Ahmed F. F. Al-Baaj et al /J. Pharm. Sci. & Res. Vol. 10(10), 2018, 2578-2581

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Total Glutathione Levels Correlation with Arginase in Serum & Urine in CA Bladder Patients in Babylon Governorate

**Ahmed F. F. Al-Baaj*, Mufeed J. Ewadh, Mohammad R.J.Abood**
*College of Medicine, University of Babylon*

**Abstract:**

The present study aims to evaluate the levels of serum total glutathione as well as, its correlation with urinary arginase level in patients with CA bladder and healthy controls in the case-control study. Forty-five patients with CA bladder and forty five apparently healthy persons as a control in Babylon province /Iraq were enrolled in this study. Results of this study were shown significant decreases in serum total glutathione as well as significant increases in the urinary arginase levels in patients with CA bladder when compared with healthy control. Also, there were significant negative correlations of serum total glutathione and urinary arginase, In conclusions, present study suggests that patients with CA bladder in Babylon province have low serum total glutathione level comparing to normal subjects and this level decrease more as the cancer progress to advance stage.as regard its antioxidant and immune supporting properties, glutathione consumed during elimination of reactive oxygen species that produced by cancer cell. Also, the present study suggests that Increased level of urinary arginase is associated with increased production of polyamine and this will enhance cancer growth and development.

**Keywords:** CA, bladder, total glutathione, urinary arginase.

## INTRODUCTION

CA Bladder is a malignant overgrowth of the cells of the bladder(1). Most commonly, the growth occurs in cells that are in the urothelium, CA Bladder is the fourth most common type of cancer in men and the eighth most common in women (2)., CA Bladder is a heterogeneous disease, with 70% of cases presenting with shallow tumors, which head for recur but are generally not life-threatening, Also 30% of cases introducing as muscle-invasive tumors connected with a high risk of mortality from separate metastases. The main submitting symptom of all CA Bladder cases is painless hematuria(3).

Cancer, including CA Bladder, evolve because of alterations in the DNA of a normal cell. DNA can be damaged by chemical exposures such as cigarette smoke, industrial chemicals, chemotherapy, and so forth (4).

The occurrence of CA Bladder climbs with age, peaking Between age 50 years and 70 years, and is three times more common in men than in women (5). There are two main types of CA Bladder: primary and metastatic. Primary CA Bladder is those that start in the bladder itself. Metastatic cancers are those that originated in another organ and then spread to the bladder. based on the stage CA Bladder classified into three categories: non-invasive cancer, invasive cancer, and carcinoma in stiu (5,6).

Most risk factors of CA Bladder are : smoking, workers in the dye, rubber, leather, textiles, and paint trades (5), personal history of CA bladder, family history of CA Bladder(1), age (higher age), gender (male more than female), life-long bladder irritation and infections.(7)

Present study aims to determine the level of serum total Glutathione in serum of patients with CA Bladder during the course of follow up , Determine the level of arginase in urine of patients diagnosed with CA Bladder during the course of follow up and study the correlation between serum level of glutathione with the level of urinary arginase in serum of patients diagnosed with CA bladder first time and for follow up for recurrence in relation to the tumor stage .

## MATERIALS AND METHODS

### Ethical Issues

The present study was approved by the local ethics committee. All persons participated in this study was agreed to participate and signed an informed consent.

### Date and Duration

The period extended from October 2017 to April 2016. This work was done in the Department of Biochemistry, College of Medicine University of Babylon and Urology Department, Al-Hila Teaching Hospital in Hilla City, Iraq.

### Study Design

This study design was a case-control study.

### Patients and Control

A total of 45 patients with CA bladder were enrolled in this study. There were 33 males and 12 females divided into two groups according to the staging of CA Bladder (**G1** non-muscle invasive and **G2** muscle invasive). 45 subjects (23 males, 22 females) who were apparently healthy control group **CG**.

### Inclusion Criteria

All patients with bladder mass, diagnosed as a primary malignant urothelial tumor of the urinary bladder. diagnosed by cystoscopy, transurethral resection of bladder tumor and histopathology.

### Exclusion Criteria

Patients with other associated malignancy in the body. Patients with other immunological diseases such as Rheumatoid Arthritis. Secondary CA Bladder invasion from cervix, prostate, or intestine carcinoma.

### Determination of Serum Total Glutathione

Determination of serum total glutathione levels in patient and control group were done by use Mybiosource ELISA kits and according to manufacturer manual(8).

### Determination of urinary arginase

Determination of urinary arginase was done by Mybiosource ELISA kits and according to manufacturer manual(9).

## RESULTS AND DISCUSSIO

According to the histopathological study for biopsy taken from patients with CA Bladder participated in this study all cases of CA Bladder with transitional cell carcinoma with different pathological stages Table 2

In this study serum total glutathione was significantly lower in CA Bladder patients groups compared with normal patients CG (Control group) P-value < 0.001. In this study, the level of (tGSH) decreases more with increase the stage of cancer. Serum total glutathione was significantly lower in G2(muscle invasive bladder cancer) compared with G1 (non-muscle invasive bladder cancer) P-value < 0.001 Table 3.

In this study urinary arginase was significantly higher in CA Bladder patients groups compared with normal patients (CG) P value< 0.001.urinary arginase was significantly higher in G2(muscle invasive bladder cancer) when compared with G1 (non-muscle invasive bladder cancer) P-value < 0.05. Table 4.

The results of the current study were found the trend toward negative significant correlations between urinary arginase and serum tGSH in CA Bladder patients P-value < 0.01.

The mean age $\pm$SD of CA Bladder patients and Control were shown in Table 2.

**Table 1:- Number of the different stage among CA Bladder patients.**

| Stage of cancer | Gender | No. of Patients |
|---|---|---|
| G1 . Non-muscle invasive | Male | 15 |
| | Female | 7 |
| G2 .Muscle Invasive | Male | 18 |
| | Female | 5 |

**Table 2: means age ±SD of bladder cancer patients compared to control.**

| | Subjects | No. | Mean ± SD | Range |
|---|---|---|---|---|
| **Age (years)** | **G1** Non invasive | 22 | 57.7 ± 11.2 | 38-75 |
| | **G2** Muscle invasive | 23 | 63.4 ± 7.9 | 50-82 |
| | **CG** Control | 45 | 51.08 ± 8.4 | 39-65 |
| **P-value** | Non - invasive versus Control group ($p > 0.05$)<br>Muscle invasive versus Control group ($p > 0.05$)<br>Non–invasive versus Muscle invasive group ($p < 0.05$) | | | |

**Table 3: The mean ±SD of (tGSH) in CA Bladder patients compared to control group.**

| Parameter | Subjects | No. | Mean ± SD | Range |
|---|---|---|---|---|
| **Total Glutathione (µmol/L)** | **G1** Non invasive | 22 | 6.6±1.92 | 4.5-11.1 |
| | **G2** Muscle invasive | 32 | 2.8±0.89 | 1.4-4.1 |
| | **CG** Control | 45 | 12.1±2.09 | 9.2-17.8 |
| **P-value** | Non - invasive versus Control group ($P < 0.001$)<br>Muscle invasive versus Control group ($P < 0.001$)<br>Non–invasive versus Muscle invasive group ($P < 0.05$) | | | |

**Table 4: The mean ±SD of Urinary Arginase (Arg) in CA Bladder patients compared to control group.**

| Parameter | Subjects | No. | Mean ± SD | Range |
|---|---|---|---|---|
| **Urinary Arginase (ng/mL)** | **G1** Non invasive | 22 | 12.3±1.0 | 10.2-13.9 |
| | **G2** Muscle invasive | 23 | 20.5±2.0 | 17.2- 24.5 |
| | **CG** Control | 45 | 5.3±0.8 | 4.1-7.1 |
| **P-value** | **Non - invasive versus Control group ($P < 0.05$)**<br>**Muscle invasive versus Control group ($P < 0.001$)**<br>**Non–invasive versus Muscle invasive group ($P < 0.05$)** | | | |

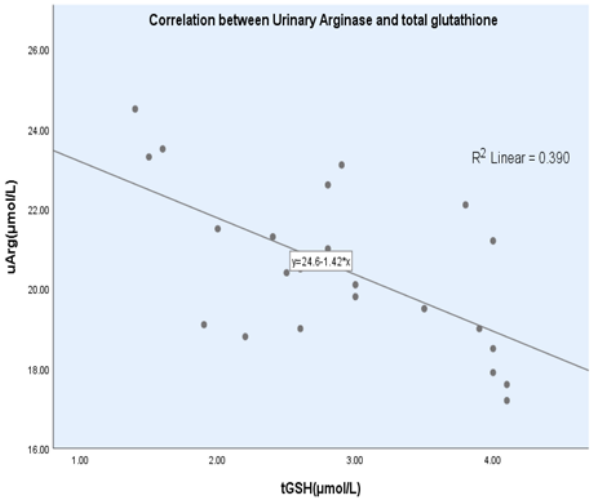


**Figure 1: Correlation between Urinary Arginase and tGSH in muscle invasive CA Bladder patients. (R2= 0.390,P value < 0.01).**

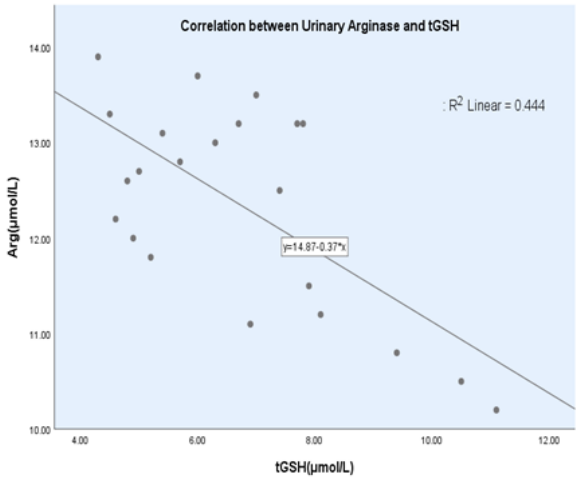


**Figure 4: Correlation between Urinary Arginase and tGSH in muscle non-invasive CA Bladder patients. (R2= 0.444,P value < 0.01).**

## DISCUSSION

The mean age of CA Bladder patients in the present study is younger than mean age of many previous studies such as Boustead GB, *et al* 2014. in United kingdoms (10) and Wyszynski A, *et al* 2014. in the United States(11), and even studies were done in neighboring countries such as Salehi A, *et al.* 2011. in Iran (12) and Abeer Abdalla El-Siddig, *et al.* 2017 in Saudi Arabia (13). This difference in patients mean age in the present study when compare to another study in other countries is related to the difference in environmental and regional conditions such as pollution, the effect of multiple wars (ex: Uranium), and direct exposure to risk factors such as heavy metal. Gender disparity in the incidence of CA bladder in this study back to hormonal differences between male and female which suggest that estrogen have a protective role in females(14,15,16) while androgen increase risk for CA Bladder (17, 18,19).

According to the history of CA Bladder patients participated in this study all 33 males' patients and 4 female patients was a heavy smoker for a long time. Cigarette smoking is the most well-established risk factors for CA Bladder (20,5).

Most patients with CA Bladder participated in this study is a heavy smoker and this goes with many studies that indicate the association between smoking and CA bladder. Smoking causes accumulation of toxic substances and increase production of reactive oxygen species (ROS) and produce oxidative stress (21). Glutathione is essential in our oxidative stress defense mechanism because of its ability to scavenge free radicals(22). Glutathione is consumed in reactions that protect the cell by removing the deleterious compound, Alternatively, Glutathione is oxidized to GSSG and/or GSSR, which are exported out of the cells, and subsequently taken up and degraded by the kidney (23). In this study the mean level of total glutathione (tGSH).Decrease level of total glutathione in CA Bladder patients is due to continuous consumption of glutathione by a tumor cell and in cancer state, there are oxidative stress and accumulation of free radical such as ROS(24).

In this study extremely low level of total glutathione in muscle-invasive CA Bladder go with the idea that says increase production of ROS In conjunction to increase the stage and grade of bladder cancer the matter that will extremely increase the consumption of total glutathione to neutralize the ROS and other free radicals. Glutathione plays essential roles in T cell function and proliferation [25,26,27]. Tamas Fulop, *et al* 2014 (28) reported that Activation of T helper cell results in decreased levels of glutathione (GSH) and production of ROS. In CA Bladder patients muscle invasive there is a demand to increase activation of T helper cell to defeat tumor cell, that may contribute the consumption of total glutathione pool in the serum of CA Bladder patients.

In this study urinary arginase was significantly higher in CA Bladder patients groups compared with normal patients (CG) P value< 0.001. urinary arginase was significantly higher in G2(muscle invasive bladder cancer) when compared with G1 (non-muscle invasive bladder cancer) P-value < 0.05. Arginase competes with nitric oxide synthase (NOS) for L-arginine(29). NO, consider a very important product that has a major role in many physiological and pathological processes such as dilates blood vessels and raising blood supply, lowering blood pressure Conversely, it helps protect tissues from damage due to low blood supply(30).

Arg can shift the metabolism of L-arginine in smooth muscle cells from nitric oxide to L-ornithine and the production of polyamines. Polyamine (which includes putrescine, spermidine, and spermine) concentrations are often increased in the blood of cancer patients(31).which can stimulate vascular lesion formation by stimulating smooth muscle cell proliferation and collagen deposition(32).

In this study the level of urinary arginase increase with increase the stage of cancer .as the cancer cell increase in size and invading more surrounding tissue in the bladder (converting from non-muscle invasive to muscle invasive), this will increase the cell degradation and release more protein molecule in the urine such as arginase. Therefore, this enzyme might serve as a useful urinary biological marker for CA Bladder while also being an indicator of cancer progression. These result support report from the previous study also found that arginase level in the different type of cancers increases as the tumor stage increase (33).

The results of the current study were found the trend toward negative significant correlations between urinary arginase and tGSH CA Bladder patients P-value < 0.01. This strong negative correlation can explain as follow: while uArg level increasing in growing CA Bladder cell more glutathione molecule consume to remove free radical produced by growing cancer cell. Muscle-invasive CA Bladder patients have more elevated Arg level . cancer cell characterized by high activity, metastasis, and increased production of free radical(34), and that will lead to extreme consumption of glutathione to near depletion level which observed in this study.

## CONCLUSIONS

CA bladder patients in Babylon province have low total glutathione level comparing to normal subjects and this level decrease more as the cancer progress to advance stage.as regard to glutathione antioxidant and immune supporting properties, glutathione consumed during elimination of ROS that produced by a cancer cell. Also, CA bladder patients in Babylon province have high urinary arginase level comparing to normal subjects and this level increase more as the cancer progress to advance stage.

Increased level of arginase is associated with increased production of polyamine and this will enhance cancer growth and development, Especially with the muscle-invasive stage. Total glutathione may serve as a biomarker in monitoring the antioxidant status in CA bladder patient and may aid the physician to treat those patients and to prescribe them the drug of choice. There were negative significant correlations between urinary arginase level and total glutathione level in CA bladder patients.

## ACKNOWLEDGMENTS

Authors would like to thank the staff of the Biochemistry Department at College of Medicine, University of Babylon, Iraq, for their efforts and facilities to complete the task of this study. Also, authors would like to thank the staff of Urology Department at Al-Hila Teaching Hospital, Hilla city, Iraq, for their assistance in the collection of samples.

## REFERENCES

1. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. European Urology. 2013, 63(2), 234-41.
2. Lin, F., & Prichard, J. (Eds.). Handbook of practical immunohistochemistry: frequently asked questions. Springer. 2015.
3. Westhoff E, Witjes JA, Fleshner NE, Lerner SP, Shariat SF, Steineck G, Kampman E, Kiemeney LA, Vrieling A. Body Mass Index, Diet-Related Factors, and Bladder Cancer Prognosis: A Systematic Review and Meta-Analysis. Bladder Cancer. 2018, 4(1), 91-112.4. Wolff K, Johnson R., Saavedra AP. Disorders of Hair Follicles and Related Disorders. Fitzpatrick Color Atlas and Synopsis of Clinical Dermatology. 2013.
4. Chen M, Rothman N, Ye Y, Gu J, Scheet PA, Huang M, Chang DW, Dinney CP, Silverman DT, Figueroa JD, Chanock SJ. Pathway analysis of bladder cancer genome-wide association study identifies novel pathways involved in bladder cancer development. Genes & cancer. 2016, (7-8), 229.

5. Wein, A. J., Kavoussi, L. R., Novick, A. C., Partin, A. W., & Peters, C. A. (2011). Campbell-Walsh Urology: Expert Consult Premium Edition: Enhanced Online Features and Print, 4-Volume Set. Elsevier Health Sciences.6. Richard P.J.B. WellerJohn A.A. Hunter JAS. Clinical Dermatology. 2008.
6. HMa B, Dorin RP, Rubino B, Miranda G, Cai J, Daneshmand S, Skinner EC. Critical evaluation of the American Joint Committee on Cancer TNM nodal staging system in patients with lymph node–positive disease after radical cystectomy. PDF hosted at the Radboud Repository of the Radboud University Nijmegen. 2015, 62(4), 157.
7. Mundy, A. R., Fitzpatrick, J., Neal, D. E., & George, N. J. (Eds.). (2010). The scientific basis of urology. CRC Press(19).8. William J. Streur, Ward B. Hurlburt SB. State of Alaska Measuring Height/Weight and Calculating BMI. 2012.
8. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Analytical biochemistry. 1969, 27(3), 502-22.
9. Wang, S. R., Chen, M. L., Huang, M. H., Lin, H. Y., Tsai, J. J., & Kuo, B. I. T. Plasma arginase concentration measured by an enzyme-linked immunosorbent assay (ELISA) in normal adult population. Clinical biochemistry, 1993, 26(6), 455-460.
10. Boustead GB, Fowler S, Swamy R, Kocklebergh R, Hounsome L. Stage, grade and pathological characteristics of bladder cancer in the UK: British Association of Urological Surgeons (BAUS) urological tumour registry. BJU international. 2014, 113(6), 924-30.
11. Wyszynski A, Tanyos SA, Rees JR, Marsit CJ, Kelsey KT, Schned AR, Pendleton EM, Celaya MO, Zens MS, Karagas MR, Andrew AS. Body mass and smoking are modifiable risk factors for recurrent bladder cancer. Cancer. 2014, 120(3), 408-14.
12. Salehi A, Khezri AA, Malekmakan L, Aminsharifi A. Epidemiologic status of bladder cancer in Shiraz, southern Iran. Asian Pac J Cancer Prev. 2011, 12(5), 1323-7.
13. Alhujaily AS, El-Siddig AA, Albasri AM, Hussainy AS. Urinary bladder cancer in adults: a histopathological experience from Madinah, Saudi Arabia.
14. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. European urology. 2017, 71(1), 96-108.
15. Bhattacharya A, Klaene JJ, Li Y, Paonessa JD, Stablewski AB, Vouros P, Zhang Y. The inverse relationship between bladder and liver in 4-aminobiphenyl-induced DNA damage. Oncotarget. 2015, 6(2), 836.
16. Al-Shakour AA, Ajeel NA, Al-Naama ML. Smoking and urinary bladder cancer: A case-control study in Basrah. The Medical Journal of Basrah University. 2014, 32(1), 1-7.
17. Godoy G, Gakis G, Smith CL, Fahmy O. Effects of androgen and estrogen receptor signaling pathways on bladder cancer initiation and progression. Bladder Cancer. 2016, 2(2), 127-37.
18. McBeth L, Grabnar M, Selman S, Hinds TD. Involvement of the androgen and glucocorticoid receptors in bladder cancer. International journal of endocrinology. 2015.
19. Li P, Chen J, Miyamoto H. Androgen Receptor Signaling in Bladder Cancer. Cancers. 2017, 9(2), 20.
20. Smith, D. R., Tanagho, E. A., & McAninch, J. W. Smith's general urology. Lange Medical Books/McGraw-Hill,. 2013.
21. Goel R, Bitzer Z, Reilly S, Trushin N, Reinhart L, Elias R, Richie JP. Tobacco Smoke Free Radicals and Related Biomarkers of Oxidative Stress. Free Radical Biology and Medicine. 2017, 112, 130-1.
22. Chang HP, Sheen LY, Lei YP. The protective role of carotenoids and polyphenols in patients with head and neck cancer. Journal of the Chinese Medical Association. 2015, 78(2), 89-95.
23. Hecht F, Pessoa CF, Gentile LB, Rosenthal D, Carvalho DP, Fortunato RS. The role of oxidative stress on breast cancer development and therapy. Tumor Biology. 2016, 37(4), 4281-91.
24. Gecit I, Aslan M, Gunes M, Pirincci N, Esen R, Demir H, Ceylan K. Serum prolidase activity, oxidative stress, and nitric oxide levels in patients with bladder cancer. Journal of cancer research and clinical oncology. 2012, 138(5), 739-43.
25. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nature Reviews Immunology. 2013, 13(5), 349.
26. Levring TB, Kongsbak M, Rode AK, Woetmann A, Ødum N, Bonefeld CM, Geisler C. Human CD4+ T cells require exogenous cystine for glutathione and DNA synthesis. Oncotarget. 2015, 6(26), 21853.
27. Yin B, Barrionuevo G, Weber SG. Optimized Real-Time Monitoring of Glutathione Redox Status in Single Pyramidal Neurons in Organotypic Hippocampal Slices during Oxygen–Glucose Deprivation and Reperfusion. ACS chemical neuroscience. 2015, 6(11), 1838-48.
28. Fulop T, Le Page A, Fortin C, Witkowski JM, Dupuis G, Larbi A. Cellular signaling in the aging immune system. Current opinion in immunology. 2014, 29, 105-11.
29. Wu G, Morris Jr SM. Arginine metabolism: nitric oxide and beyond. Biochemical Journal. 1998, 336(Pt 1), 1.
30. van Faassen EE, Bahrami S, Feelisch M, Hogg N, Kelm M, Kim-Shapiro DB, Kozlov AV, Li H, Lundberg JO, Mason R, Nohl H. Nitrite as regulator of hypoxic signaling in mammalian physiology. Medicinal research reviews. 2009, 29(5), 683-741.
31. Avtandilyan NV, Karapetyan SA, Alexanyan KA. The change of arginase activity in blood serum during breast and prostate cancer. Biochemistry. 2016, 1, 26..
32. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clinical and Experimental Pharmacology and Physiology. 2007, 34(9), 906-11.
33. PEREZ, Gabriel, et al. Arginase activity in patients with breast cancer: an analysis of plasma, tumors, and its relationship with the presence of the estrogen receptor. Oncology Research and Treatment, 2012, 35.10, 570-574.
34. Narayan, V. M., Adejoro, O., Schwartz, I., Ziegelmann, M., Elliott, S., & Konety, B. R. (2017). The Prevalence and Impact of Urinary Marker Testing in Patients with Bladder Cancer. The Journal of Urology.