ABSTRACT:

Bladder cancer is one of the most common disease treated by urologist\(^1\). In the united state, bladder carcinoma is the forth most common cancer in men after prostate, lung and colorectal cancer. In women it is the eighth most common cancer. Bladder carcinoma is more than 2.5 times common in men than in women and accounts for 2.9% of all cancer death in men and 1.5% in women.

Objectives: studying the role and efficacy of reduced glutathione as a tumor marker in diagnosis and follow up patients with urinary bladder carcinoma.

Patients and methods: Fifty seven patients with proved carcinoma of the urinary bladder were studied for the change in their GSH(reduced glutathione) levels in the blood. The studied patients were 47 males and 10 females and the control group was 30 persons of different ages and sexes, those patients were complaining from hematuria, the diagnosis of bladder carcinoma was made by Histopathological study of the biopsy taken from the bladder during cystoscopy. All patients had transitional cell carcinomas and no squamous, adenocarcinoma or other types had been found. The study had evaluated the effect of smoking, sex, residency, tumor size and grade on the level of GSH in the blood.

The mean age of the patients was 52.5 years, 44 patients (77%) were smokers, 13 patients (23%) were non smokers, 31 patients (54%) had history of urinary stone. Two patients (3.5%) had family history of carcinoma of the bladder in the first degree relatives. Four patients (7%) had second degree relatives for bladder cancer. 47 patients (82%) were males while 10 patients (18%) were females, male to female ratio was 4.7:1. The highest percentage of the patients 54% were from the periphery of ALmuthana Governorate while 46% were from the centre. 68.4% had Grade II, 17.5% had Grade I, and 14.1% had Grade III tumor. Regarding tumor size; 40 patients (70%) less than 2 cm, 12 patients (21%) between 2 and 5 cm[2-5cm], and 5 patients (9%) had tumor size more than 5cm.

Results: The study shows that carcinoma of the urinary bladder causes significant decrease in the blood levels of reduced Glutathione(GSH) in both sexes, with females had relatively higher levels than males. Smoker patients had lower GSH levels than non smokers, Urban
patients had lower blood GSH levels than rurals. Regarding the grade and size of the tumor, the study revealed that blood level of reduced Glutathione increases with the increase of the tumor grade while decreases with the increase in the tumor size.

INTRODUCTION:

CARCINOMA OF THE URINARY BLADDER:

Bladder cancer is one of the most common disease treated by urologist. In the united state, bladder carcinoma is the forth most common cancer in men after prostate, lung and colorectal cancer. In women it is the eighth most common cancer. Bladder carcinoma is more than 2.5 times common in men than in women and accounts for 2.9% of all cancer death in men and 1.5% in women, male has higher 5-year survival rate than female.

Bladder carcinoma can occur at any age even in children, it is generally a disease of middle aged or elderly persons and the incidence increases with age in both sexes with the median age at diagnosis for transitional cell carcinoma being 69 years for males and 71 years for females.

The mortality from Bladder carcinoma is also higher in elderly patients, younger patients appear to have a more favorable prognosis because they present more frequently with superficial low grade tumors, however the risk for disease progression is the same grade for grade in younger as in older patients.

ETIOLOGY AND RISK FACTORS:

Transitional cell carcinoma as for most other cancers seems to be dependant in its development on a combination of genetic and environmental factors.

Factors reported to be causally related to the development and progression include occupational exposure to chemicals like aniline dyes, chlorinated aliphatic hydrocarbons, chemical dyes in rubber industries.

Occupations reported to be associated with increased risk of bladder carcinoma include: painters, truck drivers, drill press operators, leather workers, metal workers, dry cleaners, paper manufacturers, physicians, barbers or beauticians and dental technicians.

Other risk factor is cigarette smoking, with four fold increase incidence of bladder carcinoma in smokers than non smokers, and it has been estimated that one third of bladder carcinoma cases may be related to cigarette smoking and the reduction of this risk down to the baseline take nearly twenty years after cessation.

Other risk factors are analgesic abuse, artificial sweeteners, pelvic irradiation, chemotherapeutic agents specially cyclophosphamide which have nine-fold increased risk for developing bladder carcinoma.

Chronic cystitis due to presence of chronic indwelling catheter, calculi or schistosomal chronic cystitis which usually related to development of squamous carcinoma, children with birth defects such as ectopia vesicae have increased risk of developing bladder carcinoma.

PATHOLOGY:

More than 90% of bladder cancer are of transitional cell type. Other types like squamous cell carcinomas account for 5-10%, adenocarcinoma account for less than 2%, Mixed carcinomas which account for 4-6% of all bladder cancer and are composed of combination of transitional, glandular, squamous or undifferentiated patterns. Non epithelial bladder tumors which account
approximately 1-5% of all bladder tumors like neurofibromas, pheochromocytomas, primary lymphomas, angiosarcomas, leiomyosarcomas and rhabdomyosarcomas².

Transitional cell carcinoma most commonly appears as papillary lesion, less commonly they may be sessile, nodular or ulcerated¹³.

Tumors are graded into grade(I) well differentiated tumors, grade(II) moderately differentiated tumors and grade(III) poorly differentiated tumors according to many features like number of epithelial layers, urothelial architecture, cell size, nuclear size and shape, nuclear abnormalities and number of mitosis if present¹⁴. In practice however Transitional cell carcinoma tend to occur in two principal forms low grade superficial and high grade invasive tumors¹⁵.

Figure(1) TCC. In situ

Figure(2) TCC. In situ
Figure (3) TCC. Low grade

Figure (4) TCC. Low grade

Figure (5) TCC. High grade
Figure (6) TCC. High grade

Figure (7) Adenocarcinoma of the urinary bladder

Figure (8) Adenocarcinoma of the urinary bladder
Figure (9) Squamous cell carcinoma of the urinary bladder

Figure (10) Squamous cell carcinoma of the urinary bladder

Figure (11) TCC. Of the Mixed type
DIAGNOSIS:

1. History; of painless hematuria which occur in more than 85% of patients, in reality all patients with cystoscopically detectable bladder carcinomas have at least microscopic hematuria. Other symptoms like irritative bladder symptoms like frequency, urgency and dysuria usually associated with diffuse carcinoma in situ or invasive bladder carcinomas. Very rarely symptoms of advance disease like weight loss and bone pain due to metastasis to the bone. It had been estimated that at time of diagnosis, 85% of bladder carcinomas are localized to the bladder and 15% have spread to regional lymph nodes or distant sites.

2. Microscopic cytology; of the urinary sediment or bladder wash which is highly specific test but less sensitive in detecting low grade malignant cell.

3. Flow cytometry; which measure the DNA contents of the cell again its more sensitive in high grade tumors than in low grad tumors because DNA abnormalities like aneuploidy, triploidy and tetraploidy are more common in high grade tumors.

4. Image analysis; this technique use computerized controlled fluorescent microscope that automatically scans and images the nucleus of each cell and quantitavely measure the DNA content of each cell. this technique is more sensitive than flow cytometry in detecting low grade tumors with out reduced specificity.

5. Ultrasonography; transabdominal Ultrasonography is safe and available and provide information about the tumor size, site and the upper urinary tract. while transurethral Ultrasonography before and after transurethral resection is helpful in distinguishing superficial from deep muscle invasion and extravesical spread.

6. Intravenous pyelography; unfortunately only big bladder mass can appear as filling defect on Intravenous pyelogram, it also provide information about any associated upper tract lesion and presence of ureteric obstruction.

7. Cystoscopy; all patients suspected to have bladder cancer should have careful cystoscopy and bimanual examination, abnormal areas should be biopsied.

8. Computed tomography; provide information about tumor extension, lymph node involvement and visceral metastasis which help in staging of the disease.
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 and effusions. Tumor markers are represented by small and large molecules such as peptides, proteins, enzymes, hormones, immunoglobulin, mucins, cytokeratins and low molecular weight metabolites. Most tumor markers are incidentally involved in tumorigenesis and are byproducts of malignant transformation. However, measurement 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 elevated in every patient with cancer, especially in early stage 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.

Tumor markers, in clinical practice, can be used in diagnosis of cancer, planning for treatment, staging of the tumor, predicting and monitoring the response to treatment and follow up care to check for recurrence.

Newer tests are performed on voided urine specimen, are currently being developed and validated to determine their ability to reliably predict the presence of bladder cancer. These tests include: the bladder tumor antigen [BTA] tests, determination of urinary nuclear matrix protein [NMP 22], quantification of urinary fibrinogen/fibrin degradation products, identification of Lewis antigen, determination of Telomerase activity in exfoliated cell, bladder tumor fibronectin (BTF), cytokeratins 18 (CK18), tissue poly peptide antigen (TPA) and cancer antigens (CA 19, CA 125). Most of these studies performed to date have evaluated these voided markers in patients with known bladder carcinoma, either primary or recurrent, and have compared the result with those obtained in control group without bladder cancer. Although these tests may, in many clinical situations, compliment traditional evaluation and surveillance techniques, more information on their performance is needed before they are performed routinely. Such exfoliated markers, if they prove to be both specific and sensitive, may play an important role in the initial evaluation and follow up of patients with bladder cancer. In addition to enhancing the detection, such tests may give important information on the natural history of the bladder cancer detected.

ANTIOXIDANTS:

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes. Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. It was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in biochemistry of living organisms.

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells.
THE OXIDATIVE CHALLENGE IN BIOLOGY:

A paradox in metabolism is that while the vast majority of complex life requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell.

The reactive oxygen species produced in cells include hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O$_2^-$).

These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation.

METABOLITES:

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed throughout the body. The action of one antioxidant may depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant therefore depends on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes.

OXIDATIVE STRESS:

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. In many of these cases, it is unclear if oxidants trigger the disease, or if they are produced as a consequence of the disease and cause the disease symptoms.

One case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. Here, low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally cardiovascular disease.
A low calorie diet extends median and maximum lifespan in many animals. This effect may involve a reduction in oxidative stress. Diets high in fruit and vegetables, which are high in antioxidants, promote health and reduce the effects of ageing; however antioxidant vitamin supplementation has no detectable effect on the ageing process, so the effects of fruit and vegetables may be unrelated to their antioxidant contents.

**DISEASE PREVENTION:**

Antioxidants can cancel out the cell-damaging effects of free radicals, and people who eat fruits and vegetables rich in polyphenols and anthocyanins have a lower risk of cancer, heart disease and some neurological diseases. This observation suggested that these compounds might prevent conditions such as macular degeneration, suppressed immunity due to poor nutrition, and neurodegeneration, which are caused by oxidative stress.

Many health food companies now sell formulations of antioxidants as dietary supplements and these are widely used in industrialized countries. These combinations of antioxidants, like the "ACES" products that contain beta carotene (provitamin A), vitamin C, vitamin E and Selenium, or herbs that contain antioxidants - such as green tea. Although some levels of antioxidant vitamins and minerals in the diet are required for good health, there is considerable doubt as to whether antioxidant supplementation is beneficial, and if so, which antioxidant(s) are beneficial and in what amounts.

**GLUTATHIONE:**

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiols group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants.

In healthy tissue, more than 90% of the total glutathione pool is in the reduced form and less than 10% exists in the disulfide form (GSSG). An increased GSSG/GSH ratio is considered indicative of oxidative stress. Glutathione is not an essential nutrient since it can be synthesized from the amino acids L-cysteine, L-glutamate and glycine. The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione S-transferases. This system is found in animals, plants and microorganisms.

Glutathione (GSH) participates in leukotriene synthesis and is a cofactor for the enzyme glutathione peroxidases. It is also important as a hydrophilic molecule that is added to lipophilic toxins and waste in the liver during biotransformation before they can become part of the bile. Glutathione is also needed for the detoxification of methylglyoxal, a toxin produced as a by-product of metabolism. The glutathione S-transferases are a class of glutathione-dependent antioxidant enzymes that show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism.

GSH is known as a substrate in both conjugation reactions and reduction reactions, catalyzed by glutathione S-transferases enzymes in cytosol, microsomes, and mitochondria. However, it is also capable of participating in non-enzymatic conjugation with some chemicals.
In USA Department of Investigational Therapeutics, New York. 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 that in the non-bladder cell lines. GSH was also measured in two types of control tissues: (a) normal bladder tissue from patients with TCC or a history of TCC of the bladder and (b) bladder 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.
(b) higher 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 carcinogenesis.

Hereditary GSH Deficiency:
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 life. 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.

Exogenous Causes of GSH Depletion:
Cigarette smoke contains thousands of different chemical species, and a single puff of cigarette smoke contains trillions of free radicals. 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.

Many pharmaceutical products are oxidants capable of depleting GSH from the liver, kidneys, heart, and other tissues. 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. 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 radicals.

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.
2. Ionizing radiation, whether as X-rays or ultraviolet from sunlight.
3. Tissue injury, as from burns, ischemia and reperfusion, surgery, septic shock, or trauma.
4. Iron overload, as in hemochromatosis and transfusional iron excess.
5. Bacterial or viral infections, including HIV.
6. Alcohol intake is toxic through a number of differing pathways, some of which are free radical/oxidative in character.

AIMS OF THE STUDY:
1. Studying the role and efficacy of reduced glutathione as a tumor marker in diagnosis and follow up patients with urinary bladder carcinoma.
2. Showing the relationship of Carcinoma of the urinary bladder to oxidative stress process considering (Reduced Glutathione) as Biomarkers for this tumor.
3. Illustrating the effects of age, sex, and residency on blood GSH level.
4. Studying the changes in blood GSH level in relation to the tumor size, tumor site and grade of the tumor.
5. Study the effect of smoking on blood GSH level in both patients and control group.

PATIENTS and METHODS:
From February 2015 to October 2019. Fifty seven patients (47 males and 10 females) of mean age 52.5 years were admitted to department of Urology at Al-Hussein Teaching Hospital. Although many patients have been seen in this period suffering the same problems, few of them has fulfilled our exclusion criteria which include: patients with chronic disease like hypertension, diabetes mellitus, chronic obstructive airway disease, chronic liver disease, drinking alcohol, patients on chronic medication like NSAID, steroids and anti-ischemic drugs and patients with previous history of malignancy.

All patients underwent full history including: age, sex, residence, smoking, family history of tumors, history of urinary stones, general examination and abdominal examination. Preliminary investigations have been done which included general urine examination, blood urea, serum creatinine, PCV (packed cell volume), blood sugar, ultrasound examination of the urinary system. They were proved to have bladder cancer as diagnosed by Histopathological study of biopsies taken by Cystoscopy. The indications of Cystoscopy were:
1. Radio logically proved patients with bladder mass with or without hematuria.
2. Patients above 40 years old with gross hematuria and negative radiological examination.
3. Patients above 40 years old with microscopic hematuria more than 3 times and negative radiological examination.

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

The control group were (30), they were healthy people, i.e. 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 our patients (mean age is 55.5 years).

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

Samples from patients and the control group were collected and these samples sent for quantitavely measurement of GSH level in the private laboratory the results analyzed by t-test.
RESULTS and DISCUSSION:

1. GSH in bladder cancer patients:
   The mean 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 blood GSH in control group (p<0.05) (table.1).

   Table(1): The mean blood GSH in bladder cancer patients in comparison to control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean GSH (mg/dl)</th>
<th>S.D. (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>30.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Control</td>
<td>36.0</td>
<td>6.2</td>
</tr>
</tbody>
</table>

   This difference between the mean 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 and to detoxify the toxic free radicals produced by the tumor.

   These results are similar to the results of a prospective clinical study at Baskent University in Alanya hospital in Turkey done on 52 patients with bladder carcinoma and conclude that GSH can be used for tumor detection approach and even as an indicator of biological behavior of the bladder cancer.

2. Types and grades of tumors in patients with bladder cancer:
   The research revealed presence of different grades and one type of bladder cancer among the studied patients [n=57] as shown in (fig.13). 39 patients (68.4%) with grade II TCC, 10 patients (17.5%) with grade I TCC and 8 patients (14.1%) with grade III TCC.
Fig.(13) The distribution of the tumor grades in relation to number of patients.

Regarding the reduced glutathione (GSH) level in the blood, the analysis revealed a decrease in blood GSH in all grades, and there is gradual increase of GSH with the increase grade of the tumor. This mean that the patients with low grade have low level of GSH. Then with the progression to higher grades the antioxidant defense system respond by producing more amounts of antioxidants including GSH. (table 2).

Table(2): The mean blood GSH level in bladder cancer patients in relation to the grade of the tumor.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean GSH (mg/dl)</th>
<th>S.D.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I TCC patients</td>
<td>27.3</td>
<td>7.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Grade II TCC patients</td>
<td>31.1</td>
<td>6.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Grade III TCC patients</td>
<td>32.8</td>
<td>3.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Control</td>
<td>36.0</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

3. Effect of the resident area on blood GSH level:

Thirty one (54%) patients were lived in the rural area while 26(46%) patients were in urban areas. The study show that blood GSH level is more in both patients and controls who lives in rural areas in comparison to patients and controls who lives in urban areas. The urban bladder cancer patients presented a marked decrease in their mean blood GSH in comparison to that in control urban persons, although the rural bladder cancer patients had a decrease in their blood GSH in comparison to blood GSH in control rurals, but it is less marked than that in urban bladder cancer patients(table 3), 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 that cause decrease blood GSH level. While 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. These results are similar to results of study done at Kaohsiang medical University Taiwan on a group of patients with breast cancer84.

Table(3): The mean blood GSH level in bladder cancer patients in relation to the residence in urban and rural areas.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean-GSH (mg/dl)</th>
<th>S.D.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural patients</td>
<td>30.7</td>
<td>7.3</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Gender and bladder cancer:

Regarding the sex of the patients with bladder cancer, 47 patients (82%) were males while 10 patients (18%) were females. This male to female ratio which is 4.7:1, differs from the international figures and this 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 GSH in female patients showed a marked decrease from the mean GSH of control females, which is more than the difference between male patients and control. The mean blood GSH in female patients and female control was higher than that in male (table 4). These results can be related to the effect of testosterone on GSH, because testosterone increases oxidative stress so increase consumption of GSH\textsuperscript{80,81}. In addition to that males are the main working population so have increase risk of exposure to the chemicals and industrial materials and toxins that cause depletion of blood GSH\textsuperscript{73}.

Table(4): The mean blood GSH level in bladder cancer patients in relation to their sex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean GSH (mg/dl)</th>
<th>S.D.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female patients</td>
<td>33.1</td>
<td>7.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Female control</td>
<td>38.2</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Male patients</td>
<td>28.2</td>
<td>7.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Male control</td>
<td>33.7</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

Effect of Smoking on antioxidants in bladder cancer patients:

Forty four patients (77%) were smokers while 13 patients (23%) were non-smokers. The study show that smoking decrease mean blood GSH level in both patients and control groups, the smoking effect is obvious in smoking patients in comparison to non-smoking patients as shown in table 5. These results can be explained by the effect of smoking by its content of tar and nicotine.
as a toxic free radicals that consume most of antioxidants in the defense mechanism and detoxification process 69.

These findings resembles that of a clinical trial done at the Faculty of medicine and surgery in University of Siena Italy; which conclude that: the low level of antioxidants in the blood of a smoker bladder cancer patient may be due to there increased utilization to scavenging toxic free radicals produced by the tumor as well as there sequestration by the tumor cells suggesting that normalization of the levels of antioxidants might be used to reduced tumor malignancy 83.

Table (5): The mean whole blood GSH in bladder cancer patients in relation to smoking

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean GSH (mg/dl)</th>
<th>S.D.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker patients</td>
<td>28.6</td>
<td>8.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Non smoker patients</td>
<td>32.1</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Smoker Control</td>
<td>34.2</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Non smoker control</td>
<td>37.7</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

6. Size and Site of tumor in urinary bladder:

According to ultrasonic and cystoscopic measurement of the tumor size the patients divided into 3 groups: A.(<2cm), B(2—5cm) C(>5cm). Forty patients(70%) were belong to A group[<2cm].Twelve patients(21%) were belong to B group[2-5cm].Five patients(9%) were belong to C group[>5cm].The results show that GSH level decrease with the increase in the tumor size as shown in table 6.

<table>
<thead>
<tr>
<th>Size of the tumor</th>
<th>GSH in (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2cm</td>
<td>32.6 ± 7.6</td>
</tr>
<tr>
<td>2—5cm</td>
<td>30.9 ± 6.6</td>
</tr>
<tr>
<td>&gt;5cm</td>
<td>27.7 ± 4.3</td>
</tr>
</tbody>
</table>

Table (6): The effect of tumor size on the level of GSH in bladder cancer patients.

The size of the tumor has a marked effect on GSH level, with the increase in the tumor size there is decrease in the blood GSH level and there by decrease in the defense mechanism of the body against the oxidative stress and free radicals produced by the tumor, this decrease in the body defense mechanism in turn allow the tumor to progress to more advanced stages . These results are nearly similar to the results of a study performed on deferent types of tumors in 1999 and concluded that (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 82). Regarding the 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. The site had shown non significant difference in relation to GSH.

**CONCLUSION:**

1. In patients with bladder cancer, reduced glutathione can be used as a tumor marker in addition to other marker to determine tumor progression and advancement.
2. There was relative increase in the level of GSH with the increase in tumor grade.
3. The increase in tumor size lead to decrease in GSH level.
4. Smoking lead to a marked decrease in the level of GSH in bladder cancer patients.
5. Male patients have lower blood GSH level than female patients.
6. Bladder cancer patients from urban areas had lower levels of GSH than those patients from rural areas.

**REFERENCES:**

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