatively exposed patients also had a significant difference in their whole blood GSH level in relation to the whole blood GSH in control group p<0.01 but of less significance than that of the positively exposed patients table.10.

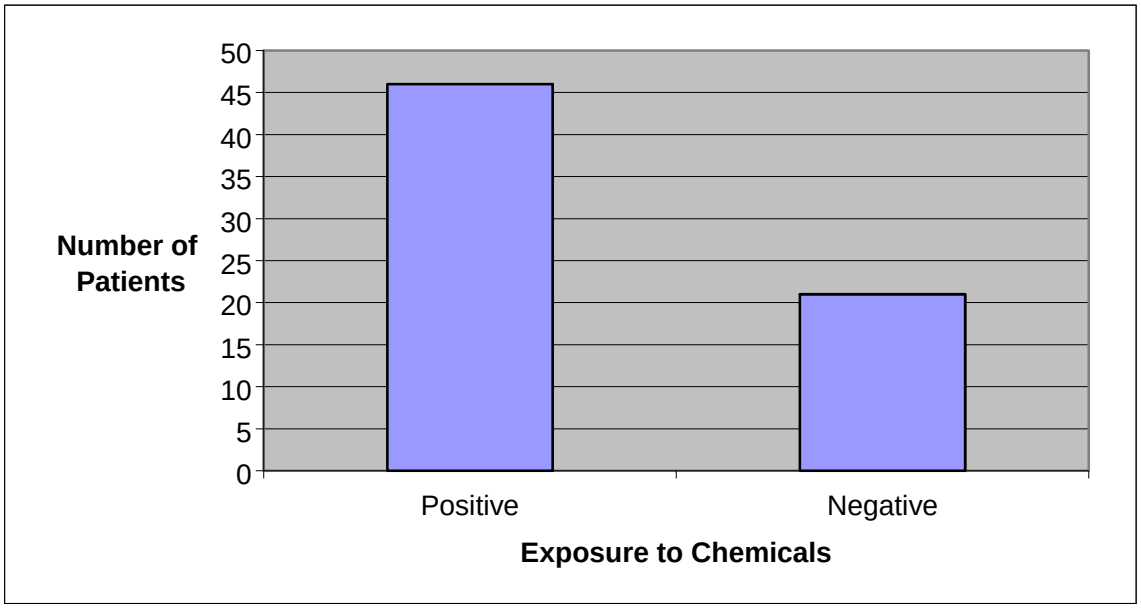


Fig.7 Number of exposed and non exposed bladder cancer patients to chemicals.

Table10:The mean le vel of GSH in whole blood of bladder cancer patients in relation to exposure to chemicals.

Group	Mean GSH mg/dl	S.D.	P value

Patients (+ve exposure	31.9	6.9	0.0005
Patients (-ve exposure	32.0	7.1	0.005
Control	37.0	6.5	

4.History of urinary stone in bladder cancer patients:

Of the total bladder cancer patients n=67,31patients had a history of urinary stone along the urinary tract , while those with negative history were 36 fig.8.

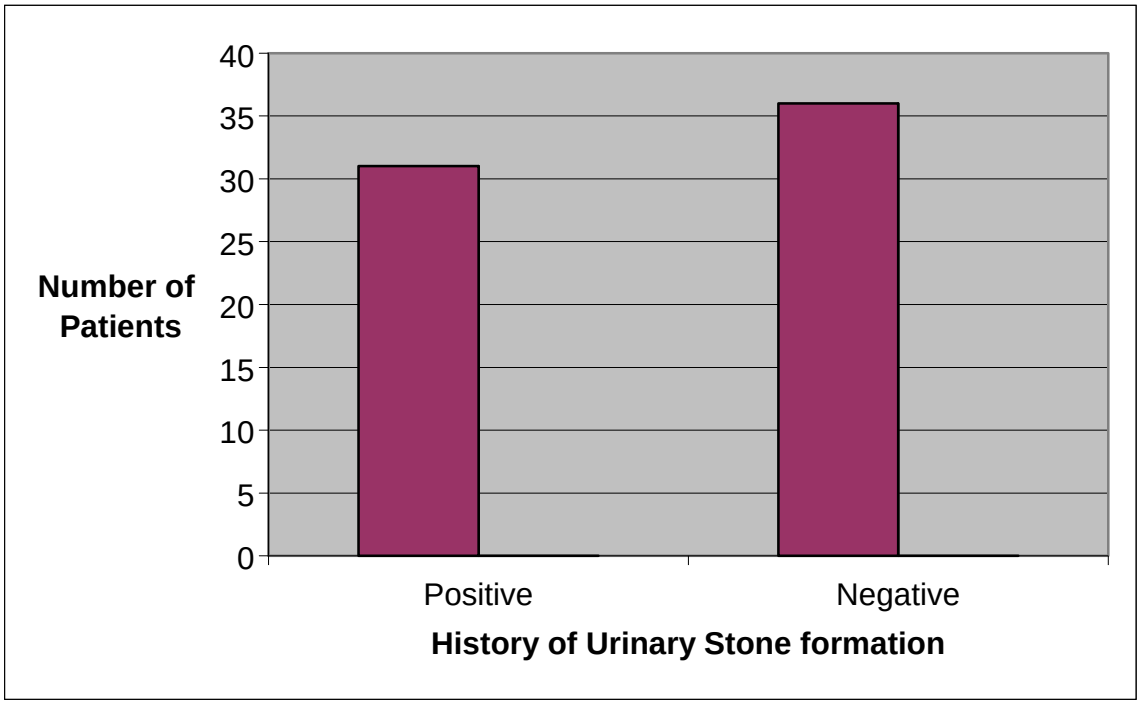


Fig.8Number of bladder cancer p atients in relation to history of urinary stone formation .

5.Residence area effect in bladder cancer patients:

The patients who resident the rural area formed 49% of the total bladder cancer patients which is nearly the same percentage as that for the urban area which formed 51% of the total patients fig.9.

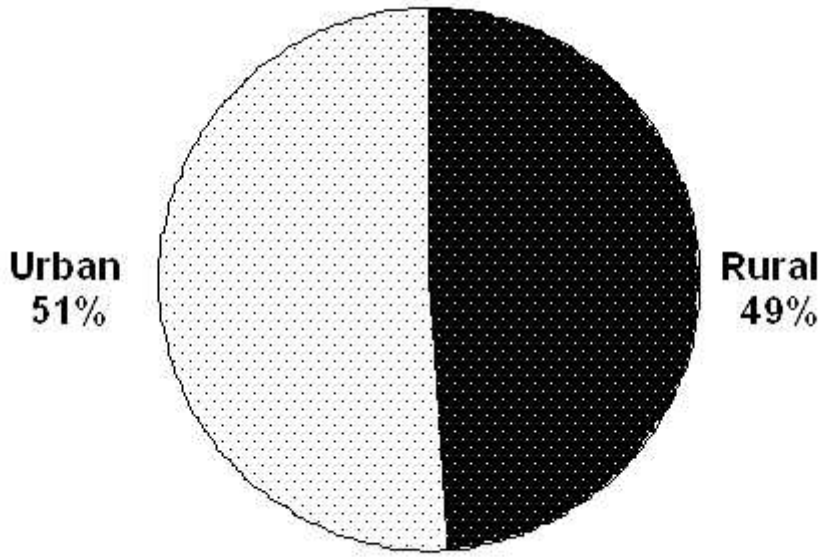


Fig.9 Percentage of bladder cancer patients according to their residence in rural and urban areas

The urban bladder cancer patients presented a marked decrease in their mean whole blood GSH in comparison to that in control urbans,although the rural bladder cancer patients had a decrease in their whole blood GSH in comparison to whole blood GSH in control rurals but it is less marked than that reduction of whole blood GSH in urban bladder cancer patients,which can be explained by the notorious effect of crowding in urban areas in association with the air pollution from the exhaust of the oil refineries,factories and car engines 72.In the rural areas there is much more chance for the availability of pure air by the presence of trees,palms and other different types of plants.To the contrary of these good conditions and natural facilities to face the cancerous agents a problem arises here which is the use of organophosphorus agents insecti cides and pesticides to protect their farms from the harmful insects or pests.The side effects of these agents

are very harmful as they induce cancer specially due to the wrong way of applying these agents to the plants by making solutions in containers and throwing the solution by their hands on the plants103,104.

The urban bladder cancer patients had shown a significant difference in their GSH whole blood level in relation to the GSH whole blood level in control group $p<0.05$.The rural bladder cance r patients also had shown a significant difference in their whole blood GSH level in relation to that of the control group $p<0.05$ table.11.

Table11:The mean whole blood GSH level in bladder cancer patients in relation to the residence in urban and rural areas.

Group	Mean GSH (mg/dl	S.D.	P value
Rural patients	32.8	7.3	0.03
Rural Control	36.1	5.1	
Urban patients	31.0	6.4	0.003
Urban Control	38.4	7.1	

The bladder cancer patients from rural areas had shown a significant difference in their erythrocyte GST in relation to the erythrocyte GST of controls $p<0.05$,while the bladder cancer patients from urban areas had shown non significant difference in their erythrocyte GST level in comparison to the enzyme level in controls $p>0.05$ table.12.

Table12:The mean erythrocyte GST level in bladder cancer patients in relation to the residence in urban and rural areas.

Group	Mean GST U/L	S.D.	P value
Rural patients	1.35	1.39	0.005
Rural Control	2.49	1.90	
Urban patients	1.40	1.76	0.08
Urban Control	2.64	3.60	

6.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.10.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 3:1which 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 patientsn=10 had shown a mild decrease from the GST erythrocyte of controlsn=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 femal es had shown non significant difference in relation to GST erythrocytes of control group $p>0.05$,While the GST in erythrocytes of male patients revealed a significant difference in comparison to the enzyme level in control group $p<0.05$ table.13.

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 GST105.

Table13:The mean GST erythrocyte lev el in bladder cancer patients in relation to their sex.

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

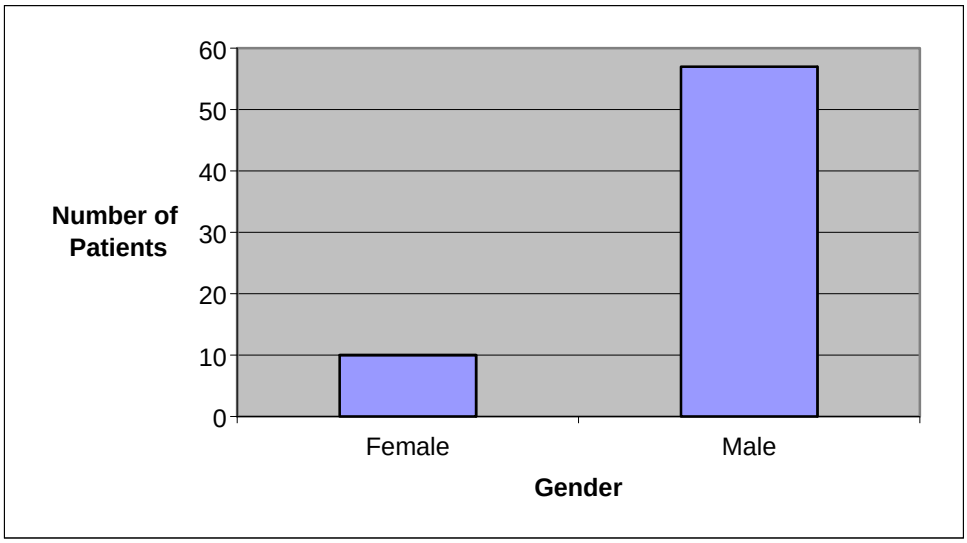


Fig.10 N umber of bladder cancer patients in relation to their sex.

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 sifnificant difference than GSH of whole blood in control group $p<0.05$ table.14.

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 estrogen during the menstrual cycle related to luteal phase and follicular phase(106,107.

Table14:The mean GSH whole blood level in bladder cancer patients in relation to their sex.

Group	Mean GSH mg/dl	S.D.	P value
Female patients	31.14	8.37	0.04
Female control	38.00	4.80	
Male patients	32.11	6.73	0.001
Male control	36.81	6.84	

7.Smoking effect on antioxidants in bladder cancer patients:

The smokers among bladder cancer patients were 84%,while the non smokers were 16% fig.11.

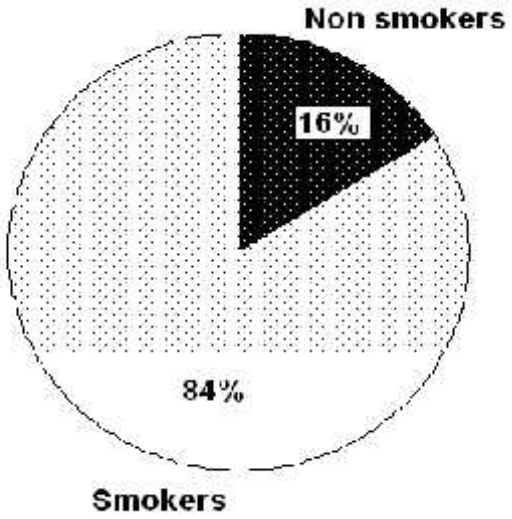


Fig.11The per centage of smokers and non smokers among bladder cancer patients.

There was a decrease in mean GST erythrocyte level of smoker patients from the mean GST erythrocyte of control group 0.7 U/L,which equals to ten folds more than the decrease shown by mean GST erythrocyte of non smoker patients compared to enzyme level of controls 0.07 U/L.The erythrocyte GST level in smoker patients had shown non significant difference in relation to the enzyme level in control group $p>0.05$.On the other hand, the GST erythrocyte level in non smoker bladder cancer patients also showed non significant difference with the enzyme level in erythrocytes of control group $p>0.05$ table.15.

Table15:The mean GST erythrocyte level in bladder cancer patients concerning smoking habit.

Group	Mean GST U/L	S.D.	P value
Smoker patients	1.71	3.82	0.16
Control	2.42	2.30	
Non smoker patients	2.35	2.18	0.4

Regarding the mean GSH in whole blood of smoker patients,it had shown a marked decrease from the level of whole blood GSH in control group6mg/dl,while the mean GSH in non smoker patients revealed less marked decreament from the mean GSH in control group2.3mg/dl.The GSH whole blood level in smoker bladder cancer patients had a very significant difference in relation to whole blood GSH of control group $p<0.05$,while the GSH in whole blood of non smoker bladder cancer patients had no significant difference in relation to the whole blood GSH in control group $p>0.05$ table.16.

Table16:The mean whole blood GSH in bladder c ancer patients in relation to smoking habit.

Group	Mean GSH (mg/dl	S.D.	P value
Smoker patients	30.93	8.01	0.0001
Control	37.00	6.50	
Non smoker patients	34.70	6.65	0.1

These results can be explained by the effect of smoking by its content of tar and nicotin to act as toxic free radicals that consume most of antioxidants in the defense mechanism and detoxification process70, such as GSH and GST,so the smokers will complain more of decrease in antioxidants than those non smoker patients who have better defense line represented by those detoxifying xenobiotics 108.

8.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.17.

Table17: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 o f 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% . These results of PCV can be explained by the effect of smoking which in turn causes secondary polycythemia,although some results of PCV may reflect a debilitating state in which the chronic illness had lead to anemia(109.

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%.

9.Age and antioxidants 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 fig.12.

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 organism110.

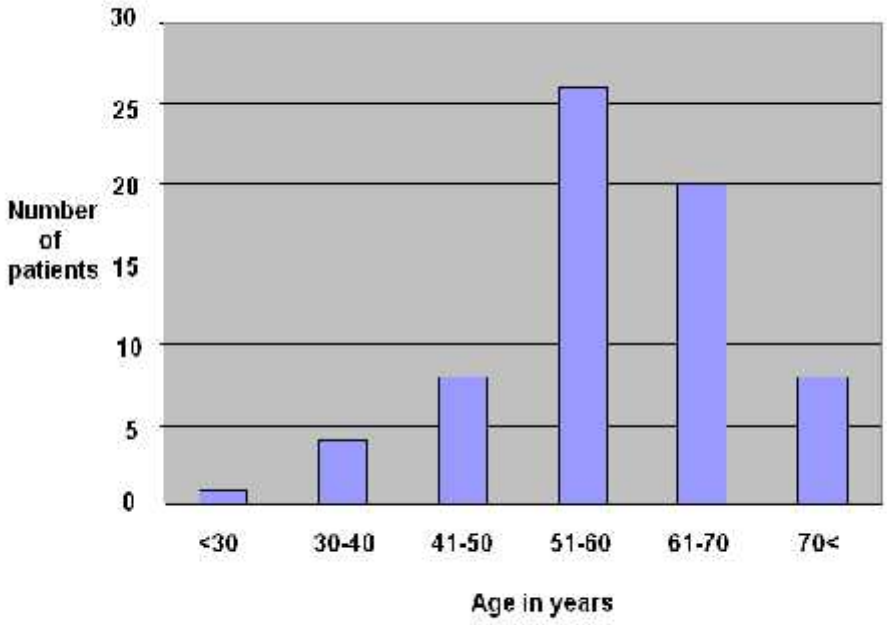


Fig.12The age group distribution of bladder cancer patients .

10.Size and Site of tumor in urinary bladder:

The size of the tumors measured by ultrasonography revealed a peak number of patients in the size between 20 -50 mm. represented by 40 patients,while the rest of the patients distributed nearly equally in the sizes below 20 m m. -{14}patients and above 50 mm. -{13}patients fig.13.

The size of the tumor had an effect on GSH,so the GSH whole blood level decreased with the increase in tumor size,while the GST had shown an increase in its erythrocyte level with the increase in size of the tumor table.18.These results are nearly similar to the effect of tumor size on antioxidants in cervical carcinoma,enzymatic and non enzymatic components of antioxidant defenses were most severely damaged in cases of large tumors which suggested a specific suppression of adaptation systems by malignancies 111.These results can be related to the consumption of more GSH with the increase in size of the tumor,while the increase of GST is to overcome the consumption of GSH by availability of the enzyme to detoxify much more of the toxic free radicals.

Table 18:The effect of tumor size on the level of GSH and GST in bladder cancer patients.

Parameter	***Size of the tumor***		
	0-20 mm.	**21-50mm.**	**>50mm.**
GSH in mg/dl*	37.35±7.6	31.38±6.69	31.71±4.38
GST in U/L *	1.07±0.78	1.17±1.02	1.56±2.49

***Results are presented as mean ± standard deviation.

Regarding the anatomoical sites, in which the tumors were grown in bladder cancer patients,they were presented mainly in the right and the left side of the urinary bladder while the least were presented with diffuse tumor growth fig.1 4.The site had shown non significant difference in relation to GSH and GST.

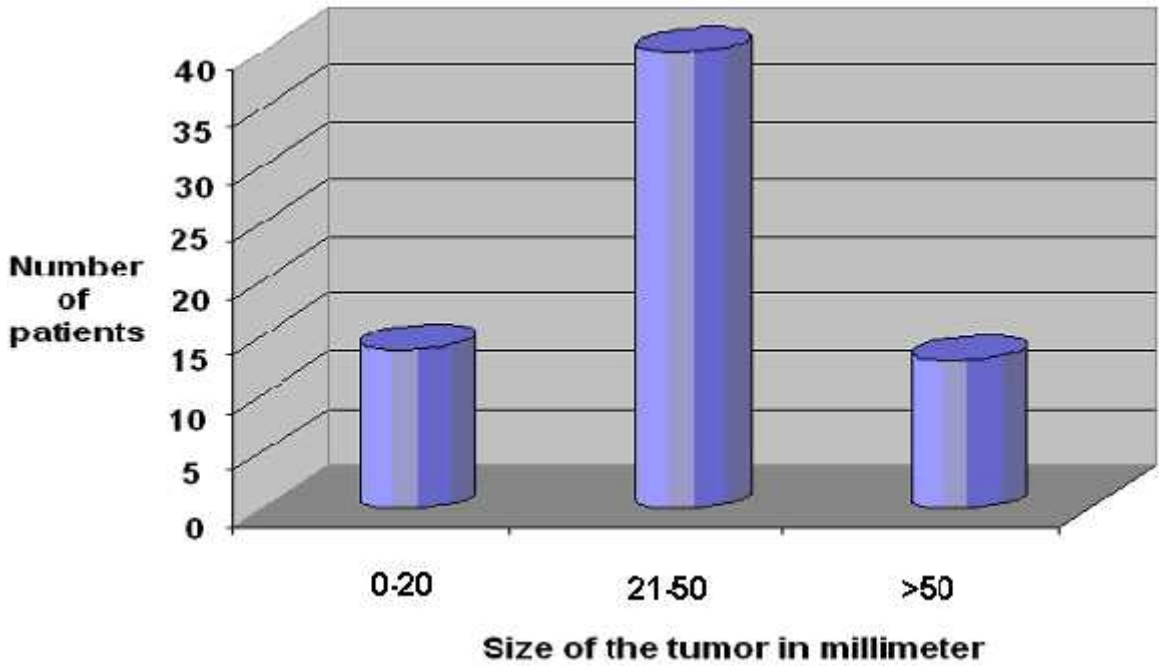


Fig.13The tumor sizes in relation to the number of bladder cancer patients.

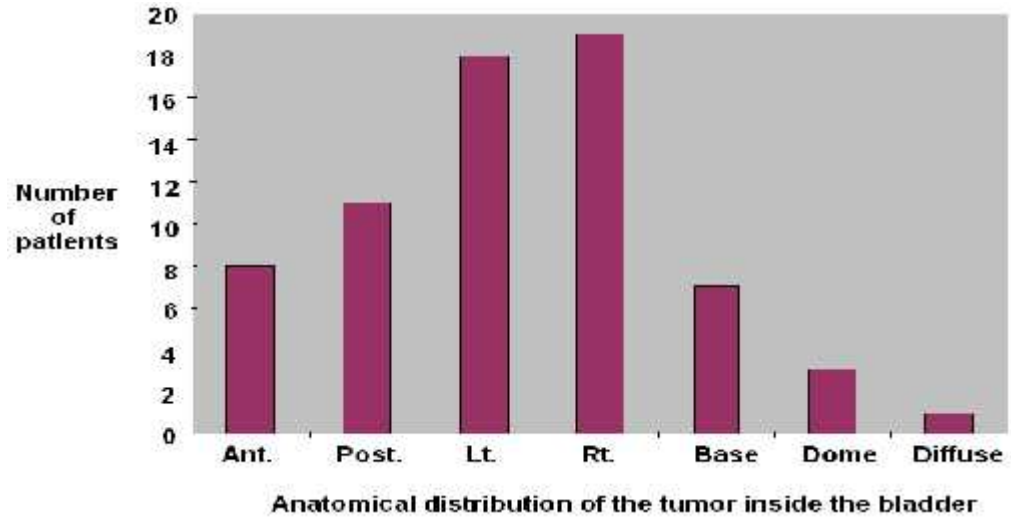


Fig.14The anato mical distribution of the tumors in the urinary bladder in bladder cancer patients.

11.Family history of tumor in bladder cancer patients:

The family history of tumors for bladder cancer patients were significantly negative i.e. 91% of bladder cancer pa tients presented with negative family history of tumors,while 9% of bladder cancer patients presented with positive family history,as shown in fig.15.

The patients who presented with positive family history had a non significant difference of their GST in relation to the control group $p>0.05$,while the patients with negative family history had a significant difference in their GST in comparison to that of the control group $p<0.05$ table.19.On the other hand, there was a significant difference betwee n the GSH level of the patients with positive family histrory in comparison to that of the control group $p<0.01$.Also there was a significant difference between the GSH of the patients with negative family history and the GSH of the control group $p<0.01$ table.20.

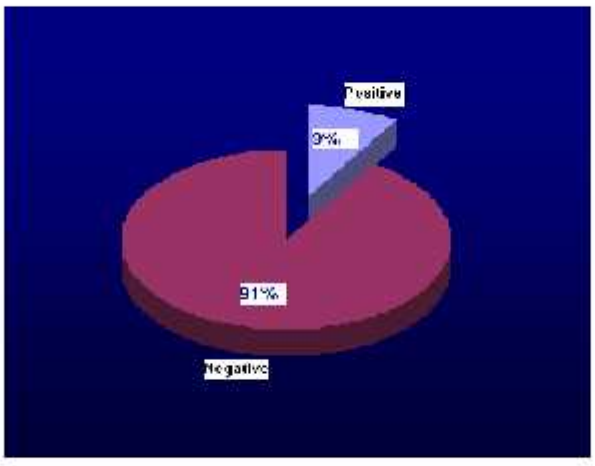


Fig.15The percentage of tumor family history in bladder cancer patients.

Table 19:The mean GST level in bladder cancer patients with positive and negative family history of tumor in relation to the control group.

Group	Mean GST (U/L	S.D.	P value
Patients with positive family history of tumors	0.98	1.01	0.07
Control	2.42	2.3	

Patients with negative family history of tumors	1.46	1.62	0.009

Table 20:The mean GSH level in bladder cancer patients with positive and negative family history of tumor in relation to the control group.

Group	Mean GSH (mg/dl	S.D.	P value
Patients with positive family history of tumors	30.11	5.6	0.009
Control	37.0	6.5	
Patients with negative family history of tumors	32.15	7.06	0.0005

12.Geographical distribution for bladder cancer patients in Babylon Governorate:

The highest percentage of bladder cancer patients were found in Hilla city which is the central part 37%.This is related to the pollution of cars engines and factories,the southern east part presented 23% of the patients which is a rural area,this is explained with the use of pesticides and insecticides,western part formed 19% of our bladder cancer patients,southern west area had 9% of total bladder cancer patients,northern part presented with 7% of total percentage of patients,while the least area with only 5% of total cases was the southern part fig.16 .

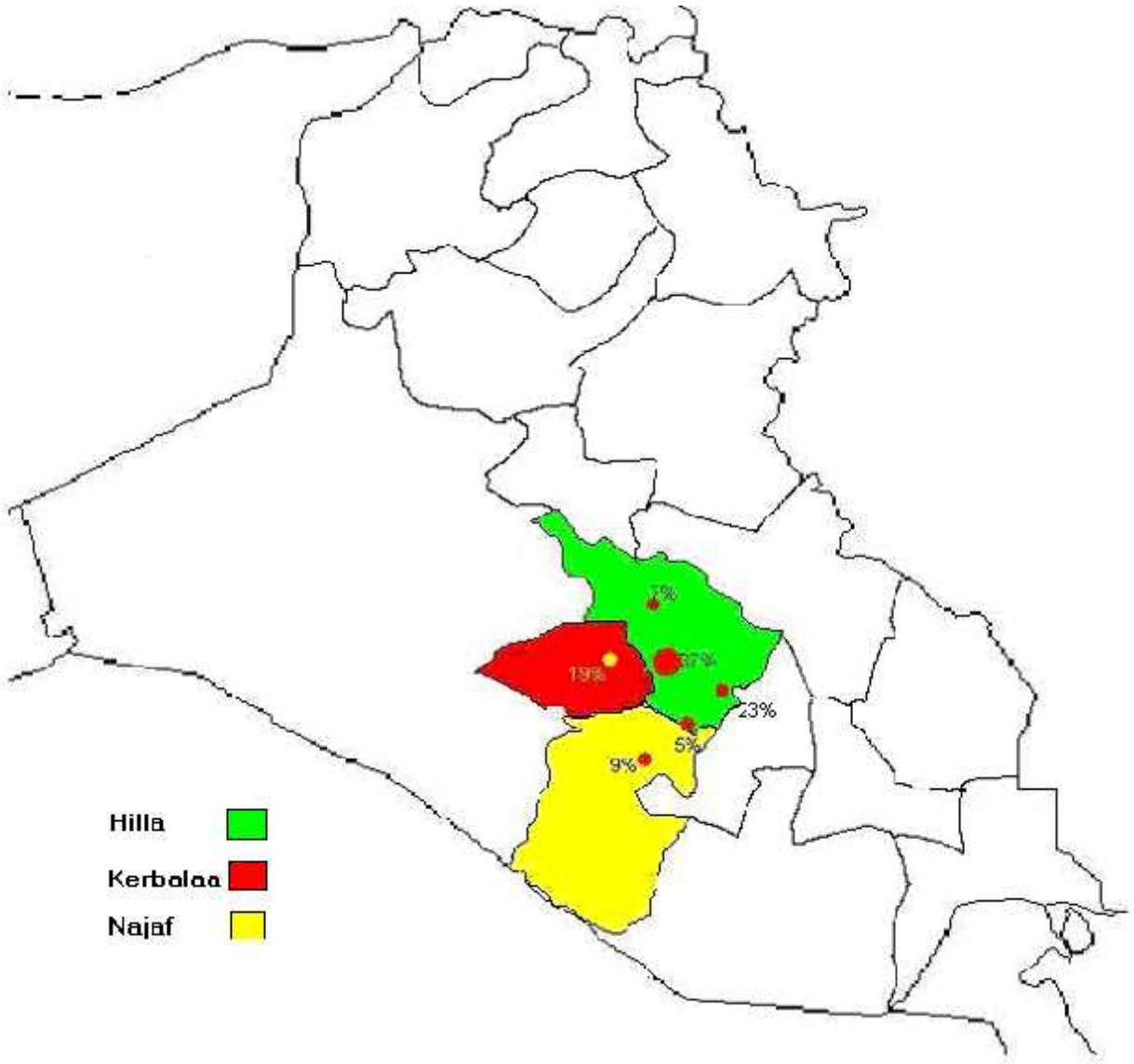


Fig.16Map diagram:Geographical distribution for bladder cancer patients in and around Hilla city according to our results for patients attended Hilla Teaching Hospital.

CONCLUSIONS

and

RECOMMENDATIONS

Conclusions and Recommendations

Conclusions

1-In patients with bladder cancer both GST and GSH levels were decreased.

2-There was an increase in the level of GSH with the increase in tumor grade.

3-The increase in tumor size lead to increase in GST level and decrease in GSH level.

4-The highest number of tumors was in the right and left sides of the bladder.

5-Positive family history of tumor lead to a decreased levels of GSH and GST.

6-Smoking lead to a marked decrease in the level of GSH in bladder cancer patients..

7-GSH had shown decreased levels in female bladder cancer patients while GST had shown decreased levels in male bladder cancer patients.

8-Chemical exposure in bladder cancer had a significant difference in relation to both GST and GSH.

9-Bladder cancer patients from urban areas had decreased levels of GSH while those patients from rural areas had decreased levels of GST.

Recommendations

1-Further studies dealing with the level of antioxidants in the urothelial tissue are important in order to be compared with their blood levels.

3-Specified tumor centers must have clear,informative and true recordings in order to help in planning correctly and successfully for the future in this field.

4-Advanced techniques and new machines have to be introduced to this work such as HPLC,PCR and atomic adsorption in order to discover the isoenzymes and their relation to the tumor grade and size.

5-Future studies on the conditions of the enzyme reaction specially those related to energy and the conjugation with toxic free radicals.

CHAPTER FOUR

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الخلاصة:

تمت دراسة التغييرات في انزيم الكلوتاثايون-أس-ترانسفريز و الكلوتاثايون المختزل في دم المرضى المصابين بسرطان المثانة البالغ عددهم سبعة و ستين مريضا (١٠ نساء و٥٧ رجلا) اما مجموعة السيطرة فقد كانت ٣٦شخصا .تم أخذ عينات الدم من المرضى في مستشفى الحلة التعليمي العام خلال الفترة (الاول من نوفمبر ٢٠٠٤ لغاية الخامس عشر من اغسطس ٢٠٠٥). كان هؤلاء المرضى يعانون من تبول دموي و كانت حالتهم تستدعي اجراء فحص ناظور المثانة من خلال الفحص السريري أو الشعاعي.

تم تشخيص المرضى بسرطان المثانة من خلال اجراء التحليل النسيجي لخزعات تم اخذها من بطانة المثانة بواسطة الناظور.

سجلت الدراسة وجود اكبر نسبة من سرطان المثانة ذو النسيج الطلائي الانتقالي من الدرجة الثانية،بينما كانت هنالك حالة واحد من سرطان المثانة ذو النسيج الحرشفي.

كما بينت الدراسة وجود تأثير لكل من:{التدخين،الجنس،التعرض للمواد الكيمياوية،و وجود التأريخ المرضي للسرطان أي ورم سرطاني) في العائلة} على مستويلت انزيم الكلوتاثايون-أس-ترانسفريز و الكلوتاثايون المختزل بدرجات مختلفة.كما كان مستوى حجم الخلايا المضغوطة مرتفعا لدى المرضى بسبب التدخين مؤديا الى فرط الكريات الحمر الثانوي.

كما وضحت الدراسة ارتفاع عدد الرجال المصابين بسرطان المثانة نسبة للنساء المصابات ١:٥ ،و كان غالبية المرضى من الفئة العمرية المحصور بين (٥١-٦٠) سنة،اما التعرض للمواد الكيمياوية فقد كان موجودا لدى ٦٨٪ من المرضى.

و من الجدير بالذكر ان ٨٤ ٪ من المرضى كانوا من المدخنين ولم يكن لدى ٩١٪ من المرضى أي تأريخ في العائلة لأية اورام خبيثة.

و بذلك نستنتج ان سرطان المثانة يؤثر بشكل واضح و يؤدي الى نقصان في مستويات الكلوتاثايون المختزل و انزيم الكلوتاثايون-أس-ترانسفريز في الدم.

بسم الله الرحمن الرحيم

و من النّاس و الدّوابّ و الأنعام مختلفٌ ألوانُهُ كذلكَ

[redacted]

[redacted]

إنّما يَخشى اللهَ من عبادهِ العُلَمَؤا إنّ اللهَ عزيزٌ غفور

صدق الله العظيم

سورة فاطر-الاية ٢٨

انزيم الكلوتاثايون-أس- ترانسفريز و الكلوتاثايون المختزل كدول ورمية لسرطان المثانة

رسالة مقدمة الى
كلية الطب – جامعة بابل
كجزء من متطلبات نيل درجة الماجستيرفي
علم الكيمياء الحياتية السريرية

من قبل الطالب

رافد سالم عوض

بكلوريوس طب و جراحة عامة - جامعة النهرين

١٤٢٦هجرية ٢٠٠٦ميلادية